Current status of the Arizona Toad (*Anaxyrus microscaphus*) in New Mexico: Identification and evaluation of potential threats to its persistence.

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Executive summary

In 2013, we assessed the population status of the Arizona toad *Anaxyrus microscaphus*, a NMDGF Species of Greatest Conservation Need under the Comprehensive Wildlife Conservation Strategy for New Mexico. The continuation of this work in 2014 allowed us to further evaluate the population trends of *A. microscaphus* and associated threats. We expanded the number of sites surveyed from 59 in 2013 to 90 in 2014. Sites were selected according to historic records from the University of New Mexico, University of Kansas, University of Arizona, United States National Museum, Los Angeles County Museum, Texas Cooperative Wildlife Collection, University of Texas at El Paso and the American Museum of Natural History. These museum collections represent the largest collections of *Anaxyrus microscaphus* from New Mexico. We conducted standardized call surveys in the spring of 2014 at all sites and increased sampling for the fungal disease chytridiomycosis caused by the pathogen *Batrachochytrium dendrobatidis* (Bd). Herein we provide site occupancy estimates based on call surveys using program PRESENCE, a summary of sampling effort, and the prevalence of Bd in *A. microscaphus.* Additionally, we report on the finding of a novel parasite/pathogen and a further evaluation of climate-related threats to *A. microscaphus.* In 2014 we detected toads using call surveys at 33 (35%) of the 90 historical sites. We report the first known incidence of Bd in *A. microscaphus* and identify drying of breeding habitats as a serious threat to local populations of *A. microscaphus*. We provide five management recommendations based on our current understanding of known threats to the species. Finally, we have compiled a species account based on the ecological and behavioral observations from 2013 and 2014 that may be relevant to implementing future monitoring or management actions. This species account is meant as an addendum to the account provided in Degenhardt et al. (1996).

Introduction

Amphibians, as a group, are the most imperiled group of vertebrates on earth with approximately 43% of all ~6,400 species threatened or declining (Stuart et al. 2004; Monastersky 2014). Many frogs of the U.S. southwest have already experienced major declines and are now considered to be high conservation priorities (Bradley et al. 2002; Schlaepfer et al. 2007; Olson et al. 2013). The loss of amphibians can have major implications on ecosystem function and those species that rely on them as food. The loss of amphibian larvae can alter stream and river food webs that negatively alter water quality and fish communities (Whiles et al. 2013). The loss of the terrestrial lifecycle of amphibians can alter nutrient cycles and the terrestrial food web (Regester et al. 2006; Best & Welsh 2014). Recent studies have shown that snakes that feed on frogs decline following amphibian declines (Ray et al. 2011; Roe et al. 2012). Evidence also suggests that game species may be affected by the loss of amphibians (Hocking & Babbitt 2014).

Climate change, habitat modification, invasive species and disease represent major threats to global amphibian populations (Hof et al. 2011). This is a major conservation crisis that is affecting local biodiversity, ecosystem function, as well as policy and management decisions (Barnosky et al. 2012). It is imperative to understand the local mechanisms that drive population declines in order to develop effective management strategies that prevent species from becoming endangered (Gratwicke et al. 2012). Identifying a species decline in its early stages, and reversing the decline, before its status becomes critical, can increase the probability of success and save money for regulatory agencies and taxpayers in the long-term (McCarthy et al. 2012).

Hybridization can increase a species susceptibility to decline and complicate recovery and conservation efforts (Schwaner & Sullivan 2009). Hybridization between *A. microscaphus* and *A. woodhousii* (Woodhouse's Toad) has been documented in Arizona, Nevada and Utah over the last 50 years (Sullivan 1995; Schwaner & Sullivan 2009) leading to population declines of *A. microscaphus* in these states (Hammerson & Schwaner 2004). Hybridization occurs when females of *A. microscaphus* mate with males of *A. woodhousii* (Schwaner & Sullivan 2005). Hybridization between these two species are thought to be facilitated by altered water flow regimes from dam construction and other anthropogenic factors (Bradford 2002; Hammerson & Schwaner 2004), but the exact mechanism is not understood. Over the last few decades *A. woodhousii* has expanded its' range up river and stream systems replacing *A. microscaphus* with hybrids, thus reducing the genetic integrity and historic range of genetically pure *A. microscaphus* (Schwaner & Sullivan 2005).

The second factor is related to climate change and forest fires that either temporarily or permanently results in loss of breeding habitat. Global increases of temperature in the southwest are increasing tree mortality, and fire risk frequency, which can be exacerbated by drought conditions (William et al. 2013). Furthermore, long-term regional drying trends are exacerbating episodic drought conditions, which can vary from year-to-year (Gutzler & Robbins 2010). Streams and rivers are sensitive to forest fires and experience large scale hydrologic changes, increased peak flows, and increased soil erosion post-fire (Luce et al. 2012). These fire-induced changes in riparian systems have devastating short-term impacts on riparian species that include high mortality and loss of annual breeding (Luce et al. 2012). If fire frequency becomes too great, natural recolonization of affected areas may not occur or take many years (Rinne 2003; Luce et al. 2012). As we have shown in 2013, fires can indirectly preclude *A. microscaphus* from breeding in fire-damaged drainages, effectively removing these drainages as breeding habitats (Ryan et al. 2013).

Annual variation in rainfall, both stochastically and during extreme drought events, can have large impacts on species detection at breeding sites (Walls et al. 2013). Under such a boom and bust scenario, it is important to have multi-year breeding surveys to understand the climate-species interactions (Alfords & Richards 1999). Many amphibian populations can persist when breeding at irregular intervals, but extended non-breeding periods can lead to eventual population declines and extirpations (Alfords & Richards 1999; Green 2003). Our 2013 surveys (Ryan et al. 2013) documented widespread declines at historically occupied Arizona toad localities that appeared to be driven by drying streams and ephemeral habitat degradation. Due to recent forest fires and subsequent heavy silt loads, toads did not breed in the Gila, San Francisco or Mimbres Rivers in 2013 (Ryan et al. 2013). Concomitantly, the drying of small streams and cattle tanks attributed to severe drought conditions resulted in further absence of breeding toads at historical sites (Breshears et al. 2005).

Here we present the results from our 2014 Arizona toad surveys in southwestern New Mexico. We summarize breeding surveys from 90 historic toad breeding sites, evaluate threats due to fires and drought and two fungal diseases. We have prepared a Species Account of *A. microscaphus* summarizing the behavioral and ecological information we have learned in 2013 and 2014 (Appendix 1). We then provide management recommendations and highlight future research needs. Finally, we provide a framework for future work that address the unknown aspects of the species biology and future threat risks that will be necessary for a robust management plan. This latter section was part of a proposal submitted to the New Mexico Department of Game and Fish in November 2014.

Methods

Field Methods

We conducted weekly breeding call surveys from 14 March to 25 April 2014 to determine presence/absence at historic Arizona toad localities in the Gila region. Historical sites were selected according to records obtained from the University of New Mexico, University of Kansas, University of Arizona, United States National Museum, Los Angeles County Museum, Texas Cooperative Wildlife Collection, University of Texas at El Paso, and the American Museum of Natural History. These museum collections represent the largest collections of *A. microscaphus* from sites in New Mexico. Our objective was to estimate the proportion of historical collection localities that are currently occupied by the Arizona Toad.

We used a standardized call-survey method to assess calling intensity of toads, which is an assay of breeding male relative abundance (Heyer et al. 1994). Sites were scored using a relative abundance metric: 0 = no toads heard calling; 1 = non-overlapping calling, individuals could be counted; 2 = calls overlapping but individuals can still be distinguished; 3 = full chorus, cannot distinguish individuals. For long-term documentation, we recorded toad choruses using digital audio recorders and these recordings are deposited with the Museum of Southwestern Biology at the University of New Mexico.

Each site was sampled 1-7 times between 14 March and 25 April 2014. Call surveys started at dusk and continued to 0200hrs. We supplemented call surveys with visual encounter surveys to obtain additional behavioral and ecological information on *A. microscaphus.* Supplemental data included: counts of adults (calling males and mating pairs), the number of egg masses, and presence of tadpoles.

The call of *A. microscaphus* is a long, 5-30 second, trill that can be heard for hundreds of meters (Ryan et al. unpubl). We used distance from a call survey site (predetermined point along road or trail with known geographical coordinates) to the point in a river, stream or tank, where toads were calling to estimate the maximum distance at which a toad can be detected.

Besides calculating the naïve occupancy rate (the proportion of sites where we detected *A. microscaphus* at least once), we used the single-season model in program PRESENCE to estimate the proportion of historical sites that were used for breeding at least once over the course of our study (Hines 2006). A non-detection of calls at a surveyed site can signify either the species was not present or we failed to detect it when it was present. PRESENCE uses a joint likelihood model to estimate the probability of missing a species when it is present at the site (detectability) and the probability that a site is occupied (occupancy) (MacKenzie et al. 2006). For purposes of parameter estimation we assumed that the probability of occupancy did not vary during the period of surveys but that the probability of detection was dependent on the date of sampling. We calculated distances between the 90 sampled sites and considered sites that shared the same water body (stream or lake) within 800m of each other to be the same for the PRESENCE analysis. This reduced the total number of sites for analysis to 77. In no instance was there conflicting information (detection or non-detection) between the sites considered to be the same for the PRESENCE analysis.

Disease

We collected Bd-disease swabs following standardized field protocols (Hyatt et al. 2007). We collected and stored *Bd* swab samples in 95% ethanol and stored them in a -80 C freezer until sent for processing (Boyle et al. 2004; Hyatt et al. 2007). Samples were submitted to Pisces Molecular Laboratory (Boulder, CO, USA) for site specific pooled analyses of *Bd* DNA using qPCR. We collected a total of 145 Bd swabs and to date have received results from 43 of the swabs; 22 swabs from West Fork of the Gila River and 21 from Indian Tank. The remaining swabs will be analyzed in early January, 2015.

We observed dead Arizona toads at two sites throughout our survey efforts. Nine dead Arizona toads were collected from Indian Tank, as well as two from O-Bar-O tank. The carcasses were collected to attempt to identify the cause of mortality via necropsy. The immediate cause of the toad's death was unknown. Histopathological examinations were done at the FWC Fish and Wildlife Research Institute in St. Petersburg, Florida.

Hybridization Between *A. microscaphus* and *A. woodhousii*

We reviewed all *A. microscaphus* and *A. woodhousii* specimens collected from Catron, Grant, Sierra, and Luna counties housed at the MSB, NMSU and WNMU museum collections. This specimen-based evaluation allowed for a morphological comparison to assess evidence of hybridization. We reviewed all adults, juveniles and tadpoles from these collections.

Invasive Species

At each site where we sampled *A. microscaphus* we conducted call and visual encounter surveys for invasive bullfrogs (*Lithobates catesbeianus*) and visual encounter surveys for crayfish. When possible, we collected the observed invasive species.

Breeding Biology, Upland Habitat Use & Diet

We recorded numerous observations on the breeding biology and habitat use of *A. microscaphus* during fieldwork that we present below. We removed stomach contents of specimens collected in 2014 and 2013. Invertebrates found in the stomach contents were identified by Grey Gustafson, an entomologist at UNM.

Results

Site Occupancy & Counts

We were able to determine that call surveys are an effective method of sampling presence of *A. microscaphus*. We found that *A. microscaphus* calls can be heard at a maximum distance of up to 933m, with an average of 536m, according to the 6 sites used for this calculation. Under most circumstances we were within 100 meters of a historical toad locality, well within the limits of the mean 536m distance of detecting calls, confirming that call surveys are a suitable and reliable method for *A. microscaphus*. Our analyses also indicate that a minimum of three call surveys is necessary to effectively determine site occupancy of toads due to temporal variation in breeding activity among sites.

Call survey sampling for *A. microscaphus* began 14 March 2014 and continued weekly through 25 April 2014 to estimate occupancy. We sampled 90 sites with a total of 231 individual site surveys (Figure 1; Table 1). Toads were detected at 31 of the 90 sites surveyed, resulting in naïve occupancy of 34% at historic sites.

The probability of occupancy calculated in PRESENCE indicates the likelihood of a given site being occupied over the course of the sampling season given the detection history of all other sites. For this analysis we present probability of occupancy for 77 sites (Table 2) based on detectability varying with each survey during the entire study. Our estimate of overall occupancy for the 77 sites analyzed in PRESENCE was 46.43% (SE = 7.42). For sites where toads were not heard calling, probability of occupancy as calculated ranges from 0.013 to 0.46. In addition, there is considerable spatial variation in the probability of occupancy throughout the study region, and we see clumps of both presence and absence of toads compared to historical sites (Figure 2). A summary of probability of occupancy shows that 35% of sites had a high occupancy with 65% of sites with low occupancy (Figure 3). This is concordant with our raw presence-absence estimates.

Although we detected toads at 31 sites there was considerable variation in the calling intensity among occupied sites (Table 3). Of the 31 occupied sites, 29 had a calling intensity of 1 or 2. This suggests the majority of occupied sites have small breeding populations. Only two sites had a calling intensity of 3, suggesting very few, known large populations of *A. microscaphus* exist in New Mexico.

During visual encounter surveys (VES) at the West Fork of the Gila River near the Gila Cliff Dwellings on 19 March 2014 we counted 244 adult *A. microscaphus* along a 220 m stretch of river, observed 22 pairs in amplexus, and counted 79 egg masses. During the VES at Indian Tank on 19 March 2014, we counted 220 *A. microscaphus*, observed 13 pairs in amplexus, and counted 124 egg masses. Refer to Table 4 for count data from additional sites.

<u>Disease and Unexplained Dead Toads</u>

Throughout the 2014 surveys, we collected 9 dead toad carcasses at Indian Tank and 2 dead toads at O-Bar-O Tank. We detected Bd in Arizona toads from Little Creek and Shooter Tank (Figure 4) and a previously unidentified fungal skin parasite from Indian Tank. None of the 20 toads sampled from Indian Tank tested positive for Bd (Ryan et al. 2014a). The lack of a positive test results likely rules out Bd as the causative agent of the dead toads at Indian Tank. This is the first record of Bd in the Arizona toad (Ryan et al. 2014b).

The results of histopathology from the dead toads detected mesomycetozoan cysts in the mucus glands containing possibly two fungal skin parasites, *Amphibiothecum sp.* or *Amphibiocystidium sp.* (Kiryu et al. 2014). Little is known about the effect of these parasites on amphibians, suggesting a need for further investigation. The specific identity of the parasites is unknown due to the need for samples preserved in ethanol or via freezing for molecular analyses (Feldman et al. 2005). If mortality should re-occur, samples should be immediately collected and preserved by either method to allow for specific determination of these parasites.

Hybridization Between *A. microscaphus* and *A. woodhousii*

We reviewed a total of 174 *A. woodhousii* and 159 *A. microscaphus* specimens and found no evidence of hybridization between these two species based on morphology (Ryan, unpublished). During the specimen review we identified specimens from Catron County erroneously identified as *A. woodhousii*. All specimens, adults and tadpoles, we reviewed which were identified as *A. woodhousii* in Catron County are *A. microscaphus*. This identification correction suggests that previous records of *A. woodhousii* from Catron County are spurious and that *A. woodhousii* is not as widespread in Catron County as has been previously reported (Degenhardt et al. 1996) and may not actually occur in the county. There are two specimens of *A. woodhousii* from extreme southern Catron County, near Glenwood, whose origin is questionable.

Upland toad observation

We observed *A. microscaphus* up to 2.5 km from active breeding events at Indian Tank in March and April. In all cases we observed gravid, adult females moving towards the calling event at Indian Tank. These toads were in grassland or open Ponderosa forests. In July, outside of the breeding season near Indian Tank, we observed toads (both male and female) up to 4km from known breeding sites, in grassland and open Ponderosa Pine forest. On 11 September 2014 we observed subadult *A. microscaphus* on the road near the Gila Cliff Dwellings, approximately 250m from the river.

Discussion

Our 2014 results support the 2013 findings of widespread declines of *A. microscaphus* in New Mexico. For our raw presence-absence surveys in 2014, we detected toads at 31 (34%) of the 90 sites surveyed representing a 16% increase in the number of occupied sites compared to 2013. The PRESENCE occupancy probability estimates for the 77 sites confirm this finding (Figure 2). We present both sets of results, but note that the PRESENCE analysis is a more robust estimate of occupancy.

Two factors explain the increase in detection between 2014 and 2013. The first is the increase in sampling effort between 2013 and 2014, with 36 additional sites sampled. The second, and most likely factor was the return of favorable stream conditions along many portions of the Gila River following the Whitewater-Baldy complex fire. In 2014, we detected toads breeding at 16 sites along the Gila River near the Gila Cliff Dwellings that went undetected in 2013 (Ryan et al. 2013). Despite the increase in number of occupied sites from 2013 to 2014 our results suggest a 66% decline in site occupancy of this species compared to the historical data. The PRESENCE results show that probabilities of occupancy calculated for sites where we did not detect toads are extremely low, often not higher than 30% or prob < 0.3.

The site-specific occupancy modeling provides valuable insights into the distribution of *A. microscaphus.* We see wide variation in occupancy estimates for sites where toads were undetected, from 0.013 to 0.46. This analysis highlights sites where toads have a moderate statistical probability of detection (17 sites at 0.2 to 0.46 probability), and should be priority sites for surveys in 2015 (Table 2). Furthermore, site-specific habitat assessments should be made at the moderate probability sites to elucidate the causes of the absence of toads in 2014.

In 2013, the majority of the Gila, Mimbres and San Francisco Rivers were heavily silted from the Whitewater-Baldy complex fire that precluded toads and other aquatic species, from breeding. A massive flash flood in September 2013 scoured the Gila River of the heavy silt loads resulting in a return to normal, clear conditions with a sandy-cobble substrate, necessary for toad reproduction in 2014. This provides strong evidence that forest fires have a strong effect on *A. microscaphus* annual breeding potential and population densities. Forest fire frequency and intensity is expected to increase in New Mexico (Dennison et al. 2014; Williams et al. 2013) and may increase the likelihood of stochastic population extirpations. If fires are too intense or increase in frequency to the level where river siltation is a regular or annual occurrence, toad populations may decline from population attrition (Alford & Richards 1999). Most arid environment anurans can forego breeding for 1-2 years with minor effects on local populations, but prolonged absence of breeding can lead to population extirpations (Wells 2007). With the likelihood of fires to become more frequent in the southwest as drying trends continue, this is a serious threat to riparian toads.

Long-term drying trends and short-term drought conditions can further exacerbate climatic stressors on toads. At Indian Tank, Alexander Cienega, Sawmill Tank, Mimbres River and Pueblo Creek, receding water levels resulted in the stranding of egg masses and tadpoles. The dry winter and spring left many water bodies in the Gila at low levels, which became lower as seasonal temperatures increased. The mean number of eggs per clutch for *A. microscaphus* is 4,500 eggs (Blair 1955) and the loss of eggs from drying can be catastrophic. For example, we counted 96 dried and stranded egg masses at Indian Tank on 8 April 2014, for an estimated loss of 432,000 eggs. At Pueblo Creek, we counted 12 dried egg masses on 14 April for an estimated loss of 54,000 eggs. This is likely an underestimate because does not account for stranded tadpoles which we did not count or egg masses that may have been overlooked or predated upon once exposed. Thus, while toads may breed in a given year in absence of fire threat, drying of water bodies is a continual threat to long-term population trends.

The observation of dead and sick toads at Indian Tank and O-Bar-O Tank are troubling because we are unsure of the cause of death of these toads. It is possible that environmental stressors (e.g. drought conditions) were directly responsible for the dead toads we observed, although we cannot rule out disease or synergistic interactions between disease and environmental conditions as the cause (Fisher et al. 2009). We have ruled out predation, as none of the carcasses showed any signs of predation. Six of the toads were found desiccated at the edge of the water bodies and three were found dead floating in in sitting postures in the water suggesting rapid death (Lips et al. 2006; Ryan pers obs.)

We report the first incidence of the fungal disease *Batrachochytrium dendrobatidis* (Bd) in *A. microscaphus*, but the role of Bd as a factor in toad declines remains unknown. Within the Gila region, Bd has caused declines in Chiricahua Leopard Frog populations, and both species have tested Bd-positive at Shooter Tank (Ryan et al. 2014a). Many toad species are highly susceptible to Bd, and toad population die-offs, declines, and local extirpations are commonly reported from this disease (Olson et al. 2013). While we have no direct link between Bd and toad declines, further work is needed to understand the Bd-toad dynamics in New Mexico.

Another fungal disease or parasite of unknown etiology we found in *A. microscaphus* belongs to the genera *Amphibiothecum sp.* or *Amphibiocystidium* sp. (AA spp. hereafter). Little is known about the extent of pathogenicity of AA spp. in amphibians, but AA spp. appear to be more common in Europe than North America (Pascolini et al. 2003). Infections of AA spp. are not commonly lethal, but frogs and toads can become debilitated and emaciated resulting in mortality (Densmore & Green 2007). In Europe, where AA spp. appears to be endemic, there is little evidence AA spp. can cause mortality, but there is general consensus more field data and lab experiments are needed to determine the ecological impacts of infection (Pascolini et al. 2003).

There are few reports of AA spp. in North America. Thus far in North America, *Amphibiothecum penneri* has been reported in American toad, *Anaxyrus americanus* (Jay & Pohley 1981) and Yosemite toad, *A. canorus* (Green & Sherman 2001). More recently *Amphibiocystidium viridescens* was recently described in Eastern red spotted newts, *Notophthalmus viridescens* (Raffel et al. 2008). Raffel et al. (2008) reported evidence of mortality from *A. viridescens*, and it is likely that as this disease complex becomes more common similar incidences of mortality will emerge and threaten North American amphibians.

This is the first documentation of this fungal skin parasite in *A. microscaphus.* It remains unknown if this parasitic infection alone was heavy enough to compromise and kill toads (e.g. Densmore & Green 2007). This infection in *A. microscaphus* indicates a new host record, or potentially a new species of mesomycetozoan. It is possible that this case is another record of *A. penneri*, although unlike the other reports, the internal cyst is not divided into septa (a species determinant) in the observed *A. microscaphus* infections (Green & Sherman 2001). Without molecular confirmation, the identity of this parasite remains undetermined. It is important to monitor this situation considering the lack of information of the ecological effects of the parasite on wild amphibians, but there is evidence of mortality in amphibians (Raffel et al. 2008). Follow-up surveys in 2015 would help determine if this disease is still present, aid in specific identification of the parasite, and monitoring will allow management agencies to track the parasite to see if it becomes more infectious and pathogenic over time, which is characteristic of many newly emerging diseases (May et al. 2001).

Our preliminary observations of upland habitat use of *A. microscaphus* suggest that toads require large buffer areas around breeding sites. While this is based off of a single site, this pattern fits with observations on upland habitat use of other aquatic breeding frogs and toads. Toads use these upland habitats for foraging during the non-breeding season and for over-wintering sites (Lamoureux & Madison 1999; Holland & Sisk 2002). It is not known where the Arizona toad over-winters, but other toad species are known to burrow in soft soils in upland habitats adjacent to riparian breeding habitats. Much knowledge of upland and over-wintering behavior could be gained from future radio-telemetry and upland habitat surveys.

Our 2013 and 2014 work has revealed that hybridization between *A. microscaphus* and *A. woodhousii* is not currently a threat to *A. microscaphus* in New Mexico. Our review of museum specimens found no historical evidence of hybridization, which was corroborated by field surveys. Two factors seem to prevent hybridization between the two species. First, *A. microscaphus* breeds from March to April, whereas *A. woodhousii* breeds May to July. This mismatch in breeding phenology appears to be a temporal barrier, although the potential for overlap in breeding phenology may occur in late April and early May if weather conditions are appropriate. We are limited with our current data to confidently support the temporal breeding phenology barrier. Second, *A. microscaphus* is primarily a stream breeding species (with some exceptions) whereas *A. woodhousii* primarily breeds in lentic bodies water or on the margins of wide river stretches. We have recorded *A. woodhousii* calling from the Gila River near the towns of Cliff and Gila in later April where there were isolated pools along the river. If hybridization were to occur between the two species this would be a high probability site. We did not find any evidence of hybridization from this site to date.

Hybridization between *A. microscaphus* and *A. woodhousii* is thought to be facilitated by altered water flow regimes from dam construction and other anthropogenic factors in Arizona, Nevada and Utah (Bradford 2002; Hammerson & Schwaner 2004). In these states hybridization is the primary threat to *A. microscaphus* (Hammerson & Schwaner 2004). It appears that the lack of major water impoundments along the Gila River and other drainages in New Mexico may have prevented hybridization between these two toad species.

Our 2014 work has elucidated the dynamic breeding plasticity of *A. microscaphus* in New Mexico. Fires and water conditions appear to strongly influence whether breeding will take place along affected rivers and streams. Results show that toads can forego breeding in one year (2013) and have large breeding success in the following year (2014) under appropriate abiotic conditions. The dynamic breeding ecology, which strongly influences detection, of *A. microscaphus* creates a monitoring and management dilemma. It has become apparent to us that even 2 consecutive years of data are insufficient to truly understand long-term population trends. Our data strongly support significant declines, although the annual variation in breeding and detection in *A. microscaphus* is greater than other species of North American frogs and toads (Alford & Richards 1999; Green 2003; Wells 2007). A longer time series may show that in any given year a maximum of 30-35% of sites are occupied, but on a 4-5 year time scale, average site occupancy and breeding are much greater. While preliminary, our 2013 and

2014 data suggest this scenario is realistic. Understanding long-term occupancy will be critical in designing a management plan and in determining whether increased protective measures are necessary for this species.

The drying of smaller streams and tanks present an additional threat as eggs and tadpoles are stranded and die from desiccation. We have learned that the fungal diseases Bd and *Amphibiothecum sp.* or *Amphibiocystidium* sp. infect *A. microscaphus*, but their roles in population declines and dynamics remain unknown.

Through our work we have learned that some of the observed threats cannot be easily mitigated for, (fires, disease) but cattle tank drying may be managed if made a priority by the appropriate agencies. Future work can focus on developing applied management strategies. We have documented a very spatially clumped distribution of toads along river drainages, but do not have an understanding of patch dynamics or movement ecology. Finally, preliminary observations show that toads may move up to 4km from breeding sites suggesting they require a large buffer zone around breeding sites. To address whether and how toads use habitat corridors and the size of upland habitat buffers, future radio-telemetry and population genetic work needs to be conducted. While our results suggest widespread declines, there are some seemingly healthy populations of *A. microscaphus* in New Mexico. The current status of *A. microscaphus* in New Mexico seemingly is not yet critical, but current and future threats make this species' status precarious. Following up and expanding on this work may help to prevent an increase in state and federal protection of *A. microscaphus* in New Mexico.

Management Recommendations

Many of the threats facing *A. microscaphus* are beyond the scope of traditional management actions due to the scale of the threats. These include disease, forest fires and long-term drying trends resulting in the loss of small streams. Yet, we can identify management strategies that can preserve the integrity of target populations. Furthermore, proactive measures such as translocation of eggs can supplement small or threatened populations.

<u>Recommendation 1</u>*: Monitoring and management of cattle tank water levels.* Indian Tank is one of our most valuable study sites because it has one of the largest populations and provided insights into the drying effects on *A. microscaphus*. As stated earlier, drying and receding of the waterline at Indian Tank resulted in the loss of an estimated 400,000 eggs.

A simple method for mitigating the loss of eggs and tadpoles would be to maintain stable water levels at tanks with breeding *A. microscaphus*. This action would only be necessary during dry years and for the development time of eggs and tadpoles. We estimate that water level management would need to occur until mid-April. This Strategy would also benefit cattle and provide additional habitat for both game and nongame species.

Recommendation 2*: Create more cattle tanks for toad habitat in A. woodhousii free areas.* This option should only be considered in areas along the Gila, Mimbres, and San Francisco Rivers that are vulnerable to siltation from forest fires and are known to be free of *A. woodhousii*. While experimental, if a series of small tanks were created and managed in years that the Gila River has a heavy silt load from fire, at least some toads may breed during these years when otherwise no breeding would occur. For this option to be feasible it will be necessary to consult with engineers and identify safe locations at a distance from the river to avoid flood damage but allow toads access. Furthermore, this should only be done in areas free of *A. woodhousii* in order to prevent hybridization. Considering the increased risk and frequency of fires and subsequent erosion in the Gila this type of experimental approach can help to naturally supplement toad populations during years with poor river conditions. By providing toad populations near the Gila River with alternative breeding sites during poor river condition years will assure at least some potential population recruitment. The addition of new tanks may also benefit populations of the federally threatened Chiricahua Leopard Frog.

Recommendation 3: *Monitoring of focal populations for disease outbreaks*. It is logistically difficult to monitor all *A. microscaphus* localities in the Gila Region, but monitoring of focal sites for sick, moribund, or dead toads can help in rapid implementation of emergency conservation actions. Focal sites should be selected based on spatial distribution, ease of access and population size. Preliminary focal sites include: Indian Tank, West Fork, Little Creek, Hell's Hole, and Pueblo Creek. All of these sites are on Public Lands and offer easy access. With cooperation from private landowners, the number of such sites may be expanded.

The most effective way to implement this action would be to train local Forest Service personnel in day time survey techniques that require walking the edge of tanks or streams to look for sick, moribund, or dead toads. If a sick, moribund, or dead toad is found the volunteer will place the toad in a bag and record relevant collection data (date, time of day, geographic coordinates, toad in water or on shore, etc). All toads should then be stored in a freezer and a designated contact person be notified. A follow-up crew should visit the site as soon as possible to collect more disease swabs at night when toads are active.

Recommendation 4: *Relocating egg masses and tadpoles from large populations to small populations.* As our call surveys show the majority of toad populations are small and because of this are more vulnerable to local extirpation from a stochastic event. It is possible to supplement these smaller populations with egg masses and tadpoles from sites such as Indian Tank and West Fork that currently have robust populations. Before this option is implemented the following considerations need to be accounted for:

1) *Breeding success in the previous year*. If a site had high egg loss or did not breed the previous year (see example from West Fork in 2013 and Indian Tank in 2014) this site should not be used as a source for other populations. First priority should be given to maintain the health of any potential source populations. If a potential source population has had successive years of high reproductive success, then a subsample of egg masses or tadpoles can be translocated to smaller populations.

2*) Emergency action*. If a tank or small stream is found to be drying out and it is determined that all eggs and tadpoles will be lost, all efforts should be made to relocate eggs and tadpoles to a more stable water body. Alternatively, drying of tanks could be mitigated through supplementation of water (Recommendation #1)

3) *Chytrid status.* Emergency translocations of tadpoles and egg masses should not be conducted from sites previously identified to be Bd-positive within the last year.

Recommendation 5: *Re-sample and conduct habitat assessments at sites with moderate to high occupancy probability, where toads were absent in 2014.* The PRESENCE occupancy probability estimates provide an ideal framework to maximize population assessment actions. There are 17 sites that have moderate probability of occupancy, but where *A. microscaphus* were not detected in 2014. As our 2013 and 2014 results show, there is significant annual site-specific variation in occupancy. As discussed above, myriad factors play a role in the dynamic annual breeding biology of *A. microscaphus*.

Monitoring of these 17 sites in 2015 may result in detection of *A. microscaphus* (inferred from moderate probability of occupancy estimates), which would improve our decline estimates. This action cannot focus solely on the 17 sites, but would have to include a replication of the 2014 work to be able to provide the same robust statistical estimates.

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Publications and Abstracts

To date we have published one paper from this work and submitted one abstract to The Wildlife Society's annual meeting of Arizona and New Mexico Chapters (Feb 2015). We are currently preparing three manuscripts for submission to peer-reviewed journals. Listed below are the citations of published and in preparation manuscripts.

Published:

Ryan, M.J., I.M. Latella, C.W. Painter, J.T. Giermakowski, B.L. Christman, R.D. Jennings, & J.L. Voyles (2014) First record of *Batrachochytrium dendrobatidis* in the Arizona toad (*Anaxyrus microscaphus*) in southwestern New Mexico, USA. Herpetol. Rev. 45:616-618.

Submitted:

Ryan, MJ, IM Latella, & JT Giermakowski. The decline of another southwestern anuran species? Recent population trends of the Arizona Toad in west-central New Mexico. New Mexico & Arizona Wildlife Society Conference (Feb 2015)

In Preparation:

Ryan, MJ, IM Latella, & JT Giermakowski. Range correction of the toad *Anaxyrus woodhousii* (Amphibia: Bufonidae) in southern New Mexico. Target Journal*: Southwestern Naturalist*.

Ryan, MJ, IM Latella, JT Giermakowski & G Gustafson. Toads eat the craziest things: diet of the Arizona Toad (*Anaxyrus microscaphus*) in New Mexico. Target Journal: *Journal of Herpetology.*

Ryan, MJ, IM Latella, & JT Giermakowski. The decline of another anuran in the southwestern United States? Recent population trends of the Arizona Toad in west-central New Mexico. Target Journal: *Biological Conservation.*

APPENDIX 1

SPECIES ACCOUNT: *Anaxyrus microscaphus*

This account summarizes the information we have learned in 2013 and 2014 and is not a comprehensive account. This account is meant to supplement the account presented in Degenhardt et al (1996) and Schwaner & Sullivan (2005). Body Size*:* (snout-to-vent length in mm; ± Std Dev)

<u>Species Description</u>. A moderately sized toad with females attaining a larger body size than males. Adult males have an average body size of 65.1±4,5mm (n=95) and reach a maximum body size of 72.4 mm. Adult females have an average body size of 74.6±7.3 (n=48) and reach a maximum of 87.9 mm.

Snout profile is truncate to slightly sloping. The dorsal surface is light to dark brown with small brown or black blotches scattered on the back. The body has numerous small raised glands that can have a brown to orange color. Some individuals exhibit an orangish coloration on head. Paratoid glands are longer than wider, and can have a brown to reddish-orange color. Some individuals may posses a hint of an incomplete mid-dorsal line. Paratoid glands are slightly longer than the upper eyelid length.

Ventral surface is white to yellowish. Throats of males may be brown during the breeding season and is white in the non-breeding season.

Fingers are not webbed and toes are extensively webbed. During the breeding season males posses a dark brown to black nuptial thumb pad. Coloration fades during the non-breeding season.

Breeding Habitat

Breeding sites in New Mexico occur along stream and rivers and in cattle tanks that do not have canopy cover. When breeding in streams *Anaxyrus microscaphus* occur in shallow water along the margins of streams, backwashes, or side pools where water flow is minimal (Schwaner & Sullivan 2005). In New Mexico mean water depth along the West Fork of the Gila River was 4.5 cm (Range 1.5-7cm). Toads appear to require clear water conditions with sand or cobble substrates.

The use of cattle tanks as breeding habitat appears to be a novel finding for this species and has not been previously reported. When breeding in cattle tanks, water conditions can be cloudy and toads were found in shallow water near the shoreline.

A. microscaphus appears to select specific stretches of river for breeding, which can have serious implications for management practices. We rely on this assessment from the West Fork of the Gila River and Little Creek. On 29 March 2014, we walked 4 river miles up the West Fork of the Gila River and counted egg masses. This survey resulted in very high densities (>80) of egg masses at a bend with shallow water. From this breeding epicenter, the number of egg masses decreased the further upstream we surveyed. We found sporadic clumps of egg masses ranging in number from 1-10, spaced 10s to 100s of meters apart, for the rest of the 4 river mile survey. On the same day we sampled Little Creek in the same manner, but for 2 river miles and found the same pattern, but with many fewer egg masses. The breeding epicenter on Little Creek yielded 15 egg masses. Similar patterns were observed at Hell's Hole along the Tularosa River, although on a smaller scale.

The cause of the observed clumped spatial distribution of *A. microscaphus* egg mass deposition along riparian habitats remains unclear. We hypothesize that a combination of stream conditions and upland habitat use play a role in breeding site selection along streams and rivers. From our observations, *A. microscaphus* requires clear, shallow, slow moving water for egg deposition. Mean water depth along West Fork was 4.5 cm (Range 1.5-7cm). Substrate conditions were sandy or pebbly with no emergent vegetation. At West Fork and Little Creek the highest densities of egg masses (and breeding toads) occurred along stream stretches where the flood plain was wide (>250m) and bordered by gentle slopes leading to upland forest. Further upstream from the breeding epicenters the floodplains narrowed into steep canyons. The upland habitat, i.e.

floodplain and adjacent slopes, of the breeding epicenter have relatively sandy soils that seem to be an important habitat characteristic. The sandy soil characteristic is also prevalent around Indian Tank and Little Creek. During the day toads make shallow burrows in the sandy soil, and it is possible they make deeper burrows in the winter. Future surveys should incorporate more detailed habitat characteristic data to allow for a better evaluation of ideal breeding habitat requirements.

Breeding Ecology

In New Mexico *A. microscaphus* breeds early in the year, typically early- to mid-March and will continue calling and egg laying into April. These toads are tolerant of cold conditions and have been observed calling and in amplexus at air temperatures as low as 30°F. This behavior is unusual for New Mexico amphibians and early breeding may be to take advantage of snowmelt and low river conditions typical of spring or to avoid competition from predatory invertebrates or tadpoles and larvae of other amphibian species.

Previous researchers have reported that *A. microscaphus* is an explosive breeder, meaning that they form large choruses with majority of seasonal reproduction occurring over the course of 10-12 days (Sullivan 1992). In New Mexico *A. microscaphus* is a prolonged breeder, with calling and egg-laying occurring for 3-5 weeks. It is unclear why New Mexican populations of *A. microscaphus* have adapted this alternative breeding strategy.

At dusk toads emerge from shallow burrows up to 20m from the breeding site. We observed numerous instances where toads emerged from burrows and measured the depth of three burrows. The burrows were 3, 3, 4.5 cm deep. When toads emerge they explode from the burrows and will hop 2-4 feet very quickly. They then orient themselves and begin moving towards the water body.

Toads will begin nighttime chorusing between 15 and 30 minutes before sunset and continue calling until at least 0200hrs. Although most calling occurs at night, we discovered *A. microscaphus* will call during the day. This behavior is uncommon in North American toads species. On 29 March 2014, along the West Fork of the Gila River, we heard males calling at 1200hrs under clear and sunny conditions. On this day we also observed one amplectant pair at 1219hrs, submerged in flowing water.

Males are the dominant sex at breeding sites on any given night. Females approach breeding sites from adjacent upland habitats, up to at least 2km away. Calling intensity increases when females approach and there can be intense competition between males for access to females. Males will wrestle and kick to prevent access to a female. In some cases when a male amplects a female, satellite males will attack the pair forming a "toad ball" with 2-5 males on top of a single female. During these fights toads are wrestling and kicking until a winner emerges. It is unclear how long these "toad ball" fights last, but in two instances we observed these interactions lasting for more than 45 minutes. The female during these fights is at the bottom of the pile, usually submerged in water.

After amplexus occurs toads may remain this way for many hours before egg laying begins. Amplectant pairs can be found on land, at the water's margin, or along the bottom of a stream. When ready to lay eggs, the pair will move to an area with shallow, slow moving water. The female then expels a clump of eggs (about the size of a table spoon) and the male fertilizes them while kicking the eggs away from the female. While being kicked away by the males, the egg mass takes on the typical string of eggs, characteristic of toads. The amplectant pair will repeat the egg laying-fertilizing process many times over the course of 45 to 70 minutes. The average number of eggs in a single clutch is 4,500 (Blair 1955). It is unknown whether *A. microscaphus* will produce more than one clutch per year, but other species are known to do this. Egg masses tend to occur spatially clumped along stream and cattle tank margins, and it is normal to see more than 40 egg masses in a 2-3 meter area.

Egg to tadpole development times range from 3-6 days and is dependent on water temperatures (Schwaner et al. 1998). It is unclear how long it takes a tadpole to develop into a metamorphic toad, but in New Mexico metamorphic *A. microscaphus* can be observed from May through June. Tadpoles presumably feed on algae and small unicellular organisms attached to the water body substrate.

<u>Non-breeding Season Ecology</u>

Very little is known about the non-breeding ecology of *A. microscaphus*. Outside of the breeding season, we have found adult toads active on roads in the Gila Region in June, July, and August on dry and rainy nights. These observations were up to 4km from known breeding sites. Outside of the breeding season we have found toads in grasslands and Ponderosa Pine forests.

It is unclear if *A. microscaphus* exhibits migratory behavior to breeding sites. Our observations of toads up to 4km from known breeding sites suggests they may have seasonal migration between upland overwintering sites and breeding sites.

<u>Feeding Ecology & Diet</u>

Breeding males appear to not feed or feed opportunistically while at a breeding site. Of 45 toad stomachs examined from male specimens captured at breeding sites, 41 had completely empty stomachs and 4 had only small remnants of prey. In contrast, all 35 toad stomachs examined from upland habitats outside of the breeding season had full stomachs.

We sampled the gut contents of 20 *A. microscaphus* specimens collected in 2013 and 2014. Previous diet analyses of *A. microscaphus* conducted by Tanner (1931) identified “bugs, beetles and moths” from 5 specimens collected in Utah.

The preliminary results are very surprising. We found *A. microscaphus* to eat a wide variety of prey and expected prey items found were crickets, grasshoppers, and various beetles. What was most surprising was that the majority of toad food items consumed were highly toxic or dangerous. Examples include: *Dasymutilla* sp. (cow killer beetle); wandering spiders; camel spiders; sun spiders; scolopendromorph centipedes; and ants.

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FIGURES

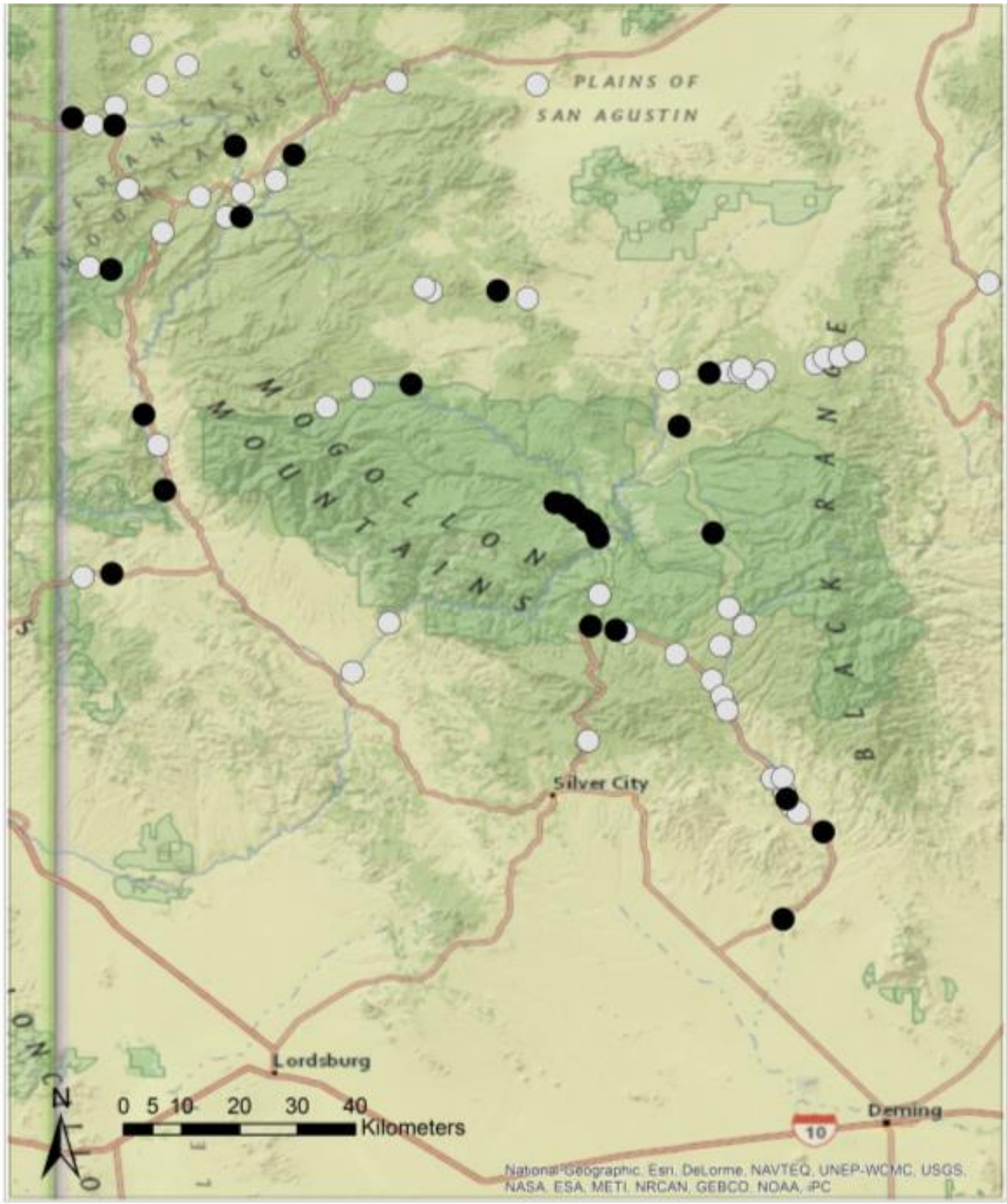


Figure 1. Map of the study area in west-central New Mexico showing occupancy patterns of the 77 sites sampled for presence of *Anaxyrus microscaphus* in 2014. Black circles denote occupied sites; white circles denote unoccupied/undetected sites.

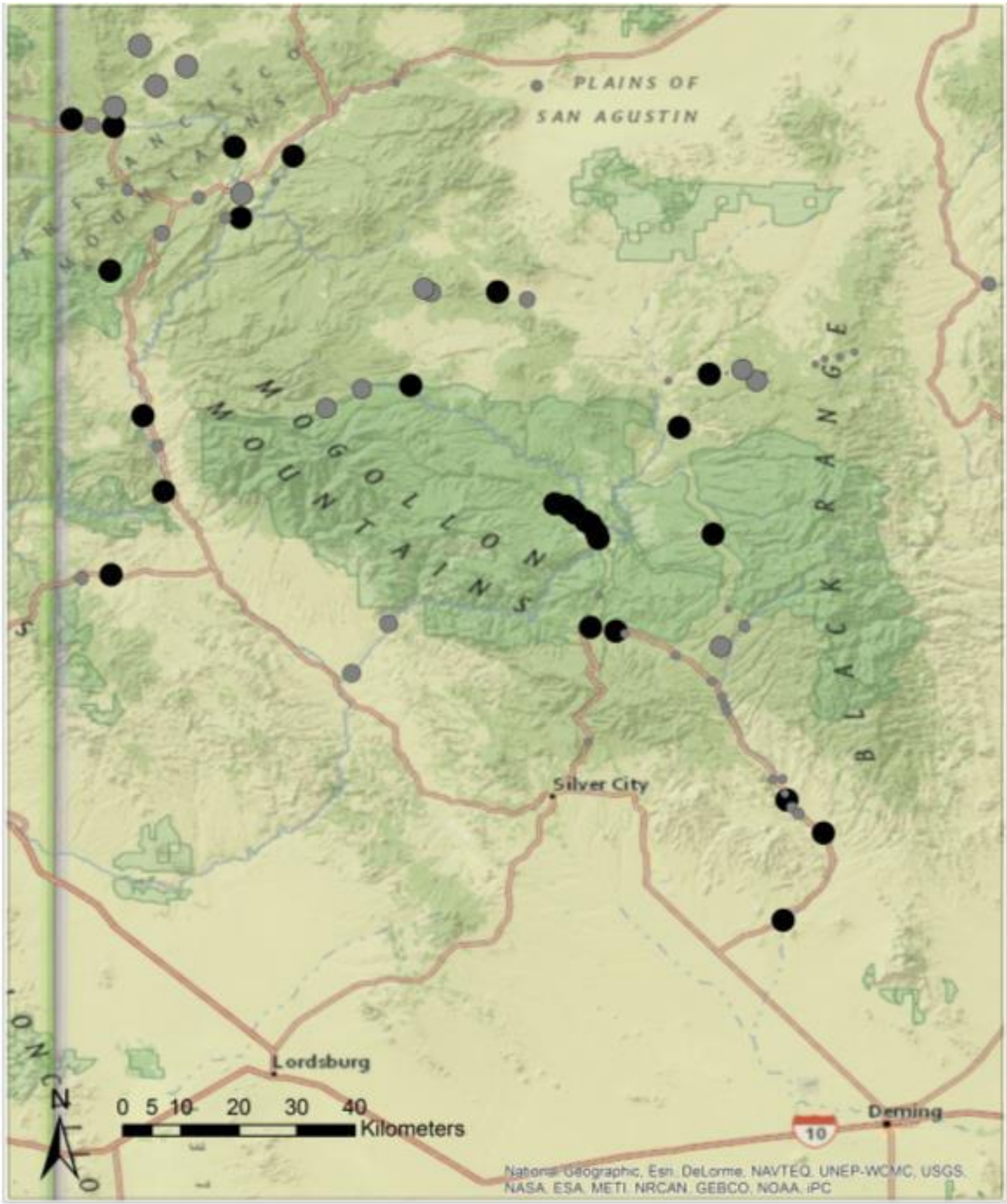
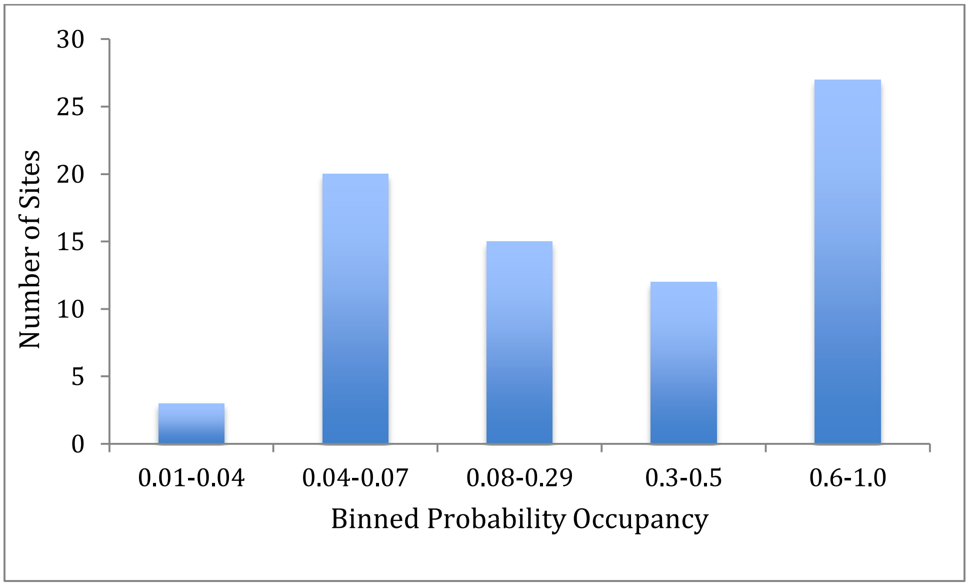


Figure 2. Map of the study area in west-central New Mexico showing per-site detection probability of the 77 sites used in PRESENCE analysis for *Anaxyrus microscaphus* in 2014. Large black circles denote an occupied site; grey circles represent an estimated probability of occupancy as calculated in PRESENCE (see methods). Size of grey circles is proportional to the probability of occupancy of that site.

A.



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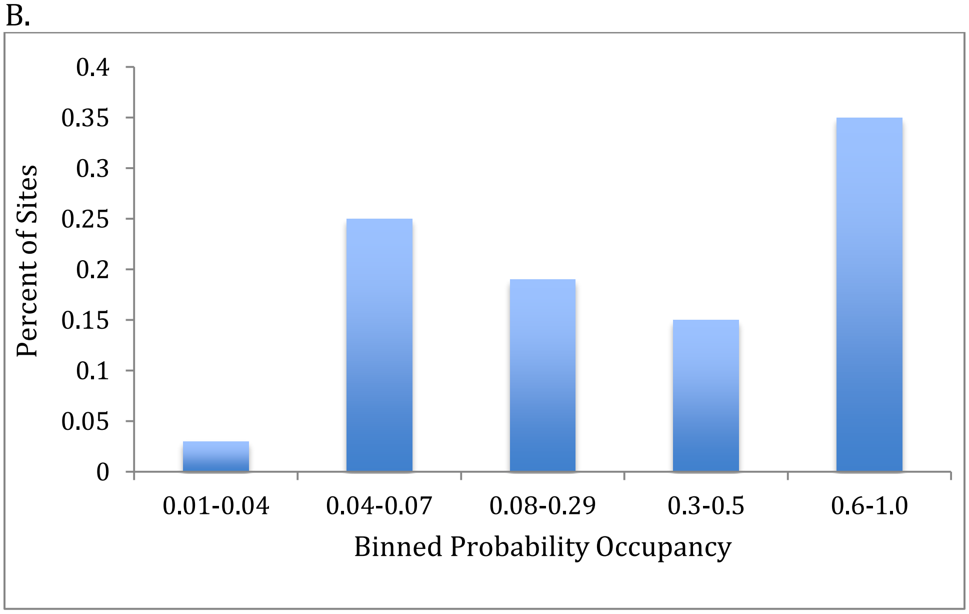

Figure 3. Summary of probability of occupancy for 77 sites used in PRESENCE analysis. Binned Probability Occupancy covers the number and frequency of sites within the value range. A) number of sites by binned probability; B) proportion of binned sites by probability. It is important to note that toad were detected only in bin 0.6-1.0 and all other bins estimates are interpolated from occupied sites. These results are concordant with 65% decline in occupied sites from raw presence-absence data.

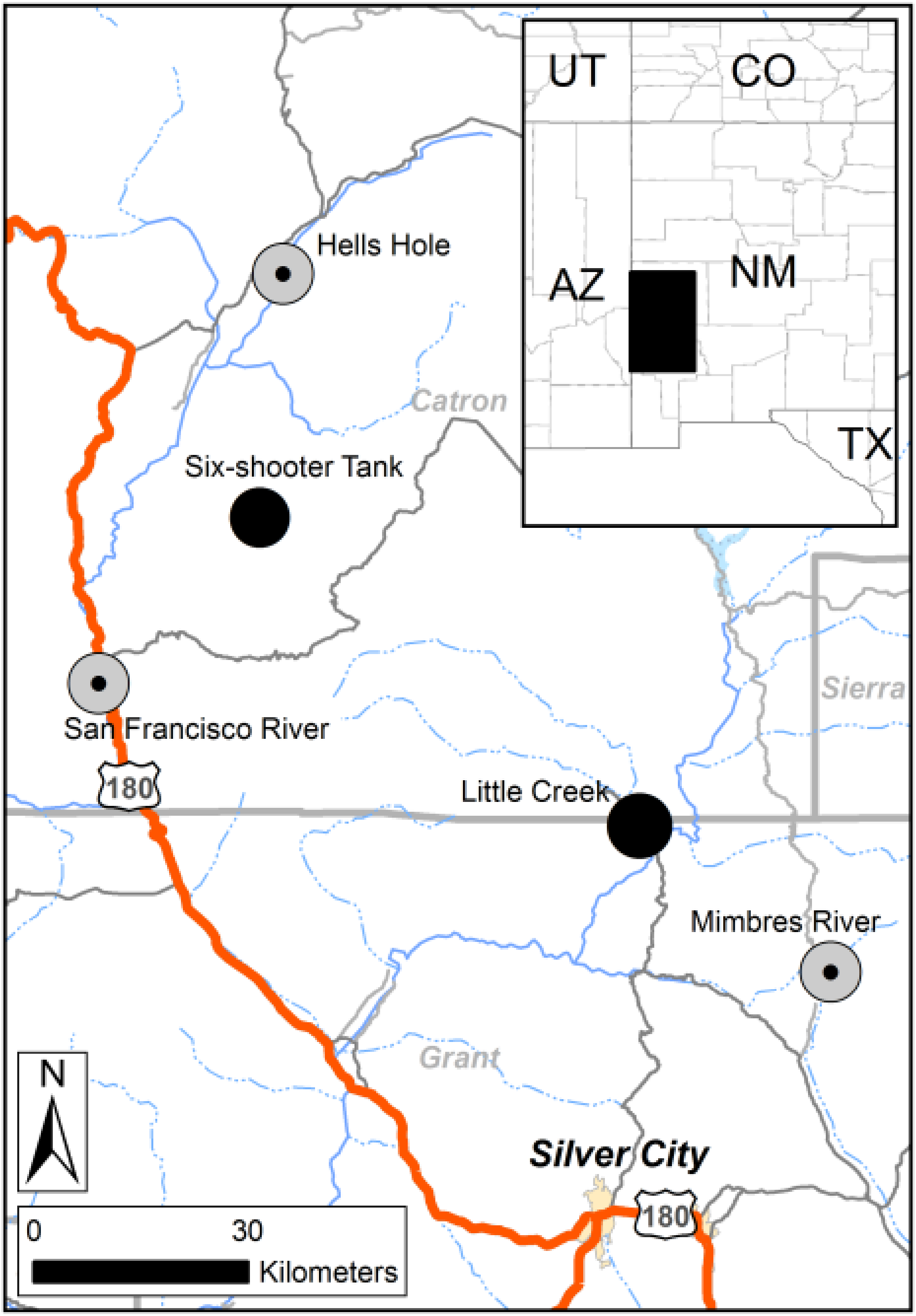

Figure 4. Distribution of localities in two counties of southwestern New Mexico from which Arizona toads (*Anaxyrus microscaphus*) were examined for *Batrachochytrium dendrobatidis* (*Bd*). Filled circles indicate sites that tested positive whereas open circles with a dot indicate sites where *Bd* was not detected. From Ryan et al. 2014.

Table 1. Summary of sampling effort of *Anaxyrus microscaphus* sampled in 2014. Calling index is an assay of abundance based off of calling males. We made a total of 251 sites visits at the 90 sites between 11 March and 8 May 2014. Calling intensity index: 0 = no toads heard calling; 1 = individuals could be counted; 2 = calls overlapping but individuals can still be distinguished; 3 = full chorus, cannot distinguish individuals.

Site #	Locality	Easting	Northing	Zone	Max Call Index	# Visits
1	NM59-01	769947	3701903	12S	0	4
2	NM59-02	775849	3703252	12S	3	5
3	NM59-03	778366	3703478	12S	0	5
4	NM59-04	222321	3703386	13S	0	5
5	NM59-05	225952	3703390	13S	0	5
6	NM59-06	233319	3704694	13S	0	4
7	NM59-07	234702	3705553	13S	0	4
8	NM59-08	236917	3705703	13S	0	4
9	NM59-09	239050	3706598	13S	0	4
10	NM61-01	226874	3630976	13S	0	4
11	NM61-02	227145	3629867	13S	1	4
12	NM61-03	227830	3628533	13S	0	3
13	NM61-04	228767	3627440	13S	0	3
14	NM61-05	232310	3624051	13S	1	3
15	NM61-06	226022	3609180	13S	1	3
16	NM78-01	690315	3666687	12S	1	3
17	NM78-02	686109	3666029	12S	0	2
18	FS150-01	221800	3659949	13S	0	2
19	FS150-02	779773	3662919	12S	0	3
20	FS150-03	777161	3675731	12S	1	2
21	FS150-04	771764	3693949	12S	0	2
22	NM12-01	749675	3752134	12S	0	2
23	NM12-02	729516	3752144	12S	0	4
24	NM12-03 (Hell Hole)	715049	3739211	12S	2	3
25	NM12-04	706572	3740606	12S	1	3
26	NM12-05	712554	3734784	12S	0	3
27	NM12-06	701656	3731749	12S	0	3
28	MSB 48680_NM12	728446	3751596	12S	0	2
29	MSB 43420_NM12	713067	3735704	12S	0	1
30	NM15-01	760060	3639560	12S	0	3
31	NM15-02	759883	3659258	12S	1	4
32	NM15-03	761017	3664717	12S	0	4
33	NM15-04	760607	3674550	12S	2	4
34	NM15-05	760322	3675481	12S	1	4
35	NM15-06	759480	3676971	12S	2	4

36	NM15-07	758762	3677438	12S	2	4
37	NM15-08	757159	3678818	12S	2	4
38	NM15-09	756620	3679347	12S	2	4
39	MSB 23153_15	757226	3679072	12S	1	2
40	MSB 78432_15	758284	3677881	12S	2	2
41	MSB 23159_15	758187	3677987	12S	2	2
42	NM159-02	720743	3695967	12S	0	1
43	NM159-03	725826	3699361	12S	0	2
44	NM159-04	732876	3700176	12S	0	1
45	NM159-05	735424	3716267	12S	0	2
46	NM159-06	744961	3716574	12S	1	2
47	Slater Tank 159-5	734340	3716886	12S	0	1
48	Snow Lake on 159	732530	3700583	12S	2	1
49	NM211-01	725490	3650449	12S	0	3
50	NM293-01	730646	3659098	12S	0	2
51	NM35-01	763551	3658654	12S	2	3
52	NM35-02	764872	3658268	12S	0	3
53	NM35-03	772355	3654725	12S	0	3
54	NM35-04	777720	3650446	12S	0	3
55	NM35-05	779732	3646425	12S	0	3
56	NM35-07	225148	3633443	13S	0	3
57	NM35-08	226644	3633506	13S	0	3
58	MSB 23148_35	772994	3654267	12S	0	3
59	USNM 320147_35	779291	3647728	12S	0	3
60	MSB 23157_35	771717	3655183	12S	0	3
61	USNM 320145_35	780064	3645311	12S	0	3
62	USNM 320146_35	779586	3647011	12S	0	3
63	USNM 320275_35	779988	3645651	12S	0	3
64	US180-01	683123	3744910	12S	2	4
65	US180-02	686083	3743881	12S	0	3
66	US180-03	689189	3743785	12S	2	4
67	US180-04	691340	3732870	12S	0	4
68	US180-05	696466	3725519	12S	0	3
69	US180-06	689173	3718920	12S	1	4
70	WF Pueblo Creek	686014	3719276	12S	0	3
71	US180-10	694389	3694115	12S	1	3
72	US180-11	696558	3688934	12S	0	2
73	CatronCoRd_B012	699399	3754323	12S	0	1
74	CatronCoRd_B080	692681	3757727	12S	0	1
75	NM435-01	705578	3728375	12S	0	2
76	NM435-02	707751	3728422	12S	1	3
77	SA Creek	695096	3750864	12S	0	1
78	Trout Creek	689228	3747011	12S	0	1
79	SF Day Use	697660	3681132	12S	1	3

80	NM52-01	258656	3717844	13S	0	1
81	WF Eggs-1	754165	3680363	12S	3	3
82	WF-1	755895	3679951	12S	2	3
83	LACM 127295_152	225113	3632795	13S	0	2
84	UAZ 54446	756468	3679402	12S	2	1
85	Sheep Tank	749258	3715352	12S	0	2
86	unnamed tank-59	224726	3702086	13S	0	1
87	Doagy Tank	222751	3704067	13S	0	1
88	North Tank	778774	3656393	12S	0	1
89	Reserve UAZ-15433	707830	3732599	12S	0	1
90	MSB 48683_12	728467	3751717	12S	0	1

Table 2. Summary of PRESENCE analysis estimating probability of occupancy for 77 sites with call index and sampling effort per site.

Site	Prob. occupancy	Prob. occupancy (SE)	95% Confidence interval	Maximum call index	Number of visits
NM59-01	0.0477	0.0618	0.0035-0.4182	0	4
NM59-02	1	0	1	3	5
NM59-03	0.013	0.0211	0.0005-0.2491	0	5
NM59-04	0.013	0.0211	0.0005-0.2491	0	5
NM59-05	0.013	0.0211	0.0005-0.2491	0	5
NM59-06	0.0477	0.0618	0.0035-0.4182	0	4
NM59-07	0.0477	0.0618	0.0035-0.4182	0	4
NM59-08	0.0477	0.0618	0.0035-0.4182	0	4
NM59-09	0.0477	0.0618	0.0035-0.4182	0	4
NM61-01	0.0628	0.0429	0.0158-0.2186	0	4
NM61-02	1	0	1	1	4
NM61-03	0.1208	0.0672	0.0382-0.322	0	3
NM61-04	0.1208	0.0672	0.0382-0.322	0	3
NM61-05	1	0	1	1	3
NM61-06	1	0	1	1	3
NM78-01	1	0	1	1	3
NM78-02	0.1515	0.1178	0.0288-0.5182	0	2
FS150-01	0.1117	0.0977	0.0179-0.464	0	2
FS150-02	0.0477	0.0618	0.0035-0.4182	0	3
FS150-03	1	0	1	1	2
FS150-04	1	0	1	0	2
NM12-01	0.1117	0.0977	0.0179-0.464	0	2
NM12-02	0.0492	0.0503	0.0063-0.2987	0	4
NM12-03	1	0	1	2	3
NM12-04	1	0	1	1	3
NM12-05	0.0492	0.0503	0.0063-0.2987	0	3
NM12-06	0.1523	0.1222	0.0273-0.5347	0	3
NM15-01	0.0709	0.0461	0.019-0.2315	0	3
NM15-02	1	0	1	1	4
NM15-03	0.0507	0.0357	0.0123-0.1861	0	4
NM15-04	1	0	1	2	4
NM15-05	1	0	1	1	4
NM15-06	1	0	1	2	4
NM15-07	1	0	1	2	4
NM15-08	1	0	1	2	4
NM15-09	1	0	1	2	4

NM159-02	0.3748	0.1221	0.1776-0.6246	0	1
NM159-03	0.3203	0.1231	0.1346-0.5879	0	2
NM159-04	1	0	1	0	1
NM159-05	0.3203	0.1231	0.1346-0.5879	0	2
NM159-06	1	0	1	1	2
SlaterTank159-5	0.4052	0.0874	0.2509-0.581	0	1
NM211-01	0.2993	0.093	0.1519-0.5047	0	3
NM293-01	0.2993	0.093	0.1519-0.5047	0	2
NM35-01	1	0	1	2	3
NM35-02	0.0785	0.0502	0.0214-0.249	0	3
NM35-03	0.0785	0.0502	0.0214-0.249	0	3
NM35-04	0.0785	0.0502	0.0214-0.249	0	3
NM35-05	0.0785	0.0502	0.0214-0.249	0	3
NM35-07	0.0785	0.0502	0.0214-0.249	0	3
NM35-08	0.0785	0.0502	0.0214-0.249	0	3
USNM320147_35	0.0785	0.0502	0.0214-0.249	0	3
USNM320145_35	0.0785	0.0502	0.0214-0.249	0	3
US180-01	1	0	1	2	4
US180-02	0.2311	0.1016	0.0893-0.4795	0	3
US180-03	1	0	1	2	4
US180-04	0.1085	0.0628	0.033-0.3029	0	4
US180-05	0.2311	0.1016	0.0893-0.4795	0	3
US180-06	1	0	1	1	4
WFPuebloCreek	0.045	0.0561	0.0036-0.3782	0	3
US180-10	1	0	1	1	3
US180-11	0.1238	0.106	0.0204-0.4898	0	2
CatronCoRd_B012	0.4643	0.0742	0.3257-0.6087	0	1
CatronCoRd_B080	0.4643	0.0742	0.3257-0.6087	0	1
NM435-01	0.1238	0.106	0.0204-0.4898	0	2
NM435-02	1	0	1	1	3
SACreek	0.4643	0.0742	0.3257-0.6087	0	1
TroutCreek	0.4643	0.0742	0.3257-0.6087	0	1
SFDayUse	1	0	1	1	3
NM52-01	0.1851	0.1339	0.0383-0.5642	0	1
WFEggs-1	1	0	1	3	3
WF-1	1	0	1	2	3
SheepTank	0.2261	0.1075	0.0806-0.4934	0	2
unnamedtank-59	0.3776	0.0851	0.2298-0.5523	0	1
DoagyTank	0.3776	0.0851	0.2298-0.5523	0	1
NorthTank	0.3776	0.0851	0.2298-0.5523	0	1
ReserveUAZ-	0.4643	0.0742	0.3257-0.6087	0	1

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Table 3. Summary of calling intensity for the 90 sampled sites. A site was assigned to a category based on the highest calling intensity recorded during the March through May sampling. Intensity criteria are as follows: 0 = no toads heard calling; 1 = individuals could be counted; 2 = calls overlapping but individuals can still be distinguished; 3 = full chorus, cannot distinguish individuals.

Call intensity category	# Sites	% of Sites
0	59	65
1	15	16
2	14	15
3	2	2
TOTAL	90	

Table 4. Summary of visual encounter surveys from selected call survey sites. Counts were conducted after call surveys are were done by slowly walking along the stream or tank edge. *indicates counts from this one specific 20 meter stretch of West Fork. The count for the 220 meter stretch was 186 on 28-Mar-14 and 244 on 4-Apr-14.

Site	Date	# Toads Observed	Egg masses
159-04 (Snow Lake)	25-Apr-14	5	Not noted
159-6 (O-Bar-O Tank)	30-Mar-14	12	Not noted
159-6 (O-Bar-O Tank)	25-Apr-14	4	5
180-01	18-Apr-14	8	5 to 6
Hells Hole	4-Apr-14	15	present
Indian Tank	14-Mar-14	110	7
Indian Tank	19-Mar-14	221	>100
NM 12-03	29-Mar-14	15	?
NM 15-06	28-Mar-14	11	6
SF Day Use	19-Mar-14	6	0
SF Day Use	29-Mar-14	14	?
US 180-01	28-Mar-14	3	0
US-180-06 (Pueblo Park Camp)	5-Apr-13	1	8 to 10
WFEggs2*	28-Mar-14	86	>60
WFEggs2*	4-Apr-14	96	>90

Arizona Toad 2014

Information Gathered in this Study

- Toads detected at 33 of the 90 (35%) historical sites using call surveys in spring
- First record of *Bd* in species
- Discovery of a novel fungal skin parasite (potentially new species)
- Documented increased period of reproductive behavior

Primary Threats

- Drying of cattle tanks and small order streams
- Loss or degradation of overwintering & upland habitat from fires
- Post-fire flash floods & siltation prior to, or during the breeding season
- Disease

Secondary Threats

- Invasive animals that prey on eggs, tadpoles, juveniles & adults (bullfrogs & crayfish)
- Invasive plants (Salt Cedar) that may modify stream channels
- Hybridization with *Anaxyrus woodhousii*

Management Recommendations

- Monitoring and management of cattle tank water levels.
- Create additional cattle tanks as toad breeding habitat in *A. woodhousii* free areas.
- Monitoring of focal populations for disease prevalence.
- Relocating egg masses and tadpoles from large populations to small populations.
- Re-sample and conduct habitat assessments at sites with moderate to high occupancy probability, where toads were absent in 2014.

Further Information Needed

- Radio-telemetry for upland habitat-use & movement ecology
- Classification of novel parasite
- Effects and prevalence of novel parasite

Primary Stakeholders

US Forest Service: contains majority of species range in New Mexico

NMDGF: wildlife agency for state

USFWS: petitioned to review for listing

Suggested Approach For Future Work

- Radio-telemetry for upland habitat-use & movement ecology
- Population genetic study to identify long distance dispersal of eggs or tadpoles & population connectivity
- Long-term call monitoring of focal populations

Photographic Appendix.
Below are photos documenting behaviors and mortality we observed in 2014.

Indian Tank
<u>Dessicated Eggs</u>

Desiccated toads

Breeding Behavior

Pair in amplexus.

Toad “ball”, males fighting for access to female. Note female on bottom of pile.

Receding shoreline at Indian Tank (26 April 2014). The surface area of this tank decreased from 5795m^2 on 19 March, 2014 to 3882m^2 on 25 May 2014. Note the stranded early stage tadpoles in the foreground.

West Fork of Gila River

<u>Breeding Behavior</u>

Calling male.

Two males at breeding site, note string of eggs in foreground.

Pair in amplexus during the day

Toad “ball” in river.

West Fork of Gila River (10 April 2014) - Clear, shallow, slow moving water with toad eggs along the margin if West Fork. This microhabitat and water conditions seem to a requirement for successful toad reproduction.

West Fork of Gila River (10 April 2014) - Day time habitat of above photo. This was the epicenter of toad breeding along this 4 river mile stretch of the Gila River, we counted >80 egg masses in this 30 meter stretch. Note the black egg strands on both sides of the sand-gravel bar.

Saw Mill Tank
11 April 2014 - Typical tank and surrounding toad breeding habitat for eastern and central Gila. Only a few toads bred in this site in 2014 and there is old fire damage surrounding tank. This tank is 12 miles from Indian Tank.

Alexander Cienega
Dried eggs & stranded tadpoles.