

Habitat Suitability Model, Population Demography, and Invasive Species
Management for the Arroyo Toad, *Anaxyrus californicus*
FINAL REPORT

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United States Geological Survey

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Abstract

The U.S Fish and Wildlife Service (USFWS) is responsible for monitoring the recovery progress of federally listed species by conducting a Species Status Assessment (SSA) every 5 years. The arroyo toad (*Anaxyrus californicus*) is an amphibian that is endemic to parts of the Pacific southwest, and has been listed as endangered since 1995. It has a 5-year SSA scheduled for fall of 2022; however, there are many unknowns that surround the toad's current status, stemming from scattered, unevaluated survey data and continuously changing habitat conditions.

We performed three separate analyses to help the USFWS determine how arroyo toad population recovery has progressed. To improve survey efficiency, we developed a habitat suitability model (HSM) that predicts where suitable toad habitat can be found within California. Additionally, to investigate trends in population recovery, we analyzed historic toad presence records in a region known to have suitable toad habitat (Piru Creek), then evaluated how these values correlate with precipitation. Lastly, acknowledging that invasive species predation contributes to toad decline, we assessed the efficacy of a controlled water release at removing invasive predators from suitable toad habitat. We found that (a) toad habitats have dwindled and (b) areas that historically supported arroyo toad reproduction have undergone decreased water availability and reproductive outputs of toads. Larger quantities of invasive predators were captured before a controlled water release took place than after, which supports its feasibility as a strategy for mitigating invasive predators in streams. Our findings suggest arroyo toad populations still face various habitat stressors that inhibit their recovery, though further validation surveys are needed to determine the full impact that sparse suitable habitat is having on toad populations. Furthermore, our water release analysis presents that this strategy may be a tangible solution for mitigating threats by invasives. Ultimately, the results of our analysis

contribute to future arroyo toad recovery assessments and therefore will be useful to the USFWS as they conduct their upcoming arroyo toad SSA.

Introduction

The arroyo toad (*Anaxyrus californicus*), native to California and northern Baja California, Mexico, is currently listed as federally endangered by the U.S. Fish and Wildlife Service (USFWS). Last reviewed in 2009, a 5-year review of the status of this species required by section 4(c)(2) of the Endangered Species Act (ESA) of 1973 is due. This 5-year review updates the accuracy of the listing status of “threatened” or “endangered”, including the potential downlisting of the species. To be downlisted, the following criteria must be met: 1) management plans are reviewed, approved and applied to land within federal jurisdiction and 2) a minimum of 20 successfully self-recruiting metapopulations or populations across the three designated recovery units must be established (USFWS, 2014). To better monitor the recovery of different toad populations throughout its range, the USFWS divided the U.S. portion of the toad’s range into two separate recovery units: The northern recovery unit (rangewide streams north of Los Angeles county) and the southern recovery unit (rangewide streams south of Los Angeles county). After being listed as endangered in 1994, there have been several attempts to downlist the status of the toad, with the latest attempt in 2014 (USFWS, 2014). However, there has not been sufficient data and analysis to reliably demonstrate that the toad was responding well to conservation efforts, thus it retains its status as endangered.

Once occupying up to 475 stream kilometers of habitat in California, stream occupancy has dwindled greatly, and arroyo toads are only found in limited headwaters of coastal streams in Central and Southern California, as well as Northwestern Baja California, Mexico (Hitchcock, 2022; Lovich, 2009). Female arroyo toads deposit eggs in the area where they are later fertilized,

making water conditions very important (Sweet, 1992; Thompson et al., 2016). After metamorphosis, which occurs during the summer months, juveniles begin to migrate away from the water's edge and participate in nocturnal migrations along 2-3km of stream (Hancock, 2009).

While working with the USFWS, U.S. Geological Survey (USGS) and United Water Conservation District (UWCD), our team has adopted a three-pronged effort to provide information that may assist the ongoing 5-year status review for the toad. First, we created the Habitat Suitability Model (HSM) that includes the toad's entire California range to identify optimal environments for the toad. Second, we conducted the first population trend analysis based on 20 years of arroyo toad data collected from Piru Creek, a stream that is included in the northern recovery unit (USFWS, 2014), to study population dynamics and the stability of this well-studied toad population. Finally, we organized and conducted a pilot study in December, 2021 and investigated the use of water releases in hydrologically managed watersheds to reduce population densities of invasive species that threaten toad survival in Piru Creek Watershed.

Habitat Suitability Model (HSM)

A key element of the USFWS's recovery plan for the arroyo toad is defining recovery criteria specific to the toad and verifying that the criteria is being met. Typically, the agency would determine species' paths to recovery by completing annual presence-absence surveys on ideal habitat within the species distribution range (USFWS, 2014). However, determining if arroyo toad populations are meeting their recovery criteria has been a challenge for the USFWS. The majority of recent arroyo toad survey efforts have been focused on a limited set of locations in the southern recovery unit of the toad's distribution (south of Los Angeles County; USFWS, 2014) and not as intensely in the northern recovery unit (within and north of Los Angeles County; USFWS, 2014). Additionally, periods of drought can temporarily, and sometimes

permanently, reduce the quality of toad breeding habitat. This variability in annual water flow makes it a challenge for the USFWS to determine whether or not ideal population recovery conditions, such as connectivity and self-sustainment (USFWS, 2014), are being achieved in designated areas of critical habitat. We address the above challenges through habitat suitability modeling.

Habitat suitability models are utilized to predict where suitable habitat is located for a given species (Hirzel, 2008) and, therefore, they can be utilized to improve survey efficiency. A habitat suitability model (or HSM) is defined as a “map composed of cells (of pixels) whose quantitative values range from 0 to 1”, where higher values indicate greater suitability and lower values indicate less suitable areas (Hirzel, 2006). Many habitat suitability models determine the ecological niche in which a species lives using a combination of spatially coarse variables such as those pertaining to climate, and fine-scale variables like soil composition (Hirzel, 2008) which can change dramatically across short distances. In general, an effective HSM can gauge the interactions between these variables, and use them to make conclusions about a given habitat’s ability to support a species. However, while a HSM can predict an area’s suitability for a given species, there is no guarantee that a species occupies the predicted habitat. HSMs have been developed for arroyo toad distributions (Treglia, 2015), however, previous models focused on specific regions of the toad’s total range and, therefore, were geographically limited.

Here, we developed a HSM that predicts where to find habitat that is ideal for toad occupancy. The model was generated by utilizing historical, verified presence records of arroyo toads and a carefully curated set of climatological, landscape, and biological variables to determine the range of suitability for toad habitat within stream areas across the toad’s known range. We used the model to quantify toad habitat, which will assist the USFWS in future

surveys and help the agency better understand the arroyo toad's current population distribution. Through this process, we determined the amount of stream that the model predicts as being high quality habitat for the arroyo toad during its breeding season. Additionally, we investigated the different land designations that the predicted high-quality habitat falls within, as well as the percentage of predicted high quality habitat that falls within and outside the area that the USFWS has determined as critical habitat for the arroyo toad. Furthermore, our model returned an index of variable importance with each environmental layer's contribution to simulated species distribution, providing insight into which factors are the most critical features of arroyo toad habitat. Overall, this model may prove to be a useful tool for the USFWS, the USGS, or any party concerned with the distribution of arroyo toad habitat to use in determining, at a very fine geographic scale, which sites have the highest probability of supporting toad populations. This information both serves to better inform current estimates of toad population status and serves as a baseline for future evaluations and status reviews.

Methods:

To create our HSM, we collated toad presence data from various sources. We used presence data from surveys done by USGS (Brehme et. al, 2010; Matsuda & Brehme, 2018) and the California Department of Fish and Wildlife (CDFW; Thomson et. al, 2016). Additionally, we included historical arroyo toad presences points (Rogers, 2020) that were extracted from a variety of sources (CFWO, 2011; ESA, 2014, 2015, 2016, 2017; Impact Sciences, 2014; Howard & Jacinto, 2018). We then compiled and organized the collated data based on the date, latitude, longitude, and development stage of each of the occurrences. We found that 97% of the presence data was collected after the year 2000, and aside from nine presence points in 1996 and 1998 in Monterey county, we only used entries from 2000 and beyond.

To distinguish breeding habitat specifically, presence data were organized into two separate datasets: total presence and breeding-only presence. The total presence dataset (5,617 entries) is composed of presences for all life stages. The breeding-only presence dataset (3,206 entries) includes presences collected during the arroyo toad breeding season (January to July) and excludes juveniles and adults, as they are known to migrate between breeding sites (Sweet & Sullivan, 2005; USGS, 2018). Because there are so few presence entries recorded in Monterey, we included the nine records in both datasets to better represent that part of the toads' range in the model.

Along with presence data, the model includes environmental variables that characterize appropriate toad habitat (Table 1). We integrated these variables into the model by creating raster layers that describe the hydrology, terrain, and climate of the area within the arroyo toad range as determined by the USFWS (USFWS, 2014).

We represented the hydrology of the areas by extracting a number of stream attributes from the National Hydrography Dataset (NHDPlus HR). Streams were filtered to only include those that were either naturally occurring (FType = StreamRiver) or ephemeral/highly seasonal (ArtificialPath). A 75 m buffer was applied on both sides of each stream to include toad occurrences that are proximal to, but not actually within, stream channels (Hancock, 2009), and to account for minor errors in georeferenced coordinates and stream movement over time. The average values of monthly stream-drainage temperatures and average precipitation values from 1991-2021 (within NHD; PRISM, 2006) were joined to these streams. Additionally, we appended average annual gauge-adjusted stream velocity (ft/sec) and volume (ft³/sec) to characterize stream flow levels at a fine scale. These naturally occurring, buffered streams with added habitat attributes were then exported as raster images for modeling.

Several physical landscape attributes were also used to model toad habitat. Topographic composition of watersheds is generally thought to be an important contributor to good arroyo toad habitat, so we assembled a suite of raster layers derived from the USGS National Elevation Dataset (NED) to represent topographic variation on several levels. A simple slope derivation was created in ArcGIS Pro from the source DEM rasters. In accordance with prior modeling efforts concerned with the flatness and evenness of valley bottoms in which arroyo toads live and breed (Treglia, 2015), two other data products were derived using SAGA GIS (Conrad et al, 2015): Multi-resolution Index of Valley Bottom Flatness (MRVBF), and Vector Ruggedness Measure (VRM) conducted at analysis ranges of 60 and 180 meters. Soil composition was also included in the model. We focused specifically on surface texture, on the percentage of sand, clay, and silt in the first 0-5 cm of soil, as analysis at this depth is most relevant for arroyo toads (Sweet & Sullivan, 2005). Normalized Difference Vegetation Index (NDVI) measures the difference between near-infrared and red light that indicates the presence and lushness of vegetation and was processed by the California Department of Fish and Wildlife using aerial remote sensing images obtained through the USGS National Agricultural Imagery Program 2020 imagery (Pettorelli, 2006). We included NDVI in our model to gauge whether highly-vegetated streams or more open, washed-out regions of streams were more likely to comprise good habitat.

Table 1: Environmental layers included in the HSM

Dataset	Variables	Description	Source
National Hydrography Dataset (NHDPlus HR)	Stream locations; stream flow rates and quantities	Indicates stream length and location; used to extrapolate stream-flow and stream-velocity. Stream rasters of each variable were created from the polygon layer with pixel sizes of 60 x 60m	U.S. Geological Survey [1]
CA NAIP 4-band Imagery (2020)	Normalized Difference Vegetation Index (NDVI)	Indicates the presence and health of vegetation.	California Department of Fish and Wildlife [2]
National Elevation Dataset	Slope; MRVBF (Multi-resolution Index of Valley Bottom Flatness); VRM60, VRM180	Indicates terrain of area; used to extrapolate Slope, Vector Ruggedness Measure (VRM), and Multiresolution Index of Valley bottom flatness (MVBFR). Each resampled to 60 x 60m,	U.S. Geological Survey [3]
PRISM Climate Data	Monthly average precipitation and temperature values (within watersheds)	Included the average precipitation and temperatures between 1991-2021	PRISM Climate Group, Oregon State University; from NHD [1]
POLARIS 30m probabilistic soil properties	Soil Surface Texture (Clay, Silt, Sand)	Percent clay, sand, and silt for top layer of soils (0-5 cm)	U.S. Department of Agriculture; Duke University [4]

[1] - <https://www.usgs.gov/national-hydrography/nhdplus-high-resolution>

[2] - <https://gis.data.ca.gov/datasets/CDFW::naip-2020-natural-color-california/about>

[3] - <https://www.sciencebase.gov/catalog/item/4fcf8fd4e4b0c7fe80e81504>

[4] - <http://hydrology.cee.duke.edu/POLARIS/>

The spatial data of each environmental variable was then transformed into a format that could be interpreted by MaxEnt. To do this, we used R to reproject, resample, and align all of the environmental layers (R Core Team, 2022; RStudio Team, 2022). This was necessary to ensure that each of the layers could be trimmed to the same extent and had the same size raster pixels. Once these conversions had taken place, we exported the files as ASCII and converted them to

the WGS 1984 Web Mercator (Auxiliary Sphere) geospatial coordinate system for use in the MaxEnt Graphical User Interface (GUI) (Phillips, 2017).

We used MaxEnt to apply maximum entropy species distribution modeling to our environmental layers and presence data with automated thinning and background-point generation. The model used predetermined settings and spatial thinning to account for data points that were in close physical proximity to account for repeated sampling in some areas. Thinning limited occurrences to only one point per 60 x 60m raster cell. After MaxEnt thinning, the total dataset included 1,907 occurrences and the thinned breeding-only dataset included 1,238 occurrences. Once assembled and trained against the suite of habitat variables, MaxEnt produced a raster image displaying the probability of toad occurrences in all eligible streams within the range (values ranging from 0-1 represent 0-100% predicted presence). This process was completed for both the 'breeding-only presence' and 'total presence' datasets, generating two prediction rasters.

Once MaxEnt had calculated predicted suitability on a continuous scale, we thresholded the data and reclassified the model into discrete classifications of habitat status. To do this, we first joined the suitability score, as predicted by our model, to each arroyo toad location used to train the model in ArcGIS. Then, we dropped the lowest 10% of suitability scores and considered them anomalous presence locations – either GPS data had been recorded incorrectly, an organism was misidentified in the field as an arroyo toad, etc. The remaining 90% of occurrences (1,715 occurrences in the total dataset; 1,113 occurrences in the breeding-only dataset) were statistically analyzed in Excel for first-quartile, mean, and third-quartile suitability scores. These statistics served as the basis for reclassification of the MaxEnt output raster in ArcGIS, and the designation of different qualities of arroyo toad habitat.

To assist different field validation or data manipulation purposes, we created two different classification systems for our habitat suitability model based on these statistics. First, our 3-class thresholded raster (Fig. 1c & 1d) depicts three different qualities of arroyo toad habitat (low, medium, and high), and was developed primarily for future model validation endeavors. In this system, “low” quality habitat is based on values between the minimum suitability score of thresholded presence point and the first quartile of the scores, “mid” quality habitat is between the first and third quartile of the scores, and “high” quality habitat is above the third quartile of the scores. Secondly, we developed a 2-class thresholded raster (Fig. 1a & 1b) to primarily be used for data analysis. The classes were defined here as follows: “low” quality or below-average habitat was defined as any habitat between the minimum suitability score of thresholded presence point and the mean suitability score for all presence points; “high” quality or above-average habitat was defined as above the mean suitability score. All thresholding and reclassifying steps were repeated for both models – ‘total presence’ and ‘breeding-only presence’.

Analyses of total stream length and estimates of habitat within public land boundaries (BLM, 2022), and within designated Critical Habitat (USFWS, 2022) were all performed on both class systems of ‘total presence’ and ‘breeding-only presence’ models, generating 4 figures for each analysis. To accomplish this, thresholded rasters were converted to polylines in ArcGIS with their suitability classification preserved as an attribute, and clipped to the agency or Critical Habitat boundary in question. Total lengths were calculated and summed using ArcGIS Spatial Analyst tools.

Figure 1: Maps developed based on results from the final habitat suitability models.

Fig 1a. Streams within the arroyo toads range (gray outline) that are based on the total presences points. This map portrays the 2-class thresholded raster (based on the mean of suitability scores), which depicts low suitability (below the suitability score mean) in yellow coloration and high suitability (above the suitability score mean) in red.

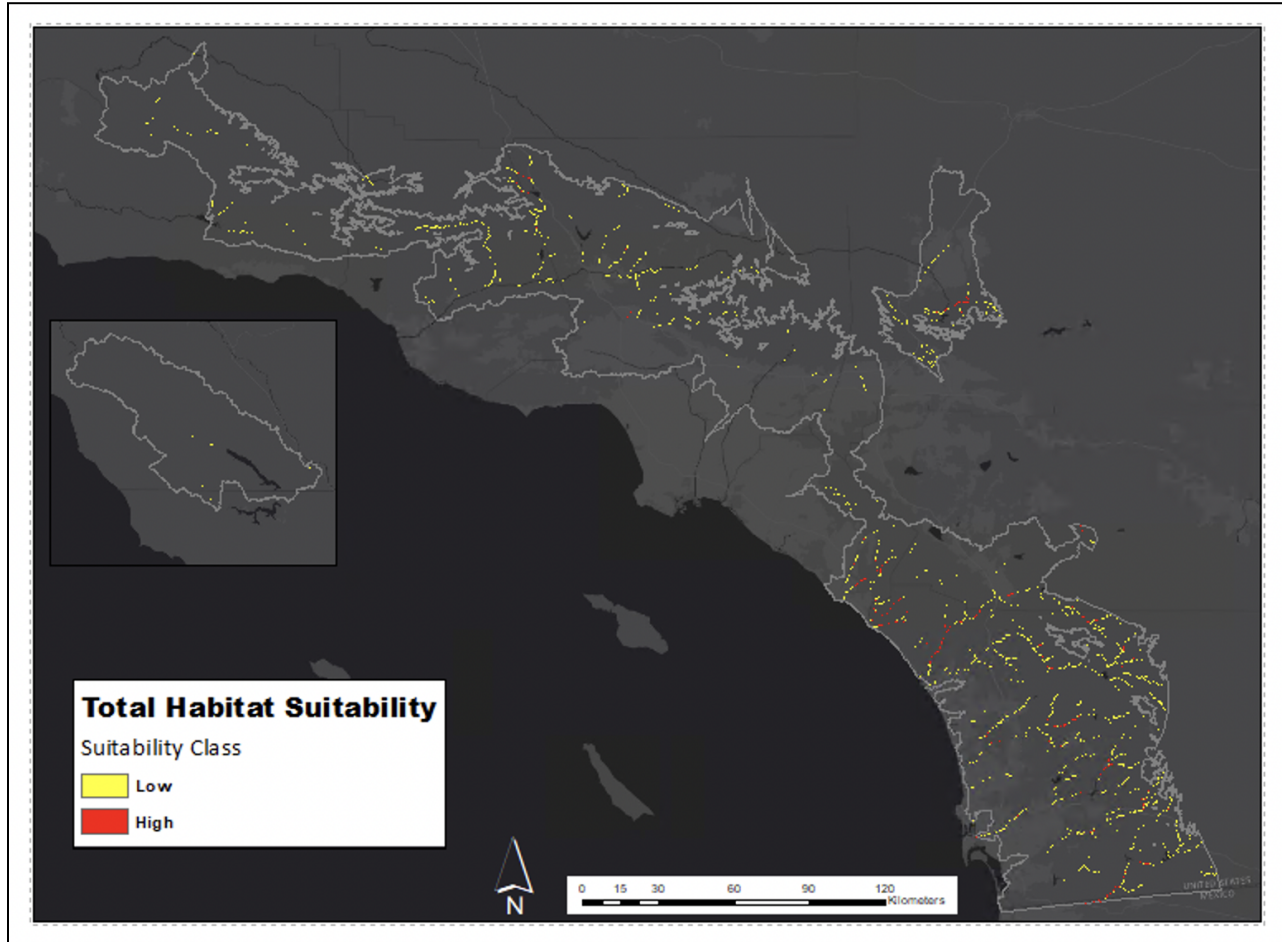


Fig1b. Streams within the arroyo toads range (gray outline) that are based on breeding-only presences points which are filtered based on the timing (Jan-July) and life stage (juveniles and adults). This map portrays the 2-class thresholded raster (based on the mean of suitability scores), which depicts low suitability (below the suitability score mean) in yellow coloration and high suitability (above the suitability score mean) in red.

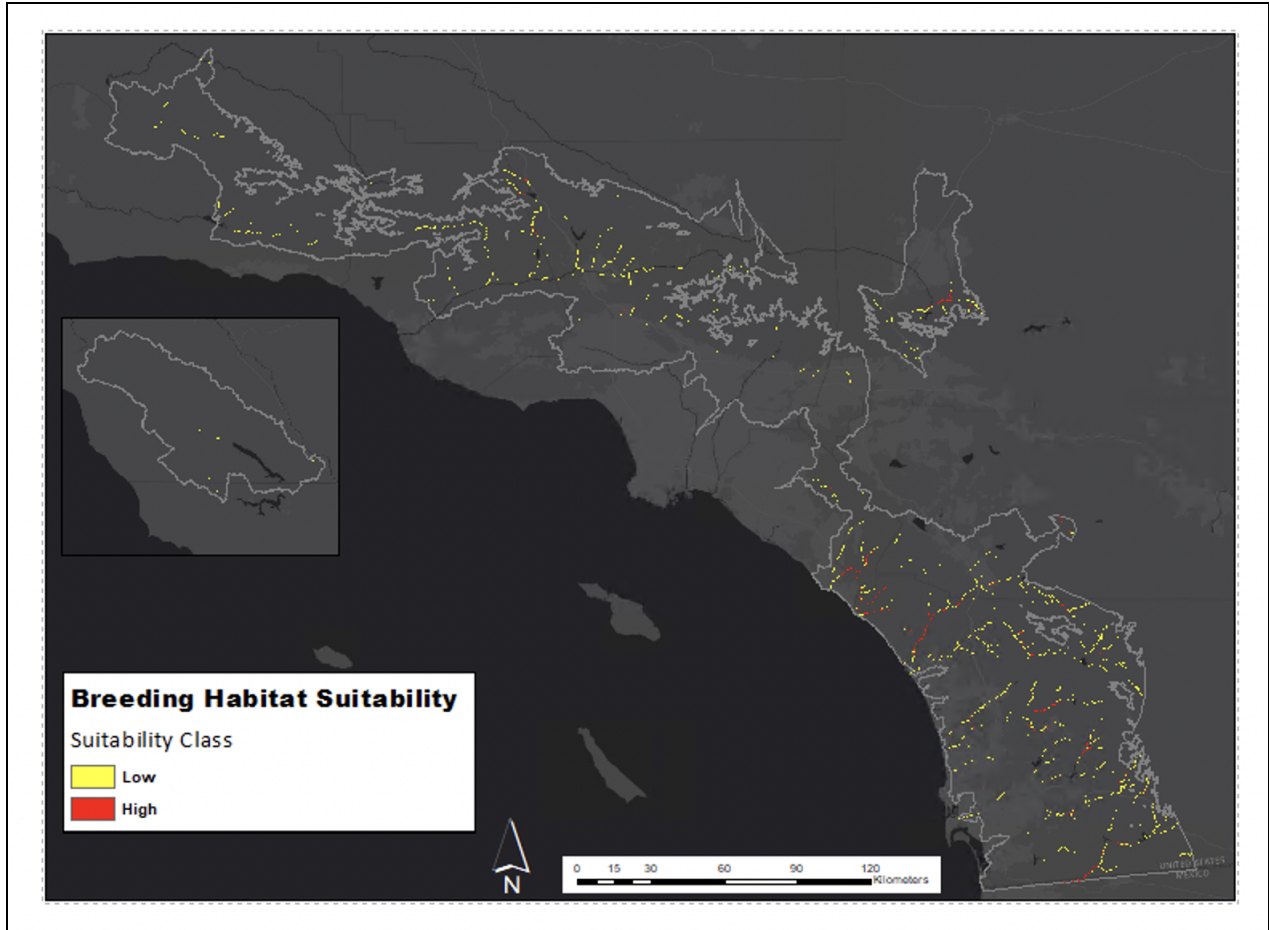
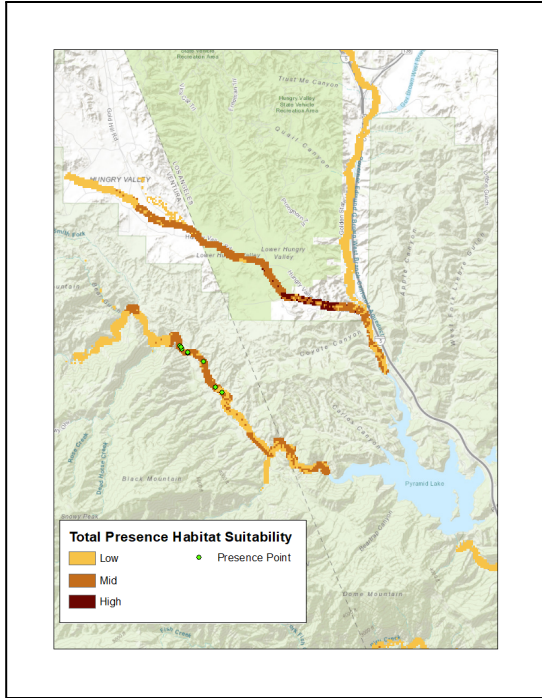
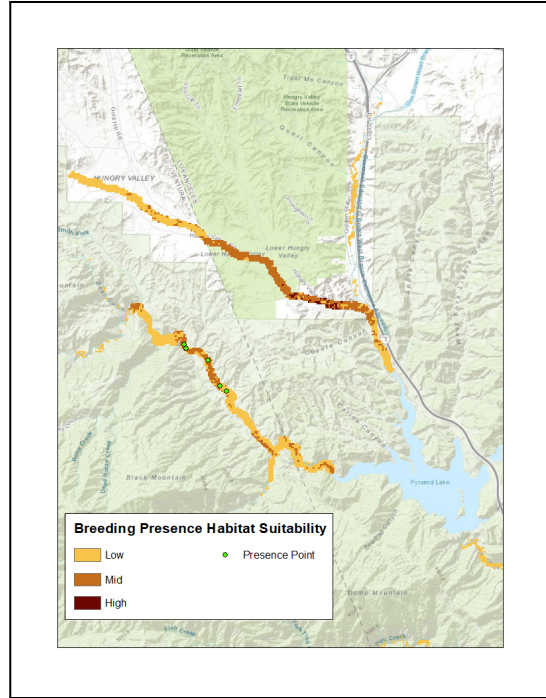


Fig. 1c & 1d. The suitability of creeks that merge into Pyramid lake (Upper Piru creek and Gorman creek) based on either the total (1c) or breeding-only (1d) presence points. The 3-class thresholded raster is based on the quartiles of the suitability scores. Each map depicts low suitability (scores between the minimum and 1st quartile) as a mustard coloration, mid suitability (scores between the 1st and 3rd quartile) as tan, and high suitability (above the 3rd quartile) as dark brown.



1c.



1d.

Results:

The most liberal estimate of arroyo toad habitat (the 2-class thresholded raster) indicated that there exists 2488.68 km of total suitable habitat. Within this, we identified 351.78 km as “above-average” habitat. The ‘breeding-only presence’ model yielded a more conservative estimate of arroyo toad habitat. This model identified 2014.45 km of habitat, and 296.61 km of above-average breeding habitat.

In each of these four estimates for arroyo toad habitat length, we found that less than 50% fell within the boundaries of designated critical habitat (Table 2). For the total presence model, 35.47% of total habitat and 39.29% of above-average habitat occurs within the critical habitat. In the breeding-only presence model, 46.67% of total and 47.20% of above-average habitat exists within critical habitat. Similar analyses for proportion of habitat on public lands (Table 3), including breakdowns by the largest public stakeholders (Local Government, State, U.S. Forestry Service, and Department of Defense).

Aside from a depiction of where suitable habitat exists within the analysis region, MaxEnt provides a number of measurements of variable importance. According to both models produced, stream flow characteristics were the most influential in determining areas of arroyo toad suitability.

Table 2: Comparison of stream lengths of suitable habitat as calculated by HSM with Critical Habitat identified by USFWS

	All suitable habitat		Above-average habitat	
	Total	Breeding only	Total	Breeding only
Stream Length (km)	2488.68	2014.45	351.78	296.61
Percent within Critical Habitat	35.47%	46.67%	39.29%	47.20%

Table 3: Proportion of predicted suitable habitat that intersects with various land management designations. Along with the proportion of predicted suitability intersecting with public land in total, public land has been divided into its four largest public stakeholders: Local government, State government, U.S. Forestry Service, and Department of Defense.

	All suitable habitat		Above-average habitat	
	Total	Breeding only	Total	Breeding only
Percent on Public Lands (Local, State, and Federal)	36.27%	37.34%	48.59%	53.43%
Local	1.04%	1.21%	4.24%	4.49%
State	3.22%	3.62%	4.26%	3.47%
US Forest Service (Federal)	19.46%	19.69%	8.73%	10.16%
Department of Defense (Federal)	7.86%	8.29%	28.53%	33.50%

Discussion:

Our final model provides a quantitative assessment of potentially suitable arroyo toad habitat in the context of land ownership across the species’ range. The 3-class thresholded

raster(low, medium, and high suitability) provides a reasonable framework for future field-based validation of the model, as well as facilitating surveys to assess toad populations.

The model predicts that there are roughly 300km of above average toad habitat found within California. This habitat is indicative of regions whose conditions satisfied the environmental preferences of the arroyo toad based on past occurrence records, including water velocity and volume, slope and precipitation. These layers were shown to be especially impactful in determining habitat suitability for the toads. Water volume was shown to have the highest regularized training gain, which is a measure of the distance between a multivariate distribution of covariates at randomly selected background sites (Hendrick, 2018). Essentially, this measures the importance of each variable in the model. The second most influential was water velocity, followed by slope, and October precipitation. Based on prior research on toad populations, the importance of these variables is understandable as they require water and slow moving streams for successful breeding.

With only 296.61 km out of the 2488.68 km qualifying as above-average breeding habitat, it is clear that limited habitat poses an issue for the long term success of arroyo toad populations. Moreover, these stream lengths seem especially low when considering the 50,156.60 km of total stream that was calculated to be within the toads range. The above-average classification was decided upon to easily set apart areas that are more likely to be good habitat, since a field validation was not feasible during the duration of this study (see below). Additionally, constraints due to private land ownership further minimize available toad habitat.

Limitations:

While our HSM model features a comprehensive evaluation of potential arroyo toad habitats, there are some limitations to our work. The first was the variability in the quality of metadata associated with presence points. Some occurrence records were thoroughly documented, including precise location coordinates, age/stage, and dates, whereas others lacked some or all of this information. Particularly for the model, which had 60m resolution, this meant that some potentially informative records had to be dropped. We suggest that all future surveys use strict universal protocols for arroyo toad research because cohesion between datasets allows for equality in extraction of specific toad, or toad habitat, attributes among datasets.

Additionally, much of the data that were available was largely concentrated in areas that are frequently surveyed, resulting in potential sampling bias. For example, the Santa Margarita River at Camp Pendleton had a very large number of occurrence records reflecting its survey frequency by the USGS. While helpful, these can create a model bias in favor of the precise habitat on Camp Pendleton, downweighting other potentially suitable areas. We accounted for this by spatially thinning the occurrences, but the bias may still be influencing the model.

Due to the specific habitat needs of the arroyo toad, environmental layers should be as accurate and as comprehensive as possible. However, due to the lack of complete data, some layers had to be excluded (greenness and soil moisture). We accounted for this by including other environmental variables (NDVI), which also helped determine the suitability of the habitats.

Time constraints also posed a limitation on our research. Originally, our team had hoped to validate the model by conducting field surveys of areas that were predicted to be very suitable for the arroyo toad. Unfortunately, this was not able to be done during the academic year, but our team intends to reconvene at the end of the quarter to conduct these surveys. This data will help us to determine how effectively the model predicted arroyo toad habitats.

Piru Creek Watershed: Population Trends in Arroyo Toad Abundance

Piru Creek is a major tributary of the Santa Clara River watershed located in Ventura and Los Angeles Counties that is inhabited by arroyo toad populations. More than 30 years of arroyo toad survey data was prepared by the California Department of Water Resources and the United Water Conservation District. Our team conducted the first analyses of these data to determine arroyo toad population dynamics and trends. We focused on two well-sampled sections of the drainage: Middle Piru Creek, regulated outflow between Pyramid and Piru lakes, and Agua Blanca Creek, an unregulated tributary that joins with Middle Piru Creek. Our main study objectives were to compare arroyo toad population trends in a stream affected by river outflow with dam presence (Middle Piru Creek) to one with unregulated flow and no dams present. unaffected by dam presence. We analyzed arroyo toad breeding activity and egg clutch presence at these two sites, as these measures are vital indicators of population health and persistence in Piru Creek. Additionally, because arroyo toads are habitat specialists, and require specific habitat conditions in order to persist and breed successfully, we studied factors such as habitat suitability, precipitation, and flow regime data in order to further support our analyses.

Methods:

While sorting and organizing the data sheets, our team identified three key variables and datasets believed to reveal the most information on local arroyo toad population trends over time: clutch, precipitation, and flow regime data. Additionally, we also believed it to be valuable to divide the data analysis into breeding season versus non-breeding season. Our team classified the breeding season months as February to June, while non-breeding seasons are July through January.

The original data on toad occurrences in this watershed consisted of a haphazard collection of formats, data, and information that had never been collated into a single format for analysis. We transformed the tables and data sets from the historic survey reports from their original form into a consistent table structure. With approximately 40 years of data available to our team, there were many instances where we would encounter “holes,” or missing information within datasets and reports. To fill in missing data, we contacted representatives from USGS and the United Water Conservation District, and were provided with additional reports, figures, articles, programs, as well as insight and advice.

In the flow regime analysis of over 1 million data entries taken in 15-minute intervals from a USGS monitoring outflow gauge, R-Studio was used to tabulate each day’s 96 entries into a single daily average value. This was done to supplement the survey reports’ outflow data as it contained gaps in reporting. We used this data to calculate monthly values that were inserted into missing monthly entries from the main outflow data set. Any other missing values were substituted by calculating the average value of the missing month using the average value of the season in which the missing value was found. This was done to account for temporal variability and summed the data into annual values divided into breeding against non-breeding seasons.

Our precipitation analysis was supplemented with rainfall data from Ventura County Public Works Agency in order to fill any gaps in data reporting within the main data set obtained from the survey reports. The monthly rainfall data was then taken into Excel and formatted into a structure that made each variable its own column and each data entry a new row. The monthly data was summed into a single value for the year as well as creating a sum value for the yearly breeding and non-breeding season. Using Excel’s visualization tools, graphs were created to display the analysis and associated trendlines.

In Excel, our team formatted the clutch survey data from the historic reports into our reformatted structure with each survey site having separate entries for each year. Survey sites were defined as breeding pools with shallow and exposed pools along the main creek channel and side channels with sandy terraces and sand/gravel flats. Additionally, once a survey site was established it had to be revisited during each following year's survey. Surveys reported the number of arroyo toad egg masses observed at that site on a specific date, and these comprised our primary data. In addition, NE and NA were sometimes included on the historic reports. NA indicates that the site had not been established as a survey area at the time of the survey, however would be habitat designated in later surveys. This value is only used so that while viewing in the survey report's table format, no cell was left empty. Meanwhile, NE signifies that there was a loss of suitable habitat to the extent that breeding could no longer be possible at designated survey sites established in years prior. . This could be due to climate conditions drying out pools or altering hydrology, vegetation or sediment overtaking the site, or human effects. However, a designation of NE marking a survey site as unsuitable did not mean that the site could not recover the following year or years later.

Clutch data were summed separately for Agua Blanca and Middle Piru for an overall number of recorded clutches for each survey year. We also calculated yearly habitat loss by taking the number of NE designated sites and dividing that by the total number of sites in the creek for a percentage loss. This resulted in the creation of two graphs: percent habitat loss by year and suitable versus unsuitable toad habitat.

Results:

Clutch Data

Using clutch data, trends were identified among habitat loss, habitat suitability, and clutch count over the years for both Middle Piru and Agua Blanca. Middle Piru has gone through the most change, having higher rates for percent habitat loss (Fig. 2). Agua Blanca however has maintained a steady rate in its trends in habitat loss and total clutches, experiencing a different effect from Middle Piru. The total overall habitat however is ultimately decreasing in suitability in recent years; we have gone from a total of 27 habitats that were all found suitable in 1991 to a total of 100 habitats with only 39 suitable habitats in 2020 (Fig. 3). Low numbers for total clutches (Figs. 4a - 4d) throughout the years have been surveyed at both locations, having lower rates for Middle Piru.

Figure 2. There are several upward trends in the percent habitat loss of the arroyo toad found in both Middle Piru and Agua Blanca. Middle Piru however is experiencing a much more rapid increase in percent habitat loss than Agua Blanca is; this is evident with their increasing trend lines. This means Middle Piru is facing higher percentages of habitat loss throughout the years, whereas Agua Blanca has maintained a steady increase instead, even facing lower percentages in recent years.

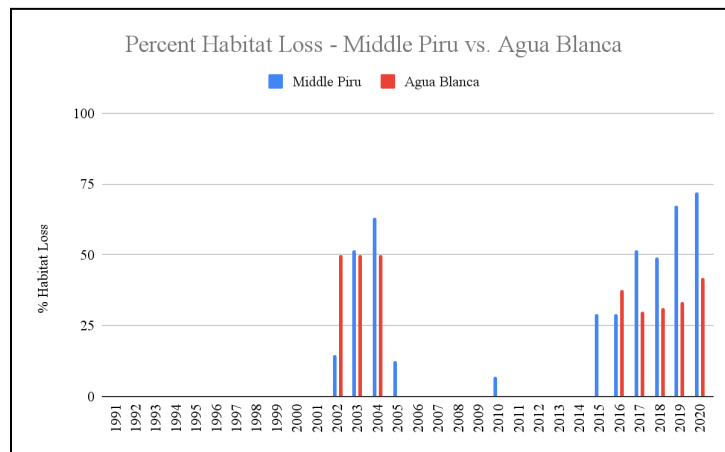
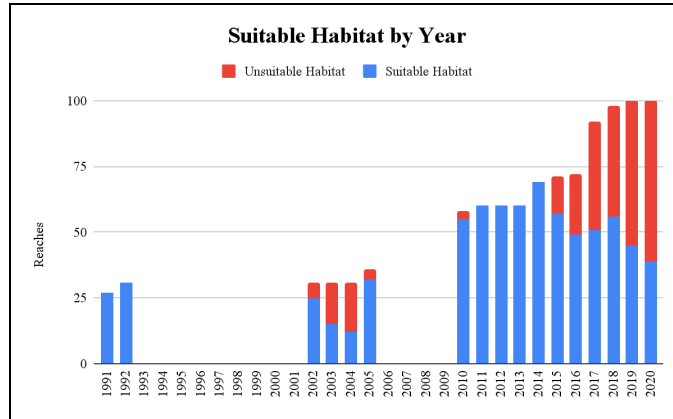


Figure 3. The total overall habitat broken down into suitability for the arroyo toad shows an increase in unsuitable habitats over the years. The number of total habitats identified, referring to total reaches found in previous survey reports, has been increasing since 1991, however from 2002 on there is a loss of suitable habitat that is also increasing in recent years.



Figures 4a-4d. Distinguishing the total number of clutches found in Middle Piru Creek and Agua Blanca shows distinct trends for total counts and zero counts.



Precipitation Data

With the total annual precipitation of the breeding and non-breeding seasons, similar trends arose, as it is decreasing overall throughout the years (Fig. 5 & 5a). Comparing these totals to habitat loss found that recent years experiencing low numbers for total precipitation had the most percent habitat loss (Fig. 6).

Figure 5. The total annual precipitation has been decreasing gradually since 1981. Despite experiencing the highest values in 1992 and 2004 with total annual precipitation of 45.78 mm and 40.84 mm, the overall rate has been decreasing with recent years experiencing the lowest rates. This is observable with the slightly negative trendline that has a slope of -0.171.

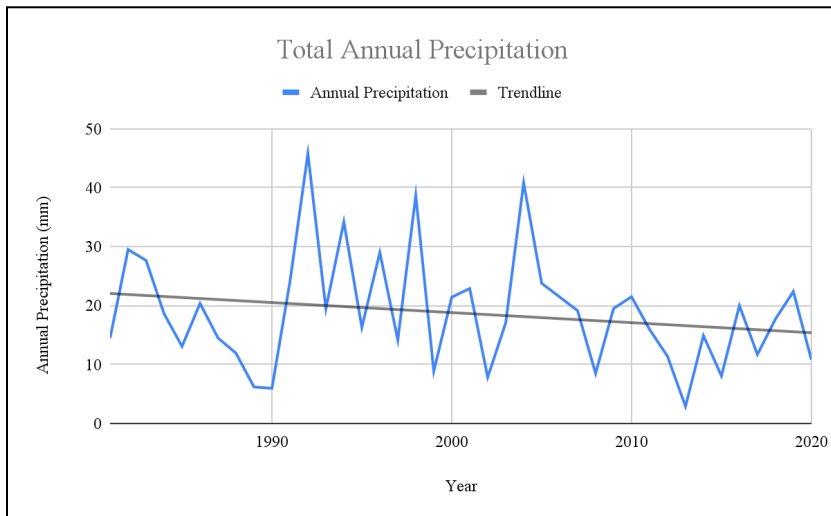


Figure 5a. Breaking down the total annual precipitation data by breeding and non-breeding season shows a similar decreasing pattern as Figure 5. We can distinguish specific impactful years for breeding seasons since 1981, revealing one good year in 1998, having the highest annual precipitation value of 34.43 mm. The most impactful year for the non-breeding seasons was 2004, having an annual precipitation value of 32.82 mm. Since its peak years, there has been a decrease in precipitation for both seasons, as evident with their corresponding negative trendlines

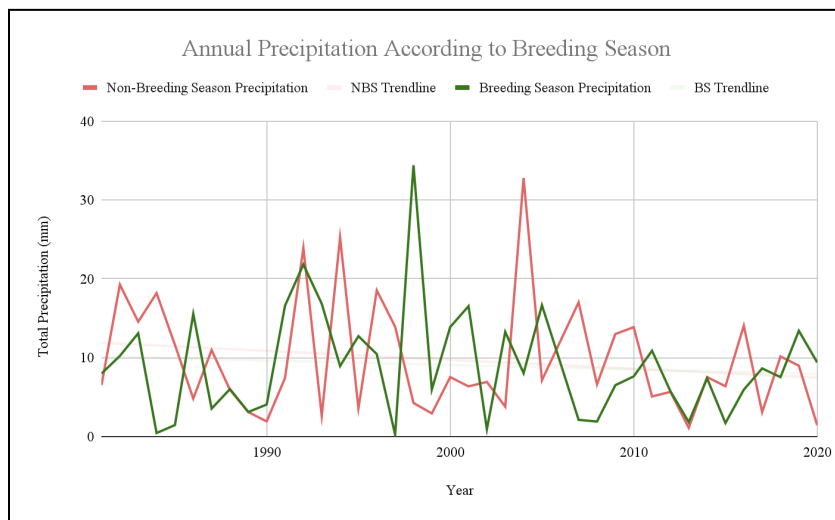
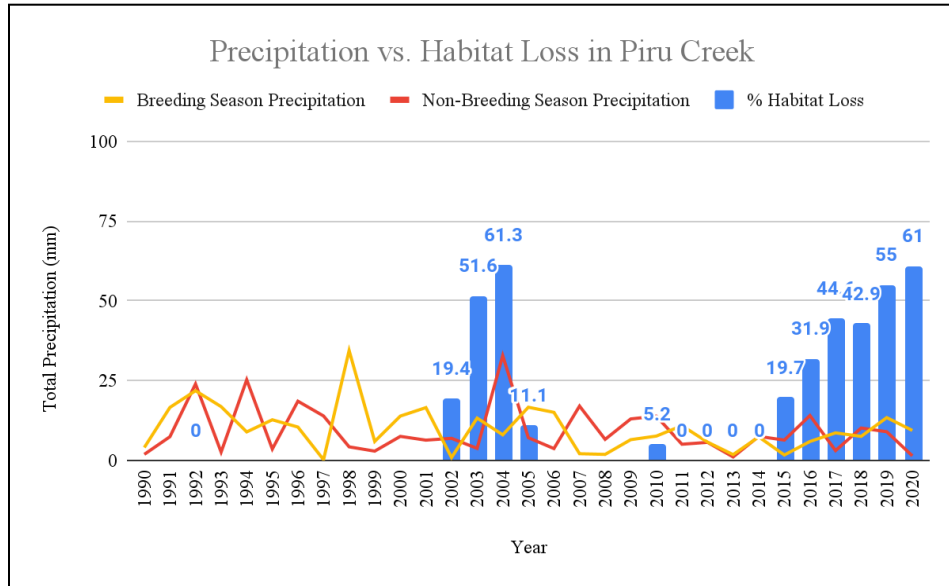


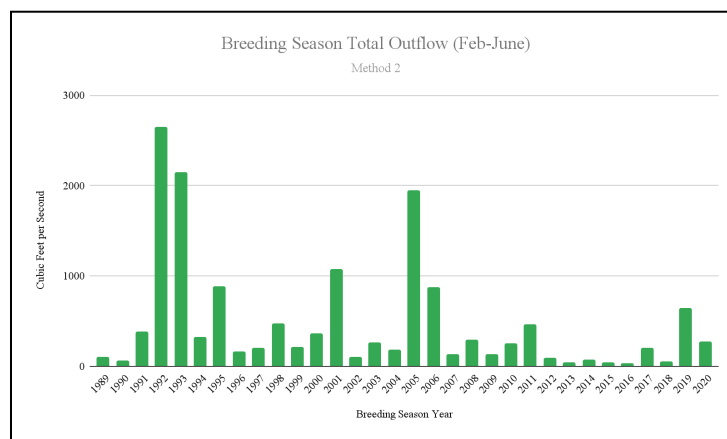
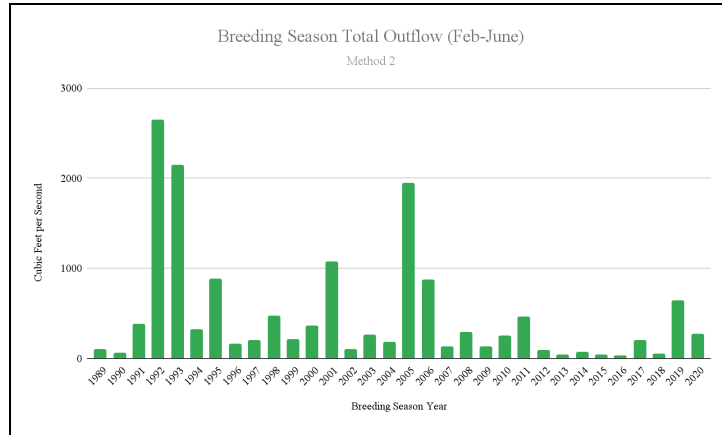
Figure 6. Comparing total precipitation for breeding and non-breeding seasons to the total percent habitat loss has found a decrease in precipitation over the years with an increase in habitat loss as well. In 2004 when the non-breeding season experienced their peak total precipitation, the highest total percent habitat loss was experienced at 61.3%.



Outflow Data

The outflow calculated for Piru found overall decreasing rates throughout the years (Figs. 7 & 8). These trends align with decreasing total clutches, habitat suitability, and precipitation totals.

Figures 7 & 8. The total outflow calculated for breeding and nonbreeding did not contrast very much and had similar trends throughout the years. Peak years were experienced in 1992 and 2005 for the breeding season and 1995 and 2005 for the non-breeding season. However, overall both seasons are experiencing a decreasing rate for the outflow present at Piru throughout the years, with recent years suffering the lowest numbers.



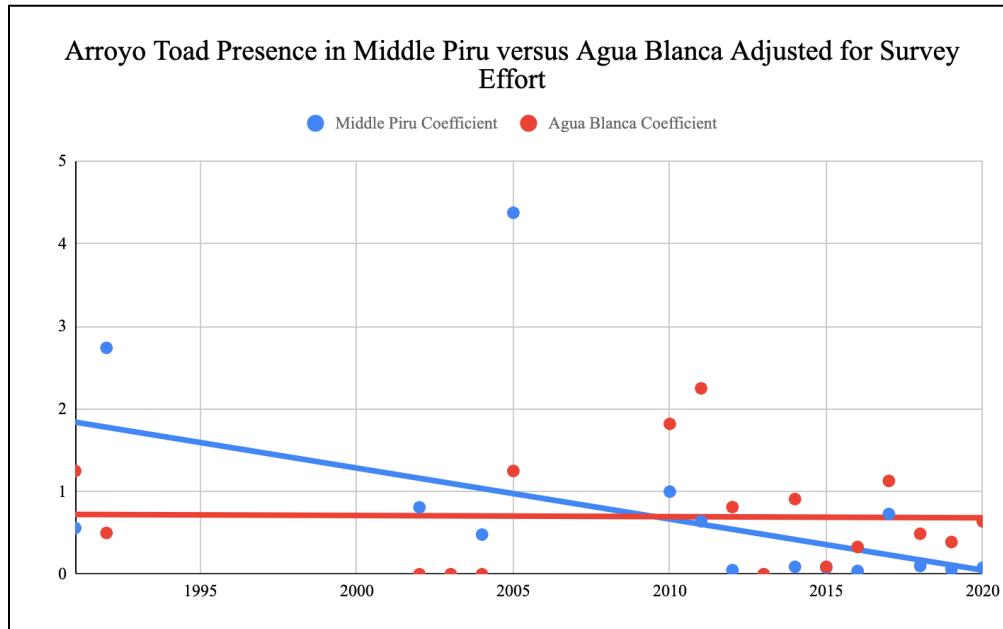
Discussion:

Through the process of sifting through the past decades of data on rainfall, outflow, arroyo toad clutch presence, habitat suitability, and even survey effort, the opportunity for a more in-depth dissection was ever present, and with that came the emergence of various trends and patterns over time. The most obvious trends our team noticed over time were an increase in survey effort, increases in percentage of habitat loss and unsuitable habitat, especially in Middle Piru since the earliest data from 1991, a general decrease in precipitation and outflow, and ultimately, an overall decrease in arroyo toad clutches in Middle Piru.

In connecting these trends to one another, there are some explanations that offer themselves more naturally. For example, the survey effort has changed drastically over time, so

tracking this evolution has been vital in contextualizing the results seen within Middle Piru Agua Blanca. To define survey effort, our team mainly looked at the number of survey locations, specifically how and when that changed over time. We also dissected the field notes for valuable context, but given inconsistencies between how data was collected, transcribed, and compiled from year to year and firm to firm, this was a largely unhelpful component of our research. At Agua Blanca, the low survey effort prior to 2010 is evident by there being only four survey locations for data collection within the entirety of Agua Blanca. Middle Piru, even in its early survey years, had nearly six times the effort that Agua Blanca ever received by researchers. It wasn't until 2010 that more survey locations within Agua Blanca were added, when the total locations surveyed quadrupled to 16. Agua Blanca saw further increases in survey effort every year from 2015-2020, with at least one new survey location being added from each year prior. Knowing that there were only four locations surveyed for arroyo toad clutches prior to 2010 contextualizes certain key data points, such as the 50% of habitat loss at Agua Blanca seen each year from 2002-2004 in Figure 2. Tracking survey effort also proved to be gravely important in assessing and discussing arroyo toad population trends. From 1991 to 2020, Middle Piru's survey effort nearly tripled, with survey locations in Middle Piru increasing from 23 to 64. This contextualizes the data regarding arroyo toad clutches found in the area, because an already dismal set of years for the Middle Piru arroyo toad population is only exacerbated when adjusted for the increased survey effort in those years, as seen in Fig. 9. With the integration of survey effort into our analyses, discussions were conducted with greater confidence, allowing that to permeate into our conclusions.

Figure 9: Arroyo Toad Clutches in Middle Piru versus Agua Blanca After Being Adjusted for Survey Effort: To factor in the inconsistent levels of survey effort across the years arroyo toad clutches were being recorded, we put the number of arroyo toad clutches found in a given year over the total number of survey locations for both Middle Piru and Agua Blanca. We then added trendlines to be able to see if there was a specific trend over time after the adjustment, and while Agua Blanca remained stable, Middle Piru saw a significant decline.



Habitat loss was another one of the most crucial factors to our analyses. Within the framework of our research, habitat loss is defined as habitat that was recorded to be suitable for arroyo toad breeding in a past year or multiple past years that does not meet the same conditions of suitability in a given year. In the event that a previously established survey location was deemed unsuitable for arroyo toads in a given year, the survey location was denoted with an “NE” as opposed to being assigned a quantitative value for toad presence. These survey locations, once established, were and still are revisited every year for gathering data on arroyo toad clutches, regardless if in a past year, or even multiple years it was deemed unsuitable, meaning that habitat that is once “lost” can become good, suitable arroyo toad habitat again. The increase of habitat loss and unsuitable habitat over time is especially noteworthy given that the

total number of survey locations since 1991 has increased by nearly a factor of four. 2020, the most recent year for which data are available, saw 61 out of 100 established survey locations deemed unsuitable habitat for arroyo toads in that year, compared to no habitat loss in the first years of which there is data on hand. While not necessarily perfectly indicative of trends in arroyo toad population, our team found habitat loss to be an interesting intersection to examine the impact of other factors, because there appears to be a connection between rainfall and thus outflow to habitat loss. For example, as seen in Figure 5, in 2002 and 2003, when rainfall across the entire year was notably low, the following years 2003 and 2004 saw overall habitat loss of over 50%, but when the non-breeding season of 2004 directly preceding the breeding season of 2005 experienced the highest aggregate non-breeding season rainfall of the past 30 years, habitat loss for 2005 decreased to 11.1%. Lastly, in assessing the data, it was important to keep in mind that Agua Blanca's outflow stems solely from the rainfall, as it is not connected to the part of Piru Creek that experiences flow from orchestrated releases.

We found it insightful to take deeper examinations at years that were significant for multiple data points, such as 2005. 2005, as mentioned before, saw the benefits of following the highest aggregate non-breeding season rainfall among all of the data our team dealt with (Fig. 5a), championed the highest outflow rate of any year since 1993, both within the breeding and non-breeding seasons (Fig. 7 & 8), saw a significantly sharp decline in habitat loss from the years preceding it (Fig. 6), and ultimately was the year 145 total arroyo toad clutches were recorded in Piru Creek, nearly twice as much as the second most bountiful year for the toads (Fig. 4a & 4b). Diving into the success of the year and taking a look at the unique outflow statistics from the year, aided with additional stream gauge data from USGS Gage 11109600, provided by Robert Fisher, outflow stood out as the key to the success of the year. Arroyo toads

need 90 days or more given the temperature of the region to successfully complete their life history from eggs to metamorphosis, so to have a year that experienced such dramatic, and most importantly, consistent outflow allowed for a population that matched the ideal conditions for a successful breeding season. Additionally, given that arroyo toads live up to six to eight years, a successful year for the population has great potential to cause rippling effects on the population for years to come. While the years directly following 2005 are missing from the overall data, 2010 and 2011, the last couple of years toads from the 2005 population burst would be expected to remain, were the last years of rather significant arroyo toad clutches recorded before the abysmal remainder of the decade.

Given all of this, the major trends our team found individually - decreases in rainfall and outflow, increase in percent habitat loss, and decrease in overall toad breeding at Middle Piru - all seemed to link up to one another quite palpably.

Piru Creek: Water Release Case Study

Every year, there is water released from Pyramid Lake running through Piru Creek into Piru Lake where the water is distributed for local use. As identified in the habitat suitability model, Piru Creek contains excellent arroyo toad habitat over much of its length. Unfortunately, it also contains many invasive species that potentially prey on arroyo toad tadpoles. We conducted an experiment to quantify the potential for using pulsed water releases to flush out invasive species in the creek. For this experiment, we performed surveys over two weekends, one before the water release and one after. We employed multiple methods to capture and release as many invasive species as possible both before and after the release.

With these data, we addressed the following questions:

1. Does the water release affect the number of invasives found within a given arroyo toad habitat?
2. Does trapping, seining, or electrofishing provide a more efficient sampling method in order to collect invasive species in Piru Creek?

Methods:

Sampling data were collected through three methods: trapping, seining, and electrofishing (e-fishing). Surveys were conducted over 11 different sections (reaches) of Piru Creek and the same survey efforts were conducted pre-release and post-release. Each reach was 250m, however only half of each reach was sampled, making the true sampling distance 125m each.

The data were analyzed using R-Studio. We conducted two analyses using general linear mixed models (GLMMs). The first analysis considered the number of invasive individuals caught before and after the water release. The model for this first analysis used the count of invasive individuals as the response and included the sampling period (pre- vs. post release) as a fixed effect. The reach represented the random effect. We used a negative binomial error structure to account for overdispersion of the count data. We ran this analysis again separately for Bullfrog tadpoles and Red Swamp Crayfish because they represented the majority of the dataset.

The second analysis considered the number of invasive individuals captured by each method (trapping, seining, and e-fishing). Here, we only used data from the 6 of the 11 reaches that were sampled by all three methods both pre- and post release. We again ran GLMMs with the count of invasive individuals as the response and a negative binomial error structure. The model included sampling method as a fixed effect. We also ran a GLMM including both sample time and sample method as interaction effects to test for interactions between the two factors. .

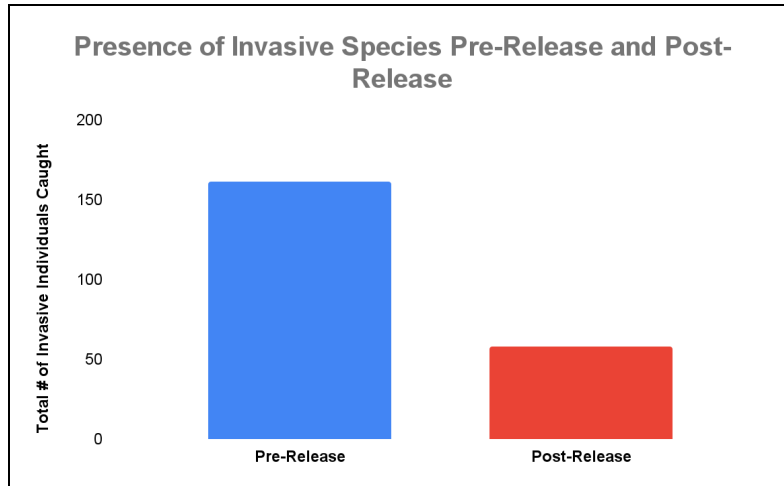
Results:

In total, we caught 2019 invasive species individuals across both pre and post water release sampling periods. This total was distributed among 8 different invasive species recorded at Piru Creek (Table 4). The number of invasive individuals caught was dependent on whether the sampling was done before or after the water release ($X^2_1 = 50.403, p = 1.252e-12$). There were significantly more invasive catches for Pre-Release sampling than Post-Release, with the Pre-Release catches being about 74% of the grand total individuals caught (Fig. 10).

Table 4. Total Number of Invasive Individuals Caught Broken Down by Species: amongst 8 invasive species recorded along Piru Creek, there were a grand total of 219 invasive individuals caught during the Pre-Release and Post-Release.

Invasive Species	Pre-Release	Post-release	Total
Bullfrog	66	18	84
Red Swamp Crayfish	31	17	48
Largemouth Bass	14	15	29
Prickly Sculpin	13	3	16
Catfish	14	4	18
Carp	16	1	17
Sunfish	4	0	4
Mosquitofish	3	0	3
Grand Total	161	58	219

Figure 10. Presence of Invasive Species Pre-Release and Post-Release: Out of 219 individual invasive species caught, there were 161 individuals caught during the Pre-Release sampling which accounts for 74% of the grand total. Only 58 individuals were caught during the Post-Release sampling, which accounts for 26% of the grand total invasive individuals.

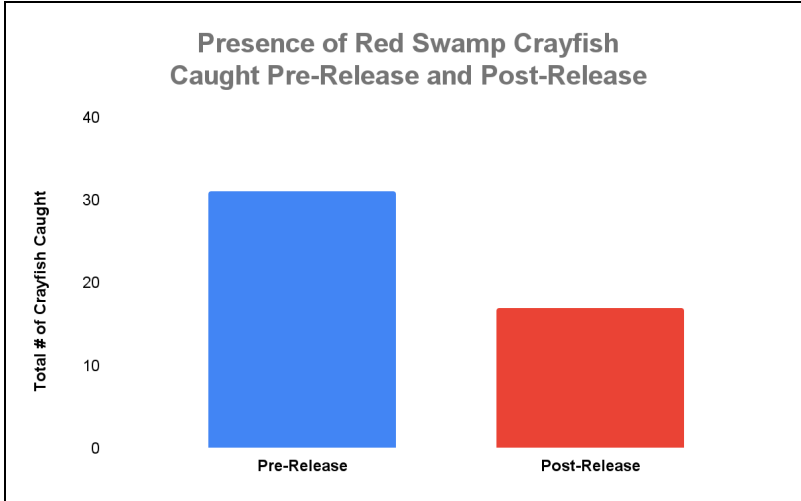


■ Pre-Release Total Number of Individuals Caught: 161

■ Post-Release Total Number of Individuals Caught: 58

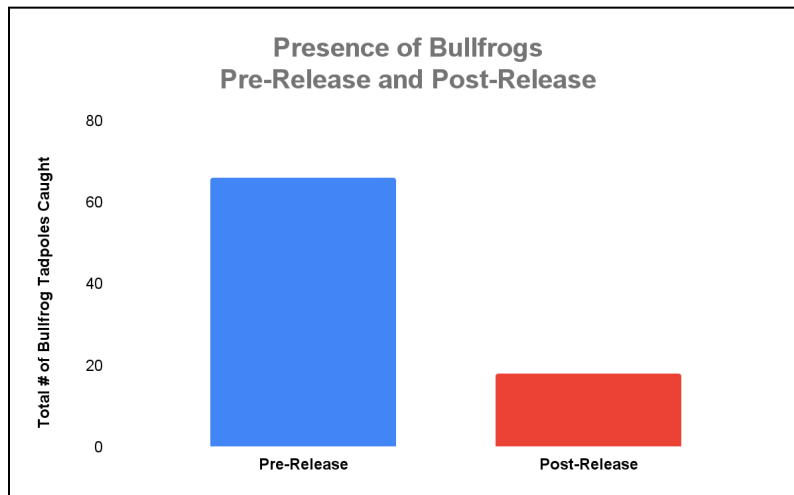
We reran the model only looking at Red Swamp Crayfish ($X^2_1 = 4.079$, $p = 0.04342$) and Bullfrog tadpoles ($X^2_1 = 29.145$, $p = 6.714e-08$). The water pulse was successful in specifically flushing out Red Swamp Crayfish and Bullfrog tadpoles. The water pulse did not have an effect on the presence of Largemouth Bass ($X^2_1 = 1.3952$, $p = 0.2375$) so was not successful in removing them (Fig. 11)

Figure 11. Red Swamp Crayfish, Bullfrog Tadpole, and Largemouth Bass Catches: Bullfrog tadpoles, Red Swamp Crayfish, and Largemouth Bass were the most caught invasive species Pre-Release and Post-Release in Piru Creek.



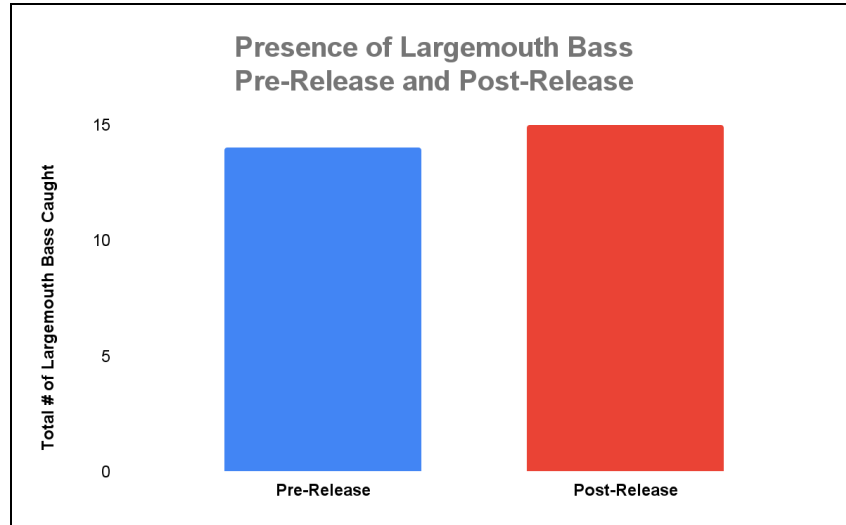
■ Pre-Release Total Number of Crayfish Caught: 31

■ Post-Release Total Number of Individuals Caught: 17



■ Pre-Release Total Number of Bullfrog tadpoles Caught: 66

■ Post-Release Total Number of Bullfrog tadpoles Caught: 18

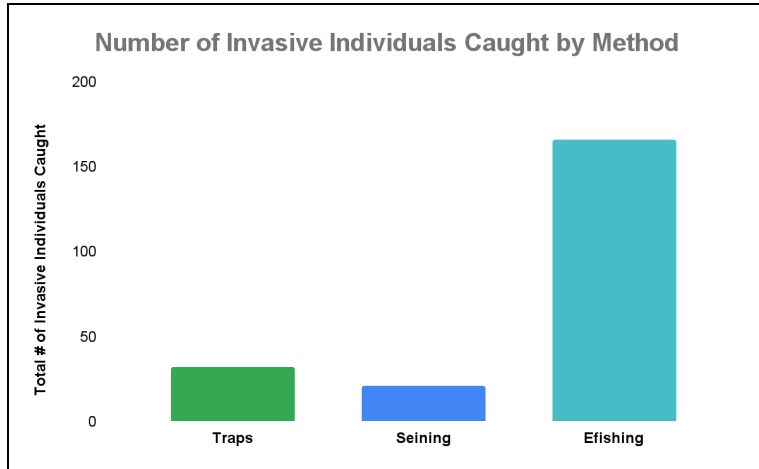


■ Pre-Release Total Number of Largemouth Bass Caught: 14

■ Post-Release Total Number of Largemouth Bass Caught: 15

The number of invasive individuals caught was dependent on the type of sampling method used with e-fishing catching a lot more individuals than trapping or seining ($X^2_2 = 28.843$, $p = 5.454e-07$). It didn't matter whether trapping or seining was used but it did matter that e-fishing was used (Fig. 12). This means we can reject the null hypothesis of there being no relationship between the type of method used. Although both the main effects of water release and method were significant, we did not detect an interaction between the two factors ($X^2_2 = 0.9586$, $p=0.6192$).

Figure 12. Number of Invasive Individuals Caught by Method: this graph shows the distribution of the 219 invasive individuals caught by sampling method. Electrofishing accounts for about 76% of all catches.



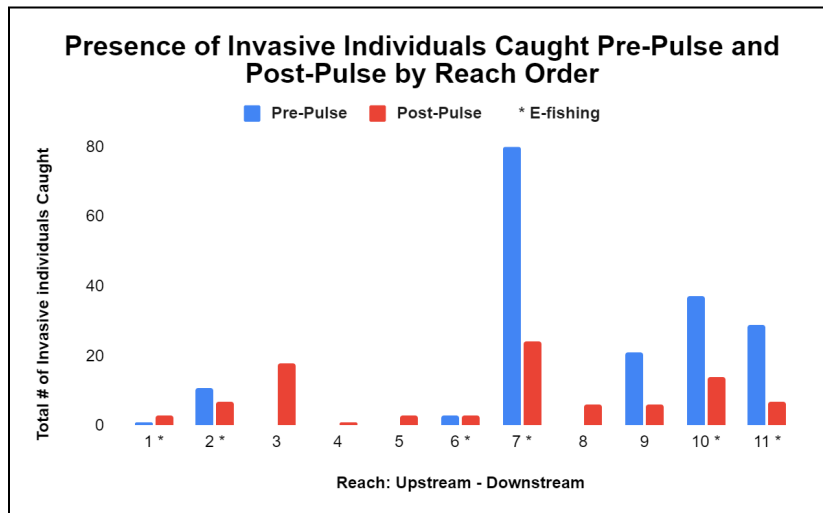
■ Total # of Individuals Trapped: 32

■ Total # of Individuals Seined: 21

■ Total # of Individuals e-fished: 166

Figure 13. Presence of Invasive Individuals Caught Pre-Release and Post-Release by Reach

Order: Each reach was ordered 1-11 (upstream-downstream) by their given GPS numbers: G8785 to G8775. Most of the individual catches were downstream of our sample reach areas in Piru Creek for both pre and post water release. It is important to note that 6 out of 11 reaches, as signified by the asterisk symbol (*) to indicate that these specific reaches were e-fished during both the pre-release and post-release phase.



Discussion:

These results have interesting implications for invasive species in Piru Creek. Our analysis has shown that the water release has an effect on the total number of invasive individuals captured. This conclusion can be used to support the conservation strategy of using high-volume pulsed water releases from Pyramid Lake into Piru Lake to flush out invasive species that may prey on the arroyo toad. Flushing them out before the breeding season could significantly benefit the arroyo toad by removing invasive predators. If the release had the potential to remove bullfrog tadpoles then it likely has the ability to remove arroyo tadpoles so the release should be done well before breeding season.

Additionally, these results show that electrofishing is the most effective method of capture for invasive species in Piru Creek. If this study were to be repeated or if there was to be a removal effort of invasive species from the creek, then electrofishing would be the best way to capture the highest number of species. However, electrofishing is also more time consuming and requires expensive equipment and trained personnel.

A potential shortcoming of the data is that the teams conducting the pre-release survey were not the exact same teams that conducted the post-release survey so efforts could be varied. Another shortcoming is that there were no GPS locations of each trap set pre-release so the post-release traps were not placed in the exact same location but were placed in the same reach. This could be addressed in future work by recording the GPS locations of the traps to ensure they are in the same location post-release as pre-release. Additionally, better planning and smaller survey groups could ensure the groups remain the same over the two weekends.

Conclusions

Our study included original data collection and analysis focused on three aspects of the current status of the arroyo toad in California. The construction of the habitat suitability model took into account many factors such as: precipitation, vegetation, percent sand, waterway velocity, and overall presence of the toad in their habitat across Southern California. This overall range-wide assessment of the habitat demonstrated that most of these territories are not satisfactory for the arroyo toads. It can be deduced that most arroyo toad habitats don't satisfy their environmental preferences because 296.61 km out of 2488.68 km of arroyo toad inhabited land was found to be suitable; thus, the toads must be facing various obstacles in their survival. In regard to the data obtained from Piru Creek, the population trend analysis reported the decrease in conditions for successful breeding over the years due to the decline in arroyo toad clutch presence. Middle Piru and Agua Blanca both faced a decline in egg clutches; however, the regulated waterway of Middle Piru reported a lower presence of egg clutches. Finally, the water release experiment showed a negative effect on the number of invasive individuals caught for this arroyo toad habitat since there were less caught after release. During this case study, it was also shown that electrofishing is the most effective method in catching species within Piru. These 2 studies were validated by our statistical analyses providing a stronger case for the release of water in regulated creeks as a method in protecting the toads from invasives. Overall, the arroyo toad faces difficulties surviving in habitats that are not suitable for their needs in addition to the decline of breeding success every year.

In response to our data analysis, we propose the following as a means to improve conservation efforts for the arroyo toad. Considering the decline of egg clutches in Piru and Agua Blanca region, we recommend implementing the use of water releases in arroyo toad

habitats in order to reduce the threat of invasive species. By scheduling the water release before the arroyo toad breeding season, many invasives can be flushed out, as indicated by the 74% total invasive caught during the Pre-Release phase. Additionally, we recommend that electrofishing become a method for other experiments of the same design or in the case that extermination of invasives is an option because it was the most productive sampling method. Although the habitat suitability model showed a lack of high-quality habitat currently available for the toads within their range, Camp Pendleton showed the most promising high-quality habitat in one location. Our team plans to conduct surveys for model validation in accurately predicting the presence of the arroyo toad. Looking ahead, our team will be providing our analysis and data to USFWS in order in hopes that our findings are able to assist them in the new 5 year review of the California Arroyo Toad.

Appendix

A. Habitat Suitability Model (HSM)

The materials used to develop the HSM and analyze the models' outputs are stored within a shared drive (https://drive.google.com/drive/folders/1SiSzop-PPI0KJy1ACdo_JpIwTVGeX2gC).

In this drive, HSM materials and deliverables have been separated based on inputs and products.

A1. Model Inputs

This folder is made up of 3 separate sub-folders: R Code, Presence Datasets, and Environmental Raster Layers. The "R Code" sub-folder contains code that was created in R-Studio version 1.4.1106 and used to filter datasets and transform the data into a format suitable for MaxEnt. The "Presence Datasets" sub-folder includes each source of presence datasets that were incorporated in our model, and a metadata document that details how the data was filtered. Lastly, the "Environmental Raster Layers" sub-folder includes ascii files that were used to describe environmental variables important to arroyo habitat prediction (hydrology, landscape, and climatic) and thus were included in our final MaxEnt model.

A2. Model Data Products

This folder is made up of 3 separate sub-folders: Thresholded Stream Analysis, Model GeoTiffs, and MaxEnt Ascii Output. The "Thresholded Stream Analysis" includes xml sheets of the analysis that we performed on the thresholded streams. These were used to determine total stream length and also used in the critical habitat analysis. Furthermore, the resulting land management percentages are described in these sheets. The "Model GeoTiffs" includes raster files of the total and breeding-only maps (saved as tif files) for both class systems (2-class and 3-class thresholded rasters). Furthermore, a "Data Analysis For Thresholding" sub-folder is included here, which includes xml sheets that detail how the thinned presence data (via MaxEnt)

was further thinned (removal of lowest 10% of suitability scores) and the calculations used to determine class ranges (low, mid, and high). Lastly, the “MaxEnt Ascii Output” includes a ascii file with the non-thresholded maxent output and thinned presence file (thinned by MaxEnt) for both the total and breeding-only occupancy datasets.

B. Piru Creek: Water Release Case Study

Below is the entirety of the data collected and used in the analysis.

<https://docs.google.com/spreadsheets/d/1tqKnfAsC8CDPP3cEhMAOiMNAJIDhBWd9eG1SXJ44IOo/edit?usp=sharing>

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