Final Report: Status of the Arizona Toad (*Anaxyrus microscaphus*) in New Mexico

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Authorship and affiliations:

Mason J. Ryan, Ian M Latella, J Tomasz Giermakowski, Howard L Snell

Division of Amphibians and Reptiles, Museum of Southwestern Biology, MSC03 2020, Department of Biology, 1 University of New Mexico, Albuquerque, NM 87131-0001, USA.

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Introduction

The decline of amphibian populations is one of the most pressing conservation issues of the 21st Century (Wake & Vredenburg 2008). The large-scale drivers of population declines include disease, anthropogenic land use and habitat modification, and climate change (Hof et al. 2011). These drivers may facilitate additional threats from hybridization and competition from native and non-native species as they expand their range (Kats & Ferrer 2003; Knapp 2005). Because threat vulnerability can vary by species and threat type, it is necessary to implement multiyear monitoring programs to determine if populations are in decline or exhibiting natural fluctuations, and to identify proximate causes (Pechmann et al. 1991; Lips et al. 2003; Storfer 2003).

Multi-year monitoring is critical for evaluating species and populations that lack baseline data and may be suspected to be in decline (Green 2003). Amphibian populations intrinsically fluctuate confounding the ability to detect decline trends, especially in absence of baseline data (Pechmann & Wilbur 1994; Alford & Richards 1999; Marsh 2001; Green 2003). To determine amphibian population trends, whether in abundance or occurrence, requires multi-year studies that ideally cover population turnover (Connell & Sousa 1983; Semlitsch 2002; Adams et al. 2013). Such long-term monitoring provides valuable insights to decline stressors, population responses to environmental variation, and population resilience to perturbations and habitat change for at-risk species (Gibbons et al. 2006; Homyack & Hass 2009).

One popular method to measure trends of populations is to use historical occurrence data as a baseline and compare it with present-day sampling to assess longitudinal changes in species presence/absence (e.g. Skelly et al. 2003; Tingley & Beissinger 2009). This approach may be biased if resurvey efforts are of short duration or if historical data is based on detections rather than detections and non-detections (Skelly et al. 2003; MacKensie et al. 2006). Studies that occur over multiple seasons and account for imperfect detection can assuage concerns of bias estimations of species occurrence and trend interpretations (MacKensie et al. 2002; 2003).

Amphibians are highly sensitive to changes in the hydrological cycle and both dry and wet extremes can impact annual breeding success and abundance (Walls et al. 2013; Mac Nally et al. 2014; Ryan et al. 2015). This issue is especially pronounced for species that live in highly variable habitats such as rivers in arid environments, which have highly variable flow regimes (Kupferberg 1996; Ocock et al. 2014). For instance, annual flow variability can lead to gradual decreases in flow that desiccate eggs and tadpoles; whereas abrupt flow increases can scour eggs and tadpoles (Kupferberg 1996) or preclude and/or shift breeding to poor quality habitats (Ocock et al. 2014). The influence of extreme hydrologic shifts can induce regional declines even among species that may not be typically considered at risk (Mac Nally et al. 2014).

In 2013, we initiated a field study to assess the status of the Arizona Toad (*Anaxyrus microscaphus*) in New Mexico. The Arizona Toad is currently listed as a Species of Greatest

Conservation Need in New Mexico with habitat modification, disease, and hybridization as the primary threats to the species' long-term persistence (Hammerson & Schwaner 2004; Schwaner & Sullivan 2005; New Mexico Department of Game & Fish 2006). The Arizona Toad is protected or considered a state 'sensitive' species in Arizona, Nevada, and Utah where introgression and hybridization with the Woodhouse's Toad (*A. woodhousii*) and habitat modification are its primary threats (Schwaner & Sullivan 2009; Schwaner and Sullivan 2005; Dodd 2013). At the time of the 2006 conservation categorization in New Mexico, threat risk was assigned based on disease-related declines of the sympatric Chiricahua Leopard Frog, and hybridization-related declines Arizona, Nevada, and Utah (Hammerson & Schwaner 2004; C.W. Painter, personal communication).

The New Mexico portion of the Arizona Toad's range is ideal for long-term monitoring because of highly variable riverine habitats. For rivers where to toad occurs, the mean annual stream flows of the Gila and San Francisco Rivers were greatest in 2013, at 299 cfs (cubic feet per second) and 142 cfs respectively, than in 2014 (Gila River, 118 cfs; San Francisco 41 cfs) and 2015 (Gila River 194.6; San Francisco 69.2 cfs). Furthermore, there is a lack of data on populations and a lack of threat risk assessments compared to neighboring states.

Within New Mexico, the Arizona Toad is restricted to the Gila, Mimbres, and San Francisco watersheds in the Gila Region of the Mogollon Rim, with disjunct populations in the San Mateo Mountains and the Black Range, approximately 40 miles east and southeast, respectively, of the Gila Region (Degenhardt et al. 1996; Kindscher et al. 2008; Jennings et al. 2010). To date potential threats to the Arizona Toad in New Mexico include climate change, forest fires, hybridization, and the disease chytridiomycosis (Ryan et al. 2014a). At this time, these threats should be considered tentative until more thorough assessments can be made with additional data. The positive chytridiomycosis record in 2014 was the first time the disease has been reported in the Arizona Toad, but it appears unlikely that the disease has caused the observed declines (Ryan et al. 2014b).

Prior to this project, the conservation status of the Arizona Toad in New Mexico was data deficient due to a lack of systematic population surveys. The only available data was limited to opportunistic and haphazard specimen collections between 1925 and 2003, which focused on the species distribution (e.g. Degenhardt et al. 1996). Furthermore, approximately 60% of the historic specimens were collected in the non-breeding season (June to August) along roads after rains, providing no insight into the size of local breeding populations. Many aspects of the toad's ecology, behavior, breeding phenology and duration, are well known in Arizona but not much is known about the toad's ecology in New Mexico. New Mexico populations of the Arizona Toad live at higher elevations than Arizona, Nevada, and Utah, and it should be expected that the higher elevation populations should differ in important ecological aspects (Ryan et al. unpublished). Additionally, little is known about the use of non-breeding habitats, tadpole habitats, adult diet, and size at metamorphosis (e.g. Schwaner & Sullivan 2005; Dodd 2013)

Herein we provide the results of three years (2013-2015) of range-wide population monitoring, a review of threat assessments, and several contributions to the basic ecology of the Arizona Toad in New Mexico. This work highlights the sensitivity of the species to abiotic factors and emphasizes the nature of annual population fluctuations in a variable environment. We provide analyses of environmental factors that influence toad reproductive behaviors, employ occupancy and detection modeling at breeding sites, and provide analyses of tadpole habitat and adult non-breeding habitat-use and movement ecology. We have built on previous threat assessments, quantitatively confirm the absence of hybridization, and assess the importance of hydroperiod on reproductive success.

Methods

Call Surveys and Site Occupancy

Our sampling protocol in 2015 followed that used in 2013 and 2014. We conducted weekly call surveys in March and April, covering the seasonal breeding period for the Arizona Toad in New Mexico at 76 historical localities (Degenhardt et al. 1996; Ryan et al. unpublished). Our call survey design was used to evaluate occupancy and provide an assay of relative abundance of males based on call intensity (Heyer et al. 1994). Each site was scored using a metric established by the North American Amphibian Monitoring Program (Weir & Mossman 2005). We listened for toad vocalizations for 3 minutes at each site and intensity was categorized as: 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. This is an ideal method for detecting species that have strong vocalizations and call regularly over the course of the breeding season (Heyer et al. 1994).

Accurate measures of detectability are critical when assessing a species conservation status because non-detection may not imply that a species is truly absent from a study site (MacKenzie et al. 2002). This issue is especially important when using call surveys where daily climatic variability can influence whether individuals may vocalize (e.g. Saenz et al. 2006). Repeated sampling of sites over the course of a single season allows for robust calculation of species detection and site occupancy estimates that may otherwise be biased by examining raw presence/absence data (MacKenzie et al. 2006).

We used program PRESENCE (MacKenzie et al. 2002) to estimate the detectability of toads and the probability of occupancy for sites where toads were not detected. This approach makes several assumptions: 1) occupancy does not change during the sampling period; 2) detections are independent of detections at other sites; 3) detectability is constant across sites and surveys or it can be modeled using site or survey covariates. To model detection probability, we considered models where detection probability was either constant or survey-specific. For purposes of occupancy analyses, sampling periods were defined as occurring within a 48-hour period. According to this criterion we had a total of six sampling periods in 2015. We then used Akaike's Information Criterion (AIC) to rank models by calculating Akaike weights (Burnham & Anderson 2002) and selected the highest ranking model to calculate probability of occupancy at each sampling site. Finally, we used ANOVA to compare site occupancy probabilities between 2014 and 2015.

Stream Flow and Calling Behavior

The reproductive strategies of stream breeding amphibians are well suited to flow perturbations at long-time scales, but are vulnerable to reproductive failure during annual disturbances (e.g. Kupferberg 1996; Ocock et al. 2014). The flow regime of the rivers in the Gila Region is highly variable with potential flashfloods from spring and summer storms (Fig 1), and conversely, to very low levels in years with low winter snow accumulation and drought conditions (Gori et al. 2014). Such variability in flow rates can be a determining factor whether riparian breeding species call or reproduce, especially during periods of above average flow (e.g. Kupferberg 1996; Bondi et al. 2013). Episodic high flow rates from severe storms or other perturbations can also influence whether frogs call and breed, further affecting annual reproduction (Kluge 1981; Fukuyama & Kusano 1992; Ocock et al. 2014). Other factors such as temperature, cloud cover, and lunar cycles have been shown to influence anuran breeding activity (Fukuyama & Kusano 1992; Saenz et al. 2006). Understanding the effect of abiotic factors, such as stream flow, on call behavior is critical for monitoring programs and management actions (Bondi et al. 2013).

We tested the effects of multiple abiotic variables on toad calling activity (presence/absence and intensity). We analyzed nightly call survey data with air temperature, wind speed (Beaufort wind scale), moon phase, index of cloud cover percentage, and river flows (cubic feet per second [cfs]; obtained from the USGS U.S. Stream Flow database (http://waterdata.usgs.gov/nwis/rt?) for the date of each survey. We limited the analyses to call survey data collected between 2013 and 2015 from the Gila, Mimbres, and San Francisco Rivers, the only rivers with USGS gauge stations. We used ordinal logistic regression with call detection (yes or no) and call intensity (ranked 0-3) as the dependent variables and the environmental factors as predictor variables. We combined data for all years and produced models for each river separately.

Breeding Success and Use of Lentic Habitats

Amphibian breeding success cannot be determined by the presence of calling males, eggs or tadpoles (Richter et al. 2003). Hydroperiod length (i.e. the number of days a breeding water body maintains water; Semlitsch 1987; Pechmann et al. 1989; Rowe & Dunson 1995) and hydrologic conditions (i.e. consistent water levels; Kupferberg 1996; Richter et al. 2003) are critical for amphibian breeding success, i.e. emergence of metamorphosed froglets. Reproductive failure can occur from drying of breeding habitats or flashfloods before metamorphosis or (Richter et al. 2003; Kupferberg et al. 2011; Bondi et al. 2013).

To disentangle toad detection and calling activity with reproductive success we monitored a subset of occupied sites to confirm the presence of emerging metamorphosed toadlets, which indicates breeding success. We detected toads calling at 23 sites and conducted post breeding season (May to August) visual encounter surveys at 12 of these sites. Supplemental surveys identified five additional sites with eggs or tadpoles, providing a total of 17 sites where reproductive success could be evaluated. We 11 occupied sites were excluded from post-calling monitoring because they were on private property.

The Arizona Toad is considered a riparian breeding species but has been occasionally reported to call and lay eggs in lentic (i.e. pond) habitats. To date, there have been no observations of Arizona Toads successfully breeding in lentic habitats despite the presence of eggs and tadpoles (B. Sullivan, personal communication; Ryan et al. unpublished). Sullivan (personal communication) hypothesizes that Arizona Toad tadpoles cannot complete development in lentic habitats when oxygen levels decrease, or they are depredated on by fish, other amphibian larvae, or invertebrates. Thus, it appears that egg laying in lentic habitats may act as a sink for local population recruitment if tadpoles do not survive to metamorphosis. Previous work in 2014 found eggs and tadpoles in nine lentic habitats, in 2015 we included these habitats in our surveys.

We conducted visual encounter surveys at 29 lentic habitat sites (e.g. cattle tanks and lakes). Twelve of these sites were part of the call survey effort and 17 were added to fill in gaps in the species distribution and to determine the extent of lentic habitat use. Sites were sampled twice per month to survey for eggs, tadpoles, and emerging metamorphosed toadlets.

Tadpole Habitat Use and Adult Movement Ecology

Little information has been reported on the habitat use of tadpoles and movement patterns of the Arizona Toad. Calling and egg laying typically occurs in shallow water along the margins of streams, backwashes, or side pools where water flow is minimal (Schwaner & Sullivan 2005). Along the West Fork of the Gila River egg masses were deposited in clear shallow water with sand or cobble substrates at a mean depth of 4.5 cm (Range 1.5-7 cm) (Ryan et al. 2014a). Habitat use of tadpoles along in streams has not yet been quantified.

The Arizona Toad is considered a habitat specialist for all aspects of their lives. For instance, adults have been found to move up to 200 m from streams, but remain within the floodplain habitats (Schwaner & Sullivan 2005). The Arroyo Toad (*A. californicus*), a phylogenetically sister species to the Arizona Toad, has been reported to rarely disperse from stream margins, but there are a few observations of adults being found up to 1,200 m from streams (Sweet & Sullivan 2005). Between these two ecologically similar species there are only a handful of upland non-breeding observations limiting the knowledge of their movement behavior. In 2015 we conducted two pilot studies to investigate tadpole habitat use and adult movement ecology.

Tadpole Habitat Use

To measure the riparian habitat use of tadpoles, we sampled five 30-meter stretches of the West Fork of the Gila River. Within each 30-meter stretch, we sampled four 5-meter sections where we recorded tadpole presence or absence and habitat characteristics along 1-meter width bands. Each sampling section was spaced 100 meters apart along the river course. We categorized habitats according to substrate type: sand or cobble/pebble; stream flow: riffle or run; and stream dimensions: wetted width, water depth at midstream and 25 centimeters from the banks. We then used logistic regression to predict presence and absence of tadpoles with habitat characteristics. Individual models were constructed with an index of percent of substrate type, stream flow designations, mean depth, and wetted width by tadpole presence or absence.

Adult Upland Habitat Movement

We conducted upland surveys (away from breeding sites) during and after the breeding season to determine non-breeding habitat use and estimate the potential distance toads may move from breeding sites. We used time-constrained visual encounter surveys, with a minimum of 60 minutes per survey per site. We randomly walked at least 1000 meters throughout areas adjacent to breeding habitats, visually scanning the ground for toads. For each survey we recorded date, time and distance surveyed, and for each toad observation we recorded body size, sex, habitat type, and the geographical coordinates of its location with a GPS unit. We estimated potential movement distance by plotting toad observation coordinates and then measuring (in meters) the distance to the nearest known water body using Garmin Basecamp.

Hybrid Analysis

Hybridization with the native Woodhouse's Toad has been identified as one of the most serious threats to the Arizona Toad in Arizona, Utah, and Nevada (e.g. Hammerson & Schwaner 2004; Schwaner & Sullivan 2005). Our previous work qualitatively assessed the threat of hybridization on the Arizona Toad in New Mexico. Using specimens collected in 2014 and 2015, as well as museum specimens from multiple institutions we investigated the threat and history of hybridization in New Mexico.

To evaluate the occurrence and extent of hybridization between *A. microscaphus* and *A. woodhousii* in New Mexico we reviewed 174 *A. microscaphus* and 406 *A. woodhousii* museum specimens from the Gila Region. The morphological review included adults, juveniles, and tadpole lots to verify species identification. To determine whether any toad specimens were *A. microscaphus* x *A. woodhousii* hybrids, we scored all specimens from the Gila Region using four characters established by Blair (1955) and Sullivan (1986). We recorded ventral spotting, cranial crests, mid-dorsal stripe, and pale bar across the eyelids with a numerical value following Sullivan (1986) and Sullivan & Lamb (1988): P=present, W= weakly present, VW= very weakly present, A= absent. Ventral spotting, mid-dorsal stripe, and cranial crest were scored as P = 3, W = 2, VW = 1, A = 0; while pale eye bar across the eyelids was scored conversely: P = 0, W = 1, VW = 2, A = 3. Numerical scores were then summed and used to calculate a hybrid index from 0 to 12, with low scores representing *A. microscaphus* and high scores representing *A. woodhousii* (Blair 1955; Sullivan 1986). We only examined toads greater than 45 mm SVL to avoid trait ambiguities of juveniles associated with ontogenetic changes (e.g. Sullivan 1986).

We used logistic regression to compare hybrid index scores between specimens of both species for the entire Gila Region in New Mexico. We then repeated the logistic regression for Grant and Sierra counties where the two species co-occur. This use of logistic regression provides a test of whether the hybrid scores can be used to predict trait overlap between the two species. We did not do this analysis for Catron County because during the specimen review, all specimens previously identified as *A. woodhousii* were found to have been misidentified (see Results).

Disease and Die-offs

We collected 45 chytridiomycosis samples from the Arizona Toad but these samples have not yet been analyzed. We observed 36 toads with red ventral spotting which in 2014 was preliminarily identified as *Amphibiothecum sp.* or *Amphibiocystidium* sp. fungi (Kiryu et al. 2014). We collected 23 of the infected toads for further analyses and sent three of these samples to the Fish and Wildlife Research Institute Pathology Lab in Florida.

Results

Call Survey Summary and Site Occupancy

Between 11 March and 14 April 2015 we sampled 76 localities where we conducted a total of 248 call surveys (Table 1, Fig 2) and detected toads at 22 (29%) of the sampling localities. Each site was visited an average of 3.9 times (median 4, range 2-6). Of the 22 occupied sites, 12 (54%) had a maximum call intensity of 1; 6 (27%) had a maximum call intensity of 2; and only 2 sites (9%) had a maximum call intensity of 3. In 2015, the number of occupied sites and the number of sites with maximum call intensity of 3 were similar to results in 2014, but greater than in 2013 (Figs 3 and 4). This suggests that regional population status was stable in 2014 and 2015, but the naïve number of occupied sites has decreased compared to the number of historically known occupied sites, i.e. a ~70% decline range-wide.

In 2013 and 2014, we did not detect toads at historic localities in Alamosa Creek near Monticello Box or at West Red Canyon in the San Mateo Mountains. In 2015, we did not detect toads at these two sites through call surveys, but on March 24th we did find a single male Arizona toad in Alamosa Creek on Socorro County Road C033, approximately ¾ miles East of Monticello Box. This observation confirms that Arizona toads are extant in the San Mateo Mountains and warrant further investigation in order to determine the species' current distribution in this isolated mountain range.

We used all 76 sites in model construction to estimate probabilities of detection and occupancy for 2015 (Table 2). The best model included sampling period as a covariate affecting detectability. The naïve occupancy estimate for all surveyed sites is 0.329 whereas the estimated proportion of sites occupied was slightly higher (0.398) but within the range of the standard error. Probability of occupancy estimates for sites where toads were not detected ranged from 0.0128 to 0.2931, suggesting a low probability of presence of toads at unoccupied sites. The estimated mean detection probability for all sites was 0.444, and ranged from 0.0128 to 1, with 1 representing the sites where toads were detected. Estimates varied considerably between periods which may be attributed to short-term weather variation or seasonal shifts in calling behavior (Fig 5). Estimates of detection probabilities can be used to estimate the number of sampling occasions necessary to declare that a species is absent during a given year. Based on the mean detection probability in 2015, at least five sampling periods per site are required to conclude with 95% probability that toads were absent. In spatial analyses, we did not find a pattern in individual site detection probability or occupancy across the Gila Region. Instead, both detection and occupancy varied relatively uniformly across the region, suggesting a large-scale connective metapopulation within each river system (Fig 6).

The mean detection probability estimates for all sites did not vary between 2014 and 2015 (ANOVA: *P*=0.36; R^2=0.006; F-ratio = 0.836). Combined with our raw call data results, this suggests that regional population status was stable for 2014 and 2015.

Stream Flow and Calling Behavior

Our analyses of Arizona Toad calling behavior predictors (i.e. environmental variables) found that stream flow (i.e. cubic feet per second, cfs) was the only significant predictor influencing call behavior (Table 3). Both call detection and call intensity was best predicted by low cfs in the Gila, Mimbres, and San Francisco Rivers (Table 4; Figs 7 and 8). These results suggest that Arizona Toad breeding behavior is highly sensitive to water levels and stream flow rates.

All three rivers had significant variation in mean flow rate on the dates we conducted call surveys (ANOVA: P=0.0001; F-Ratio=242.77), with the Gila River having the greatest mean flow (149.3 cfs), followed by the San Francisco (18.0 cfs) and Mimbres River (12.3 cfs). While each river varies in mean flow rate during the breeding season, the impact of flow rates on toad breeding behavior scales with each river. Our model results indicate that toads do not call when flow rates exceed a flow rate threshold: 79.6 cfs for the Gila River, 20.4 cfs for the San Francisco, and 5.9 cfs for the Mimbres River (Table 4; Figs 7 and 8).

Reproductive Habitat & Success

We detected toads at 31% (nine tank) of the 29 tank habitats sampled, four by calling surveys and five by visual surveys (Table 5). This represents a small subset of total tanks in the Gila Region and suggests Arizona Toads will occasionally use tanks. The caveat with the use of tanks as breeding sites is that there is a very low reproductive success rate compared to stream habitats.

Reproductive success (emergence of metamorphosed toadlets) was 35% at our 17 focal sites 2015 (Table 6). This included nine stream sites and eight tank sites, and streams had a higher success rate (55%) than the tank habitats (12%). Reproductive failure at two stream sites (Pueblo Creek and US 180-6) was caused by a flashflood in July, which washed away tadpoles before metamorphosis. Our Mimbres River site, at Cooney Camp, dried out in April resulting in mortality of tadpoles. Reproductive failure at tanks was caused by drying out of tanks at four sites and undetermined factors at another four sites.

These observations suggest that the Arizona Toad is highly vulnerable to changing water levels and the presence of calling males, eggs, and tadpoles are not a reliable indicator of successful reproduction. The 2015 results are consistent with the results observed in 2014 (Ryan et al. 2014a).

Tadpoles Habitat Use

Our assessment of tadpole habitat use at the West Fork of the Gila River showed that water depth, wetted width and the flow type, i.e. riffles, best predicted the presence of tadpoles along stream stretches, with no relationship with substrate type (Table 7; Fig. 9). The water flow characteristics suggest tadpoles prefer shallower and slower flowing stream stretches and microhabitats when flow rates are low. This same section of stream was monitored in 2013 during flows >222 cfs, with no tadpoles observed.

Our habitat preference analysis suggests that Arizona Toad breeding and tadpole development sites are based on specific stream habitat features. This pilot study should be expanded to other rivers and streams to better understand that habitat association and requirements to better inform management actions. If our preliminary results remain consistent with greater sample sizes, it may be possible to model the distribution of toads within river systems (i.e. Treglia et al. 2015).

Adult Upland Habitat Use and Movements

We conducted 24 upland surveys around occupied breeding sites, two during the breeding season and 22 in the non-breeding season yielding a total of 154 individual toad observations. During the breeding season (March) we made 14 observations of toads moving towards Indian Tank with mean distance of 122.1±73.8 m (31.9 – 289.6 m) from breeding sites. During the non-breeding season we made 140 observations with a mean distance of 178.8±143.0 m (Range: 8.5 – 965 m) from nearest water body. We made 22 observations at Indian Tank and 142 observations near streams and found tank toads to move slightly further than stream toads (Fig. 10).

One toad, an adult male, was excluded from the non-breeding season calculation because it was found an estimated 2011 m from the nearest water body on Highway 15 on 12 June 2015. This single observation appears to represent the extreme distances this species is capable of covering.

The upland vegetation types where toads were observed consisted of meadows, mixed meadow-woodlands, or ponderosa pine woodlands. There does not appear to be any upland vegetation preference according to our data for the months of June, July, August, and September. During this time period, toads appear to be foraging in order to gain mass to prepare for overwintering and the breeding season. We infer that summer activity is related to foraging based on the gut content analyses of 49 toads, of which 78% (38 individuals) had ingested prey items. Conversely, during the breeding season (March and April) 2.5% (2 individuals) of 78 toads had ingested prey items.

Our upland and distance from breeding site observations provide a tentative estimate that toads require, at a minimum, a buffer of approximately 1,900 m around breeding sites for non-breeding foraging area. This is likely an underestimate constrained by our sampling design, and future surveys should focus on areas <1000 m from breeding sites. It is still unknown where toads are overwintering, which would alter the estimated buffer around breeding sites. The final piece of the movement ecology of this species is to determine overwintering grounds which will require radio-telemetry.

We do not have sufficient data for analyses, but rainfall appears to have a major influence on whether toads are observed and active during the non-breeding season. For example, on 26 June 2015 we observed 67 toads following a late afternoon rain, whereas surveys on dry days and nights yielded less than 20 observations per night.

Hybrid Analysis

Prior to this review, museum records indicated that *A. microscaphus* and *A. woodhousii* co-occurred in four counties: Catron, Grant, Sierra, and Socorro; and within these counties specimens of both species were found in relatively close proximity (within 4 kilometers of each other) at 20 localities: 17 in Catron County and three in Grant County (Fig. 11).

Our initial specimen review found that all previously identified *A. woodhousii* from Catron County were misidentified and should be assigned to *A. microscaphus*. The misidentifications included two adults and 71 tadpole lots from 15 unique collection localities. Additionally, we found one misidentified juvenile *A. woodhousii* from Grant County and one from Luna County, which should be assigned to *A. microscaphus*. Finally, we found three misidentified *A. microscaphus* specimens from Grant County that should be assigned to *A. woodhousii*. Refer to Figure 12 for the revised distribution of both species in the Gila Region.

The hybrid index scores indicate that there is no morphological evidence of hybridization between *A. microscaphus* and *A. woodhousii* throughout the Gila Region, and Grant and Sierra Counties, specifically, where the two species co-occur (Table 1). The logistic regression further supports this finding for the entire Gila Region (P = 0.0001; Chi Square = 348.75; Estimate = -6.51; Fig. 13A), Grant County (P = 0.001; Chi Square = 125.66; Estimate = -7.29; Fig. 13B), and Sierra County (P = 0.0001; Chi Square = 59.40; Estimate = -5.82; Fig. 13C). The hybrid index logistic regression plots show a strong separation between *A. microscaphus* and *A. woodhousii* (Fig. 13).

Disease and Die-offs

The 2014 histopathology results tentatively identified the red ventral spotting and mortality was caused by infection with *Amphibiothecum sp.* or *Amphibiocystidium sp.* In 2015 we sent three additional specimens for confirmation of the 2014 findings but the results were conflicting. The 2015 histopathology results suggest the cause of the red ventral spotting is due to chigger mites, possibly from the genus *Hannemannia* (Kiryu et al. 2015). Duzynski and Jones (1973) and Grover et al. (1975) reported *Hannemannia* spp. in New Mexico anurans from Sierra County in the Arizona toad and *Hyla arenicolor*. To our knowledge infections of *Hannemannia* spp. do not cause mortality in amphibians (e.g. Duzynski and Jones 1973; Grover et al. 1975). It is unclear why there was a discrepancy between the 2014 and 2015 histopathology analyses and does not elucidate the cause of the observed dead and moribund toads.

In 2015, we found two dead Arizona Toads and four dead *Ambystoma tigrinum* at Indian Tank. We could not perform necropsies on carcasses this year because all were collected in late stages of decomposition. This issue of unexplained die-offs remains enigmatic and warrants further attention and monitoring. We are going to contact a second laboratory to conduct histopathology to assist in determining the cause of observed mortality.

Endangered Species Observations

Much of the range of the Arizona Toad overlaps with the Federally Threatened Chiricahua Leopard Frog (*Lithobates chiricahuensis*). We observed approximately 5-8 Chiricahua Leopard Frog tadpoles at Rain Creek in the western Gila (N33° 11.282', W -108° 40.134') on 15 August 2015. Tadpoles were observed along the banks of the creek and co-occurred with *H. arenicolor* tadpoles. Photographs of the tadpoles were taken and Michelle Christman of the U.S. Fish and Wildlife Service was contacted immediately after this observation. Dr. Randy Jennings conducted a follow-up survey on 22 August 2015 to confirm this observation. This represents a new, previously unknown population of Chiricahua Leopard Frogs in the Gila. This site is approximately 6 Euclidian miles from Bud's Hole, a historic Chiricahua Leopard Frog locality that is now extirpated.

Discussion

Our raw call survey data collected between 2013 and 2015, and results form detection and occupancy modeling from 2014 and 2015, show consistent results among years, with an approximate 70% decline in the number of occupied Arizona Toad localities (Figs. 3 & 4; Table 2; Ryan et al. 2014a). The largest annual variation in occupied sites was between 2013 and 2014-2015, which was due to high river flow rates along the Gila River (Fig. 1), which appears to influence detection and breeding activity. The below-average rainfall in recent years has led to the drying of many small tributaries along the river systems in the Gila excluding many potential breeding sites. The reduction in available breeding sites may be the driving factor in the low number of occupied sites we have found over the last three years. The current El Niño brought high amounts of winter precipitation in the region and many of the previously dried tributaries are now flowing (Ryan personal observation, December 2015). Considering the sensitivity of the Arizona toad to flow rates and breeding habitat desiccation, the wet conditions expected for 2016 may result in an increase in the number of occupied small streams. Conversely, there may be a decrease in reproductive activity in larger order streams and rivers due to higher spring flow rates.

Consistent with the number of occupied sites between 2013 and 2015, is the proportion of occupied sites that have small numbers of breeding males. Our call intensity assays show that in 2014 and 2015, 6% and 18% of sites, respectively, had a call index of 3, indicating a small number large breeding congregations. While there was an increase in large congregations between years, this confirms that the majority of occupied sites are relatively small and therefore vulnerable to extirpation from stochastic events. It is likely that this is a long-term stable strategy for the Arizona toad considering the highly dynamic nature of their riparian breeding habitats. Flashfloods are a common occurrence for the rivers of the Gila Region, which can alter riparian habitats in a shifting mosaic. We hypothesize that the small number of large breeding congregations act as core sources for colonization of the smaller satellite congregations following flashfloods. Under this scenario, river systems that lack large source congregations are at greater risk of local extirpation. To date, we are unaware of any large breeding congregations along the San Francisco River, Whitewater Creek, and Willow Creek, which all consist of small breeding congregations. Conversely, the Gila River, Mimbres River, and Black Canyon Creek have large breeding congregations, which may preserve the integrity of the metapopulation dynamics within these rivers. Our call intensity assay data and inferences are in need of testing and quantification using population genetic analyses to determine dispersal patterns within drainages in relation to large breeding congregations. This information will be invaluable to conservation managers in planning any recovery or management plans.

The disease chytridiomycosis (*Bd*) has been responsible for many enigmatic amphibian population die-offs and declines (Wake & Vredenburg 2008), and is responsible for declines in some New Mexico species (e.g. Ryan et al. 2014b). *Bd*-driven amphibian population declines are typically associated with mass die-offs (dozens to hundreds of individuals) at breeding sites, often occur at middle elevations, may effect stream species more than terrestrial species, and occur uniformly across the landscape (Bradley et al. 2002; Lips et al. 2003; 2006). The apparent declines we have observed in the Arizona Toad do not necessarily fit the pattern of a *Bd* outbreak. For example, during the period of time when *Bd* moved through New Mexico causing declines in the Chiricahua Leopard Frog, there were no reported incidents of mass die-offs of the Arizona Toad (e.g. Ryan et al. 2014b). In addition, extant toad populations occur in scattered localities across the Gila Region from low to high elevations (Fig 6), which do not conform to a *Bd*-decline spatial pattern. Furthermore, even though *Bd* has been detected in the Arizona Toad, prevalence rates are low (Ryan et al. 2014b) and many amphibian species can exist with *Bd* but not show signs of decline (e.g. Lannoo et al. 2011; Olson et al. 2013). While we cannot conclusively rule out *Bd* as a causative or contributing agent of the apparent declines in the Arizona Toad, other factors such as land-use change or climatic factors appear to be driving declines (Ryan et al. 2014a).

One major contribution provided by the 2015 work is the call behavior – environmental variable analyses. For the first time we were able to quantify which abiotic factors regulate call behavior and intensity for the Arizona toad. Of the factors we measured, only stream flow (cfs) regulated calling behavior. For each river analyzed, (i.e. the Gila, Mimbres, and San Francisco Rivers), we estimated a maximum flow threshold for call detection (Table 4). These analyses confirm that the Arizona toad is highly sensitive to water levels and cease calling and breeding if flows reach this threshold. The lack of calling during high flow rates may be due to adult toads and/or freshly laid eggs being vulnerable to displacement downstream.

Tadpoles are also highly vulnerable to extreme shifts and increases in stream flow caused by flashflood events. Arizona toad tadpoles appear to require relatively shallow, slow flowing stream stretches, avoiding more rapid run types of flow habitats under normal flow conditions (Table 5). The avoidance of rapidly flowing habitats in our habitat selection analysis supports the inference of tadpole vulnerability to shifts in flow. This was confirmed in 2013 and 2014 when we observed a complete loss of tadpoles along the Mimbres and San Francisco Rivers following floods (Ryan et al. 2014a); and again in 2015 along the San Francisco River and Pueblo Creek when a series of severe thunderstorms moved across the western Gila in July causing flooding. From our post-flood surveys all tadpoles were lost at these sites resulting in reproductive failure.

It is important to emphasize that call detection and tadpole presence does not equal reproductive success in a given year (e.g. Richter et al. 2003). Of the 17 sites we monitored through the summer of 2015, only six had toadlets emerge, confirming reproductive success. The combination of summer flooding, and conversely drying, of aquatic habitats in the spring often resulted in reproductive failure. This has important implications for long-term local population trends and persistence if reproductive failure occurs over consecutive years. Because of the year-to-year variability in water levels in the Gila Region, the Arizona Toad epitomizes importance of occasional highly successful reproductive years as a long-term breeding strategy (e.g. Alford & Richards 1999; Green 2003).

Prior to the 2015 work, there was little information on non-breeding upland habitat use in Arizona toads. Schwaner and Sullivan (2005) reported that Arizona toads could be found up to 200m from rivers. Our upland, non-breeding season surveys show that toads can be found up to 965 meters from their aquatic breeding habitats. This is significant because it greatly expands the buffer areas around breeding habitats that may need protection or management.

The results presented in this report need to be examined in the context of the proposed Gila River Diversion Project and subsequent flow changes to the Gila River. The proposed diversion project would occur at Turkey Creek, and can potentially impact flow rates and habitat change up to 60 miles upstream (Schwaner & Sullivan 2009). This has the potential to negatively impact the largest Arizona toad populations in New Mexico. A side effect of riverine diversions in the southwest is the facilitation of the spread of the native Woodhouse's toad, which may hybridize with, and threaten the persistence of the Arizona toad (Hammerson & Schwaner 2004; Sullivan et al. 2015). Currently, there is no evidence of hybridization between the Arizona and Woodhouse's Toads in New Mexico.

Future Directions

The work presented in this report was collected over 3-years that experienced similar climatic conditions. The fact that 2013-2015 were climatically similar is useful because it provides inferences on toad occupancy and call behavior under comparable environmental conditions. Now that we have established a 3-year baseline, we are uniquely prepared to test the Arizona Toad's response to the current extreme El Niño climatic event. To date, the current El Niño has produced high levels of rain and snow across the Gila Region; this is expected to continue through 2016 (NOAA Climate Prediction Center; http://www.cpc.ncep.noaa.gov/products/predictions/long_range/seasonal.php?lead=2; accessed 16 Dec 2015).

In December 2015, we visited five streams in the western Gila that have not had aboveground water flow for the last 4-years. These five streams are all flowing now due to the recent increase in precipitation. In addition, many tanks that were dried or had low water levels in 2015 are now filled with water. The fall and winter precipitation associated with the 2016 El Niño appears to have greatly increased the number of potential Arizona Toad breeding sites. Additionally, the excess water may prevent tanks and smaller streams from drying during the breeding season, potentially increasing regional reproductive success.

The continuation of sampling in 2016 will also allow us to further test the effects of stream flow on breeding behavior. We will continue to expand upon the tadpole habitat use study and further elucidate the movement patterns of adults in the breeding and non-breeding season.

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Publications and Abstracts

To date we have published one paper in Herpetological Review and presented one talk at The Wildlife Society's annual meeting of Arizona and New Mexico Chapters (February 2015). We have a manuscript in review and are preparing three additional manuscripts for submission to peer-reviewed journals. Listed below are the citations of manuscripts that are published or in preparation.

Published:

Ryan, MJ, IM Latella, CW Painter, JT Giermakowski, BL Christman, RD Jennings, JL Voyles (2014) First record of *Batrachochytrium dendrobatidis* in the Arizona toad (*Anaxyrus microscaphus*) in southwestern New Mexico, USA. *Herpetological Review* 45:616-618*.*

In Review:

Ryan, MJ, IM Latella, JT Giermakowski, G Gustafson & HL Snell. *Anaxyrus microscaphus*. Diet. *Herpetological Review.*

In Preparation:

Ryan, MJ, IM Latella, JT Giermakowski. Range correction of the toad *Anaxyrus woodhousii* (Amphibia: Bufonidae) in southern New Mexico. Target Journal*: Herpetological Conservation and Biology*.

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.*

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 Chapters of The Wildlife Society annual meeting (Feb 2015).

Tables

Table 1. Summary of calling intensity for sites sampled in 2015. A site was assigned to a category based on the highest calling intensity recorded during the March through April sampling. Intensity criteria are: 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	*2014 # Sites (% of sites)*	*2015 # Sites (% of sites)*
0	59 (65%)	54 (71%)
1	15 (16%)	12 (16%)
2	14 (15%)	6 (8%)
3	2 (2%)	4 (5%)
TOTAL	90	76

Table 2. Summary of PRESENCE analyses estimating probability of occupancy for 2015.

Site	*Prob. Occupancy*	*Prob. Occupancy (SE)*	*95% Confidence interval*	*Maximum Call Index*	*Number of visits*
FS 150-1	0.0153	0.0171	0.0017-0.126	0	5
FS 150-2	0.0153	0.0171	0.0017-0.126	0	5
FS 150-3	1	0	1	1	5
FS 150-4	0.0153	0.0171	0.0017-0.126	0	5
FS 150-5	1	0	1	1	4
FS 150-6	1	0	1	1	4
FS 150-7	0.0239	0.0258	0.0028-0.1758	0	4
NM 12-2	0.2148	0.0723	0.1056-0.3879	0	3
NM 12-3	1	0	1	1	3
NM 12-4	0.2079	0.0701	0.1023-0.3767	0	3
NM 12-5	0.2079	0.0701	0.1023-0.3767	0	3
NM 12-6	0.1476	0.0612	0.0626-0.3099	0	4
NM 15-01	0.0153	0.0171	0.0017-0.126	0	5
NM 15-02	1	0	1	2	5
NM 15-03	0.0153	0.0171	0.0017-0.126	0	5
NM 15-04	1	0	1	1	5
NM 15-05	0.0153	0.0171	0.0017-0.126	0	5
NM 15-06	1	0	1	1	5
NM 15-07	0.0153	0.0171	0.0017-0.126	0	5
NM 15-08	0.0153	0.0171	0.0017-0.126	0	5
NM 15-09	0.0153	0.0171	0.0017-0.126	0	5
NM 15-10	0.0153	0.0171	0.0017-0.126	0	5
NM 15-11	1	0	1	1	3
NM 15-12	1	0	1	1	3
NM 15-13	0.025	0.0268	0.0029-0.1816	0	3
NM 159-3	0.2931	0.0756	0.1687-0.4587	0	2
NM 159-4	1	0	1	3	3
NM 159-5	1	0	1	1	2
NM 211-1	0.0128	0.0154	0.0012-0.1236	0	3
NM 293	0.0128	0.0154	0.0012-0.1236	0	3
NM 35-01	1	0	1	2	4
NM 35-02	0.0228	0.0245	0.0027-0.1677	0	4
NM 35-03	0.0153	0.0171	0.0017-0.126	0	5
NM 35-04	0.0153	0.0171	0.0017-0.126	0	5
NM 35-05	1	0	1	3	5
NM 35-07	0.0153	0.0171	0.0017-0.126	0	5
NM 35-08	0.0153	0.0171	0.0017-0.126	0	5

NM 35-09	0.2148	0.0723	0.1056-0.3879	0	3
NM 35-10	0.2148	0.0723	0.1056-0.3879	0	3
NM 435-1	1	0	1	2	3
NM 435-2	1	0	1	2	3
NM 59-01	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-02	1	0	1	3	4
NM 59-03	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-04	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-05	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-06	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-07	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-08	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-09	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-10	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-11	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-12	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-13	0.1476	0.0612	0.0626-0.3099	0	4
NM 59-14	0.1476	0.0612	0.0626-0.3099	0	4
NM 61-1	1	0	1	3	5
NM 61-2	1	0	1	2	5
NM 61-3	1	0	1	1	5
NM 61-4	0.0153	0.0171	0.0017-0.126	0	5
NM 61-5	1	0	1	2	5
NM 61-6	0.0153	0.0171	0.0017-0.126	0	5
NM 78-1	1	0	1	1	4
NM 78-2	1	0	1	2	4
North Star Tank	0.2148	0.0723	0.1056-0.3879	0	3
North Tank	0.2148	0.0723	0.1056-0.3879	0	3
Rock Core Tank	0.2833	0.736	0.1627-0.4457	0	2
Sawmill Tank 1	0.2918	0.0754	0.1678-0.4571	0	2
US 180-01	0.0358	0.0369	0.0045-0.2316	0	3
US 180-02	0.0358	0.0369	0.0045-0.2316	0	3
US 180-03	1	0	1	1	3
US 180-04	0.0358	0.0369	0.0045-0.2316	0	3
US 180-05	0.025	0.0268	0.0029-0.1816	0	3
US 180-06	0.1476	0.0612	0.0626-0.3099	0	4
US 180-10	1	0	1	1	5
US 180-11	0.2145	0.0707	0.107-0.3833	0	2
US 180-12	1	0	1	2	4

Table 3. Ordinal logistic regression results of Arizona toad call intensity and call detection by environmental variables for all rivers and years combined (cfs = stream flow rate as cubic feet per second, P = p-value, asterisk denotes significant relationship).

	Predictor variable	*Whole Model $P_{df, N}$*	*Whole Model X^2*	*Parameter P*	*Parameter X^2*	*Parameter Estimate*
Call Intensity	cfs	$*0.0047_{1,223}$	7.98	0.0110	6.47	0.008
	Cloud Cover	$0.0863_{1,307}$	2.94	0.1030	2.66	0.007
	Temp C°	$0.7466_{1,184}$	0.10	0.7480	0.10	-0.01
	Wind speed	$0.0945_{1,179}$	7.92	NA	NA	NA
	% Moon Visible	$0.0594_{1,286}$	3.55	0.0611	3.51	0.007
Call Detection	cfs	$*0.0029_{1,223}$	8.90	0.0075	7.14	0.008
	Cloud Cover	$0.1344_{1,307}$	2.24	0.1476	2.10	0.006
	Temp C°	$0.7504_{1,184}$	0.10	0.7499	0.10	-0.01
	Wind speed	$0.1282_{1,179}$	7.14	NA	NA	NA
	% Moon Visible	$0.\ 0867_{1,172}$	2.93	0.0874	2.92	0.007

Table 4. Ordinal logistic regression results of Arizona toad call intensity and call detection for Gila, Mimbres, and San Francisco Rivers for all years combined with stream flow summaries (asterisk denotes significant relationship; *P* = p-value; SD = standard deviation). **CFS values are estimated maximum stream flow for toad calling, above this level toads are not detected.

	Predictor variable	*Whole Model* $P_{df,\ N}$	*Whole Model* X^2	*Parameter Estimate*	*Mean CFS±STD (Range)*	***Mean CFS±SD for detection*
Call Intensity	**Gila**	$0.0001_{1,106}$	36.06	0.04	149.3±51.0 (222.6-4.67)	NA
	Mimbres	$0.0009_{1,50}$	10.93	0.34	12.3±9.2 (23.3-3.2)	NA
	San Francisco	$0.0258_{1,66}$	4.97	0.21	18.0±23.1 (88.1-3.2)	NA
Call Detection	**Gila**	$0.0001_{1,106}$	38.51	0.05	149.3±51.0 (222.6-4.67)	79.6±9.0
	Mimbres	$0.0009_{1,50}$	10.93	0.34	12.3±9.2 (23.3-3.2)	5.98±6.0
	San Francisco	$0.0262_{1,66}$	4.94	0.21	18.0±23.1 (88.1-3.2)	20.4±15.3

Table 5. Cattle tank and lake sites sampled for Arizona toads in 2015.

Site Name	*Survey Type*	*Call survey result*	*Eggs/tadpoles observed*	*Bullfrogs detected*
Tennessee Creek @ NM78	Call	Present	No	Yes
Indian Tank	Call	Present	Yes	No
Lake Roberts	Call	Absent	No	Yes
North Star Tank	Call	Absent	No	No
North Tank	Call	Absent	No	No
O-Bar-O Tank	Call	Present	No	No
Rock Core Tank	Call	Absent	No	No
Sawmill Tank 1	Call	Absent	No	No
Sawmill Tank 2	Call	Absent	No	No
Snow Lake	Call	Present	No	No
Unnamed Tank on NM-59	Call	Absent	No	No
Wall Lake	Call	Absent	No	Yes
Alexander Cienega	Visual	NA	Yes	No
Alexander Tank South	Visual	NA	No	No
Aspen Tank	Visual	NA	No	No
Baney Park Tank	Visual	NA	No	No
Bull Pass Tank	Visual	NA	No	No
Burnt Cabin Tank	Visual	NA	No	No
Cabin Tank	Visual	NA	Yes	No
Collins Pasture Tank	Visual	NA	No	No
Cooney Tank One	Visual	NA	Yes	No
Deep Canyon Tank	Visual	NA	No	No
Dutchman Tank	Visual	NA	No	No
Five Spring Tank	Visual	NA	No	No
Gap Tank	Visual	NA	No	No
Gillette Tank East	Visual	NA	No	No
Gillette Tank West	Visual	NA	Yes	No
Gwynn Tank	Visual	NA	No	No
Kennedy Mesa Tank	Visual	NA	No	No
Loco Mountain Tank	Visual	NA	No	No
Miner Tank	Visual	NA	No	No
NF-94 West Tank	Visual	NA	No	No
Pipe Tank	Visual	NA	No	Yes
Potato Patch Tank	Visual	NA	No	No
Sacaton South Tank	Visual	NA	No	Yes
Slater Tank	Visual	NA	No	No
Steer Mesa Tank	Visual	NA	No	No
T Bar Duck Tank	Visual	NA	No	No
Tennessee Tank	Visual	NA	No	No
Trail Tank	Visual	NA	No	No
Unnamed Tank (FR28 & 141)	Visual	NA	Yes	No

Table 6. List of 17 sites monitored to determine whether eggs survived to metamorphosis, indicating successful reproduction in 2015. Emerging toadlets were observed at 37% of sites.

Site Name	*Eggs or tadpoles observed*	*Metamorphosed toadlets observed*	*Reason for failure*
Indian Tank	Yes	No	Tank dried
Snow Lake	Yes	No	?
Alexander Cienega	Yes	No	Tank dried
Cabin Tank	Yes	No	?
Gillette Tank	Yes	No	Tank dried
Unnamed Tank (FR28 & 141)	Yes	No	?
NM 78-2	Yes	No	Tank dried
Pueblo Creek	Yes	No	Flashflood
Cooney Camp	Yes	No	Stream dried
US 180-6	Yes	No	Flashflood
Mule Creek	Yes	No	?
Cooney Tank 1	Yes	Yes	
West Fork 1	Yes	Yes	
West Fork 2	Yes	Yes	
Black Canyon 1	Yes	Yes	
Black Canyon 2	Yes	Yes	
Hell's Hole	Yes	Yes	

Table 7. Stream characteristics at different sections of habitat available to tadpoles.

Section	*% Cobble*	*% Sand*	*Water Depth (Left-Center-Right)*	*Mean Water Depth*	*Mean Wetted Width*	*% Riffle*	*% Run*	*Tadpoles Present*
A	75	25	2.9 – 16.7 – 3.6	7.7±1.0	694.7±67.5	75	25	Yes
B	26	74	4.4 – 18.9 – 4.7	9.3±1.3	1072±54.0	48	52	Yes
C	100	0	5.2 – 23.0 – 5.0	11.1±0.8	451±24.4	100	0	Yes
D	100	0	7.5 – 16.4 – 8.1	10.7±1.1	285±56.0	100	0	Yes
E	73	27	4.8 – 20.1 – 5.7	10.2±1.9	1320±68.3	6	94	No

Table 8. Logistic regression results of tadpole presence, which was set as the dependent variable. The results indicate whether a habitat characteristic increases likelihood of observing tadpoles. According to these analyses tadpoles prefer narrow stream sections that are dominated by riffle type flows. Substrate type is not a factor in use of habitat by tadpoles.

Habitat Characteristic	*Whole Model $P_{df,\,N}$*	*Whole Model X^2*	*Parameter Estimate*	*Association*
Mean Depth	$0.0292_{1,83}$	4.75	0.32	+
Wetted Width	$0.0001_{1,83}$	93.89	0.37	+
% Cobble	$0.5838_{1,83}$	0.03	0.00	-
% Riffle	$0.0001_{1,83}$	62.97	-0.08	+

Table 9. Summary of the means, standard deviations (SD), range, and sample sizes of four traits, and hybrid index of *A. microscaphus* and *A. woodhousii* from Grant and Sierra Counties, the only counties in New Mexico where the two species co-occur.

	A. microscaphus			*A. woodhousii*		
	Mean ± SD	***Range***	***Sample Size***	***Mean ± SD***	***Range***	***Sample Size***
Gila Region						
Dorsal Stripe	0.16 ± 0.44	0-3	202	3.00 ± 0.00	0	85
Throat Spots	0.20 ± 0.46	0-2	202	1.57 ± 0.91	0-3	85
Cranial Crest	0.73 ± 0.46	0-2	202	2.91 ± 0.27	2-3	85
Pale-bar	0.18 ± 0.41	0-2	202	3.00 ± 0.00	0	85
Hybrid Index	1.29 ± 0.87	1-4	202	10.49 ± 0.98	9-12	85
Catron Co.						
Dorsal Stripe	0.17 ± 0.43	0-3	123	NA	NA	NA
Throat Spots	0.23 ± 0.61	0-4	123	NA	NA	NA
Cranial Crest	0.69 ± 0.46	0-2	123	NA	NA	NA
Pale-bar	0.17 ± 0.39	0-1	123	NA	NA	NA
Hybrid Index	1.21 ± 0.84	0-4	123	NA	NA	NA
Grant Co.						
Dorsal Stripe	0.23 ± 0.60	0-3	34	3.00 ± 0.00	0	63
Throat Spots	0.20 ± 0.47	0-2	34	1.52 ± 0.94	0-3	63
Cranial Crest	0.88 ± 0.40	0-2	34	2.95 ± 0.21	2-3	63
Pale-bar	0.32 ± 0.53	0-2	34	3.00 ± 0.00	0	63
Hybrid Index	1.64 ± 1.01	1-4	34	10.47 ± 0.98	9-12	63
Sierra Co.						
Dorsal Stripe	0.04 ± 0.20	0-2	23	3.00 ± 0.00	3	20
Throat Spots	0.30 ± 0.55	0-3	23	1.65 ± 0.81	0-3	20
Cranial Crest	0.65 ± 0.48	0-1	23	2.85 ± 0.36	2-3	20
Pale-bar	0.08 ± 0.28	0-1	23	3.00 ± 0.00	3	20
Hybrid Index	1.08 ± 0.90	0-3	23	10.50 ± 1.00	9-12	20

Figures

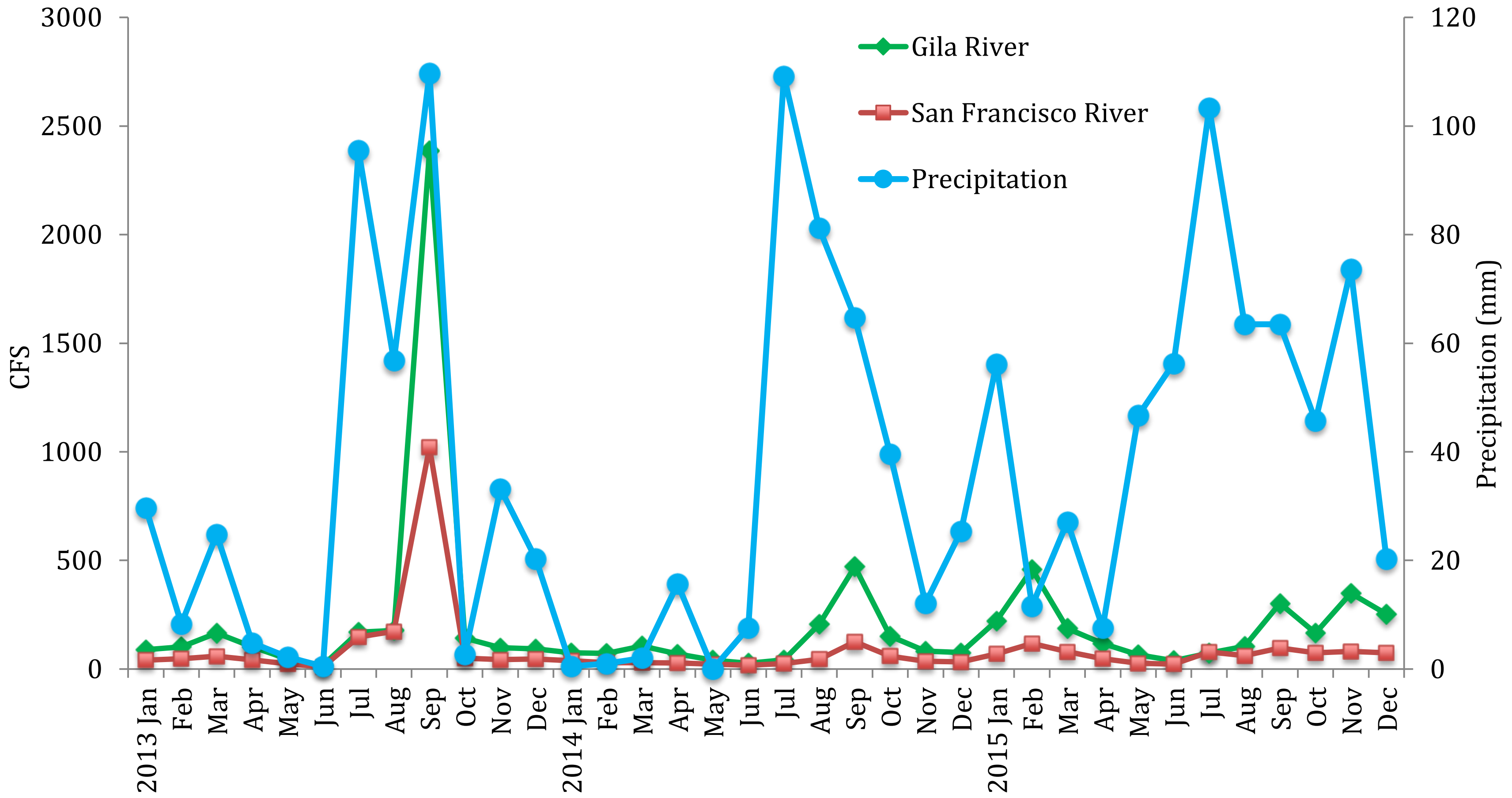


Figure 1. Summary of flow (cubic feet per second [cfs]) and precipitation for 2013—2015 for major rivers in the Gila region.

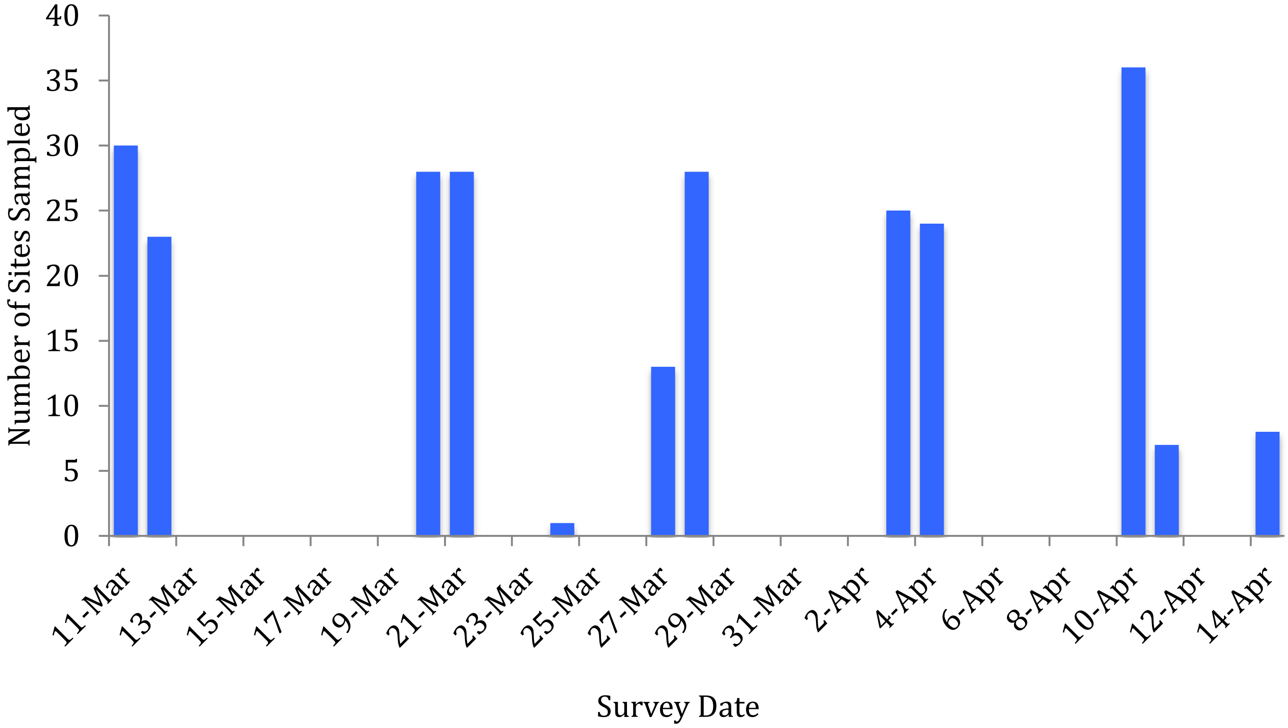


Figure 2. Call survey sampling summary by date for 2015.

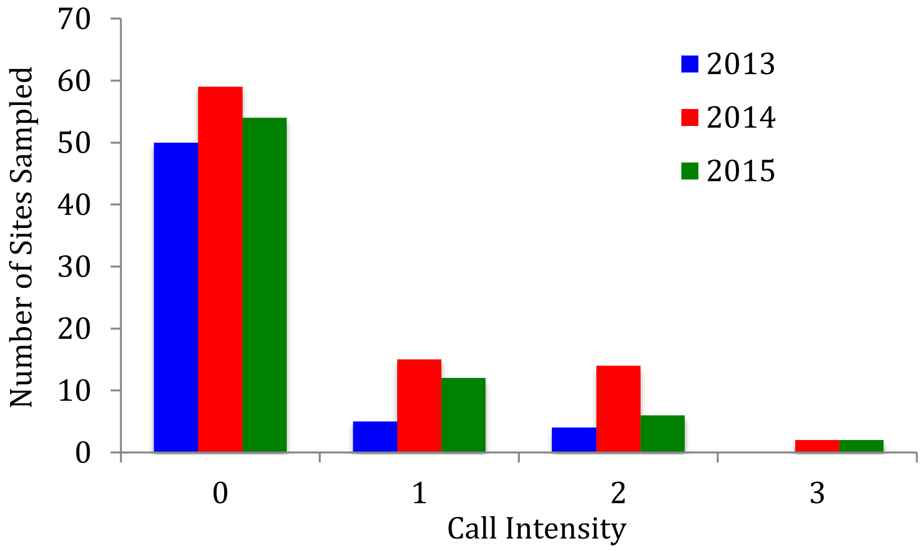


Figure 3. Summary comparison of *A. microscaphus* call intensity surveys from 2013 to 2015. Call intensity represents the maximum intensity observed at a sampling locality. The number of sites sampled varied per year (2013: 59; 2014:90; 2015:76) but maximum call intensity is consistent among years.

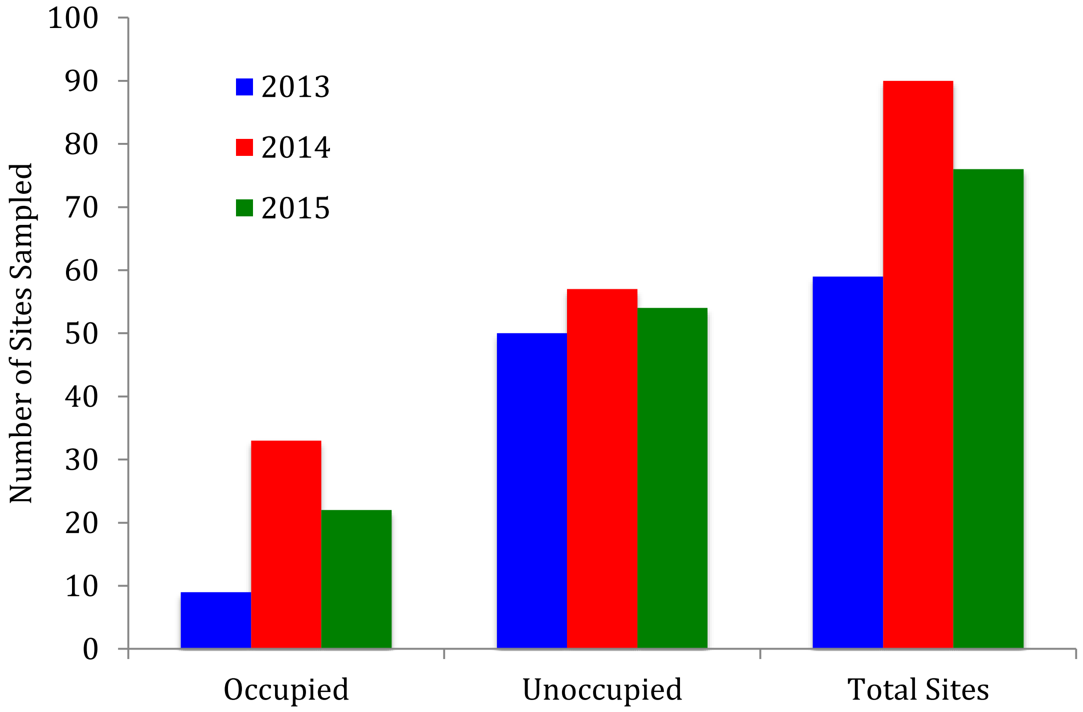


Figure 4. Summary comparison of occupied and unoccupied sites from 2013 to 2015. Site occupancy was assessed from call surveys.

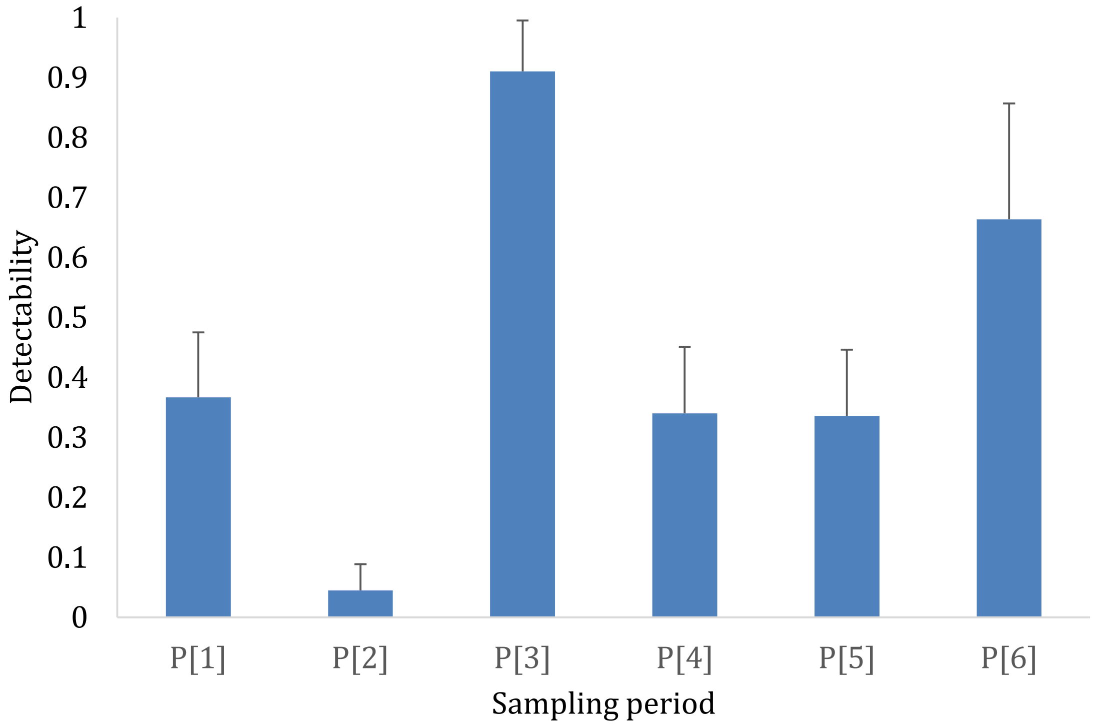
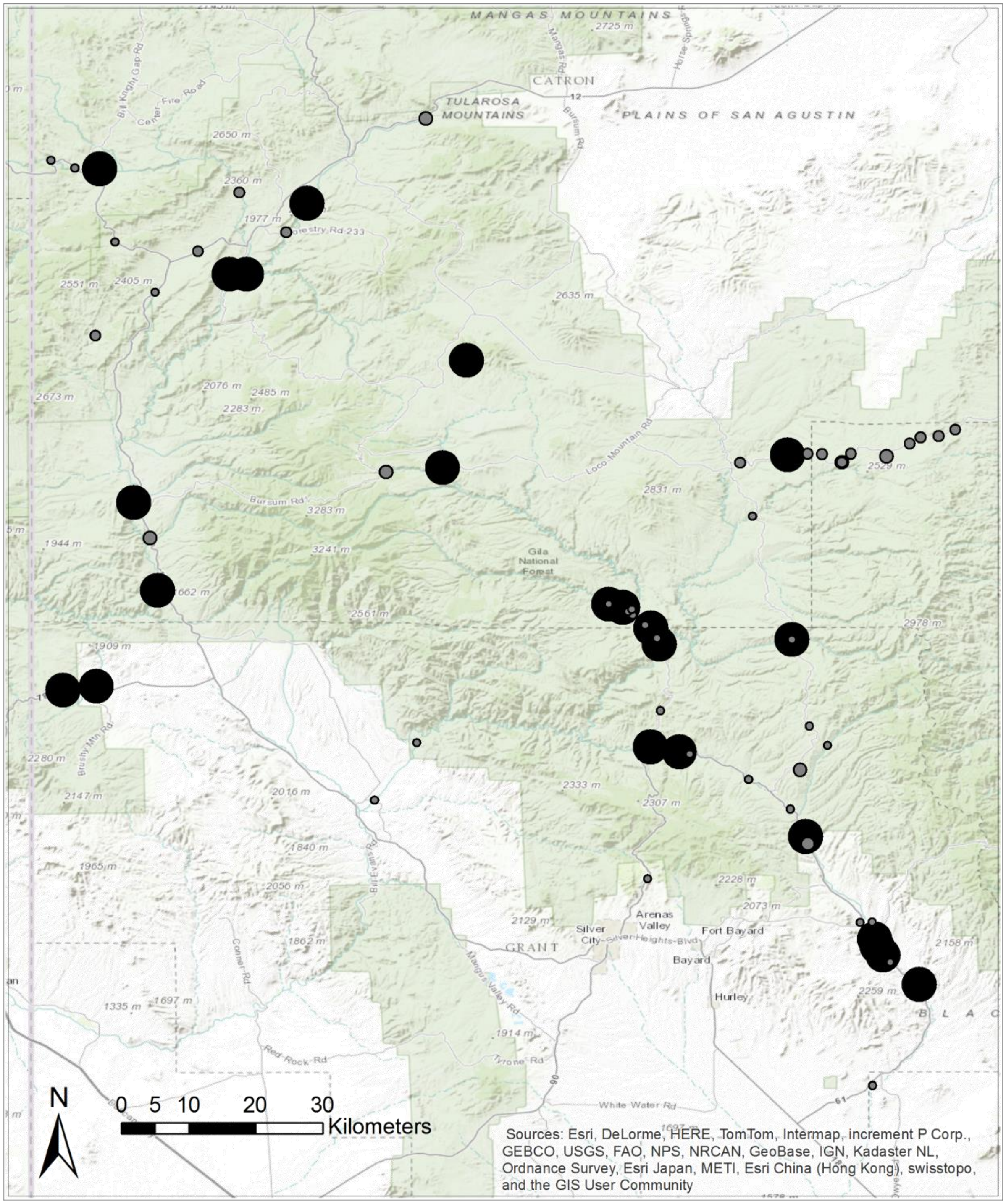


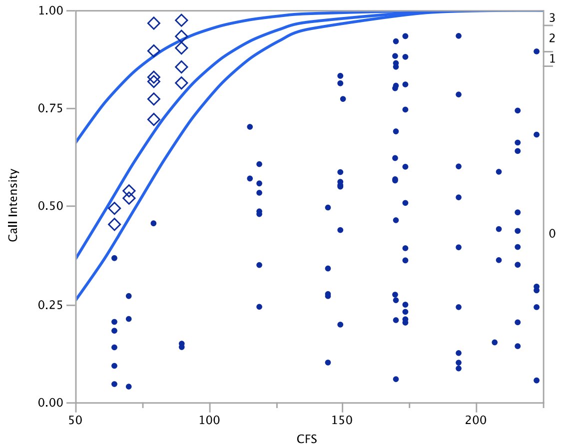
Figure 5. Estimates of detectability per each sampling period during 2015. Bars indicate standard errors.



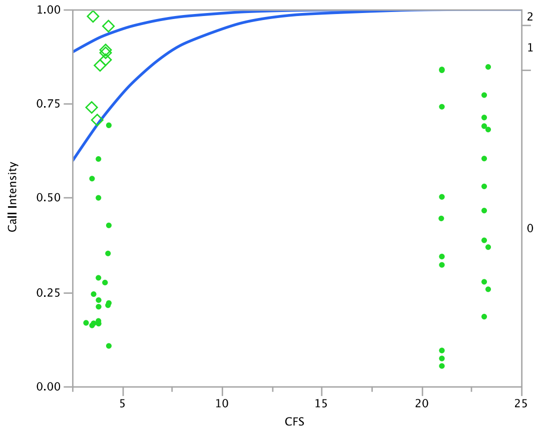
Sources: Esri, DeLorme, HERE, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, and the GIS User Community

Figure 6. Map of the study area indicating per-site detection probability. Black circles denote an occupied site (probability of occupancy is 1), whereas grey circles represent an estimated occupancy (see Results). Size of grey circle is proportional to the probability of occupancy. Note that majority of sites where toads were not detected have a low probability of occupancy (<0.25).

A. Gila River



B. Mimbres River



C. San Francisco River

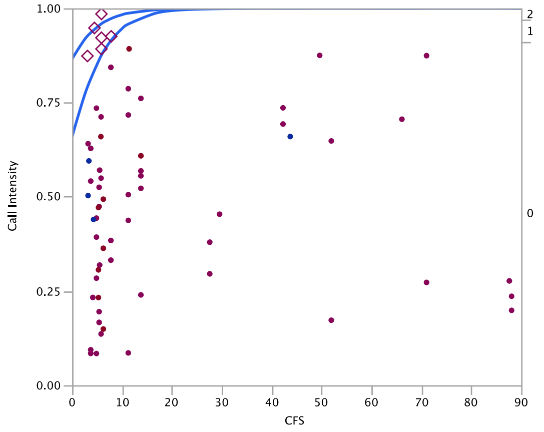
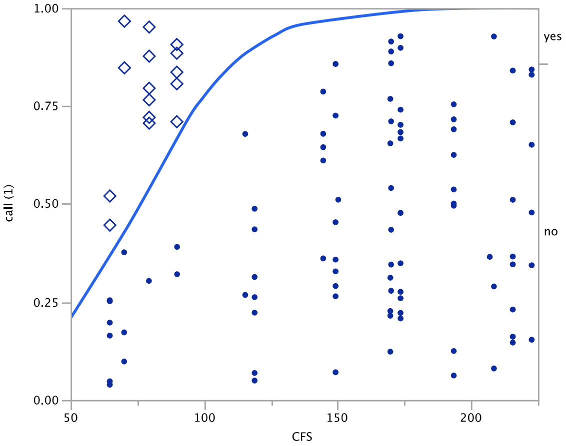
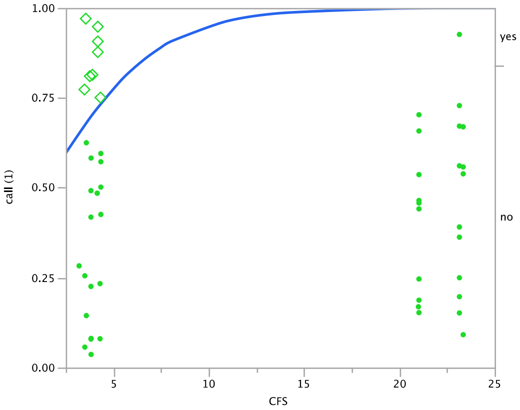


Figure 7. Ordinal logistic regression plots of toad call intensity and stream flow for (A) Gila, (B), Mimbres, and (C) San Francisco Rivers. Closed circles are surveys where toads were not detected, open diamonds are surveys where toads were detected. See Table XX for results.

A. Gila River



B. Mimbres River



C. San Francisco River

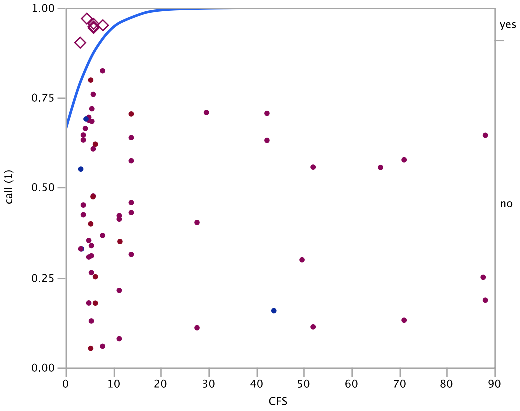
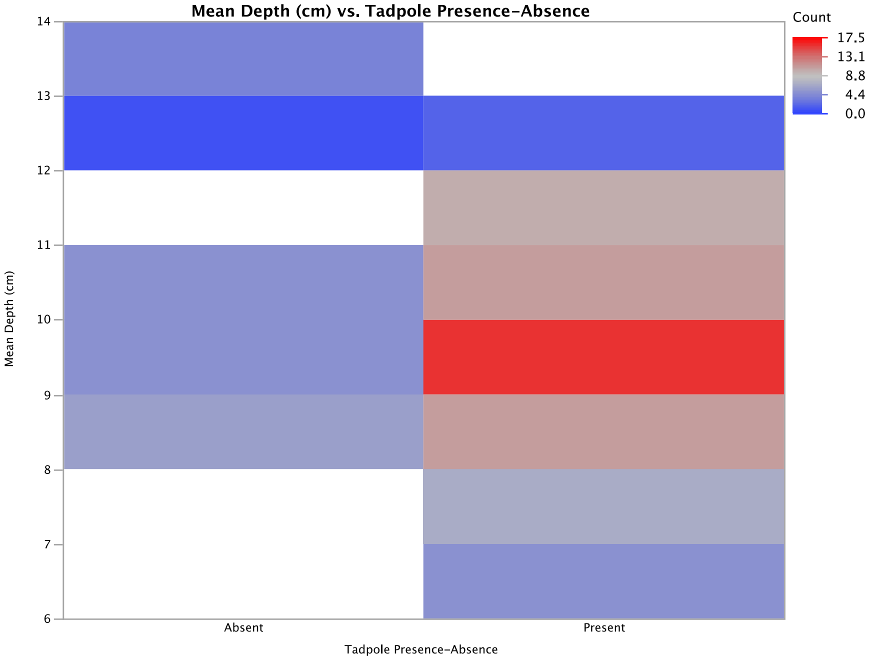
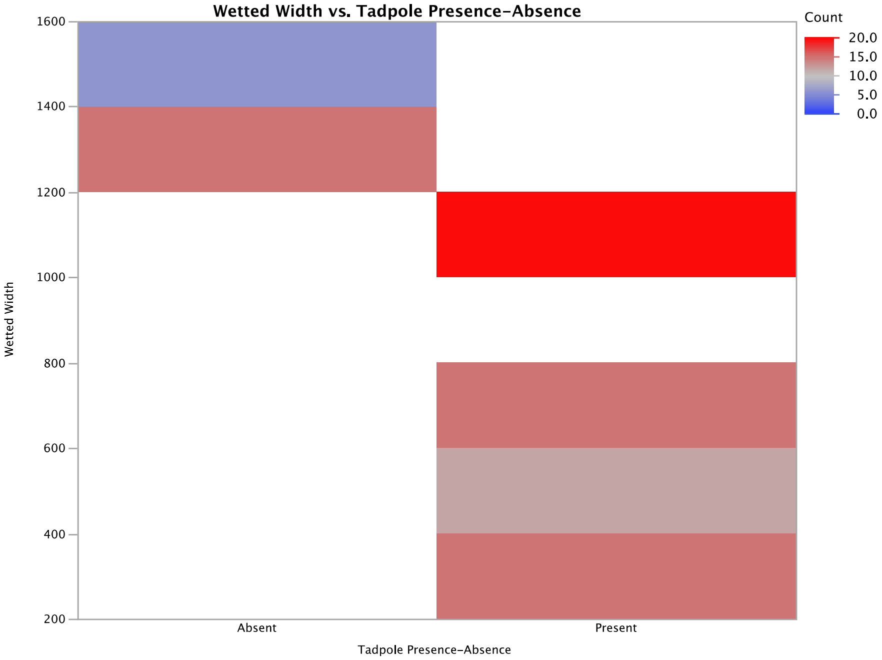


Figure 8. Ordinal logistic regression plots of toad call detection and stream flow for (A) Gila, (B), Mimbres, and (C) San Francisco Rivers. Closed circles are surveys where toads were not detected, open diamonds are surveys where toads were detected. See Table XX for results.

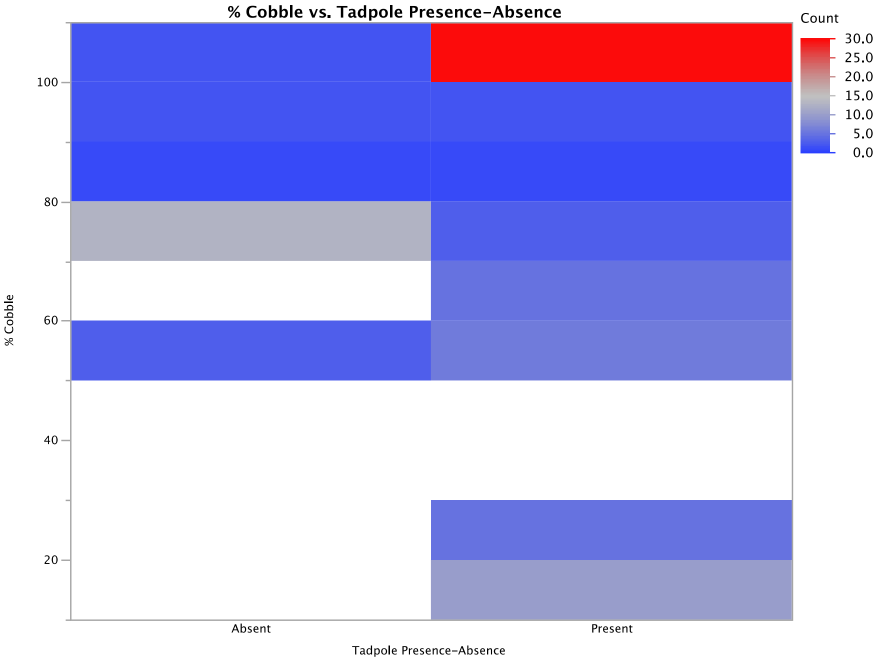
A. Mean Depth



B. Wetted Width



C. % Cobble



D. % Riffle

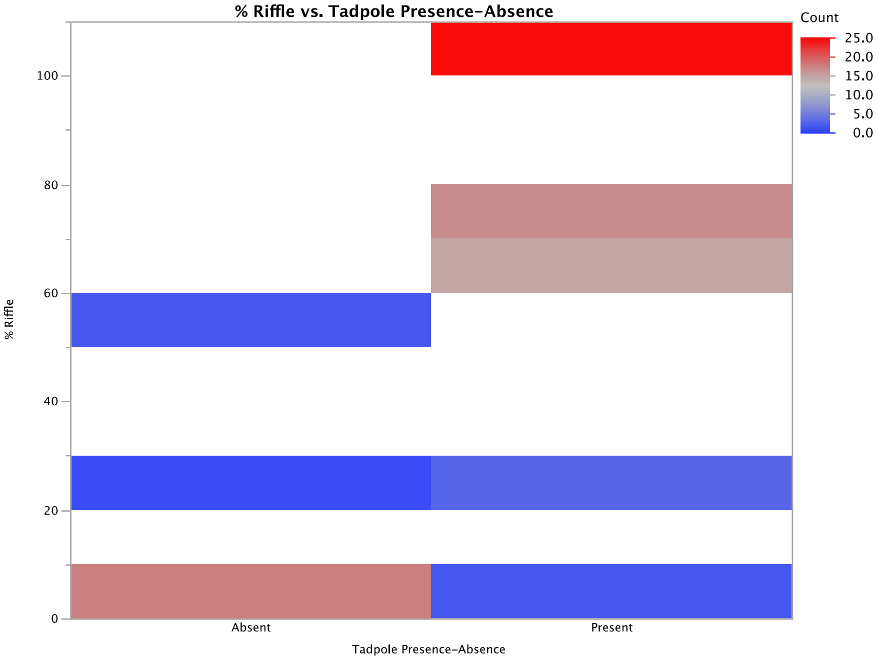


Figure 9. Heat maps of tadpole presence-absence per meter of stream by habitat characteristics. Red indicates high frequency of occurrence, blue indicates low frequency of occurrence, white indicate moderate frequency of occurrence.

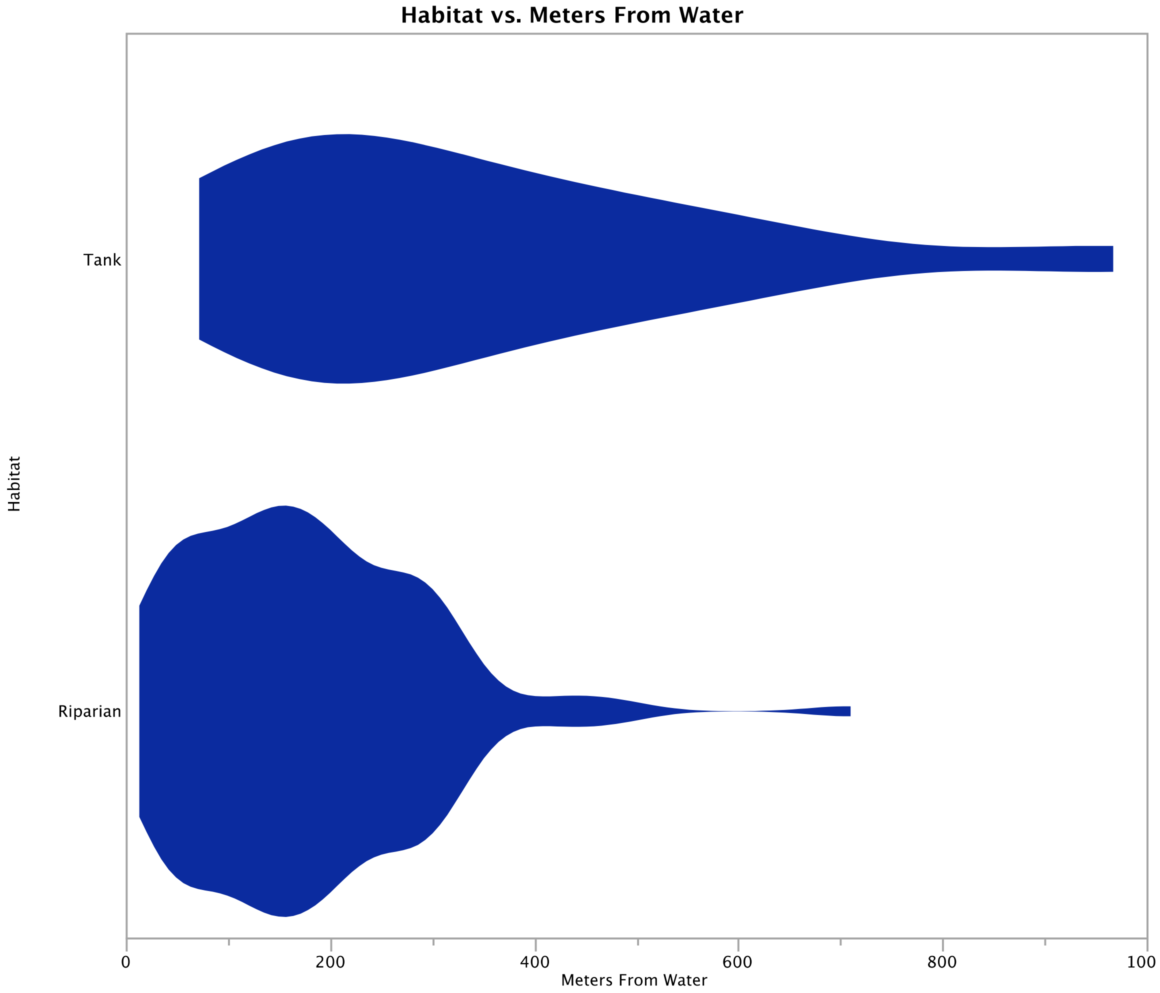


Figure 10. Contour graph showing density of observations by meters from water for non-breeding season (June to August) Arizona Toad observations separated by type of habitat.

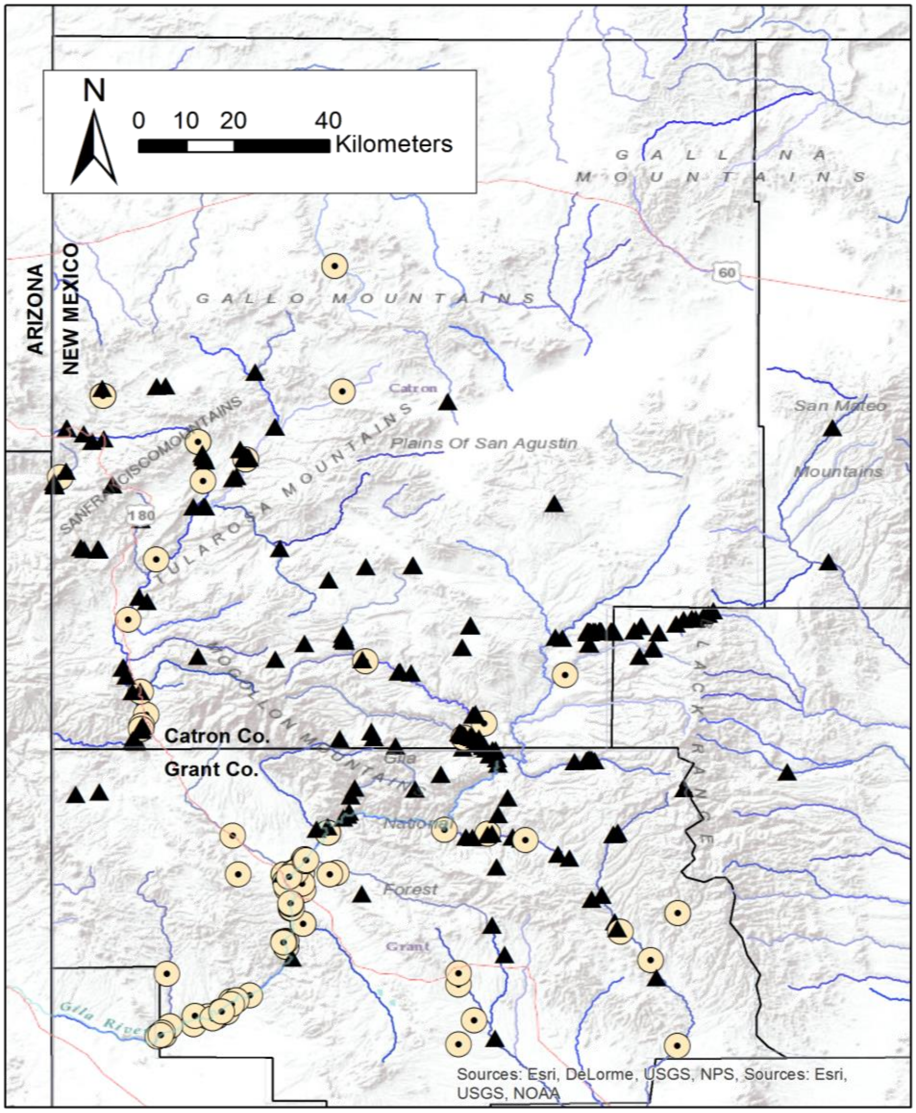


Figure 11. Currently recognized distribution of *A. microscaphus* (black triangles) and *A. woodhousii* (white circles) in Catron, Grant, and Sierra Counties.

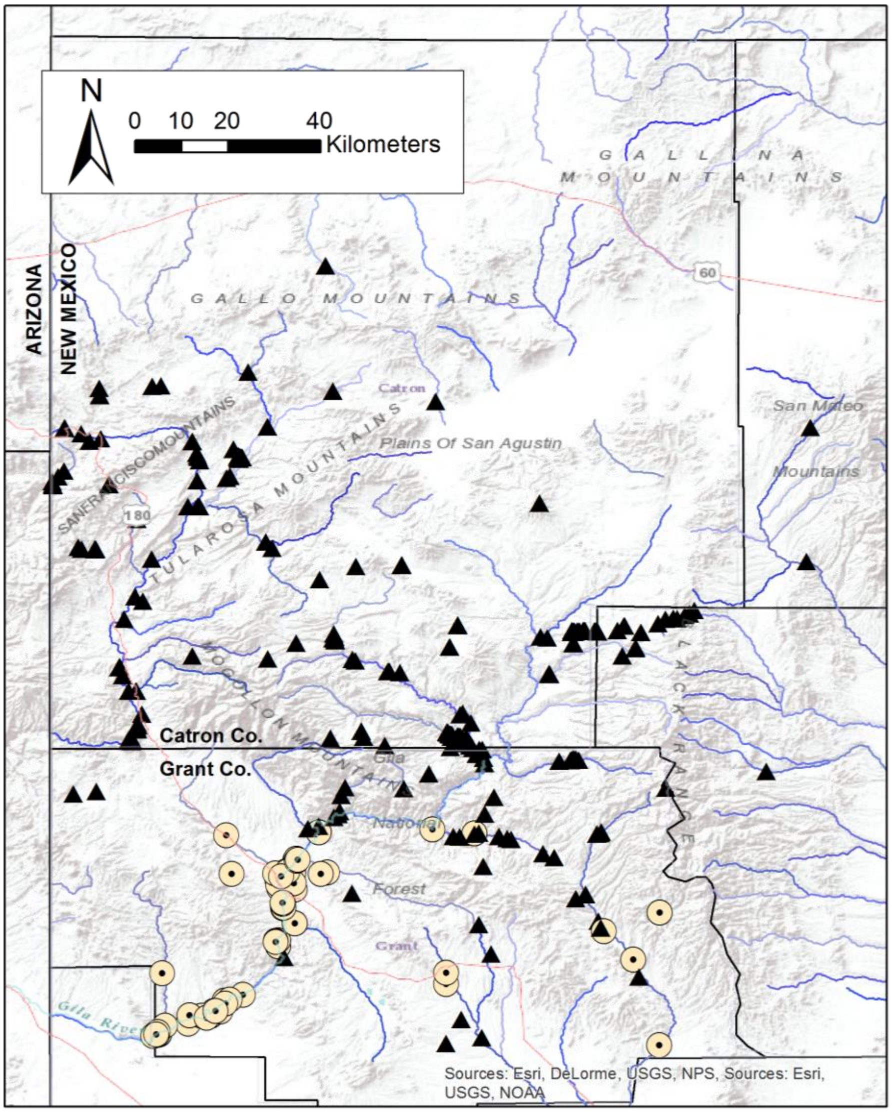
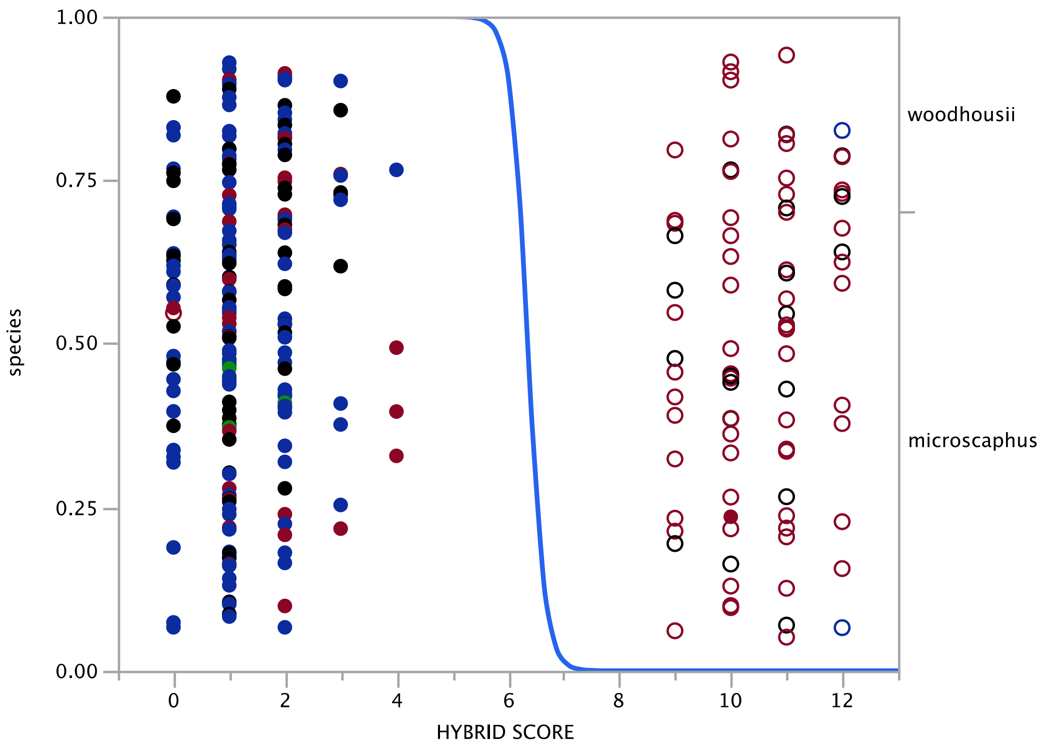
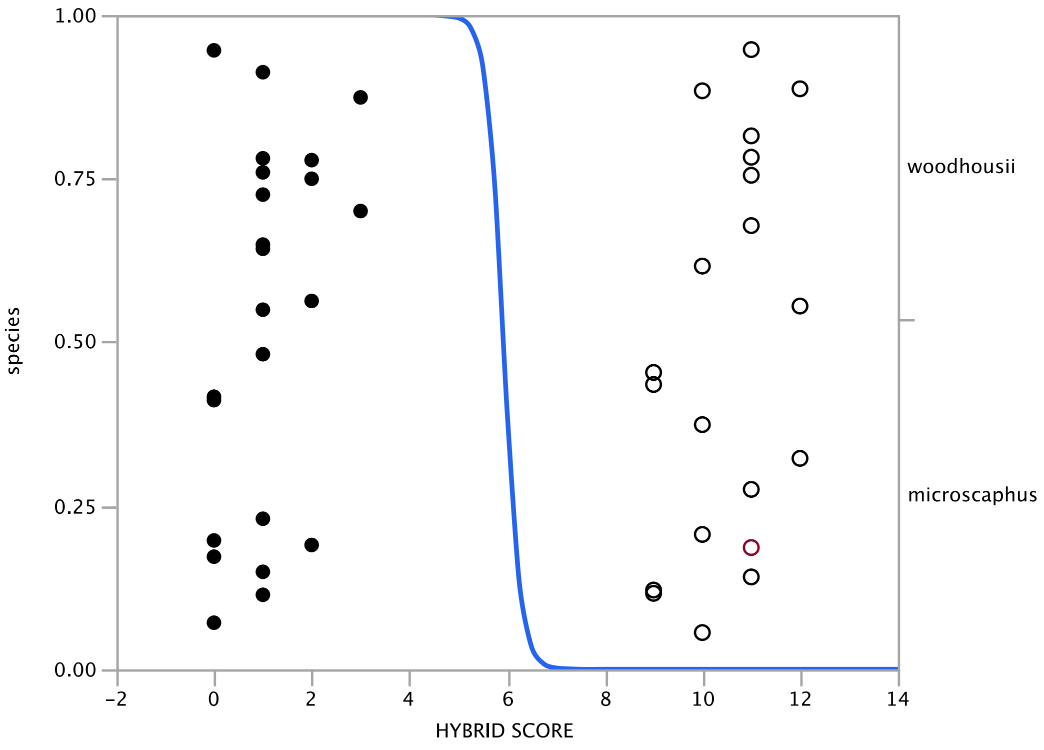


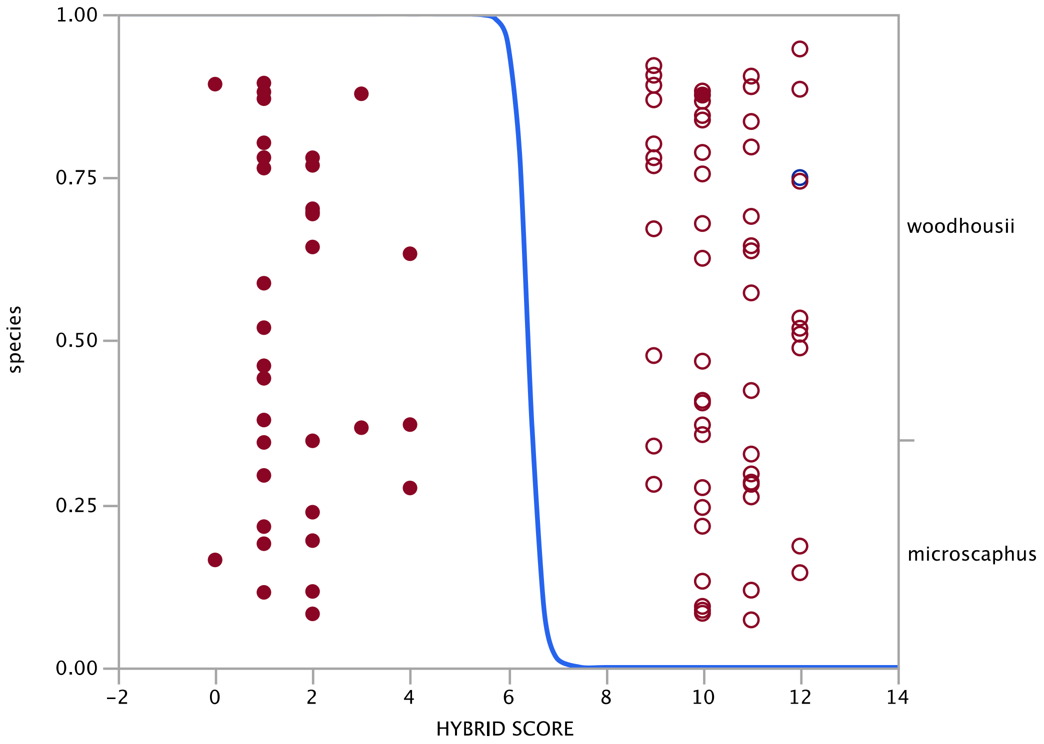
Figure 12. Revised distribution of *A. microscaphus* (black triangles) and *A. woodhousii* (white circles) in Catron, Grant, and Sierra Counties.



A. All specimens (*P* = 0.0001; Chi Square = 348.75; Estimate = -6.51)



B. Grant County (*P* = 0.001; Chi Square = 125.66; Estimate = -7.29)



C. Sierra County (*P* = 0.0001; Chi Square = 59.40; Estimate = -5.82)

Figure 13. Hybrid index logistic regression plots of: A) all scored *A. microscaphus* (closed circles) and *A. woodhousii* (open circles) from the Gila; B) *A. microscaphus* (closed circles) and *A. woodhousii* (open circles) from Grant County; C) *A. microscaphus* (closed circles) and *A. woodhousii* (open circles) from Sierra County. These figures show the hybrid index break between the two species and logistic regression results in parentheses. The four *A. microscaphus* specimens with a hybrid score of four are discussed in the text.