# California/Nevada Amphibian Populations Task Force 2018 Meeting

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## ABSTRACTS

Foothill Yellow-legged Frogs, photo Craig Seltenrich

## ORAL PRESENTATIONS

**BACKLIN, ADAM R.*, ELIZABETH A. GALLEGOS, and ROBERT N. FISHER**. US Geological Survey, Western Ecological Research Center, San Diego, CA, abacklin@usgs.gov.

### Southern California's Mountain Yellow-legged Frog (*Rana muscosa*): Status, Research, and Conservation Activities

USGS has been monitoring all known extant populations of the mountain yellow-legged frog in southern California annually from 2000 to present. During this time, we have coordinated with partners to identify and conduct restoration activities to stabilize declines and rebuild populations. Restoration activities include limiting public interaction with frog populations, installing fish barriers, removing non-native trout, implementing research, conducting translocations, and developing and maintaining a captive breeding program. This presentation will highlight activities to date and partners involved, and provide a current status of the mountain yellow-legged frog in southern California.

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### Update on Restoration of Endangered Mountain Yellow-legged Frogs in Sequoia and Kings Canyon National Parks

Both species of endangered mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) occur in Sequoia and Kings Canyon National Parks (SEKI) but are vulnerable to extirpation, primarily due to nonnative trout and *Batrachochytrium dendrobatidis* (*Bd*). Many historic populations are no longer extant, and most remaining populations are small, isolated, often restricted to small habitats, and still adapting to *Bd*. In response to the large decline and the frogs' currently limited ability to persist with disease, SEKI recently completed a long-term restoration plan with a goal of recovering dozens of extant and recently extirpated frog populations. In 2016-2017, staff from SEKI, UCSB, Oakland and San Francisco Zoos, and USGS collaborated to conduct many actions, including 1) initiating two large fish removal sites, 2) captive-rearing, immunization, and reintroduction of frogs from four populations, 3) studying garter snakes in restoration sites, 4) measuring results of an antifungal treatment, and 5) disease surveillance monitoring. Progress is being made that we hope will eventually contribute to frog recovery at a landscape scale.

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### Foothill Yellow-legged Frog (*Rana boylii*) Growth, Longevity, and Population Dynamics from a 9-Year Photographic Capture-Recapture Study

We studied growth, longevity, and population dynamics of a Foothill Yellow-legged Frog (*Rana boylii*) population in a section of the Pit River, Shasta County, California from 2007 through 2015. We made 1254 *R. boylii* captures during annual surveys and identified 665 individuals using the ExtractCompare pattern recognition software program specifically modified for *R.*

*boylii* chin patterns. Accuracy of the program was 99.4% and greatly reduced identification time and identification errors by researchers. Recapture data indicate a typical lifespan of 3-4 years for males and 5-6 years for females; the oldest male and female are 5 and 9 years old, respectively. We observed newly metamorphic frogs as small as 18 mm SVL and as early as July. Females reached reproductive size, based on gravidity, as small as 48.1 mm SVL and as early as two years post-metamorphosis. Males reached reproductive size, based on nuptial pads, as small as 31.4 mm SVL and as early as the spring post-metamorphosis.

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### Effects of Drought on an Ephemeral Water Breeding Amphibian, the Yosemite Toad

Montane amphibians like the Yosemite toad (*Anaxyrus canorus*) inhabit variable, stochastic environments as demonstrated by the recent California drought (2012-2015), one of the warmest and driest periods on record. Examining the Yosemite toad's response to the drought may provide insights into those characteristics that lead to vulnerability and resiliency to these unpredictable environments. We compiled long-term habitat and demographic monitoring data to describe the Yosemite toad's response to California's drought contrasting breeding habitats and wet versus dry years. Monitoring data encompassed pre, during, and post-drought years. During the breeding period at snowmelt, although all habitats dried considerably during the drought, pothole habitats retained some water whereas many flooded vegetation habitats were completely desiccated. Both water table depths and surface water extent were much reduced during the drought. The timing of spring breeding was relatively well correlated with snowmelt in normal and high water-years, but data suggests there may be a minimum threshold. In flooded vegetation habitats, Yosemite toads did not breed during several drought years but resumed breeding in 2016, the first year after the drought. In pothole habitats, breeding was consistent and normal during the drought. These data suggest that Yosemite toads are vulnerable to breeding habitat loss during drought conditions but that certain types of habitat may confer some resiliency, the long-lived adults may survive periods unsuitable for breeding, and that the phenology of Yosemite toad breeding may be adaptive to avoid risks of early and late breeding.

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### Comparison of California Tiger Salamander Breeding Pool Types and Preserve Viability in Sonoma County

The Sonoma California tiger salamander (SCTS) is a federally endangered vernal pool-breeding distinct population segment, and the subject of conservation efforts consisting primarily of protecting existing populations in remnant habitat patches –preserves– in a rapidly urbanizing landscape. We conducted a 14-year (2002 – 2015) study of SCTS breeding activity at 112 natural and constructed vernal pools on eight preserves using standardized dipnet sampling. Natural and

constructed breeding pools did not differ significantly in depth, surface area, dry date, frequency of breeding, larval relative density, or growth rate. Newly constructed pools had relatively low larval densities initially and in later years greater densities relative to natural pools. The time required for the colonization of constructed pools ranged from 3 to 12 years. At the preserve level, our surveys showed a significant decrease in total SCTS larval abundance and number of pools used for breeding at preserves. Larval abundance was highly variable across years, with variability inversely correlated with preserve size and number of pools. The decline in larval abundance, likely indicative of a rapidly shrinking population, appears to result from habitat loss and fragmentation associated with urbanization, increasingly dry conditions over the study period, and mostly non-native vertebrate predators in some deeper breeding pools. Although constructed pools have enhanced SCTS habitat, our results suggest that a conservation strategy of preservation of remnant habitats and populations is unlikely to successfully conserve SCTS and additional active conservation management may be necessary to avoid extinction.

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**Cascades Frogs in Northern California: Threats, Priorities, and Opportunities**

Cascades frogs (*Rana cascadae*) have declined in California due to multiple factors, including predation by introduced trout, habitat degradation, and disease caused by an introduced chytrid fungal pathogen. These declines prompted the recent designation of the species as a candidate for protection under the California Endangered Species Act. The Cascades frog occupies two geographically distinct areas in California: the southern Cascades in the vicinity of Lassen Volcanic National Park (LVNP), and the Klamath Mountains. The Cascades frog is no longer present in LVNP, where it was once widespread. Almost all of the 11 known remnant populations in the surrounding region (all of which are in meadow/stream habitats) are declining in size, likely due to disease, drought, and degraded habitat. Populations in the Klamath Mountains are larger and more numerous, though many are likely at-risk due to disease and introduced trout. Recent work has emphasized that the chytrid fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is ubiquitous in the southern Cascades region and is driving recruitment failure in remnant populations. Further reproductive failure is driven by egg mass and larval desiccation caused by drought and stream incision. The top priorities for Cascades frog conservation in this region are to address disease and drought. Previous efforts to treat Cascades frogs for chytrid have been successful using anti-fungal drugs to decrease disease loads and increase juvenile over-winter survival. Habitat modifications include stream channel and breeding pond alterations to increase seasonal water retention in addition to raising water tables with beaver dam analogs in wet meadows. Recent proposals have suggested reintroducing Cascades frogs to LVNP now that we have some understanding of the main threats to the species. Reintroduction will require healthy donor populations identified by ongoing genetic and demographic work, appropriate site selection that will impart resilience in the face of disease and climate change, and active management to ensure transplant success.

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**Managing Invasive Crayfish to Restore Stream Habitat in the Malibu Creek Watershed, Los Angeles Co., USA**

Mountains Restoration Trust (MRT), a local land trust organized as a 501(c)(3) public benefit CA corporation, has been working in the community for over 35 years conserving the natural and cultural resources of the Malibu Creek Watershed located in the Santa Monica Mountains. The presence of the invasive red swamp crayfish (*Procambarus clarkii*), native to southern USA, in regional streams has resulted in significant negative impacts to local aquatic ecosystems and native populations. Through predation and habitat alteration, red swamps are thought to have contributed to the local extirpation of the California Red-Legged Frog (*Rana draytonii*, CLRF) and reduction of Baja California Treefrog (*Pseudacris hypochondriaca*) populations. MRT's aquatic invasive species (AIS) and crayfish control strategy consists of three major components: management, research, and outreach/education. For management, MRT staff is currently employing passive trapping methods in target streams. For research, MRT has conducted both a benthic macroinvertebrate assessment survey in managed and non-managed streams and a crayfish trap comparison study to identify the most effective method for catching crayfish while limiting bycatch such as native fish, frogs and tadpoles. Finally, our education and outreach methods include facilitating volunteer participation in trapping and removal efforts and conducting social media outreach. This presentation will showcase some interesting results from implementing our AIS strategy, including new observations of several CRLF individuals in one of our crayfish-managed streams during 2017. With help from project partners and volunteer contributors, MRT aims to restore stream form and function encouraging native species recruitment aligning with state and regional recovery plans.

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***In Situ* Population Enhancement of an At-Risk Population of Foothill Yellow-Legged Frogs, *Rana boylii*, in the North Fork Feather River, Butte County, California**

The Cresta reach of the North Fork Feather River historically had a robust population (30+ breeding females) of foothill yellow-legged frogs (*Rana boylii*). The population in this regulated river reach has been steadily declining since the early 2000's, due to a suite of factors including recreational whitewater flows, hydroelectric dam operations, predation from introduced predators and increased riparian vegetation in breeding habitats. The U.S. Forest Service is working in collaboration with Garcia and Associates, Pacific Gas and Electric Company, and California Department of Fish and Wildlife in an effort to increase the population size of foothill yellow-legged frogs. The current population includes at least five adult females based on 2017 egg mass counts. Two egg masses were salvaged for captive rearing in early June, as they would have otherwise desiccated due to rapidly dropping water levels following high spring runoff flows. Tadpoles were reared *in situ* utilizing flow-through cages. Within two weeks of collection the eggs hatched into tadpoles, which were visited every 2-3 days for feeding, cage cleaning, and

repositioning due to fluctuating water levels. Tadpoles grew rapidly in flow-through cages and 2,400 were periodically released as a preventative measure to avoid the possibility of a mass-casualty event. The final release in mid-August included 407 metamorphosing frogs, many of which had fully developed legs and were in the process of reabsorbing their tails. These individuals were released nearby at the three most suitable historic breeding sites. Surveys two months later revealed higher numbers of young-of-year juvenile frogs (n=15) present than in any previous year on record since 2007. These promising results demonstrate that *in situ* rearing may be a viable management option for small populations of amphibians at risk of extirpation.

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

The federally threatened California red-legged frog (*Rana daytonii*) has been reintroduced to Yosemite National Park. In summer 2017, the park continued its effort to restore this frog by reintroducing 400 captive reared adult-sized *R. draytonii* throughout Yosemite Valley in partnership with the San Francisco Zoo and the Yosemite Conservancy. Efforts to remove the invasive American bullfrog (*Lithobates catesbeianus*) from another infested area of the park were initiated in 2017, this time in wilderness and a historically occupied *R. draytonii* site (Swamp Lake). We will discuss the long-term strategy for reintroducing *R. draytonii* as well as efforts to reduce and eradicate *L. catesbeianus*. In addition, we will also present findings of long-term population viability and uncertainty for the federally threatened Yosemite toad (*Anaxyrus canorus*). Furthermore, we will present results of reintroduction efforts for the federally endangered Sierra Nevada yellow-legged frog (*Rana sierrae*) which have seen an increase in numbers in higher elevation populations in Yosemite National Park while lower elevation populations have experienced a decrease.

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**Occurrence of California Red-legged Frogs (*Rana draytonii*) and Northern Red-legged Frogs (*R. aurora*) in Timberlands of Mendocino County, California, Examined with Environmental DNA**

Effective species management requires knowledge of species distributions, but surveys for cryptic species near the boundaries of their geographical ranges can be difficult. We used environmental DNA (eDNA) and occupancy modeling to examine the distribution of Northern Red-legged Frogs (*Rana aurora*) and federally threatened California Red-legged Frogs (*Rana draytonii*) in a sample of 60 forested stream sites near where their ranges meet in southern

Mendocino County, California, USA. For both species, the probability of occurrence (ψ) in forest streams in our study area was very low: California Red-legged Frog ψ was <0.01 (95% credible interval = <0.01–0.05), and Northern Red-legged Frog ψ was 0.07 (0.02–0.15). DNA from both species was found at one pond site, suggesting either co-occurrence or introgression. Our results suggest that abundance, stream use, or both are very low for red-legged frogs in forested streams in southern Mendocino County.

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### An Improved Camera Trap for Amphibians, Reptiles, Small Mammals, and Large Invertebrates

Camera traps are valuable sampling tools commonly used to inventory and monitor wildlife communities but are challenged to reliably sample small animals. We introduce a novel active camera trap system enabling the reliable and efficient use of wildlife cameras for sampling small animals, particularly reptiles, amphibians, small mammals, and large invertebrates. It surpasses the detection ability of commonly used passive infrared (PIR) cameras for this application and eliminates problems such as high rates of false triggers and high variability in detection rates among cameras and study locations. The system, which employs a HALT trigger, is capable of coupling to digital PIR cameras and is designed for detecting small animals traversing small tunnels, narrow trails, small clearings, and along walls or drift fencing.

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### Drivers of Survival and Recruitment in Translocated Populations of the Endangered Mountain Yellow-legged Frog in Yosemite National Park

Mountain yellow-legged frogs are endangered across their historical range due to the impacts of introduced trout and the amphibian chytrid fungus (*Bd*), and reestablishing populations is a top conservation priority. Translocations, in which frogs are moved from a population that is persisting with *Bd* to suitable unoccupied habitat, can be an effective method to accomplish this goal, but the factors affecting population establishment remain poorly understood. In 2006 and 2008 we reestablished two frog populations in Yosemite National Park using translocations from a single donor population, and surveyed these populations through 2017 using mark-recapture methods. Over the 10-12 year study period, the two populations showed markedly different dynamics: one population became self-sustaining but persistence of the other required additional supplemental translocations. We used a Bayesian multistate mark-recapture model to identify the drivers of these contrasting patterns of survival and recruitment, after accounting for imperfect detection. Survey air temperature and *Bd* infection intensity (load) both increased the probability of detection. Survival of adult frogs during over-winter and over-summer periods was negatively affected by *Bd* load and positively affected by precipitation (snow) during the previous winter. In contrast to the positive effect of snow on survival, recruitment was reduced in summers

following snowy winters. In addition, when *Bd* loads in infected adults were high, new recruits transitioning from the subadult population into the adult population were more likely to be *Bd* positive. Novel insights gained from this analysis will allow us to better manage translocated populations to maximize their chances of successful establishment.

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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 work to protect the newly described Dixie Valley toad in Nevada, using CEQA to challenge projects threatening rare amphibians in California, fighting to preserve critical habitat for endangered frogs in the Sierra Nevada, and filing petitions to list imperiled amphibians under the California Endangered Species Act.

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**Preliminary Findings on the Age Determination of the Coastal Tailed Frog (*Ascaphus truei)* in the Trinity Alps Wilderness, California**

The Coastal Tailed Frog (*Ascaphus truei,* family Leiopelmatidae) belongs to the most basal lineage of extant anurans. Previously described larval periods in this species range from 1 year in the coastal regions of California and Oregon to 3-4 years in montane regions of northwestern Washington. However, little is known of larval periods in montane areas of California near their elevational range limit. We report preliminary data on the life history of a high-elevation (> 2000 m) population of Coastal Tailed Frogs in the Trinity Alps Wilderness of northern California. We present age data using two approaches: length-frequency analysis of larvae to identify the number of cohorts, and skeletochronology of adult phalanges to estimate age. Our preliminary results suggest Coastal Tailed Frogs in this high elevation population have a prolonged (4-year) larval period and can attain an adult age of at least 10 years, identical to the maximum age previously reported for high elevation Montana populations of the sister species, *A. montanus*. This study highlights the importance of using multiple methods to age Coastal Tailed Frogs, which display wide variation in both larval period and size of post-metamorphic frogs across populations.

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**Dammed Rivers: Genetic Signatures of Altered Flow Regimes in a River Breeding Frog (*Rana boylii*)**

River regulation via flow alteration and impairment can have pervasive effects on ecosystem function and thus the biota that have evolved within these systems. Small populations with limited connectivity may have reduced adaptive potential and difficulty responding to current conditions as well as future environmental change. Identifying these populations is crucial for effective conservation prioritization and management. One tool for addressing these ecological genomics questions is restriction site-associated DNA sequencing (RADSeq). A new method called RAPTURE adapts RADSeq to target desired loci and allows highly efficient genotyping across large numbers of individuals. To test the effects of river regulation (hydropower) on population structure, genetic variation (adaptive capacity), and population connectivity, we used RAPTURE to genotype hundreds of frogs from the Northern Sierra Nevada. We characterized and quantified population structure and genetic variation in *Rana boylii* populations across different levels of regulated and unregulated rivers, and show clear patterns of population structure at both fine and broad watershed scales. We identified distinct patterns of increased isolation and bottlenecking in populations of *R. boylii* found in regulated river reaches compared with those in unregulated reaches, and provide conservation context with range-wide *R. boylii* genomic data.

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**Ranaviruses Infect Mountain Yellow-legged Frogs (*Rana muscosa* and *Rana sierrae*)**

Disease is one of the leading causes of worldwide amphibian declines, and while *Batrachochytrium dendrobatidis* (*Bd*) is the major driver of documented disease-driven frog declines, other diseases including ranaviruses can contribute. We found ranaviruses present in mountain yellow-legged frog tadpoles (*Rana muscosa* and *Rana sierrae*) in Kings Canyon National Park. Epizootics caused mass die-offs of tadpoles and short-term declines in tadpole abundance, but did not lead to overall, long-term frog population decline or local extinction. Overall, ranavirus and related epizootics were rare in sampled frog populations. Although present in mountain yellow-legged frogs, ranaviruses appear to play a minor role in the overall decline of these species, especially relative to *Bd*. We also comment on disinfection protocols to prevent dispersal of *Bd* and ranavirus.

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**How Can We Make Meadow Restoration Work for California's Mountain Frogs?**

Intensive land uses have transformed many of the Sierra Nevada's meadows from multi-thread channels with annually inundated floodplains into single-thread, incised channels that store less water and have reduced habitat quality for a diverse suite of meadow-associated wildlife. Among the organisms that inhabit montane meadows, amphibians are especially threatened. Meadows may serve as refugia for amphibians, including the declining *Rana sierrae* and *R. cascadae*, due to their complex and varied habitat conditions. We evaluated the relationship between meadow hydrological conditions and habitat use by these frogs to understand the conditions that promote population persistence. The frogs tend to occur in meadows with consistently high water tables maintained by ground water springs, snowmelt, or beaver. Within meadows, frogs occur in the more open, fluvially active regions where they can bask along the open shoreline, see the approach of predators, and quickly evade them by hopping into nearby aquatic refuges. Frogs avoid the more stable, densely vegetated meadow flats. After breeding, adult frogs tend to use meadow streams to feed and take refuge, while juvenile frogs reside in secondary channels and oxbows, and larvae develop in off- or on-channel pools. In the winter, the frogs move to deep water or groundwater springs to wait for the spring thaw in relative warmth. Thus, effective meadow restoration should consider the processes that create and maintain the diverse habitat conditions needed by all life stages. While raising the water table and thereby increasing the amount of surface water and length of the hydroperiod are part of the solution, restoration of dynamic processes, including erosion and deposition, are an equally important part. It is the hydrological complexity and dynamism of mountain meadows that create a resilient landscape for these montane frogs.

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**Relict Leopard Frog Susceptibility to Chytridiomycosis Varies by Collection Site**

The disease chytridiomycosis caused by the aquatic fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has emerged as a factor in worldwide amphibian declines, including some that occurred decades ago. For some species, the declines occurred with little documentation and are thus poorly understood. The relict leopard frog (*Rana onca* = *Lithobates onca*) has experienced such a decline and by the latter part of the 20th century only occurred in two general areas in southern Nevada. The *Bd* pathogen has been found in *R. onca*, but recent research suggests that the species may have resistance to chytridiomycosis. That conclusion, however, was derived using *Bd* strains that were not from the local environment and therefore may not relate the actual situation experienced by *R. onca* in the wild. We addressed this concern by isolating *Bd* from anurans in southern Nevada, including *R. onca*, and used these isolates to challenge juvenile frogs. Our results indicate that *R. onca* is susceptible to chytridiomycosis from two local *Bd* isolates, as well as from an isolate acquired from a commercial amphibian vender.

We also found that juvenile frogs derived from a currently *Bd* infected area cleared infections and survived in much higher proportions than those from a *Bd*-free area, indicating that the species may be able to adapt to the presence of *Bd*. This finding may create new opportunities for conservation efforts aimed at establishing populations of the species across a landscape where the pathogen may be present.

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**A Case for Drawing Down Fishless Ponds: Interactions Between California Red-legged Frogs (*Rana draytonii*) and Two Species of Newt (*Taricha torosa* and *T. granulosa*) at a Coastal Breeding Site**

In February 2017, we observed unusually large salamander larvae eating the eggs of California red-legged frogs (*Rana draytonii*) in an artificial stock pond on the coastal slope of the Santa Cruz Mountains at Cotoni-Coast Dairies National Monument. Using mitochondrial DNA, we confirmed the presence of larvae of two species of salamander, the California newt (*Taricha torosa*) and the rough-skinned newt (*Taricha granulosa*), and determined the large larvae to be individuals of *T. granulosa* that had likely overwintered. Further sampling throughout the following 12 months determined that *T. torosa* larvae appeared at approximately the same time as eggs of *R. draytonii* were deposited and did not attain large size soon enough to pose a risk to frog eggs. Newly hatched *T. granulosa* larvae appeared for the first time in May. We found evidence for inter-and intra-species interactions between the two newt species. Overwintering newt larvae may pose a unique threat to successful *R. draytonii* reproduction that can potentially be managed by drying down ponds in late fall. However, overwintering in *T. granulosa* expresses natural variation in the reproductive cycle of that species that may warrant conservation itself, posing a potential tradeoff in conservation priorities between two native species.

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**Using Environmental DNA to Detect Amphibians - A Case Study in Southern California**

Environmental DNA (eDNA) detection methods are becoming a powerful tool to determine the presence of species in complex aquatic environments, replacing more traditional labor-intensive surveys. As these methods become more common, it is imperative to understand the advantages and disadvantages of these techniques when used to establish the presence/absence of a specific amphibian species or to establish general amphibian biodiversity at a site. We tested a previously published species-specific primer and its ability to detect an invasive amphibian, the African

clawed frog (*Xenopus laevis*), at 40 sites throughout southern California. The eDNA field survey results appeared to confirm the presence or absence of *X. laevis* at the majority of sites. Some sites were inconclusive based on the molecular analysis and would require further testing, or a traditional survey, to confirm the species presence/absence. In addition, some sites were included in metabarcoding analysis, which used a previously published universal amphibian primer to detect all Anuran and Urodela species in the samples. The metabarcoding analysis detected nearly all species expected to be present in the water system. In all, eDNA was a highly efficient method to evaluate the amphibian diversity, especially given the large size of the study areas, which would have made traditional amphibians survey methods unrealistic to perform in one season. While eDNA survey methods are powerful tools to detect species presence, best practices and sample controls must be properly considered to limit false negatives and positives.

## POSTERS

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### Human Traffic and Habitat Complexity are Strong Predictors for the Distribution of a Declining Amphibian.

Invasive species and habitat modification threaten California's native pond-breeding amphibians, including the federally threatened California Red-legged Frog (*Rana draytonii*). The relative contributions of invasive species, including the American Bullfrog (*Lithobates catesbeianus*), and of habitat changes to these declines are disputed. I conducted a field study over several years in central California to examine the presence/absence of these two species at 79 breeding ponds to determine the predictive role for occupancy of factors including vegetation, pond characteristics, and measures of human activity. I used a boosted regression tree approach to determine the relative value of each predictor variable. Increased measures of human activity, especially proximity to trails and roads, were the best predictors of the absence of California Red-legged Frogs and some other native taxa. Historical factors and habitat conditions were associated with the extent and spread of the American Bullfrog. The extent and complexity of aquatic macrophytes and pond surface area were good predictors for the presence of these and other amphibian species. Surprisingly, invasive species played a relatively small role in predicting pond occupancy by the native species. These findings can inform conservation and restoration efforts for California Red-legged Frogs, which apparently persist best in small vegetated ponds in areas of low human disturbance.

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**Understanding the Detection of Environmental DNA from Yellow-legged Frogs to Develop a Recommended Sampling Protocol**

The capture of environmental DNA (eDNA), or genetic material shed into water, could help augment methods to assess occupancy of species that are difficult to find using traditional surveys. To determine the most effective eDNA sampling protocols for stream and lake systems, we conducted a survey for two hard to find species: the Sierra Nevada yellow-legged frog (*Rana sierrae*) and the foothill yellow-legged frog (*R. boylii*). In streams, we collected samples at 100 m intervals in two different volumes at seven points along five locations in Plumas National Forest. In five lakes, we collected water at sites spaced 50 m apart and combined five consecutive sites together in two different final volumes. We took samples at four time points over the summer of 2016 using single use funnels for a total of 470 samples. We analyzed samples in triplicate using species-specific quantitative PCR (qPCR) assays we developed and validated, then analyzed detection results using generalized linear mixed effect models. Filtering double the volume of water (2L) increased the odds of unambiguous detection in streams by 5.53X (95% CI: 3.24-9.45X). Lake sample volumes were limited by filter clogging and we found no effect of volume or filter type. Sampling later in the season increased the odds of detection in streams by 1.71 per 30 days between May and August, but had no effect for lakes. Spatial autocorrelation of the amount of yellow-legged frog eDNA captured in streams peaked at 200 m indicating that sampling at close intervals is important for detection.

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**Integrating Environmental DNA Sampling into Long-term Monitoring Strategies: A Case Study for Detecting *Rana sierrae* in Streams**

Environmental DNA (eDNA) detection techniques are gaining popularity as a means of surveying for rare and often cryptic amphibians. Land managers and regulatory agencies are beginning to request collection of these data as part of routine biological surveys for long-term monitoring, such as on hydroelectric relicensing projects. We present methods and results of an initial trial for one season of eDNA sampling efforts on stream reaches in the Plumas National Forest, targeting the Sierra Nevada yellow-legged frog (*Rana sierrae*), a federally endangered species. We utilized both visual encounter surveys and eDNA riverine sampling to infer species presence/absence on three high-gradient intermittent stream reaches. Results from eDNA sampling were consistent with lack of detections in two reaches that were surveyed visually. At a third site, the species was detected visually but not with eDNA in August, and with eDNA but not visual surveys in September. This highlights the importance of identifying optimal methods for eDNA sampling based on site-specific environmental conditions, as well as the current benefits of continued incorporation of traditional methods when making management recommendations that may affect amphibian habitat.

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**Predation by Gartersnakes (*Thamnophis elegans*) on Translocated and Reintroduced Mountain Yellow-legged Frog Populations (*Rana muscosa* and *Rana sierrae*)**

Predator impacts on managed species can reduce the effectiveness of conservation actions. For example, mountain yellow-legged frogs (*Rana muscosa* and *Rana sierrae*) released during translocations and reintroductions face potentially heavy predation by mountain gartersnakes (*Thamnophis elegans elegans*), which can reduce the likelihood of frog population establishment. To enhance the success of our frog restoration efforts, we are studying 1) snake abundance and movement patterns before and after mountain yellow-legged frog translocations and reintroductions, 2) snake prey preferences, and 3) the effectiveness of snake translocations away from the frog populations to reduce predation pressure on frogs. Here we describe our ongoing study and share preliminary results.

* Indicates presenter in multi-authored presentation