



Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Relict Leopard Frog Monitoring and Management

2013 Annual Report



April 2014

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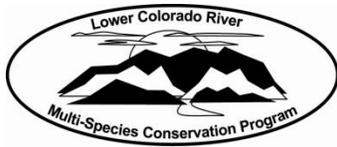
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Lower Colorado River Multi-Species Conservation Program

Relict Leopard Frog Monitoring and Management

2013 Annual Report

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¹ Under an agreement with the Lake Mead National Recreation Area, National Park Service, and Nevada Department of Wildlife.

Lower Colorado River
Multi-Species Conservation Program
Bureau of Reclamation
Lower Colorado Region
Boulder City, Nevada
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ACRONYMS AND ABBREVIATIONS

<i>Bd</i>	<i>Batrachochytrium dendrobatidis</i> (a pathogenic fungus)
BLM	Bureau of Land Management
CAS	conservation agreement and strategy
Lake Mead NRA	Lake Mead National Recreation Area
NDOW	Nevada Department of Wildlife
NPS	National Park Service
Reclamation	Bureau of Reclamation
RLFCT	Relict Leopard Frog Conservation Team
SBD	swim bladder disorder
T ^A	ambient air temperature
UNLV	University of Nevada, Las Vegas
VES	visual encounter survey

Symbols

°C	degrees Celsius
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EXECUTIVE SUMMARY

This report summarizes efforts under a project designed to implement monitoring and translocation actions described in the voluntary conservation agreement and strategy (CAS) (Relict Leopard Frog Conservation Team [RLFCT] 2005) for the relict leopard frog (*Rana onca* = *Lithobates onca*). The intent of the CAS is to increase both overall abundance as well as the number of separate populations of the species 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 were conducted at all natural and experimental sites known to contain the relict leopard frog. 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 the objectives determined by the RLFCT. Assistance was provided to agency partners to identify potential translocation sites and to conduct associated conservation actions, including assisting with the coordination of the RLFCT meetings. The following information summarizes observations made during monitoring surveys and results from the headstarting and translocation actions in 2013. Other conservation actions are also noted when appropriate.

- Surveys were completed at all natural and active experimental sites, with multiple surveys associated with a mark-recapture project at one site.
- The high count of the relict leopard frog was larger this spring than last year; however, the high count in autumn was quite low, predominately because of a lack of frogs observed at Tassi Spring.
- Overwintering was documented for the first time at Perkins Pond, Bearpaw Poppy Spring, Horse Spring, and Lime Spring. Breeding activity was observed at Horse Spring.
- Eggs and hatchlings were collected for headstarting from Upper Blue Point Spring and from two sites within Black Canyon.
- A total of 275 headstarted animals from Black Canyon were released at Goldstrike Canyon, Lime Spring, Red Rock Spring, and Union Pass Spring. A total of 444 headstarted animals from Upper Blue Point Spring were released into Perkins Pond, Bearpaw Poppy Spring, Horse Spring, and back to Upper Blue Point Spring. An additional 60 animals were transferred to the University of Nevada, Las Vegas (UNLV), for a *Batrachochytrium dendrobatidis* (*Bd*) challenge study.

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- Small-scale vegetation reductions were conducted at Boy Scout Canyon and both Upper and Lower Blue Point Springs. The Bureau of Land Management conducted habitat efforts at Horse Spring, and the Nevada Department of Wildlife conducted habitat efforts at Perkins Pond.
- A site visit was conducted at Chill Heal Spring to assess the site for possible translocation.
- Sampling for *Bd* was continued at several sites.
- A study to assess the impact of *Bd* on the leopard frog was initiated by UNLV.

INTRODUCTION

Background

The relict leopard frog (*Rana onca* [= *Lithobates onca*]) appears to be a narrowly distributed endemic (Jaeger et al. 2001; Oláh-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), and taxonomic confusion once led to the declaration that the relict leopard frog was extinct (Jaeger et al. 2001). Natural populations of these frogs now occupy only a few spring sites within two general areas of southern Nevada within the Lake Mead National Recreation Area (Lake Mead NRA). Translocation efforts have established new populations at additional sites (see below).

Conservation efforts for this species began in earnest in the early 1990s, as additional information on population dynamics and distribution was being gathered, including phylogenetic studies. The first interagency meeting focused on the relict leopard frog 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. Listing was considered warranted but 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. Despite the success of some conservation efforts, the relict leopard frog remains imperiled.

The information contained herein represents a summary of management, monitoring, and conservation actions implemented by the National Park Service (NPS) and Nevada Department of Wildlife (NDOW) toward meeting objectives outlined in the CAS. This document represents a final report for field efforts during 2013. Major efforts under this project were performed under the task agreement by personnel at the School of Life Sciences, University of Nevada, Las Vegas (UNLV). Jef Jaeger, at UNLV, was the principle investigator in collaboration with Ross Haley at the Lake Mead NRA and Jon Sjöberg at the NDOW. Other actions conducted by cooperating agencies, such as the Bureau of Land Management (BLM) and the Bureau of Reclamation (Reclamation), are also summarized when appropriate.

Goal and Objectives

The main goal of the project was the conservation of existing relict leopard frog 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 managers with translocations to these new sites

Reporting Format

The “Results and Discussion” section presented in this document follows a reporting format stipulated by the RLFCT. The format is intended to provide meaningful summaries of actions conducted at each site for seasonal reviews by team members.

METHODS

The methods implemented in this project are specified in the Relict Leopard Frog Protocol and Techniques Manual included in the CAS (RLFCT 2005). The protocols and techniques detail the various procedures used for 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 (VESs) were conducted at all natural and experimental sites known to contain the relict leopard frog. Surveys were conducted in early spring and again in autumn. All frogs and egg masses observed were counted, but tadpole numbers represented estimates up to 300, after which a plus sign was 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. Nocturnal surveys conducted during spring and autumn were used to better assess numbers of adult frogs; these frogs are more readily observed at night using spotlights when they can be seen in less densely vegetated patches.

Headstarting and Translocations

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, were determined during meetings of the RLFCT. Eggs were processed in a laboratory facility maintained by the Lake Mead NRA. Tadpoles were grown out at this facility as well as at the Willow Beach National Fish Hatchery, maintained by the U.S. Fish and Wildlife Service, and the Lake Mead State Hatchery, maintained by the NDOW. Eggs were collected in the wild during February and released as late-stage tadpoles or juvenile frogs from mid-April through June and again in October.

Other Activities

This report includes references to provisional mark-recapture results for population estimates at some targeted sites (Jef R. Jaeger, unpublished data). The mark-recapture efforts were conducted under a separate agreement and funding; provisional results are provided in discussion for context. Testing for the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*), the causal agent of chytridiomycosis (Lips et al. 2006), was also conducted earlier in the season as part of a regional study mostly funded by the NDOW; this report contains summary information of the results pertaining to relict leopard frog populations sampled. Some habitat maintenance activities were also performed or facilitated at important breeding pools, and these efforts are noted along with the habitat activities conducted by collaborating entities.

STUDY AREAS

Eight sites containing historical relict leopard frogs were monitored during 2013. Sites are defined for monitoring purposes and recordkeeping but do not necessarily represent separate demographic units. The historical sites occur in two general areas of southern Nevada, in Black Canyon below Hoover Dam, and in the Northshore Springs Complex along the edge of the Muddy Mountains. In addition, 11 experimental translocation sites in Nevada and northwestern Arizona were active in 2013 (figure 1).

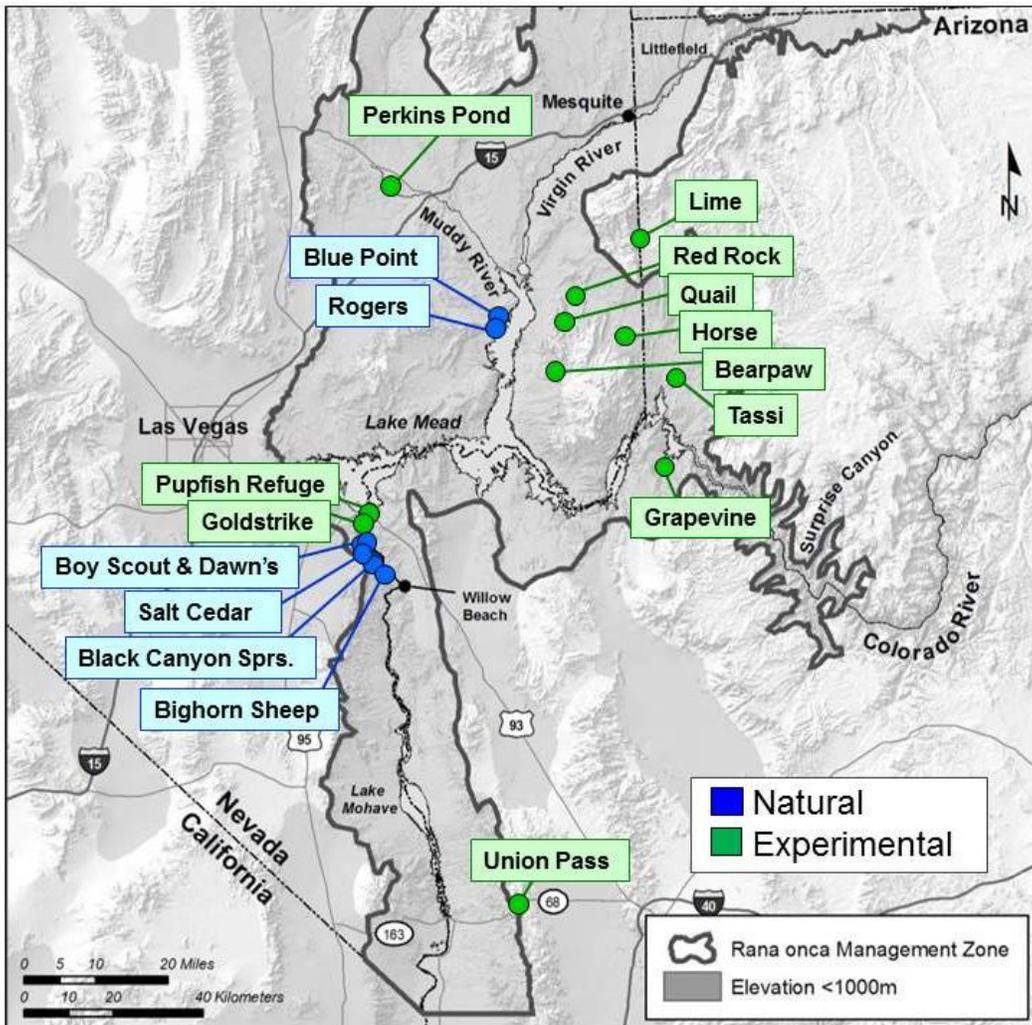


Figure 1.—Locations of sites containing historical populations of the relict leopard frog (in blue) and sites containing active experimental translocation sites (in green).

The potential management zone for the species as identified in the CAS (RLFCT 2005) is indicated, although the area identified in western Grand Canyon may not be a viable region for translocations because of the documented presence of a closely related leopard frog species.

RESULTS AND DISCUSSION

The total number of adult and juvenile relict leopard frogs seen during VESs at sites represents a minimum count for the entire population. The highest seasonal count in 2013 was 745 frogs during the spring survey (table 1). This count represents a minimum estimate, as not all individuals were detected during the survey period. The count in autumn was quite low and resulted predominately from a lack of frogs observed at Tassi Spring. The disappearance of frogs at this site in autumn has occurred before, and subsequent surveys the following year indicated that the population had not actually declined.

Table 1.—Summary of the maximum number of adult and juvenile relict leopard frogs seen during visual encounter surveys at sites in 2013, with results from 2012 presented for reference

(At some sites [indicated by asterisks], multiple mark-recapture surveys were conducted, and the number presented represents the highest count from these surveys.)

Site type	Site	Spring 2012	Autumn 2012	Spring 2013	Autumn 2013
Natural	Bighorn Sheep Spring	11	71*	29	17
	Boy Scout Canyon	34	37	49	31
	Dawn's Canyon	6	4	6	3
	Black Canyon Springs	22	30	18	19
	Salt Cedar Canyon Spring	13	13	21	39
	Upper Blue Point Spring	26*	22*	14*	11*
	Lower Blue Point Spring	12	7	11	19
	Rogers Spring	14	11	18	11
Experimental	Bearpaw Poppy Spring	Intro.	11	20	35
	Goldstrike Canyon	30	15	26	15
	Grapevine Spring, Arizona	189*	94	125	88
	Horse Spring	Intro.	2	17	11
	Lime Spring	Intro.	6	5	6
	Pupfish Refuge Spring	47*	29	39	24
	Perkins Pond	0	4	2	1
	Quail Spring	114	121	154	91
	Red Rock Spring	15*	10	7	3
	Tassi Spring	122	116	128	1
	Union Pass Spring	34	37	56	73
Totals		689	640	745	498

Monitoring of Natural Sites

Bighorn Sheep Spring, Nevada

High recruitment has been noted over the last couple years from artificial pools created in 2011 to mitigate storm damage in 2006; however, these pools washed out before the 2012 autumn surveys. The high number of juveniles noted in spring 2013 (table 2) likely can be attributed to those pools. During the April survey, 10 smaller relict leopard frogs were captured to measure snout to vent length. These animals measured from 38 to 44 millimeters, and the larger animals were likely to have overwintered as juveniles. Two partial egg masses were collected for headstarting during the survey. During the autumn survey, surface water flow appeared to have decreased.

Table 2.—Summary of relict leopard frogs observed at Bighorn Sheep Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}\text{C}$] during the survey)

Survey type	Date	T^A ($^{\circ}\text{C}$)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/14/2013	23.7	0	1	> 200	5
Nocturnal	04/12/2013	24.6	18	11	28 ^a	0
Nocturnal	10/21/2013	22.9	15	2	2	0

^a Observed 16 large tadpoles.

Boy Scout Canyon Spring, Nevada

Observations at this site (table 3) were similar to counts over recent years. Breeding again was observed in a side channel located low in the system as well as at the highest breeding pools. During the nocturnal spring survey, about a third of the frogs observed appeared to be smaller adults, with the majority of these animals seen near the base of the canyon. Most likely, these frogs were recruited from the side channel. Recently metamorphosed frogs were observed during the autumn nocturnal survey. In March, some minor habitat maintenance was done by UNLV personnel at important breeding pools. Vegetation and silt were cleared from known breeding pools to maintain waterflow and quality. Calling by the relict leopard frog was heard during that time.

Table 3.—Summary of relict leopard frogs observed at Boy Scout Canyon Spring during visual encounter surveys conducted in 2013

(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}C$] during the survey)

Survey type	Date	T^A ($^{\circ}C$)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/14/2013	15.0	3	0	> 100	0
Diurnal	03/01/2013	24.2	12	0	5 ^a	0
Nocturnal	04/19/2013	21.6	44	5 ^b	> 100 ^c	0
Nocturnal	10/11/2013	24.0	29	2	0	0

^a Two of these were large tadpoles.

^b One was a metamorph.

^c Observed 31 large tadpoles.

Dawn's Canyon Spring, Nevada

The number of frogs counted during the nocturnal survey this year (table 4) is consistent with numbers observed over previous years. No egg masses were seen, but hatchlings and older tadpoles were observed at the plunge pool located at the end of the survey area. During the April survey, surveyors climbed 25 meters past the plunge pool (usual survey stopping point) and saw a small frog.

Table 4.—Summary of relict leopard frogs observed at Dawn's Canyon Spring during visual encounter surveys conducted in 2013

(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}C$] during the survey)

Survey type	Date	T^A ($^{\circ}C$)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/14/2013	15.6	2	0	28	0
Nocturnal	04/19/2013	21.7	6	0	5 ^a	0
Nocturnal	10/11/2013	23.0	2	1 ^b	1 ^c	0

^a Observed three larger tadpoles.

^b Metamorph.

^c Large tadpole.

Black Canyon Spring and Black Canyon Side Spring, Nevada

These two areas represent components of the same system, although they are treated as separate sites for reporting. This year, several adult relict leopard frogs were observed along the main channel (table 5) near the confluence with the drainage leading to the side spring (in general, few animals are recorded along the main stream). The relict leopard frog likely exists at sites above the large waterfall that is at the end of the survey area along the main stream. At Black Canyon Side Spring, vegetation had increased since the last visit. All life stages were again observed in Black Canyon Side Spring (table 5), and in spring, hatchlings (still clinging to the egg mass) were collected for headstarting.

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Table 5.—Summary of relict leopard frogs observed at Black Canyon Spring (main channel) and Black Canyon Side Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}C$] during the survey)

Site	Survey type	Date	T^A ($^{\circ}C$)	Adult	Juvenile	Larvae	Egg masses
Main stream	Diurnal	02/22/2013	14.4	0	0	0	0
	Nocturnal	04/27/2013	25.5	1	0	0	0
	Nocturnal	10/11/2013	24.0	6	0	0	0
Side spring	Diurnal	02/16/2013	18.1	0	0	10	1
	Diurnal	02/22/2013	14.4	1	0	13	1
	Nocturnal	04/27/2013	24.7	16	1	21 ^a	0
	Nocturnal	10/11/2013	19.7	10	3	0	0

^a Mostly large tadpoles.

Salt Cedar Canyon Spring, Nevada

This year, the autumn survey (table 6) provided the second highest count of relict leopard frogs observed since monitoring began at this site. Most of the adults observed were larger animals, thus not likely recent recruits. Minor flooding had occurred at the site sometime before the early season surveys, but later, observers noted that vegetation had grown in densely, affecting visibility. During a diurnal survey, observers went approximately 60 meters further up the drainage beyond the usual ending point and saw one adult relict leopard frog. There is an animal trail that can be used to avoid the dense vegetation at the usual end point, but there is also some tricky rock climbing required. During the April survey, one adult frog was seen low in the system in the channel near the river, and interestingly, during the autumn survey, an adult frog was spotted jumping into the Colorado River.

Table 6.—Summary of relict leopard frogs observed at Salt Cedar Canyon Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}C$] during the survey)

Survey type	Date	T^A ($^{\circ}C$)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/16/2013	20.2	0	0	4	0
Diurnal	02/22/2013	18.5	2	0	6 ^a	0
Nocturnal	04/27/2013	25.5	18	3 ^b	8 ^c	0
Nocturnal	10/11/2013	19.3	33	6	0	0

^a Observed one overwintering tadpole.

^b Metamorphs observed.

^c All larger tadpoles.

Upper and Lower Blue Point Springs, Nevada

Surveys at Upper Blue Point Spring were conducted as part of a mark-recapture estimation effort, and observations from these surveys are reported herein as VESs (table 7). Most of the frogs observed at this site were likely the individuals released to the site as part of augmentation efforts in 2008, 2010, and 2013; a total of 10 juveniles were released this year in June (see table 21). This spring, a large relict leopard frog tadpole was found in the main stream. Calling was heard multiple times in February and March. Young animals (some possibly naturally recruited) were noted on several occasions during both seasons.

Table 7.—Summary of relict leopard frogs observed at Upper and Lower Blue Point Springs during visual encounter surveys conducted in 2013

(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}\text{C}$] during the survey)

Site	Survey type	Date	T^A ($^{\circ}\text{C}$)	Adult	Juvenile	Larvae	Egg masses
Upper	Diurnal	02/05/2013	17.5	0	0	0	1
	Diurnal	02/05/2013	22.6	1	0	1 ^a	1
	Diurnal	02/06/2013	17.9	0	0	0	0
	Diurnal	02/13/2013	17.5	0	0	0	0
	Diurnal	02/21/2013	18.5	0	0	25 ^a	0
	Diurnal	03/07/2013	24.7	3	0	0	0
	Diurnal	03/12/2013	15.1	4	0	1 ^a	0
	Nocturnal	03/22/2013	13.1	12	0	4	0
	Nocturnal	03/25/2013	20.0	14	0	30	0
	Nocturnal	03/30/2013	16.0	14	0	1	0
	Nocturnal	04/10/2013	13.8	10	1	0	0
	Nocturnal	04/21/2013	23.9	5	0	2	0
	Nocturnal	05/27/2013	22.9	8	0	4	0
	Diurnal	06/26/2013	41.7	3	0	1	0
	Nocturnal	09/27/2013	20.0	8	0	0	0
	Nocturnal	10/17/2013	15.0	6	0	0	0
	Nocturnal	10/22/2013	15.8	11	0	0	0
Nocturnal	11/01/2013	15.7	7	1	0	0	
Lower	Diurnal	02/06/2013	25.5	0	0	0	0
	Diurnal	02/13/2013	22.5	1	0	0	0
	Diurnal	02/21/2013	17.2	0	0	50	0
	Diurnal	03/07/2013	23.2	0	0	23 ^b	1
	Diurnal	03/12/2013	25.2	3	0	22	0
	Nocturnal	03/22/2013	14.5	11	0	0	0
	Nocturnal	10/17/2013	8.8	16	3	0	0

^a Species uncertain; later confirmed to be the relict leopard frog.

^b Four observed were of uncertain species.

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Efforts to maintain open areas along the spring were conducted by personnel from UNLV and the NDOW and were concentrated on reducing vegetation at existing artificial pools. Approximately 51 and 46 linear meters of vegetation were cut in early February and mid-November, respectively. Late in spring, burro grazing was noted on new shoots in areas where vegetation reductions had occurred. Prior to vegetation reductions, surveys were conducted for egg masses. In February, one egg mass was found (in the lower artificial pool). The egg mass was collected for headstarting and hatched successfully in the laboratory. Afterwards, multiple site visits were conducted, searching for additional eggs, but none were found. Young tadpoles, however, were seen.

At Lower Blue Point Spring, habitat efforts focused on rehabilitating the large, artificial fish-free pond. The pond was cleared of some cattails, and the flow re-established, but silt built up quickly, covering the openings on the intake pipe and decreasing waterflow. Regular clearance of the pipe would be needed to keep an adequate waterflow to the pond. By autumn, vegetation surrounding the fish-free pond was tall and dense, but the center of the pond remained clear. At that time, silt was again cleared from the intake pipe to increase waterflow.

Multiple surveys were conducted at Lower Blue Point Spring in search of egg masses. While no egg masses were seen within the fish-free pond, a cohort of young relict leopard frog tadpoles were observed over multiple surveys of the pond. An egg mass was located along the main channel in an area where there was heavy evidence of burro activity. Swabbing for *Bd* occurred again at this site, with 11 frogs sampled. As in recent years, *Bd* was again detected.

Rogers Spring, Nevada

Habitat conditions at this site remain relatively poor, with dense vegetation covering the vast majority of the system (mostly sawgrass and *Phragmites*). As in the recent past, frogs were only observed at, or just above, the powerline road where some open habitat remained. There appears to be an increasing trend in frogs seen over recent years (table 8), which likely results from augmentations. There was, however, evidence of breeding again this season. Calling by the relict leopard frog was heard during the diurnal survey.

Table 8.—Summary of relict leopard frogs observed at Rogers Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/07/2013	25.5	0	0	35	1
Nocturnal	04/04/2013	27.4	18	0	0	0
Nocturnal	10/30/2013	11.8	11	0	0	0

Vegetation along the south fork near the powerline road was well grazed by burros (and possibly cows) during spring. An egg mass was observed in this area in February, but vegetation had grown noticeably denser by the autumn survey. No tadpoles or juveniles were seen in autumn.

Monitoring of Experimental Translocation Sites

Goldstrike Canyon, Nevada

The number of frogs observed this year is similar to counts from last year (see table 1). All life stages of relict leopard frogs were observed prior to augmentation (table 9). During the April survey, smaller adults were observed, and four of these frogs were caught to take snout to vent length measurements. The frogs measured from 46 to 50 millimeters. In June, a naturally recruited juvenile was observed.

Table 9.—Summary of relict leopard frogs observed at Goldstrike Canyon during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T^A (°C)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/19/2013	18.2	3	0	0	5
Nocturnal	04/22/2013	27.8	26 ^a	0	> 100 ^b	0
Diurnal	06/13/2013	33.1	1	1	4	0
Diurnal	06/28/2013	38.5	1	0	0	0
Nocturnal	10/07/2013	25.3	15	0	5 ^c	0

^a Five of these were smaller adults.

^b Observed eight large tadpoles.

^c Large tadpoles.

Augmentation was conducted this year, even though the site was off the augmentation list because frogs had been released at the site for 5 years previously. The augmentation was used to release excess headstarted Black Canyon animals (see table 21). In June, 83 frogs and 5 tadpoles were released. Surprisingly, the autumn nocturnal survey numbers were quite low given the releases. Only one small adult was seen at the time. An additional 23 frogs were released following the autumn survey.

During the autumn survey, it was noted that the water level had decreased in an important breeding pool located low in the system where larger tadpoles have often been observed. During the survey, tadpoles were observed in the now smaller pool, and the water level appeared sufficient to allow successful metamorphosis of these animals. The structural change in this pool, however, may limit recruitment in the future.

Grapevine Spring (Meadview), Arizona

Surveys associated with mark-recapture efforts last year resulted in higher counts at this site than those normally returned for VES. Counts this year (table 10) were more consistent with results from earlier years. Vegetation has also been increasing since the last major flooding event in November 2010, hindering visibility during surveys. All life stages were observed this year and calling was heard during the March survey. Also during the March survey, 24 *R. onca* were swabbed for *Bd*; all the animals tested negative for the fungus.

Table 10.—Summary of relict leopard frogs observed at Grapevine Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Nocturnal	03/14/2013	18.5	51	0	6 ^a	12
Nocturnal	05/03/2013	19.4	124	1	> 110 ^b	1
Nocturnal	10/04/2013	18.2	85	3	9 ^a	0

^a All large tadpoles.

^b Ten of these were larger tadpoles; the rest were of uncertain identification.

Pupfish Refuge Spring, Nevada

This site continues to maintain a robust population of frogs, and as usual, all life stages of the relict leopard frog were observed (table 11). Egg masses and overwintered tadpoles were again seen in pools along the drainage ditch. Calling was heard by the species during the nocturnal survey in spring. Two dead, adult frogs were seen in the lower stretch of the drainage ditch, one during each of the spring surveys. Neither of these dead animals showed any clear indication of physical trauma, and the causes of death were unknown.

Table 11.—Summary of relict leopard frogs observed at Pupfish Refuge Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/25/2013	17.6	0	0	> 1,000 ^a	0
Nocturnal	03/28/2013	23.7	39	0	> 300 ^b	4
Nocturnal	10/25/2013	25.9	23	1	1	0

^a Observed six large, overwintered tadpoles.

^b Observed 10 overwintered tadpoles.

Quail Spring, Nevada

Translocation to this small spring ended last year with the release of some juvenile frogs. All life stages of the relict leopard frog were seen again during the spring surveys, including a metamorph and many overwintered tadpoles within the main pool (table 12). Calling by the species was heard during each of the surveys. Natural recruitment at this site, however, cannot yet be confirmed because of recent augmentations.

Vegetation along the edge of the main pool has been well maintained by cattle, leaving the center of the pool dense with cattails. During the autumn survey, however, the cattails were reduced to two small clumps, and the banks were heavily trampled. Also noteworthy was the unusually low amount of insect activity, which could be the reason why some frogs appeared skinny at that time.

Table 12.—Summary of relict leopard frogs observed at Quail Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/10/2013	15.9	2	0	0	1
Nocturnal	04/28/2013	21.0	143	11*	53	0
Nocturnal	10/06/2013	20.4	83	8	0	0

* One was a metamorph.

Red Rock Spring, Nevada

Counts from VESs were lower this year than in previous years, and additional nocturnal surveys were conducted during both seasons (table 13). Although the initial translocations to this site were completed in 2010, no recruitment into the adult population has been documented. Because excess Black Canyon animals were available from headstarting, the RLFCT decided to augment this site, and 60 juveniles were released in June (see table 21). Surprisingly, none of the young animals were observed during the autumn surveys, and only three adult frogs were counted. The ambient temperature during the first autumn survey was cold, but the weather conditions during the later survey were well within the conditions encountered in previous years.

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Table 13.—Summary of relict leopard frogs observed at Red Rock Spring during visual encounter surveys conducted in 2013

(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}C$] during the survey)

Survey type	Date	T^A ($^{\circ}C$)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/10/2013	9.2	0	0	0	0
Nocturnal	04/28/2013	15.8	5	0	0	1
Nocturnal	05/21/2013	22.0	7	0	0	0
Diurnal	06/12/2013	45.2	2	0	0	0
Nocturnal	10/14/2013	11.5	0	0	0	0
Nocturnal	10/24/2013	18.6	3	0	0	0

In April, the stream was already intermittent at stretches, and there was no obvious flow to the upper mud pools where overwintered relict leopard frog tadpoles had been seen last year. Tadpoles inhabiting the mud pool this year appeared to be a toad species, most likely Woodhouse’s toad (*Bufo [Anaxyrus] woodhousii*) or possibly red-spotted toad (*Bufo [Anaxyrus] punctatus*). A relict leopard frog egg mass was observed at the waterfall plunge pool where water is permanent during the hotter months, but in April, no tadpoles were observed, and waterflow into the pool had ceased; waterflow returned to the plunge pool by the time of the autumn survey.

Tassi Spring, Arizona

Survey results this year were odd, with a record high count in spring and only one frog counted in autumn (table 14); however, similar discrepancies in counts occurred in 2009 and 2010. Weather conditions during the first autumn survey were suitable; however, earlier that day, the region did have heavy winds (20–25 kilometers per hour), and the week before, temperatures had cooled substantially. During the later survey, weather conditions were considered appropriate. During spring, all life stages of relict leopard frogs were observed, and calling was heard. The large tadpoles observed were all within the lower portion of the spring (areas in the main wash). An area behind the old farmhouse was searched, and a few frogs were seen. This area has some water but is densely covered with *Anemopsis*.

Sometime in early spring, prior to the initial survey, vegetation was cut by NPS personnel. Areas were cut from around the house, along the path leading to the stream, and parallel to the stream channel near the springhead. With the vegetation removed near the springhead, the outflow from the spring created a small marshy area where relict leopard frog egg masses and tadpoles were

observed. By late April, emergent vegetation had grown dense in the marshy area. No tadpoles from the previous survey were seen, but this could have been because visibility was minimal in the dense vegetation.

Vegetation was also cut from around an old rusting spring box, which was fed by an old pipe protruding from the hill above the main stream (not far from the main springhead). A shallow pool (approximately 1 meter x 1 meter) was formed by the spring box, with water flowing down to meet the main channel. Several frogs were seen in this area during both spring surveys.

In February, the pipe supplying water to the horse trough had mostly stopped flowing, with only drips supplying a much reduced pool in the trough. By late April, the trough still had water, but the water had a brown tint from debris and rust from the metal trough.

Table 14.—Summary of relict leopard frogs observed at Tassi Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/28/2013	22.0	20	0	> 400 ^a	2
Nocturnal	04/24/2013	16.7	125	3 ^b	21 ^c	2
Nocturnal	10/13/2013	20.5	0	0	4	0
Nocturnal	10/23/2013	19.5	1	0	11 ^d	0

^a Observed three overwintered tadpoles.

^b One metamorph.

^c All large tadpoles.

^d Species uncertain.

Perkins Pond, Nevada

Translocations of the relict leopard frog to this artificial pond began in 2010, and overwintering was documented for the first time this spring when chuckling was heard (table 15). Two individual relict leopard frogs were heard calling in April, and one was heard during May. The site was augmented in May, June, and October with 242 frogs and late-stage tadpoles (see table 21).

A bullfrog was heard calling from the pond, although how the bullfrog got through the exclusion fence is unclear. Personnel from the NDOW saw a subadult bullfrog in the pool. In March, chorus frogs (*Pseudacris* sp.) were swabbed for *Bd*, with some of the animals testing positive. Earlier this year, NDOW personnel cut vegetation from around the exclusion fence and added a few cottonwood logs to the northwest corner of the pond. In December, vegetation was further cut around the fence line, mainly along the north side.

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Table 15.—Summary of relict leopard frogs observed at Perkins Pond during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Nocturnal	03/25/2013	13.3	0	0	0	0
Nocturnal	04/30/2013	25.5	2 ^a	0	0	0
Nocturnal	05/15/2013	27.1	1 ^a	0	0	0
Diurnal	05/24/2013	29.2	0	0	0	0
Diurnal	06/25/2013	35.5	0	0	0	0
Nocturnal	10/10/2013	12.5	1	0	0	0

^a Frogs only heard, not observed.

Union Pass Spring, Arizona

Translocations to this site began in 2011, and while natural recruitment cannot yet be confirmed, egg masses were observed this year (table 16). The nocturnal survey in April was not a complete survey; the survey was stopped at the meadow just above the “pipe pools” where large tadpoles were observed. Augmentation with 71 tadpoles and frogs occurred prior to a full nocturnal survey conducted in May (see table 21). One juvenile frog was caught at that time above the pools where animals were released this year, although late-stage tadpoles were released at the pool in early 2012. This animal measured 40 millimeters. Tadpoles from egg masses observed in February at the “upper pools” were seen in May and October. Adult frogs continue to be seen in watered areas well below where releases have occurred.

Table 16.—Summary of relict leopard frogs observed at Union Pass Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Diurnal	02/11/2013	4.8	0	0	0	7
Nocturnal	04/18/2013	12.3	8	0	8 ^a	0
Nocturnal	05/17/2013	20.6	55	1	21 ^b	1
Nocturnal	10/03/2013	18.3	70	3	17 ^b	0

^a Observed four small tadpoles but uncertain if they were relict leopard frogs.

^b All large tadpoles.

Lime Spring, Nevada

This site was established by translocation in 2012, and overwinter survival of both frogs and tadpoles were observed this spring (table 17). Release of headstarted animals occurred this year just after the May survey (see table 21), but by that time, streamflow had declined substantially and had become intermittent.

Table 17.—Summary of relict leopard frogs observed at Lime Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}\text{C}$] during the survey)

Survey type	Date	T^A ($^{\circ}\text{C}$)	Adult	Juvenile	Larvae	Egg masses
Nocturnal	03/26/2013	9.6	0	0	0	0
Nocturnal	05/02/2013	11.5	5	0	3	0
Nocturnal	10/01/2013	16.2	6	0	0	0

In October, minor flooding was noted, and segments of the spring that were previously dense with vegetation were clear and easier to survey. No relict leopard frog tadpoles were observed in pools where they had been previously seen, but instead, the pools were filled with small aquatic beetles. A dead, adult chorus frog was encountered during the autumn survey. The relict leopard frog observed, however, all appeared healthy. During spring, *Bd* swabbing was conducted on chorus frogs, with the test results negative for *Bd*.

Horse Spring, Nevada

Translocations to this site also began in 2012, and a large number of frogs were observed to overwinter (table 18). Calling and amplexus by relict leopard frogs were observed during the spring nocturnal survey, and several clusters of newly hatched tadpoles were present, with old remnants of an egg mass nearby. The identity of numerous small tadpoles was not confirmed, but the remnants of one egg mass appeared to be that of the relict leopard frog. Red-spotted toads, however, were also actively breeding at the site.

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Table 18.—Summary of relict leopard frogs observed at Horse Spring during visual encounter surveys conducted in 2013
(Also provided is the ambient air temperature [T^A] in degrees Celsius [°C] during the survey)

Survey type	Date	T ^A (°C)	Adult	Juvenile	Larvae	Egg masses
Nocturnal	04/26/2013	21.2	17	0	> 500 ^a	0
Diurnal	04/27/2013	31.5	6	0	0	0
Nocturnal	10/14/2013	12.6	10	1	25 ^b	0

^a Uncertain of species.

^b Twelve of these were larger tadpoles.

During the spring survey, the flow was running strong down the wash for about 60 meters, and the artificial pool (under the willow tree) was deep with water, although occupied by a large number of small boatmen. Before the autumn survey, the main stream was again redirected from the artificial pool, leaving the pool dry with a few cattails established near the far side of the bank. Recent cattle activity was observed at this time.

Personnel from the BLM supervised a Boy Scout troop in habitat maintenance at the site on Save the Frog Day. The work entailed removing cattails from the large, shallow pool. Before maintenance, BLM personnel noted that there did not seem to be any recent cow activity at the site. Headstarted frogs were released after habitat maintenance finished as part of the event (see table 21).

Bearpaw Poppy Spring, Nevada

Translocations to this site also began last year, and overwintering was confirmed (table 19). Several of the frogs observed were quite large. After the diurnal survey in May, 100 small frogs were released (see table 21), and several “smaller adults” were noted later during the October survey. Burro trampling and grazing was heavy throughout the stream. There was little activity within the fenced springhead area where vegetation was growing dense, although at least one burro had gotten through. The artificial pools within the enclosure area were open, except for some reeds growing from holes previously made in the liner. By October, a section of the exclusion fence was leaning outward where a burro had apparently attempted to get out of the enclosed area; all other sections of the fence were still intact.

Table 19.—Summary of relict leopard frogs observed at Bearpaw Poppy Spring during visual encounter surveys conducted in 2013

(Also provided is the ambient air temperature [T^A] in degrees Celsius [$^{\circ}\text{C}$] during the survey)

Survey type	Date	T^A ($^{\circ}\text{C}$)	Adult	Juvenile	Larvae	Egg masses
Nocturnal	05/12/2013	31.5	20	0	0	0
Diurnal	05/16/2013	36.2	2	0	0	0
Nocturnal	10/16/2013	16.5	35	0	0	0

Other Monitoring Actions

Chill Heal, Arizona

A diurnal survey was conducted at this site on April 11, by personnel from both the BLM and UNLV, along with Dr. David Bradford. This spring is located near Grand Wash on NPS land. Emergent vegetation at the site included *Eleocharis/Juncus*, *Phragmites*, and a small amount of *Typha*. The *Phragmites* covered a substantial portion of the upper system. Other vegetation included grapevine, arrowweed, mesquite, *Bacharis*, and dead tamarisk. Riparian width averaged between 21–50 meters, with a lotic width between 0–2 meters. Several, larger pools observed at the time were thought to potentially hold permanent water. Canyon treefrogs (*Hyla arenicolor*) and Woodhouse’s toads were observed. At the time of the survey, surface water was present over several hundred meters.

Both Dr. Bradford and Jef Jaeger questioned the potential usefulness of the site for translocation. They were skeptical about whether the site maintained enough permanent water for successful development of relict leopard frog tadpoles. They noted the limited amount of emergent vegetation, pointing out that most of the riparian vegetation had deeper roots.

Chytridiomycosis Assessment

Testing for the pathogenic fungus *Bd* continued this year, and a total of 35 relict leopard frogs were sampled from Lower Blue Point Spring and Grapevine Canyon. *Bd* was detected again at Lower Blue Point Spring. Chorus frogs were tested at Perkins Pond and Lime Spring, with positive detection of *Bd* in the samples from Perkins Pond. Also, UNLV personnel began a study funded by the BLM to challenge relict leopard frogs with *Bd* to help determine susceptibility of the species to chytridiomycosis. The initial experiment was initiated with 60 juvenile relict leopard frogs.

Headstarting and Translocations

Collections

For headstarting, 2 partial egg masses and 1 complete egg mass, along with forty-three 1-day-old hatchlings were collected (table 20). The collections from Black Canyon and that from Upper Blue Point Spring were kept separate.

Table 20.—Collection sites and dates collected of partial egg masses of relict leopard frogs for headstarting and translocation in 2013

Area	Site	Date	Partial egg masses
Black Canyon	Bighorn Sheep Spring	02/14/2013	2 (small, 1/4 collected)
	Black Canyon Side Spring	02/22/2013	43 hatchlings
North shore	Upper Blue Point Spring	02/05/2013	1 (small, full collection)

Translocations

A total of 719 animals were released to 7 experimental sites as well as being returned to the 1 site of egg collection (table 21). Lime Spring and Union Pass Spring received Black Canyon animals. Because more Black Canyon animals were raised than could be released to these sites, releases were also conducted to augment Goldstrike Canyon and Red Rock Spring as decided by the RLFCT. Blue Point Spring animals were released at Bearpaw Poppy Spring, Horse Spring, Perkins Pond, and back to Upper Blue Point Spring.

Hatchery Issues

Raceways at both the Lake Mead State Fish Hatchery and Willow Beach National Fish Hatchery were used again this year to grow out tadpoles. Feeding protocols followed those used in the NPS laboratory at Hilltop, and food was supplied from common stocks. As per protocol, only Blue Point Spring animals were reared at the Lake Mead State Fish Hatchery and Black Canyon animals only at the Willow Beach National Fish Hatchery.

For the most part, both systems produced healthy tadpoles and juvenile frogs; however, at the Willow Beach hatchery, there were several tadpoles and metamorphs that again showed bloating. The first bloated metamorph was observed on April 29 amongst a group of healthy metamorphs. The bloated animal actively swam around the tank; however, it died several days later. All the other metamorphs developed into healthy frogs. On May 15, amongst a group of otherwise healthy tadpoles, two bloated tadpoles were discovered. The entire batch of tadpoles was kept in a separate tank at Hilltop to finish metamorphosis. The tadpoles were fasted for the first 2 days and afterwards given the regularly scheduled diet. The two bloated tadpoles were eventually isolated and housed

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Table 21.—Numbers of late-stage tadpoles and post-metamorphic relict leopard frogs raised from eggs collected in Black Canyon or Lower Blue Point Spring and released at sites in 2013

Stocks	Translocation site	Date	Tadpoles	Juveniles	Event totals
Black Canyon	Goldstrike Canyon	06/13/2013	–	60	60
		06/28/2013	5	23	28
		10/07/2013	–	23	23
	Lime Spring	05/02/2013	–	63	63
	Red Rock Spring	06/12/2013	–	30	30
	Union Pass Spring	04/18/2013	56	15	71
	Cumulative subtotal		61	214	275
Blue Point Spring	Bearpaw Poppy	05/16/2013	–	100	100
	Horse Spring	04/27/2013	29	63	92
	Perkins Pond	05/15/2013	61	49	110
		05/24/2013	62	31	93
		06/25/2013	–	24	24
		10/10/2013	–	15	15
	Upper Blue Point Spring	06/26/2013	–	10	10
	Cumulative subtotal		152	292	444
Column totals			213	506	719

in treated water of 0.3-percent salinity. Both animals eventually lost their symptoms and were treated with Sporanox and Benzylkonium Chloride before being released into Goldstrike Canyon in late June.

Bloating has been seen in the past but was most dramatic in 2006. The prognosis at that time was a kidney disease called oxalate nephrosis. Crystals had formed in the kidney, blocking fluid passage, which caused fluid accumulation. This disease may be caused by the ingestion of ethylene glycol, which is found in certain pesticides, fertilizer, spinach, and other products. The veterinarian suggested a potential dietary problem and recommended a diet high in alfalfa. Later it was discovered that some Willow Beach hatchery personnel were feeding the tadpoles with extra supplements that were not approved. Alfalfa pellets, however, are part of the current diet, and spinach is no longer used. The current staff at the Willow Beach hatchery appeared to be carefully following the current feeding protocol.

At the Hilltop laboratory, a few tadpoles were again seen with the symptoms of buoyancy difficulty; these tadpoles tended to hang at the water surface and struggled to stay on the bottom of the tank during feeding. These symptoms were similar to those of swim bladder disorder (SBD), which can be caused by compression, deformity, or a diseased swim bladder. SBD can also be caused by

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high levels of nitrites or nitrates in the water. Ammonia tabs are used for monitoring in the laboratory, but the conversion of ammonia to nitrite then to nitrate would not be indicated on the tabs. The tadpoles were not isolated, but water changes were increased in the affected tanks (approximate 75-percent change weekly). Organic shell-less green peas were also added to the diet, which is supposed to help with SBD. None of the tadpoles died, and all symptoms disappeared over time.

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