GENERAL DISTRIBUTION AND ABUNDANCE OF CHIRICAHUA LEOPARD FROGS ON THE LADDER RANCH INCLUDING PREVALANCE OF CHYTRID FUNGUS

Final Project Report
Professional Services Contract # 07-516-0000-03604
New Mexico Department of Game and Fish Share with Wildlife

TURNER ENDANGERED SPECIES FUND

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November 15, 2007

INTRODUCTION

Chiricahua leopard frogs (*Rana chiricahuensis,* CLF) have experienced a pattern of decline similar to that reported for amphibian populations worldwide including western North American species (Beebee and Griffiths 2005). In July 2002, citing significant declines and continued threats, the CLF was listed as a threatened species under the Endangered Species Act (ESA) by the US Fish and Wildlife Service (USFWS 2002). Several factors have been discussed as reasons for amphibian declines in general, including climate change, contaminants, habitat loss, and non-native invaders; however, in the case of the CLF, particularly within the scope of this study, two factors appear especially important. Chytrid fungus (*Batrachochytrium dendrobatidis*) is a disease that appears particularly virulent in some amphibian species (see for example Bell et al. 2004; Ouellet et al. 2005). The disease, chytridiomycosis, affects amphibians by feeding on keratinized skin cells; however it is unknown exactly how death occurs from this pathogen. American bullfrogs (*Rana catesbeiana*) are a non-native invasive species in New Mexico that have been implicated in the decline of native ranid frogs and other riparian obligate herpetofauna in the west. American bullfrogs likely impact other species through intense predation, predatory or competitive larval interactions, and the transmission of parasites or pathogens (Kupferberg 1997; Kiesecker and Blaustein 1998; Lawler et al. 1999). However, native frogs have also declined in the absence of American bullfrogs.

The Ladder Ranch (Ranch), owned by Turner Ranch Properties L. P., is located in Sierra County, New Mexico, and has some of the last known CLF populations remaining in New Mexico. Of the currently known populations of the Mogollon form of the CLF in New Mexico, approximately 1/3 exists on the Ranch and adjacent Gila National Forest (Forest) grazing allotments. Additionally, populations of CLF on the Ranch are on the eastern edge of their range, thus they are potentially genetically important in a range-wide perspective, although overall genetic characteristics of CLF are poorly understood.

The Turner organization is acutely concerned with the welfare of native species and in light of amphibian declines in the Southwest conducted survey, research, and conservation work on the Ranch from 2001 until 2004 with assistance from the New Mexico Department of Game and Fish State Wildlife Grant program. This effort ultimately provided a much clearer picture of CLF distribution and abundance, as well as chytrid fungus prevalence (Kruse and Christman 2005) in the study area. For example, 17 of the 57 aquatic sites visited on the Ranch over the four year period contained CLF during at least one visit. Additionally, 6% of 111 skin scrapes collected from ten consistently occupied sites (the same sites that are considered priority one in this report) in 2003-04 were positive for chytrid fungus, however, all positive samples came from just two locations (Cave Creek [4 positives] and Cuchillo Warm Springs [3] – see discussion below). Because of the continued decline of CLF and the critical importance of remaining populations, we felt it imperative to re-survey the primary habitats known to contain frogs in 2001-04. The objective of this study is to update the distribution, relative abundance and disease information within the focus area to continue to assist in guiding management and conservation activities not only on the ranch, but also range-wide. This information will be especially valuable in planning and implementing a proposed propagation facility at the Ranch for CLF restoration in habitats across its New Mexico range.

This Share with Wildlife contract between the New Mexico Department of Game and Fish and the Turner Endangered Species Fund required that a minimum of 15 suitable sites on the Ranch and adjacent Forest lands be surveyed for presence of CLF. Additionally, a minimum of 30 skin scrapes are to be collected to test for presence of chytrid fungus. We prioritized survey sites based on past records of occurrence of CLF within the study area: priority 1 sites are ten locations known to contain CLF on a consistent basis, including: North Seco, Pague, Fish, Davis, and LM Bar wells; Cuchillo Warm Springs, Cave Creek, Cuchillo Negro Creek, Sucker Ledge, and Seco Creek between Davis and N Seco wells; priority 2 sites are two locations where deliberate translocations were conducted in the past – Artesia and George Mee wells; priority 3 sites are any locations where CLF have been found at least once previously on the ranch from 2001-04 (see Table 1); and priority 4 sites are other sites where CLF have not been found previously but contain habitat that might support CLF. These sites might include those unoccupied locations listed on Table 1, as well as other habitats encountered opportunistically. Priority 4 sites were included because significant moisture occurred

on the ranch during summer 2006 and spring 2007, thus there are more sites that might potentially support CLF, at least in the short term, than were present during the previous surveys, a relatively dry period.

STUDY AREA

The Ladder Ranch encompasses 62,950 hectares of arid landscape in southwestern New Mexico and is bordered on the west by the Gila National Forest (Forest). The Ranch lies within the Mexican Highland physiographic province and elevations across the ranch range from 1980-2135 m in the western foothills of the Black Range to 1520 m on the eastern plain. Precipitation ranges from 17.5-20 cm per year, with the majority falling during brief but heavy (monsoon) storms from June to October. Terrain generally varies from mountainous foothills, with rolling hills and mesas, dominated by ponderosa pine along the western boundary, grading to juniper and gray oak, mesquite shrublands, and grama grasslands with creosote bush as the elevation decreases. Riparian communities along wetted reaches are dominated by cottonwoods, willows, and Arizona sycamores, while dry tolerant shrubby species occupy more ephemeral sections.

Four drainages with headwaters in the Forest (ca. 2800 m) drain easterly across the ranch to the Rio Grande River (ca. 1280 m). These streams, Cuchillo Negro, Palomas, Seco, and Animas (Figure 1), from north to south, can be categorized as perennial interrupted, with extended sections of reduced or subsurface flows during low precipitation periods. Perennially wetted sections are often associated with springs or bedrock confined areas, where flow returns to the surface. Summer rains, and occasional snowmelt events, produce intermittent and often localized (to a given drainage) periods of high flows that reconnect the reaches of permanent surface water for short periods of time. Wetted sections ebb and grow depending on moisture patterns, but are fairly consistent from year to year. The dynamic flow regime in these systems produces variable habitat patches and conditions. Canyon reaches are dominated by bedrock and boulder pools that remain fairly stable, while unconfined reaches are characterized by migrating channels and riffle-scour pool sequences. Course substrates are most common, but finer materials and organic debris are present on pool bottoms, especially in more stable areas. Channel gradient is low across drainages on the ranch (< 2%), with the exception of a few canyon step pool reaches, but increases with elevation onto Forest lands. Agricultural and increasing domestic water use have significant impact on surface flows and have likely reduced the frequency of connection between the Rio Grande River and habitats on the Ranch and Forest.

Water sources across the Ranch include not only perennial sections of stream, but also developed livestock and wildlife watering facilities, most supplied by wind or solar powered pumping of groundwater. These sites often include either or both of a steel storage tank and an earthen overflow pond, and are scattered across the Ranch. Some sites are active only on a seasonal basis; however, those that are maintained year round are often important sources of amphibian habitat, especially the earthen ponds. The importance of these man-made habitats to CLF conservation and recovery was recognized in a special 4d rule during the ESA listing process (USFWS 2002).

Previous surveys (Nature Conservancy 1995, Calamusso 1998) documented the presence of five amphibian species within the four primary drainages on the Ranch and the surrounding Forest lands. These surveys documented CLF in the Seco drainage, and noted their presence in Cuchillo Negro Creek. American bullfrogs appeared to be concentrated in the lower Animas (especially in ponds around headquarters) and Palomas drainages, but were also found in Bulldozer and Artesia springs. Tiger salamander (*Ambystoma tigrinum*) and canyon tree frogs (*Hyla arencolor*) are relatively widespread, and the presence of red spotted toads (*Bufo punctatus*) was documented in the Palomas and Animas drainages. More recent surveys from 2001-05 have generally confirmed the previous species accounts, but have provided a more extensive and complete description of species distributions across the Ranch (Kruse and Christman 2005). Additionally, Kruse and Christman (2005) were able to differentiate the presence of Plains leopard frogs (*R. blairi*) in the Cuchillo Negro and lower Palomas drainages. The native Rio Grande sucker (*Catostomus plebeius*) and Rio Grande chub (*Gila pandora*) are found in the Animas, Seco, and Palomas watersheds, while hybridized remnants of native Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*) occur in Animas Creek. These fishes likely represent the entire historical array of native fishes that occurred in the study area, even

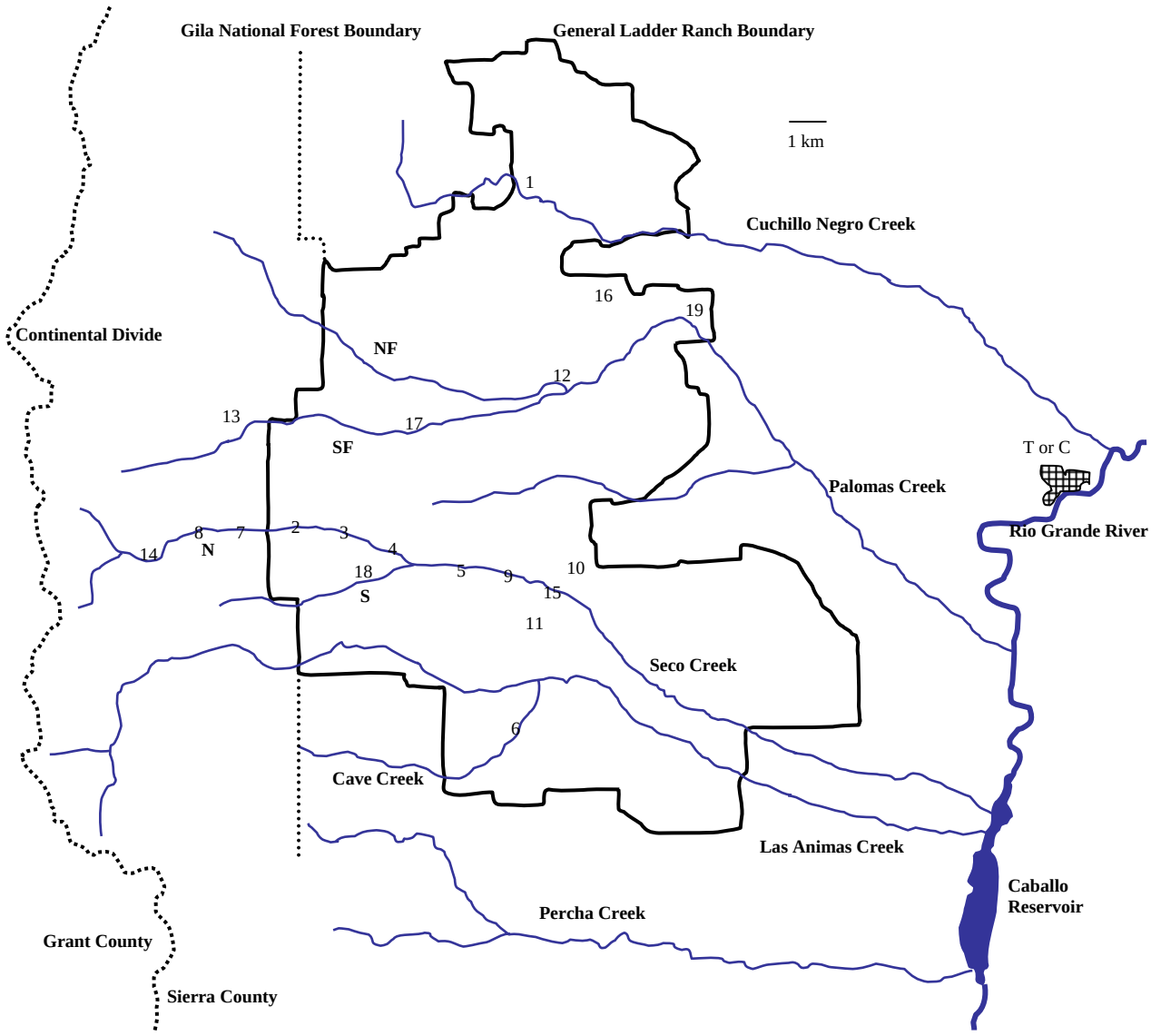
though at least 14 taxa of native fishes have been documented in the lower Rio Grande of southern New Mexico. Non-native rainbow trout (*O. mykiss*) and long-fin dace (*Agosia chrysogaster*) are both present in Animas Creek, while dace are sympatric with the two native species in Palomas Creek, and is the only fish found in Cuchillo Negro Creek, at least within the boundaries of the Ranch. Both rainbow trout and Yellowstone cutthroat trout (*O. c. bouvieri*) have been stocked in Animas Creek in the past, and remnants of these introductions are apparent in both morphological examination and genetic analyses of the current trout population. Rio Grande sucker on the Ranch represents the southern most known locality of the species within the Rio Grande basin, as most populations are found in northern New Mexico.

METHODS AND RESULTS

Surveys for the presence of CLF at each site followed USFWS survey protocols developed from Arizona Game and Fish Department guidelines and involved any or all of the following activities – walking the perimeter of livestock watering ponds and stream habitats observing individuals, watching for jumping frogs (plop counts), listening for calls, or dip-netting under banks and in vegetation for adult and larval individuals. Binoculars were used to count basking frogs, if present, at a distance prior to approaching ponds. Egg masses were also noted. If CLF were confirmed at a location in April or May, a secondary evening or night capture event was sometimes conducted within 24 hours to sample CLF for chytrid fungus. Seine hauls or dip nets were used to capture adult and juvenile frogs (species other than CLF were also captured) and individuals were sampled for chytrid presence by scraping the ventral skin surface of the body and thighs of individual frogs using a sterile wooden swab. The swab end was cut off and placed into a buffer solution and labeled with date, locality, and species. All specimens were handled separately using nitrile gloves and clean zip-type bags to minimize cross contamination between frogs and samples. A few individuals that were captured opportunistically during normal site surveys were also scrapped for chytrid analysis. All sampling gear was routinely sterilized prior to moving from one sampling site to another by spraying with or dipping into a 10% bleach or quaternary ammonia solution and allowed to air dry.

In 2007 surveys for CLF were conducted 47 times (not including the six capture events) at 25 unique sites during April, May, August, or September survey periods (Table 2). All priority 1 sites were surveyed at least once, and eight of the ten were surveyed both early (April or May) and late (August or September) season. The May 29th and 30th surveys along N Seco Creek that included portions of the channel from Davis Well up to and above Sawmill Well was the only time that priority 1 sites Sucker Ledge and N Seco Creek from N Seco to Davis wells were surveyed. Artesia and George Mee wells, the two priority 2 sites, were surveyed at least twice. Additionally five priority 3 sites (South Fork Palomas – Hermosa; SF Palomas – Ranch Boundary; Seco Box, Avilas Well, and N Seco above Sucker Ledge) and eight priority 4 sites (Bulldozer Spring; Torres Tank; Johnson Well; South Seco Well; S Seco Creek; Sawmill Well; N Seco Creek above Sawmill Well; and Ash Canyon above Artesia Well) were surveyed.

CLF presence was documented at nine priority 1 sites (Table 2), but not at the Cave Creek site. Cave Creek had consistently contained low, but declining, numbers of frogs in past surveys (Kruse and Christman 2005) but chytrid fungus was present and the status of the population has generally been considered tenuous. Low numbers of CLF were found at Cuchillo Warm Springs and nearby Cuchillo Negro Creek, but larger numbers of multiple life stages were present at the remaining priority 1 sites. Over 100 adult and juvenile frogs were counted at Pague and LM Bar wells on multiple occasions, while counts at Davis, N Seco, and Fish wells exceeded 50 adult and juvenile individuals. Artesia Well, a 2004 recipient of frog stockings contained large numbers of CLF; however, only a single frog was observed at George Mee Well. CLF were documented at two (Seco Box and N Seco above Sucker Ledge) of the priority 3 sites where frogs had been found (or introduced) at least once in the past. CLF have never been known to naturally occur in Avilas Well, but this site was the recipient of an emergency translocation of CLF tadpoles from SF Palomas Creek in 2003. CLF have not been observed in Avilas Well since the translocation. No frogs were found in the two upper SF Palomas sites where they have been occasionally, but not consistently, seen in the past (Hermosa and Ranch Boundary). Five of the eight priority 4 sites surveyed in 2007 – Sawmill Well, S Seco Well, S Seco Creek above S Seco Well, Torres Tank, and Bulldozer Spring – have been surveyed in the past, but this



1. Cuchillo Warm Springs
2. N. Seco Well
3. Pague Well
4. LM Bar Well
5. Fish Well
6. Cave Creek
7. Davis Well
8. Sucker Ledge
9. Johnson Well
10. George Mee Well
11. Artesia Well
12. Gallinas Springs
13. Hermosa
14. Sawmill Well
15. Seco Box
16. Bulldozer Spring
17. Avilas Well
18. South Seco Well
19. Torres Tank

Figure 1. Ladder Ranch boundary and 2007 Chiricahua leopard frog sampling sites.

was the first year CLF were observed at any of these sites (first three). We speculate that the wetter conditions during summer 2006 and this spring have allowed CLF to move out of core areas into these habitats. Frogs in the S Seco drainage likely migrated up from LM Bar Well, while the single individual seen at Sawmill Well, if not missed during previous surveys, might have moved up the N Seco Creek channel from Sucker Ledge. Bulldozer Spring, an isolated, spring-fed pond in the Palomas Creek drainage, contained bullfrogs until 2002 when the pond was drained and a concerted effort to remove bullfrogs was completed. Nothing was observed at Bulldozer in 2007. Similarly, Torres Tank has been occupied by bullfrogs in past, which have been opportunistically removed. No bullfrogs were observed in 2007; however, somewhat surprisingly, plains leopard frogs were documented – likely immigrants from lower Palomas Creek. The other three priority 4 sites were not surveyed prior to 2007. Johnson Well, dry for several years but now holding water due to wetter conditions and a new pump on the well (in 2006), has been colonized (likely) by juveniles dispersing from larger upstream sub-populations in the Seco drainage. CLF were seen at this site for the first time in May of 2007 and numbers rose by the end of the summer. CLF presence in N Seco Creek above Sawmill Well and in Ash Canyon above Artesia Well are likely the result of frogs taking advantage of wetter conditions and colonizing, however temporarily, previously inhospitable areas.

Table 3 lists the dates, numbers, and sites where chytrid samples were taken. Skin scrapings were collected from 45 individuals (including five *R. blairi* and one salamander) and shipped to the University of Arizona Human Origins Genotyping Laboratory for analyses by starch gel electrophoresis. Logically, the majority of the samples were from the Seco Creek drainage, but samples were also collected from Cuchillo Warm Springs (n=8) and Artesia Well (n=7; which ultimately drains into Seco Creek downstream of the Seco Box). Seven, or 16%, of the samples were positive for chytrid fungus, including 5 of the 8 from Cuchillo Warm Springs. Single samples from Artesia and LM Bar wells were also positive. Individuals captured at Cuchillo Warm Springs have tested positive for chytrid fungus in the past; however, this is the first time chytrid has been documented in the Seco drainage upstream of the Seco Box or in any of the core sub-populations that make up the Seco metapopulation (Kruse and Christman 2005). Both positive samples from Artesia and LM Bar wells had a weak gel band, suggesting that while the disease was present, it was a lighter infection relative to other samples - we are unsure of the significance, if any, of this result.

DISCUSSION AND SUMMARY

These data suggest that for the most part priority 1 sites on the Ladder Ranch and nearby Forest lands continue to support CLF. The exception is Cave Creek, a population that was considered at risk in 2004. Because of the difficulty of detecting a population of only a few individuals it may be premature to conclude that the Cave Creek population has gone extinct; however these results suggest that few, if any, individuals remain in Cave Creek. Because it was relatively isolated from other CLF populations on or off the Ranch, the loss of the Cave Creek population could represent a significant genetic loss for the species in New Mexico.

The N Seco Creek complex (from upstream to downstream) of Sawmill Well, Sucker Ledge, Davis Well, N Seco Well, Pague Well, LM Bar Well, Fish Well, Johnson Well, and the Seco Box (as well as ephemeral channel habitat between these sites) continues to support large numbers of frogs in a pattern of habitat patches logically viewed as a metapopulation (e.g., Hanski and Gilpin 1997; Pope et al. 2000; Figure 2). A previous movement study (Kruse and Christman 2005) supports the assertion that these sites occasionally exchange individuals as juveniles disperse from natal habitats or adults migrate. The core area from Sucker Ledge down to Fish Well has supported relatively consistent and large numbers of CLF over the past seven years. Recent moisture seems to have allowed the species to expand both up and downstream within the drainage. Furthermore, Johnson Well may become an important habitat in the future if the pond habitat is consistently maintained. On the other hand, it is a concern - potentially realized this year - that Johnson Well may provide a conduit for the movement of chytrid fungus into the core CLF habitat in Seco Creek. CLF have been documented sporadically in low numbers (including 2007) in the Seco Box, which is downstream

of Johnson Well and the other frog habitats in the drainage (see Figure 1). Chytrid fungus was putatively histologically identified in a single tadpole collected in the Seco Box area in 2001. In fact, this was the first suggestion of chytrid presence anywhere on the Ranch. However, until this year no other tadpole, juvenile, or adult from the Seco drainage ever tested positive for chytrid. If chytrid continues to be present in the Seco Box area and Johnson Well allows greater and more frequent movement of CLF (or other amphibian species) between the Seco Box and upstream habitats, the risk of a consistent infusion of infection into core CLF habitat increases. We are unsure if the positive chytrid tests from Artesia and LM Bar wells in 2007 are the result of frogs moving from or through the Seco Box or another infected area. Other explanations include inaccurate test results, cross contamination of samples, or disease transmission by other routes, including humans (e.g., contaminated sampling equipment or ranch activity). The Ranch proposes to monitor this situation closely.

Overall numbers of CLF at Cuchillo Warm Springs and in nearby Cuchillo Negro Creek are of concern. Monitoring over the past several years, while not statistically validated, suggests that CLF are declining at these sites. Chytrid fungus has been chronically present in this area, was confirmed again this year, and may be depressing population numbers. In fact, the percentage of samples testing positive for chytrid in 2007 was higher than any previous year. Further, plains leopard frogs are sympatric with CLF at these sites. It is unknown whether both species have always been present or if the plains leopard frog more recently invaded. If it is a recent invasion, interactions between the species may negatively impact CLF, either independently or synergistically with chytrid fungus. Numerous tadpoles were observed at the Warm Springs site in 2007; however it is unknown whether they were *chiricahuensis* or *blairi*. Regardless, the presence of larvae did not later translate into larger numbers of juveniles and adults. The Ranch will continue to monitor population status of CLF at Cuchillo Warm Springs and in Cuchillo Negro Creek.

The 2004 translocation of CLF to Artesia Well appears to have been a success. Large numbers of all life stages were observed and the presence of frogs further up Ash Canyon suggest that juveniles are immigrating out of the earthen pond at Artesia and attempting to find and colonize other habitats. Less successful was the translocation to George Mee Well. While the habitat quality at this site is not as good as Artesia, we felt it could support CLF. While frogs are present, only a single or handful of frogs has survived at George Mee. We did not consider the emergency translocation of tadpoles to Avilas Well a true translocation. While it was the nearest off channel refugia site available for stocking during a 2003 ash flow, we considered it relatively poor frog habitat and are not surprised that stocked tadpoles did not survive or stay at the well after metamorphosing. The lack of bullfrogs at Bulldozer Spring is encouraging and the Ranch will likely propose translocation of CLF to this high quality habitat in the near future if an adequate source population is available or inter-basin translocation is allowed.

It is encouraging that the Ranch's core CLF metapopulation in the Seco drainage appears to have maintained and even expanded over the past three years, however, the two positive chytrid samples are disconcerting and suggest that this large, formerly secure metapopulation is now at risk. Although it is more difficult for individuals, and presumably disease, to move out of or into Artesia Well to and from other sub-populations in the Seco drainage (approximately 4.5 km overland or 8.5 km along an ephemeral channel to reach, for example, Johnson Well), LM Bar Well sits in the middle of the Seco metapopulation and infected individuals from this site could presumably access other sub-populations more regularly, especially during precipitation events (see movement information in Kruse and Christman 2005). Furthermore, even if chytrid fungus only caused localized population effects at these two sites, their decline or extinction would represent a significant loss as both Artesia and LM Bar wells are important and high quality habitats for CLF on the Ranch. Following several years of drought in the first half of the decade, average or above average precipitation over the last two years has allowed CLF populations on the Ladder Ranch increased opportunity to move between habitats or into previously unoccupied areas (for example Sawmill Well and Ash Canyon). This should be viewed as a positive outcome; however, greater mobility, exploration, and exchange of individuals among populations may also accelerate the spread of pathogens such as chytrid fungus. This may be one plausible explanation for the recent positive chytrid tests at Artesia and LM Bar wells. However, the source for this

recent disease invasion is unknown and not easily explained, and other chytrid vectors should not be dismissed without further investigation.

These results diametrically argue for locating a propagation facility and breeding program at the Ladder Ranch. First, relatively large numbers of CLF remain in the Seco drainage and could be used to support an artificial propagation program dedicated to CLF restoration in New Mexico. Obviously, individuals collected from the Seco drainage, or anywhere else on the Ranch, would need to be quarantined and confirmed disease free prior to a translocation event or integration into a breeding program. Second, the need for a CLF propagation facility on the Ranch may have a renewed urgency and potentially different focus if chytrid presence negatively affects the Seco metapopulation to an extent where extinction seems likely. If so, then a propagation facility at the Ranch would have a parallel (to wider population restoration) goal of maintaining and rebuilding the Seco metapopulation.

By facilitating completion of this project, the Ladder Ranch and New Mexico Department of Game and Fish have made a significant contribution to the conservation of Chiricahua leopard frogs, especially when considering the strategy elements of the Chiricahua Leopard Frog Recovery Plan: 1) protect and manage remaining populations and habitats; 2) restore and create habitat, and establish additional populations as needed to build viable metapopulations and isolated robust populations in each recovery unit; 3) monitor progress towards recovery; 4) research the conservation biology of the frog with the objectives of facilitating efficient recovery; 5) develop support and build partnerships to facilitate recovery; and 6) practice adaptive management in which the recovery plan is revised to reflect new information developed through research and monitoring. Much of what was accomplished through this project will provide useful information for consideration under these strategy elements.

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Figure 2. Earthen pond CLF habitat (with livestock exclosure) at LM Bar Well – a typical habitat patch in the Seco drainage.

TABLE 1. Locations of sites surveyed 2001-2004 (Kruse and Christman 2005).

Site	Description	Drainage	Year surveyed				Species observed			
			2001	2002	2003	2004	2001	2002	2003	2004
3	Western ranch boundary	S.F. Palomas	-	+	-	-		Rc	Ha	
4	Cuchillo Warm Springs	Cuchillo	+	+	+	+	Rc, Rb	Rc, Rb	Rc, Rb	Rc, Rb
5	Middle Box or Seco Box	Seco	+	+	-b	ob	Rc	Rc		
6	N Seco Well to Davis Well	N. Seco	+	o	+	+	Rc		Rc	Rc
7	Fish Well	Seco	+	+	+	+	Rc, At	Rc	Rc	Rc, At
8	LM Bar Well	N. Seco	-	+	+c	+	At	Rc, At	Rc, At	Rc, At
9	Pauge Well	N. Seco	+	+	+	+	Rc, At	Rc, At	Rc, At	Rc, At
10	N. Seco Well	N. Seco	+	+	+	+	Rc	Rc	Rc	Rc, At
11	Rouse Well	S.F. Palomas	-	-	o	o				
12	Avilas Well	S.F. Palomas	-	-	+a	-	Rct, At, Ha	Ha, At	Rc, At	At
13	Sissel Well	S.F. Palomas	od	o	o	o				
14	Middle Seco Creek	M. Seco	-	o	o	-	Ha			
15	South Seco Creek	S. Seco	-	o	o	-	Ha			Ha
16	Bodine Spring/Long Canyon	Seco	-	o	o	o				
17	George Mee Well	Seco	-	o	o	+a				Rc
18	Artesia Well	Ash/Seco	-	-	o	+a	Rct, Ha	Rct, Ha, At		Rc
19	S. Seco Well	S. Seco	-	o	o	-				
20	Turkey Spring	S. Seco	-	o	o	-	At			
21	Johnson Well	Seco	-	o	o	o	At			
22	Davis Well	N. Seco	-	+	+	+	At	Rc, At	Rc, At	Rc, At
23	Emrick Spring	S.F. Palomas	-	-	o	o		Ha		
24	Bear Canyon Tank and tributary	S.F. Palomas	-	-	o	o				
25	Yellow Spring	Palomas	-	o	o	o				
26	Cross O Canyon	N.F. Palomas	-	o	o	o	Ha			
27	Bulldozer Spring	Indian/Palomas	-	-	-	-	Rct, At	Rct, At		
28	Torres Tank	Indian/Palomas	-	-	-	o	Rct	Rct	Rct	
29	Salado Creek	Palomas	-	o	o	-	Rb, Rct			
30	Palomas below Gallinas Springs	Palomas	-	o	o	-	Rb, Rct, Ha			Rb, Rct, Ha
31	S.F. Palomas upstream from Hermosa	S.F. Palomas	-	o	-	o	Ha			
32	N.F. Palomas in Spring Canyon vicinity	N.F. Palomas	-	o	o	-				
33	Cross O Spring and Spring Canyon	N.F. Palomas	-	o	o	o	Ha			
34	Apodaca Spring	N.F. Palomas	-	o	o	-				
35	Morgan Creek	S.F. Palomas	-	o	o	o				
36	Easter ranch boundary to Myers Spring	Animas	-	o	o	o	Rct			
37	Diversion to Cave Creek	Animas	-	o	o	-	Rct			Rct
38	Kelsey to Negro Bill Spring	Animas	-	-	-	o		Ha	Ha	
39	Cave Creek	Animas	+	+	+	+	Rc, Rct	Rc, Rct	Rc, Rct	Rc
40	Warm Springs to Old Mill Canyon	Cuchillo	-	+	+	+	Rb, Ha, Rct	Rb, Rc, Ha	Rc, Rb	Rc, Rb, Ha
42	S.F. Palomas 2 km above Gallinas Springs	S.F. Palomas	o	o	-	o				
43	Circle Seven Well and nearby Creek	S.F. Palomas	o	-	o	-		Ha		Ha
44	N.F. Palomas upstream of Forest boundary	N.F. Palomas	o	-	o	o				
46	Upstream of Negro Bill Spring	Animas	o	-	o	o				
47	Lower Cave Creek	Animas	o	o	-	-				
48	S.F. Palomas 1.5 km below Hermosa	S.F. Palomas	o	o	+	-			Rc	Ha

TABLE 1. Continued.

Site	Description	Drainage	Year surveyed				Species observed			
			2001	2002	2003	2004	2001	2002	2003	2004
49	Rouse Spring	S.F. Palomas	o	o	-	o			Ha	
50	Curtis Well	S.F. Palomas	o	o	-	o				
51	Sucker Ledge	N. Seco	o	o	+	+			Rc	Rc, Ha
52	N. Seco above Sucker Ledge	N. Seco	o	o	-	+			Ha	Rc
55	Cave Creek confluence to Kelsey	Animas	o	o	-	o				
56	Wild Cow Spring	N. Seco	o	o	-	o				
57	Middle Seco Spring	M. Seco	o	o	-	o				
58	Geronimo Spring	S. Seco	o	o	-	o				
59	Conley Spring	S. Seco	o	o	-	o				
60	N. Percha Creek	Percha	o	o	-	o				
61	6B Spring	Cave/Animas	o	o	-	o				
64	Turkey Well	S. Seco	o	o	o	-				Ha
65	Sawmill Well	N. Seco	o	o	o	-				Ha

+ Chiricahua leopard frog seen at site at least once during survey year (some sites only surveyed once, others multiple surveys)

- Site was visited at least once during survey year, but Chiricahu leopard frogs were not observed

o Site not visited during survey year

a - Chiricahua leopard frogs observed at these sites only after a translocation of frogs during current year

b - A single frog was seen in the Middle Box in 2003 during fish survey activity in May; however, it was not seen and could not be verified during specific herpetological surveys

c - A single frog was present at LM Bar Well during the first four monitoring events, prior to translocation of 32 sub-adult frogs in late summer 2003

d - site visited in 2000

Rc - *Rana chiricahuensis* (Chiricahua leopard frog)

Rb - *Rana blairi* (Plains leopard frog

Rct - *Rana catesbeiana* (American Bullfrog)

Ha - *Hyla arenicolor* (Canyon treefrog)

At - *Ambystoma tigrinum* (Tiger salamander)

Site numbers are not consecutive in some cases due to duplication and combination of sampling sites

TABLE 2. Locations of sites surveyed 2007.

Date	Site	Watershed	Other species	Rana chiricahuensis			Comments	Time	Northing	Easting	Priority	Site # in Table 1 - a
				Adult/Juv.	Tadpoles	Egg mass						
04/19/07	Cuchillo Warm Spr	Cuchillo	Rana blairi		20		Tadpoles either blairi or chiricahuensis	1715	261385	3684508	1	4
04/19/07	Cuchillo Warm Spr	Cuchillo	Rana blairi	1			Night survey; collected 4 chytrid samples (3 blairi adults)	2125			1	4
04/20/07	Cuchillo Warm Spr	Cuchillo	Rana blairi	3	50+		Tadpoles either blairi or chiricahuensis; collected 4 cytrid samples (2 blairi adults)	1015			1	4
08/16/07	Cuchillo Warm Spr	Cuchillo	Rana blairi	4				1854	261305	3684991	1	4
09/13/07	Cuchillo Warm Spr	Cuchillo	Rana blairi	4				2045	261310	3684990	1	4
04/17/07	Fish Well	Seco		4		1	Frogs in steel rim tank	1652	258144	3664680	1	7
05/16/07	Fish Well	Seco		5	20		2nd year tadpoles; 1 chytrid sample	1057			1	7
08/17/07	Fish Well	Seco		53				1155			1	7
09/30/07	Fish Well	Seco		35			In steel rim tank; dirt tank too shallow?				1	7
04/17/07	LM Bar Well	Seco		125	20+		Earth tank low; 1 chytrid sample	1416	254644	3665037	1	8
04/18/07	LM Bar Well	Seco		9			Seining survey; 9 chytrid samples	1540			1	8
05/16/07	LM Bar Well	Seco	Ambystoma tigrinum	150	500+		Few 2nd year tadpoles; most new	1025			1	8
09/30/07	LM Bar Well	Seco		160							1	8
04/17/07	Pague Well	Seco	Ambystoma tigrinum	240			One dead adult Rana chir.; 2 chytrid samples	1312	251992	3666396	1	9
04/18/07	Pague Well	Seco		5			Night survey; 6 chytrid samples	2100			1	9
05/16/07	Pague Well	Seco	Ambystoma tigrinum	125		6		951			1	9
09/30/07	Pague Well	Seco		120							1	9
04/17/07	N. Seco Well	Seco		37	200+		At least 4 2007 clutches	1203	249086	3666982	1	10
04/18/07	N. Seco Well	Seco		10			Night survey; 2 chytrid samples	2035			1	10
05/16/07	N. Seco Well	Seco		33	500+	6		858			1	10
09/30/07	N. Seco Well	Seco		90	500+		Late season tadpoles				1	10
04/17/07	Davis Well	Seco		25	100+	1	Mostly small adults	1107	245356	3666868	1	22
04/18/07	Davis Well	Seco	Ambystoma tigrinum	6	3		Seining survey; 2nd yr tadpoles; 6 chytrid samples (1 salamander)	1802			1	22
05/16/07	Davis Well	Seco	Ambystoma tigrinum	60	1			822			1	22
09/30/07	Davis Well	Seco		37							1	22
04/16/07	Cave Creek	Animas					Last siting here in May 2006	1725	259382	3656612	1	39
05/29/07	Cave Creek	Animas						1500	259598	3656870	1	39

TABLE 2. Continued.

Date	Site	Watershed	Other species	Rana chiricahuensis			Comments	Time	Northing	Easting	Priority	Site # in Table 1 - a
				Adult/Juv.	Tadpoles	Egg mass						
05/30/07	Cave Creek	Animas					Frogs here in past	1608	258774	3655981	1	39
04/19/07	Cuchillo Negro Crk	Cuchillo			50+		Downstream of warm springs (sec 16); either blairi or chiricahuensis	1610	261842	3684071	1	40
08/17/07	Cuchillo Negro Crk	Cuchillo		1			Lower box area	1735	269007	3682130	1	40
05/29/07	N. Seco Crk - b	Seco		14			Several frogs from Sucker Ledge to Upper Box along crk channel above Davis Well; mostly small adults; 3 chytrid samples between Sawmill and Davis		244029	3667061	1,1,3	6,51,52
08/17/07	George Mee Well	Seco		1			Swirl in tank - frog?	935	264564	3663592	2	17
09/13/07	George Mee Well	Seco		1				1710			2	17
04/17/07	Artesia Well	Seco	Ambystoma tigrinum	10	100+			1804	262915	3660666	2	18
04/17/07	Artesia Well	Seco		20			Night survey; 7 chytrid samples	2120			2	18
05/15/07	Artesia Well	Seco		44	200+	4		1409			2	18
08/17/07	Artesia Well	Seco		95	300+		Metamorphs dispersing from pond	1103	262904	3660678	2	18
09/30/07	Artesia Well	Seco		100	present		Emergent and submerged vegetation present, difficult to survey and likely underestimated numbers; tadpoles seen but not counted				2	18
05/30/07	SF Palomas Crk	Palomas	Hyla arenicolor				Falls at ranch boundary		248719	3672832	3	3
08/16/07	Seco Box	Seco		5	6			1315	261977	3663713	3	5
05/30/07	Avilas Well	Palomas	Rana catesbeiana Ambystoma tigrinum						256087	3672435	3	12
05/30/07	SF Palomas Crk	Palomas					Downstream of Hermosa		247082	3672735	3	48
04/17/07	S. Seco Crk	Seco		6			Likely upstream migrants from LM Bar	1540	250966	3664296	4	15
04/17/07	S. Seco Well	Seco		2			Frogs in drinker	1515	252247	3664346	4	19
04/17/07	Johnson Well	Seco		2			Tank refilled in 2005, migrants from Fish Well	1732	260553	3664054	4	21
05/15/07	Johnson Well	Seco	Ambystoma tigrinum	1		1		1536			4	21
05/16/07	Johnson Well	Seco		3			Introduced aquatic veg from LM Bar	1130			4	21
09/30/07	Johnson Well	Seco		12		1	Likely immigrants				4	21
08/16/07	Bulldozer Spring	Palomas					No bullfrogs	2100			4	27

TABLE 2. Continued.

Date	Site	Watershed	Other species	Rana chiricahuensis			Comments	Time	Northing	Easting	Priority	Site # in Table 1 - a
				Adult/Juv.	Tadpoles	Egg mass						
09/13/07	Torres Tank	Palomas	Rana blairi				Adult plains leopard frogs; likely immigrants from lower Palomas Creek; bullfrogs killed here in the past	1317	270226	3678571	4	28
05/30/07	Sawmill Well	Seco	Hyla arenicolor	1			First observation at Sawmill Well, frog in drinker		242510	3666811	4	65
05/30/07	N. Seco Crk	Seco	Hyla arenicolor	3			Above Sawmill Well		240436	3665404	4	-
09/13/07	Ash Canyon	Seco		20+			Above Artesia Well, small spring flows in wet years	1405	262827	3660872	4	-

a - the last column provides a cross reference to Table 1. The site surveyed in 2007 and reported in this table corresponds to the same site in Table 1 listed in the last column here.

b - this survey effort along the N Seco Creek channel covered multiple sites listed in Table 1, thus those corresponding site numbers and 2007 priorities are listed in the last two columns of this table.

TABLE 3. Chytrid collections from Ladder Ranch in 2007.

Date	Location	Date Extracted	Species	Results	Comments	Sample ID
17 April 07	Artesia Well	13-Jun-07	Rach	NEGATIVE		CHY00221
17 April 07	Artesia Well	13-Jun-07	Rach	NEGATIVE		CHY00222
17 April 07	Artesia Well	13-Jun-07	Rach	NEGATIVE		CHY00225
17 April 07	Artesia Well	13-Jun-07	Rach	POSITIVE	Weak gel band	CHY00226
17 April 07	Artesia Well	13-Jun-07	Rach	NEGATIVE		CHY00227
17 April 07	Artesia Well	13-Jun-07	Rach	NEGATIVE		CHY00229
17 April 07	Artesia Well	13-Jun-07	Rach	NEGATIVE		CHY00230
17 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00228
17 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00223
17 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00224
18 April 07	Davis Well	13-Jun-07	Amti	NEGATIVE		CHY00304
18 April 07	Davis Well	13-Jun-07	Rach	NEGATIVE		CHY00361
18 April 07	Davis Well	13-Jun-07	Rach	NEGATIVE		CHY00362
18 April 07	Davis Well	13-Jun-07	Rach	NEGATIVE		CHY00365
18 April 07	Davis Well	13-Jun-07	Rach	NEGATIVE		CHY00368
18 April 07	Davis Well	13-Jun-07	Rach	NEGATIVE		CHY00370
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00301
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00302
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00303
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00305
18 April 07	LM Bar Well	13-Jun-07	Rach	POSITIVE	Weak gel band	CHY00306
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00307
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00308
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00309
18 April 07	LM Bar Well	13-Jun-07	Rach	NEGATIVE		CHY00310
18 April 07	N. Seco Well	13-Jun-07	Rach	NEGATIVE		CHY00363
18 April 07	N. Seco Well	13-Jun-07	Rach	NEGATIVE		CHY00364
18 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00313
18 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00318
18 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00320
18 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00366
18 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00367
18 April 07	Pague Well	13-Jun-07	Rach	NEGATIVE		CHY00369
19 April 07	Cuchillo Warm Springs	13-Jun-07	Rabl	POSITIVE		CHY00311
19 April 07	Cuchillo Warm Springs	13-Jun-07	Rach	POSITIVE		CHY00312
19 April 07	Cuchillo Warm Springs	13-Jun-07	Rabl	NEGATIVE		CHY00314
19 April 07	Cuchillo Warm Springs	13-Jun-07	Rabl	NEGATIVE		CHY00316
20 April 07	Cuchillo Warm Springs	13-Jun-07	Rach	POSITIVE		CHY00240
20 April 07	Cuchillo Warm Springs	13-Jun-07	Rabl	POSITIVE	Weak gel band	CHY00315
20 April 07	Cuchillo Warm Springs	13-Jun-07	Rach	POSITIVE		CHY00317
20 April 07	Cuchillo Warm Springs	13-Jun-07	Rabl	NEGATIVE		CHY00319
16 May 07	Fish Well	13-Jun-07	Rach	NEGATIVE		CHY00234
29 May 07	N. Seco Creek	13-Jun-07	Rach	NEGATIVE		CHY00231
29 May 07	N. Seco Creek	13-Jun-07	Rach	NEGATIVE		CHY00238
29 May 07	N. Seco Creek	13-Jun-07	Rach	NEGATIVE		CHY00239