California/Nevada Amphibian Populations Task Force 2017 Meeting

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ABSTRACTS

Arroyo Toad (*Anaxyrus californicus*)

ORAL PRESENTATIONS

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Historical Rise in Fungal Pathogen Prevalence Coincides with Rapid Southern California Extirpation of *Rana boylii*

Invasive species—especially pathogens—are an increasingly significant cause of biodiversity loss. As extinctions continue across the globe, repatriation programs are also increasing; however, a fundamental prerequisite to repatriation is determining the causes of declines. We hypothesized that *Batrachochytrium dendrobatidis* (Bd), the causative agent of the deadly amphibian disease chytridiomycosis, was an important factor in the rapid extirpation of *Rana boylii* from southern California, which is purported to have occurred over a period of less than 10 years. We used a combination of interviews with herpetological experts, field notes, and museum specimens to determine 1) historical relative abundance of *R. boylii*; 2) potential causes of *R. boylii* declines; and 3) historical prevalence of Bd. Increase in Bd prevalence coincided with *R. boylii* declines and a time of rapid change in post-World War II southern California, wherein fish stocking, backcountry recreation, development, and the amphibian pet trade were all on the rise. Extreme flooding in southern California during the winter of 1968-69 marked localized extirpations in *R. boylii* populations observed by respondents. We conclude that Bd likely played a role in the rapid extirpation of *R. boylii* from southern California, and that multiple natural and anthropogenic factors may have worked in concert to make this possible in a relatively short period of time.

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Attempting to Recover Endangered Mountain Yellow-legged Frogs in a *Bd*-Naïve Landscape – Everything but the Kitchen Sink?

Both species of endangered mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) are vulnerable to extirpation in Sequoia and Kings Canyon National Parks (SEKI). Primarily due to impacts from nonnative trout and the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), populations have disappeared from most historic sites, and nearly all remaining populations are small, isolated, struggling to adapt to *Bd*, and often restricted to small ponds. The large scale of declines and the frogs' currently limited adaptation to disease have necessitated a recovery approach involving numerous complementary actions. A newly completed long-term restoration plan aims to help recover dozens of extant and recently extirpated frog populations via habitat restoration, captive-rearing, immunization, reintroduction, translocation, antifungal treatment, predator relocation, and monitoring. In 2015-2016, staff from SEKI, UCSB, Oakland and San Francisco Zoos, USGS, and USFWS collaborated to conduct many of these actions. Results thus far are encouraging and will hopefully contribute to frog recovery at a landscape scale.

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Statewide Assessment of Road Related Risks to Reptile and Amphibian Species

Our primary goal was to provide Caltrans and other transportation planning agencies guidance to prioritize mitigation efforts for amphibian and reptile species in California. This involved assessment of over 160 species of frogs, toads, salamanders, snakes, lizards, and chelonids (turtles, tortoise). Subspecies were included if they had special federal or state protection status. Rankings such as these can be easily biased according to the availability of species road-related literature or toward iconic and favored study species. We attempted to assess risk across all species in an objective manner informed by current road ecology literature. Therefore, scoring was based upon a suite of life history and space-use characteristics associated with negative road effects. For amphibians, 55% of toad species, 45% of frog species and 17% of salamanders were ranked as high or very high risk from negative road impacts within their terrestrial and/or aquatic habitats. This assessment helped to confirm the presumed risk for many species but also highlighted the need to focus on previously overlooked species. In conjunction with this, we are in the process of identifying specific roads of concern and existing underpasses or culverts for field studies of high risk species. We invite the APTF attendees to collaborate and provide any information from past or current studies to inform this effort, as well as to comment on and identify potential study areas and species. This information will be used to inform future road planning and mitigation efforts such as need for wildlife crossings and barrier structures.

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Using Pond Hydroperiod to Attenuate the Spread of Invasive Tiger Salamander Alleles

Progressive globalization is leading to rapid rates of hybridization throughout the world. Hybridization can lead to loss of local genetic diversity and negatively impact species biodiversity in affected regions. It is therefore necessary to evaluate mechanisms that slow, stop, or even reverse hybrid introgression. The California tiger salamander (*Ambystoma californiense*) is a threatened species that is experiencing rapid introgression from non-native alleles in the center of its range. Efforts to control this spreading hybridization are largely ineffective, necessitating research in novel methods of management. Previous mesocosm experiments coupled with field observations suggest that artificially longer hydroperiods select for hybrid genotypes, though the application of this is purely speculative. We propose an experiment that will evaluate the effectiveness of manipulating pond hydroperiod as a means to attenuate the spread of invasive genes. We will investigate shifts in the non-native allele frequencies using modern genomic tools. Additionally, we will collect demographic data to be used in conjunction with an integral projection model to determine the effects these altered ponds will have on the life history of natives, hybrids, and the interaction between them. The end result of this project will be a generalizable model that will give the effective change in non-native allele frequencies in any part of the range, given an altered hydroperiod. This information should help management decide if and where this strategy is viable to prevent further erosion of native genotypes.

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Restoring the Western Pond Turtle (*Emys marmorata*) to Yosemite Valley

Western Pond Turtles (*Emys marmorata*) occurred in Yosemite Valley until the 1950's. Anthropogenic influences such as increased predators (native and invasive), the removal of woody debris from the river channel, and the destruction of wetlands, likely resulted in the extirpation of the turtle from the area. Now that these factors have been alleviated, we have begun restoring Western Pond Turtles to their former range in Yosemite National Park. We translocated 10 male turtles from nearby populations to Yosemite Valley during the summer of 2016. We monitored translocated turtles using radiotelemetry. Herein, we present preliminary results on survivorship, health, and movement patterns immediately after release, throughout the summer, and during the fall. Given that many habitats could support Western Pond Turtle populations in the future and introductions to bolster population numbers are occurring, we hope that our results are relevant and valuable to other managers working to establish turtle populations.

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Long-term Success of Experimental Assisted Migration of the Western Spadefoot (*Spea hammondii*) in Southern California During Drought

The western spadefoot (*Spea hammondii*) is currently a species of special concern within the state of California, and will be assessed for listing under the Endangered Species Act by the US Fish and Wildlife Service in 2018. It is closely tied to vernal pools, tinajas, and ephemeral creeks/arroyos for breeding. Climate change predictions indicate that environmental suitability for this species will change over the next few decades; independent of the presence of future breeding sites. Throughout southern California much of this breeding habitat has been destroyed through land use changes. Recent research shows that this species is more sessile as adults than previously thought, which could impact migration to novel breeding habitats. Experimental construction of aquatic habitats took place in 2005 in Orange County at sites where no historic breeding pools occurred although simple habitat suitability models showed high potential. The movement of spadefoots to these pools took place in 2005 and 2006 from a site approximately 5 miles away. During 2016 we revisited the long-term success of this experiment and compared these results to current year success at other spadefoot sites that were within their natural range. Surprisingly the novel habitats had exceptional success during this drought year, with the natural pools almost entirely failing for recruitment. More aggressive assisted migration and habitat creation for this species within remaining reserves may be necessary to build climate resiliency for this species.

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Amphibian Update for California Red-legged Frog, Yosemite Toad, and Sierra Nevada Yellow-legged Frog in Yosemite National Park

The federally threatened California red-legged frog (*Rana daytonii*) was once found in Yosemite National Park and believed to be extirpated by the end of the 1970s. In 2016, after over 30 years of its disappearance, reintroductions have begun with the release of *R. draytonii* tadpoles into Yosemite Valley following the eradication of the invasive American bullfrog (*Lithobates catesbeianus*). In summer 2017, the park plans to introduce additional captive reared adult-sized *R. draytonii* throughout Yosemite Valley and initiate *L. catesbeianus* removal in another infested area of the park. We will discuss the long-term strategy for reintroducing *R. draytonii* as well as efforts to reduce and eradiate *L. catesbeianus*. Prior to summer 2016, the federally threatened Yosemite toad (*Anaxyrus canorus*) was previously thought to have experienced irreversible impacts from the five-year drought leaving long-term population viability in Yosemite uncertain. We will present new data from 2016 with evidence to support the resilience of *A. canorus* to prolonged drought. The federally endangered Sierra Nevada yellow-legged frog (*Rana sierrae*) has seen a boost in some populations in Yosemite National Park in recent years while other populations have felt the effects of the ongoing drought, specifically desiccation and total cohort mortality. We will summarize highlights from recent restoration actions including salvage efforts to remove desiccating tadpoles from one meadow site and attempt to establish two new *R. sierrae* populations from this opportunity. We will present upcoming management actions for *R. sierrae* as well as outline future planned restoration efforts in the park.

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Adopting Molecular Techniques for Yellow-legged Frog (*Rana muscosa* and *R. sierrae*) Restoration and Management

Analysis of aquatic environmental DNA (eDNA) obtained from filtered water samples is a promising tool for monitoring invasive species, but the application of this method is hindered by the inability to distinguish whether sources are alive or dead. We analyzed how the detection of eDNA from dead and live model organisms (goldfish; *Carassius auratus*) differs depending on collection method and applied the resulting method in the field. We sampled microcosms containing dead or live fish using different filter membrane types and pore sizes and at varying depths. We detected DNA from dead individuals less frequently and in lower quantities compared with live individuals. DNA from dead individuals was found only at the bottom of the water column. As pore size increased, the quantity of DNA captured decreased for both treatments. Because dead individuals were associated with less DNA, using filters with larger pore sizes decreased detection of dead individuals. We applied our findings to completed (n=21) and active (n=9) restoration sites across the Sierra Nevada where non-native fish are being removed to create habitat for two yellow-legged frog species (Southern Mountain Yellow-legged Frog, *Rana muscosa*, and the Sierra Nevada Yellow-legged Frog, *R. sierrae*). Our field sampling accurately indicated the status of each site, with the exception of one low-level false positive and one false negative at a low-density site. Our results highlight that collection methods for eDNA can be tailored to maximize the utility of eDNA techniques in aquatic habitat conservation. Yosemite National Park has incorporated these eDNA techniques in their aquatic wildlife program, including yellow-legged frog restoration, bullfrog eradication, and red-legged frog introduction.

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Against the Odds: Recovering Endangered Mountain Yellow-legged Frogs in the Sierra Nevada

The once abundant mountain yellow-legged frog (*Rana sierrae* and *Rana muscosa*) has declined precipitously during the past century. Major drivers of this decline include introduced trout, and the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Bd). Reversing the effects of these stressors will require disparate methods, some aimed at removing the stressor or, when the stressor cannot be removed (Bd), methods that increase the probability of frog persistence in the presence of the stressor. During the past 15 years, implementing these actions as carefully designed field experiments has been essential for understanding their efficacy and limitations. Trout removals are highly effective at recovering frog populations, although recovery in the presence of Bd can be slow. Physical removal methods were originally considered effective only in relatively small lakes, but recent efforts indicate their much broader utility. The challenges posed by Bd are complex due both to an inability to remove Bd from ecosystems and to difficulties in affecting either epizootic or enzootic disease dynamics. Recent attempts to prevent population extirpation following the initial arrival of Bd in a population (epizootic dynamics) produced promising results, and will be important in preventing the extirpation of remaining Bd-naïve frog populations. Frog translocations and reintroductions can allow the establishment of populations despite the ongoing presence of Bd (enzootic dynamics), but establishment can be a lengthy and unpredictable process. Collectively, these methods, if widely implemented, would allow considerable progress to be made in reversing the decline of this iconic amphibian.

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Field Anti-fungal Treatments Conducted During Bd-caused Mass Mortality Events Dramatically Increase Frog Survival and the Likelihood of Population Persistence

Mountain yellow-legged frogs (*Rana sierrae* and *Rana muscosa*) have declined precipitously during the past century. A major driver of these declines is the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Bd), which causes the disease chytridiomycosis. The spread of this pathogen across the frogs' range since the 1970s has resulted in mass die-offs and extirpations of many *R. sierrae* and *R. muscosa* populations. As such, a method to mitigate the negative effects of Bd is critically needed. In 2015, we conducted a field experiment in Kings Canyon National Park in which we treated hundreds of *R. sierrae* adults with an antifungal drug during a Bd-caused die-off event. Samples collected immediately before and after treatment indicated that Bd loads were reduced by 2-3 orders of magnitude. During multiple visits to the treatment sites in 2016, we recaptured as many frogs as possible and collected samples to quantify Bd loads. Results indicated that treatment dramatically increased frog survival: 50% of treated frogs were recaptured compared with <1% of untreated control frogs. In addition, Bd loads on treated frogs remained low, well below levels associated with frog mortality from chytridiomycosis. These results are consistent with treated frogs having developed an immune response that is protective against chytridiomycosis. Long term monitoring will be necessary to determine whether the study population is persisting despite the ongoing presence of Bd. If so, this would represent a major advance in efforts to mitigate the devastating consequences of Bd to mountain yellow-legged frogs and other susceptible amphibian species worldwide.

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Genetic Structure of the California Giant Salamander (*Dicamptidon ensatus*) Based on mtDNA and Nuclear Loci

The California Giant Salamander (*Dicamptodon ensatus*) is a large forest dwelling salamander found in the California Coast Ranges from Mendocino County to north of Monterey Bay. We generate a molecular phylogeny for the species based on mtDNA and an anonymous nuclear marker to identify three putative distinct population segments based on mtDNA. Two of these populations occur North of San Francisco Bay and form a unique pattern to this species. We then verify this pattern with the nuclear marker and attempt to determine if distinct population segments appear to be independent evolutionary distinct units. We also identify potential historical biogeographical causes for the genetic patterns found in this species based on past differences in the California geography such as seaways, assembly of the Coast Ranges, and drainage patterns. A comparison to other species that occupy the coast ranges is made in order to examine common patterns among herpetofauna as well as the uniqueness of biogeographical patterns in *D. ensatus*.

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An Update on the Center for Biological Diversity's Work to Protect Amphibians in California and Nevada.

The Center for Biological Diversity works to secure a future for all species, great and small, especially those hovering on the brink of extinction. The Center has a dedicated campaign focused on the protection of imperiled amphibians and reptiles and works to obtain federal and state safeguards and protected habitat for herps in California and Nevada and across the country. The Center also works to insure compliance with the Endangered Species Act for species that are already listed under the ESA and uses advocacy at the local, state, and federal levels in its campaign to address the amphibian and reptile extinction crisis. In this presentation, Jenny discusses the Center's work to protect frogs and salamanders in California and Nevada. These efforts include petitioning to restrict live bullfrog imports into California, using CEQA to challenge projects threatening rare amphibians, litigating to insure public agencies are fulfilling their duties under the ESA, and filing petitions to list imperiled amphibians under the California Endangered Species Act.

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Long-term Occupancy Monitoring of the Endangered Arroyo Toad: Findings, Feedback Loop to Management, and Implications of Climate Change

Since 2003, Marine Corps Base Camp Pendleton (MCBCP) has supported an integrated endangered arroyo toad (*Anaxyrus californicus*) monitoring strategy across 87 km of habitat in three watersheds on Base. Across MCBCP, arroyo toads have recently experienced five years of decline in all watersheds. There has been no breeding since 2011 in two ephemeral watersheds due to lack of surface water availability tied to extremely low rain years. Continued drought is a major concern for persistence of the arroyo toad in these and other ephemeral watersheds. The youngest toads in these habitats will be six years of age in 2017 while the estimated lifespan of the arroyo toad is only six to eight years. In an effort to sustain populations of toads within ephemeral watersheds range-wide during this extended drought, we suggest a collaborative action for species-wide rescue efforts through development of a head-start model program *in-situ* in 2017. Within the perennial watershed on MCBCP, drought conditions along with the associated lack of substrate scouring events, water-use needs, beaver population expansion, and non-native aquatic species all impact the toads. Over the past 15 years, occupancy models have shown a consistent negative association between the presence of arroyo toad larvae and non-native aquatic species, including bullfrogs, predatory fish, and crayfish. Long term persistence of the toads in perennial watersheds will likely require continued non-native species removal as well as adaptive water and habitat management. As part of a monitoring and management feedback loop, MCBCP has supported annual non-native removal efforts that have greatly helped to reduce this threat and are currently working on adaptive water management plans to address the needs of toads during breeding season.

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California Red-legged Frog Use of Anthropogenic and Natural Refugia During Late Summer Dry Conditions

Pre-construction surveys were conducted in eastern Alameda County, California immediately prior to structural bridge repairs in September 2016. Very little standing water was present near the project site. Nine large adult California red-legged frogs, one adult foothill yellow-legged frog, one large adult western toad, and several juvenile California newts and Sierran tree frogs were located within a damp crevice beneath a bridge abutment. The crevice measured about 1.0 by 5.0 meters, and was about 30 cm high at the highest point. High flows had scoured the crevice, threatening structural damage to the bridge and triggering the repairs. All amphibians found in the crevice were translocated to a small remnant pool a short distance upstream and monitored for the duration of the project. At least three of the California red-legged frogs remained in the immediate vicinity of the upstream pool and entered natural scour holes within

undercut stream banks. Although much smaller and within native substrate, these holes were otherwise similar to the undercut crevice where the frogs were originally found and they retained moisture. Such scour holes are commonly located near bridges or where bedrock outcrops constrain stream flow, and they may be used as amphibian refugia at inland sites where late summer conditions can become xeric. Careful flashlight searches of similar scour-induced refugia can be a productive dry season amphibian survey technique.

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Spatial Genetic Structure and Environmental Niche Modeling Identify Two Conservation Units for the Western Spadefoot (*Spea hammondii*)

The western spadefoot (*Spea hammondii*) is a Species of Special Concern in California and is now under review by the U.S. Fish and Wildlife Service for listing under the Endangered Species Act. We explored the potential for conservation units within *S. hammondii* by analyzing spatial genetic structure using five nuclear loci and one mitochondrial locus. With both nuclear and mitochondrial loci, we found that *S. hammondii* consists of two genetically distinct, allopatric clusters divided by the Transverse Ranges. The mitochondrial DNA analysis further suggests Santa Barbara County is host to its own genetic sub-cluster. To corroborate the northern and southern genetic clusters as conservation units from an ecological perspective, we additionally applied a niche identity test to environmental niche models of the two clusters. We found that the niches of the northern and southern clusters are statistically significantly different, suggesting the clusters may be ecologically non-exchangeable. With the demonstration of significant genetic and ecological differentiation between allopatric clusters of *S. hammondii*, we recommend each cluster be considered as its own conservation unit with potentially unique management needs.

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California Department of Fish and Wildlife: Drought Response Actions and Updates

California has experienced an exceptional drought throughout most of the state, and as a result, the California Department of Fish and Wildlife was tasked with rapidly assessing and responding to drought-related impacts to sensitive species. Several amphibians and aquatic reptiles were considered high priorities, and drought funding was used to improve habitat, assess population status, and undertake emergency actions. This presentation will focus on drought-related activities performed by the Department and our contractors to support conservation of imperiled amphibians and reptiles.

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Application of New Genomic Sequencing Methods to Assess Effects of River Regulation on Population Structure and Diversity in a Hydrologically Sensitive Species (*Rana boylii*)

As freshwater systems and biota are rapidly declining globally, conservation efforts will require assessment of the adaptive capacity of populations to rapid environmental change. Small populations with limited genetic diversity may have reduced adaptive potential and difficulty responding to future environmental change. Identifying these populations is crucial for effective conservation prioritization and management. One tool for addressing ecological genomics questions at scales that were previously impossible is massive parallel sequencing (MPS) and restriction site-associated DNA sequencing (RADSeq). A new method adapts RADSeq to target desired loci and recovers more unique RAD fragments. Regulated (hydropower) rivers have experienced rapid environmental change as part of substantial alterations to flow regime hydrology (i.e., changes in water temperature, flow timing, magnitude), and offer an opportunity to compare a gradient of hydroecologic and genetic responses to rapid environmental change with the few unregulated, undammed systems remaining in California. The imperiled foothill yellow-legged frog (*Rana boylii*) is uniquely linked to hydrologic change, because they link aquatic and terrestrial ecosystems and have evolved strong breeding associations with hydrologic cues. Using MPS, we are evaluating *R. boylii* at multiple genetic scales. Of particular interest is assessing the effects of river regulation on population structure and adaptive capacity (genetic diversity) and contemporary effective population sizes. Preliminary assessment of this method successfully shows detailed patterns of structure at both fine and broad scales in populations in the American and Alameda watersheds.

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Stream-breeding Amphibians of The Geysers Region of Sonoma and Lake Counties: A Summary of 19 Years of Aquatic Monitoring

The Bear Canyon/West Ford Flat aquatic monitoring program (BC/WFF) and Squaw Creek aquatic monitoring program (SCAMP) were initiated in 1975 and 1984, respectively, to monitor in-stream fisheries health and water quality parameters within The Geysers geothermal field. With few deviations, the lead researchers have annually surveyed the same 6 stream reaches in Lake County (BC/WFF) and 6 stream reaches in Sonoma County (SCAMP) during the same week in late summer since 1998. Elements of the regular monitoring program include fish population sampling using standard electrofishing methods, benthic macroinvertebrate sampling, 35 water quality parameters, and an assessment of overall stream condition. Outside of the formal program, in 1999, researchers began collecting quantitative data on foothill yellow-legged frogs (*Rana boylii*) and California giant salamanders (*Dicamptodon ensatus*) that were encountered during electrofishing surveys. This discussion will focus on habitat use by instream amphibian populations and changes in the physical environment that appear to affect them. In addition, each of the 6 BC/WFF field sites was within the burn area of the 2015 Valley Fire in Lake County and post-fire amphibian responses to the burn will be discussed.

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Assessing the Impact of Illegal Pesticide Use on National Forest Headwater Stream Communities

The illegal cannabis industry continues to expand in California with "trespass grows" on US Forest Service lands also expanding dramatically. Unregulated grow operations on both public and private land are having dramatic, acute effects on the local ecology. In addition to drawing down headwater streams at the peak of the dry season, cannabis cultivators use numerous pesticides to prevent crop loss to herbivory and to kill nuisance animals. Dangerous pesticides such as anticoagulant rodenticides have been found to kill terrestrial mammals including fisher (*Pekania pennanti*) and black bear (*Ursus americanus*). Traces of Diazinon, one of the pesticides found on illegal grow sites, have been found in stream water directly downstream of recently eradicated grow sites on USFS lands. Here we present on an ongoing study into the effects of toxicants on headwater stream communities. In the fall of 2016 we established access to six cultivation sites on the Shasta-Trinity National Forest, delineated survey reaches up- and down-stream of the site run-off footprint, and deployed water quality monitoring devices in each reach. To characterize reach habitat conditions and benthic macroinvertebrates, we implemented the Surface Water Ambient Monitoring Program (SWAMP) bioassessment protocol. For amphibians, we collected Environmental DNA samples and conducted amphibian belt surveys. Samples are being processed this winter. Species encountered during initial surveys include coastal giant salamander (*Dicamptodon tenebrosus*), foothill yellow-legged frog (*Rana boylii*), coastal tailed frog (*Ascaphus truei*), and southern torrent salamander (*Rhyacotriton variegatus*). Spring resurveys will be conducted in March and April 2017.

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Investigation of Beaver Dams as Tools for Protecting Amphibians from Climate Change and Introduced Trout at High Elevations

Under climate change, mountains in western North America may receive less snowfall, preventing many small, fishless water bodies from holding water long enough to support amphibian reproduction. Trout-sensitive species depend heavily on these marginal habitats because their reproduction is prevented or impaired in large water bodies by native or introduced predatory trout. To investigate beaver dams as a solution, we surveyed a wet meadow in the eastern Oregon Cascade Range with beaver-dammed, undammed, and isolated pools and three species of amphibians: Pacific treefrogs (*Hyliola* [*Pseudacris*] *regilla*), Cascades frogs (*Rana cascadae*), and long-toed salamanders (*Ambystoma macrodactylum*). We observed water flowing over and through every dam, suggesting that trout can pass these dams easily. Indeed, we visually observed trout in 75% of dammed pools and in 82% of stream-pool sections bordered by dams, suggesting that the beaver dams excluded trout from little or none of the aquatic habitat potentially used by amphibians for breeding. Few stream-connected pools

supported breeding of any of the amphibian species, regardless of the presence or absence of dams. Isolated, seasonal pools lacking fish were more likely than stream-connected pools to be used by amphibians for breeding, although this pattern was significant only in Pacific treefrogs. Overall, our results suggest that beavers influence amphibians by changing the amount of isolated, fishless pool habitat rather than by restricting trout distribution or causing habitat changes that allow amphibians to better reproduce in the presence of trout. We are now testing this hypothesis in other beaver-occupied meadow systems.

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Landscape Genomics of California Tiger Salamander and Foothill Yellow-legged Frog: You Really Do Get More from Genomics

Over the last several years the conservation, systematics, and population biology communities have increasingly moved toward large-scale genomic-level data sets based on (tens of) thousands of molecular markers rather than a few dozen genes to construct questions and provide results. Amphibians, and particularly salamanders, have fallen behind on this new agenda largely because their extremely large genomes have posed challenges for efficient, cost-effective genomic research. Here, we present two ongoing projects from our lab, one on foothill yellow-legged frog (*Rana boylii*, estimated genome size of ~7-10 GB) and one on the California tiger salamander (*Ambystoma californiense,*~32 GB) that use RADSeq and target capture, respectively, to explore the potential gain from using these large data sets. For *R. boylii*, our analysis of 25,565 RADseq loci (present in at least 50% of our samples) provides strong evidence for four or five primary clusters of populations across the species' range, and points to the critically endangered southern populations as harboring the greatest genetic diversity. For *A. californiense*, our 5237-gene data set indicates both that major structuring of the species is along a west-east division, and that the primary genomic admixture from non-native barred tiger salamanders has been largely, but not entirely restricted to the Salinas Valley. In both cases, the gain in resolution from genomic-level analyses has been both worthwhile and relatively cost-effective, and we encourage others to utilize these new tools in population, phylogenetic, and conservation genetic amphibian research.

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Success and Failure with Endangered Amphibian Species

This is a tale of two federally-listed Endangered species: arroyo toads (*Anaxyrus californicus,* listed in December 1994); and the Santa Barbara population of California tiger salamanders (*Ambystoma californiense*, emergency listed in January 2000). Most formal events are similar, but the outcomes have been very different. Listing each species drew heavy criticism, threats and vandalism. Each has had critical habitat designated, revised and reinstituted; each has a Recovery Plan; each has had a 5-year review; and each has been proposed by the USFWS for downlisting to Threatened, and in each case it was overturned. The recent drought has severely affected both species, but otherwise arroyo toad populations have been stabilized and were recovering, whereas Santa Barbara County tiger salamanders are in much worse shape than they were at listing. The principal reason for this disparity is that arroyo toads occur almost exclusively on public lands, whereas tiger salamanders are found almost entirely on private lands in Santa Barbara County. To benefit arroyo toads on public lands off-road trails, roads and campgrounds have been closed, military activities and gold and gravel mining restricted, and dam water release schedules altered; only firefighting has continued without real oversight while causing massive habitat destruction. By contrast, USFWS has been essentially incapable of preventing tiger salamander extinction via breeding and upland habitat conversion to intensive agricultural uses. A major problem is that federal prosecutors hesitate to file take cases without dead bodies, and USFWS is unwilling to take action to collect bodies when habitat is being destroyed. The agency has also proved incapable of getting other federal agencies to eliminate a large breeding population of nonnative tiger salamanders within dispersal distance of native salamander ponds.

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Population Dynamics of the Santa Barbara County Distinct Population Segment of the California Tiger Salamander, *Ambystoma californiense*

The Santa Barbara County population of the California tiger salamander, *Ambystoma californiense*, is recognized as a Distinct Population Segment (DPS) under the Federal Endangered Species Act and is one of the most endangered populations of salamanders in California. Current recovery and management efforts have two main foci: (1) maintenance and development of suitable and connected habitat to support population growth among breeding ponds in six geographically isolated populations, and (2) evaluation of the level of introgression and halting the spread of non-native genes from the invasive barred tiger salamander, *Ambystoma mavortium.* However, our current understanding of population genetic structure, connectivity and landscape use is limited, but will be essential for evaluating the current status of these populations and guiding these management efforts. To this end, we examined the landscape genetic structure, connectivity and level of non-native introgression of 23 breeding ponds across the Santa Barbara range, sampled from 1986-2001.

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The Rise and Fall of *Rana draytonii* in Little Panoche Valley

Little Creek contains one of the very few extant populations of California red-legged frogs, *Rana draytonii*, in the San Joaquin Valley. In 2009, BLM repaired two historic cattle ponds that had not held water for at least 10 years. One pond (avg. depth >2 m) was immediately colonized by adult *R. draytonii* (>5 frogs). The other pond did not initially hold sufficient water in 2009 or 2010 to provide habitat for *R. draytonii* but in 2011 the pond filled to a depth of ~ 0.3 m and was found to contain larval *R. draytonii*, which transformed into more than 50 terrestrial frogs. The pond system continued to function as a productive breeding site until 2014, at which time neither adult nor larval *R. draytonii* were observed at either of the ponds. As of winter of 2016 no *R. draytonii* have been detected at the ponds. The observed extirpation of *R. draytonii* was not associated with lack of water as the ponds maintained water throughout 2012-2015. At least one nearby pond that had contained breeding *R. draytonii* also experienced a local extirpation. Our hypothesis is that frogs emigrated en masse from the sites then could not recolonize them due to a drought-induced paucity of nighttime rain events. Future work will continue to see if ponds are naturally recolonized; alternatively, we may actively re-stock the ponds then monitor them in subsequent years. Our observations suggest that workers should be careful about interpreting sources of extirpation in pond-breeding frogs.

POSTERS

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Effects of Bullfrog (*Lithobates catesbeianus*) Removal on Arroyo Toad (*Anaxyrus californicus*) from Remote Training Site Warner Springs

In 2014, Naval Base Coronado (NBC) contracted the United States Geological Survey (USGS) to continue an ongoing bullfrog eradication program at Remote Training Site Warner Springs (RTSWS). The eradication program was designed in 2006 to enhance habitat for the endangered Arroyo Toad (*Anaxyrus californicus*). We focused our efforts within the portion of the West Fork San Luis Rey River that is within the RTSWS boundaries. Prior to 2014, arroyo toads had only been documented in the San Luis Rey River and the Cañada Aguanga, both of which are areas on the property that were free of bullfrogs. Survey methods consisted of several surveys that generally occurred between spring and fall and were conducted in any pool with the potential of harboring bullfrogs and bullfrog tadpoles. Daytime surveys focused on tadpoles and egg masses while nighttime surveys focused on adults and juveniles. Methods used for eradication were air rifles, rimfire rifles, gigs, hand nets, hand captures, minnow traps and seines. In addition, stomach contents were also analyzed to identify what species were being consumed by bullfrogs. Overall, bullfrog numbers have decreased since implementation of the program and in 2015 the first arroyo toad was detected in the West Fork San Luis Rey.

* Indicates presenter in multi-authored presentation