California/Nevada Amphibian Populations Task Force 2024 Meeting

University of California, Merced
Merced, CA
January 11–12, 2024

ABSTRACTS

Sierra Nevada Ensatina (*Ensatina eschscholtzii platensis*) from near Alder Springs, Fresno County, CA. Photographed by Robert W. Hansen.

ORAL PRESENTATIONS

* Indicates presenter in multi-authored presentation

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A Successful Method to Translocate California Red-legged Frogs in Central California

Effective translocation of wildlife species requires significant understanding of the species' natural history. In addition, translocation techniques should mimic the conditions that natural populations experience. We used a technique to translocate California red-legged frog egg masses that expediently reflected the ovipositioning behavior, hatching conditions, and initial larval development conditions that we witnessed in wild populations of frogs. A floating rearing pen that supported egg masses at the surface and subsequently allowed hatchling larvae to shift to deeper conditions among pond bottom substrate was designed to mimic natural conditions. Egg masses were monitored three times per week until hatching. Post-metamoprhic frogs were monitored weekly and PIT tagged. Over two breeding seasons 2,310 eggs hatched from four translocated egg mass halves and resulted in 449 post-metamorphic frogs. These frogs showed secondary sexual characteristics in 7 months and were adult size (length and weight) in 15 months. We contend that a critical aspect of the success of this translocation was frequent monitoring during the egg mass placement, and that marking post-metamorphic frogs confirmed success rates.

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Applying Novel Genetic Methods to Rapidly Scan for Non-Native Genes in Sonoma California Tiger Salamanders

The persistent threat of invasive species requires the development of early detection and rapid response (EDRR) methods. Cryptic invasions are especially challenging, often requiring expensive and time-consuming molecular methods. Here we present a novel application of the Fluidigm microfluidic SNP-Type Assay to identify non-native alleles using pooled samples, significantly reducing the cost (1/3) and time (1/30) to results. We demonstrate the efficacy of this method using experimental Fluidigm pools (97.8% accuracy) and with Next Generation Sequence data (96.6% accuracy). We apply this method to a population of California tiger salamanders (CTS) in Sonoma County where only two non-native barred tiger salamanders (BTS) hybrids have been detected over the past 30 years. We screened 5,805 larvae from across the population in 387 sample-pools containing 15 larvae each. We did not find any verifiable detections of non-native hybrids, a result that was confirmed with sequence data. While the apparent lack of BTS alleles is encouraging, additional years of sampling are needed to confirm the absence of hybrids in Sonoma. We believe this approach will make genetic screening for critical alleles more widely available in conservation, facilitating EDRR in myriad new taxonomic systems.

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Distribution and Habitat Use of the Santa Clara County Population of Red-bellied Newts (*Taricha rivularis*)

Anthropogenic habitat loss and degradation has increased extinction rates in amphibians worldwide, yet little is known about many remaining populations. In 2009, a disjunct population of Red-bellied Newt (*Taricha rivularis*), an endemic California species, was discovered 130 km south of its previously known range. Here we document the range and breeding phenology of this population as of 2019 and contrast its habitat use with that of other sympatric newts. Surveys across two years suggest that the southern population of *T. rivularis* is confined to one 1-km reach of Stevens Creek and a tributary informally called Twitty Creek, and the population follows an early-March to late-April migratory breeding pattern. Spatial analysis shows that breeding male *T. rivularis* aggregate only in Stevens Creek, likely dispersing through Twitty Creek, and that they associate with riffle and run mesohabitats rather than pools. Sympatric *T. granulosa* and *T. torosa* populations tended to associate more with woody debris cover types and cobble substrates than *T. rivularis*. *T. rivularis* oviposition site selection was most influenced by large substrate size. Protecting large substrate and complex instream habitat in sensitive breeding reaches, as well as upland habitat along dispersal routes, should be an important consideration for land managers. Understanding the nuances of range, temporal behavior, and habitat needs for this disjunct population is critical to ensure the survival of this California Species of Special Concern.

DENNHARDT, ANDREW J. Ventura Fish and Wildlife Office, U.S. Fish and Wildlife Service, Ventura, CA.

Five-year Status Review for the Arroyo Toad, *Anaxyrus californicus*.

Five-year status reviews give the U.S. Fish and Wildlife Service the opportunity to periodically review the best available scientific and commercial information about a species and assess progress made toward species recovery. The arroyo toad is managed across three recovery units, and a final recovery plan was published in 1999, which provided both quantitative criteria and priority actions to help recover the species. Here, I present findings from an evaluation of population status and threats to the species, progress made toward achieving recovery criteria, and future recommended conservation actions. After reviewing the best available scientific and commercial information, we conclude that the arroyo toad remains an endangered species, with increased threat from wildfire, drought, and climate change. Recommendations for future conservation actions include conducting spatiotemporally replicated surveys at historical and recent occurrence locations, implementing a rangewide genetic and genomic study, and establishing new conservation easements to advance toad recovery—to name a few. Five-year status reviews assist us and our partners in identifying conservation needs and better targeting and prioritizing conservation efforts for listed species.

DENNHARDT, ANDREW J. Ventura Fish and Wildlife Office, U.S. Fish and Wildlife Service.

Proposed Listing of the Northwestern Pond Turtle (*Actinemys marmorata*) and Southwestern Pond Turtle (*Actinemys pallida*) as Threatened under the Endangered Species Act.

In July 2012, the U.S. Fish and Wildlife Service was petitioned to list 53 herptile species, including the western pond turtle (*Actinemys marmorata*), under the Endangered Species Act. In April 2015, we published a 90-day finding that the petition presented substantial information that listing may be warranted for the western pond turtle. The western pond turtle was then split into two separate species: northwestern pond

turtle (*Actinemys marmorata*) and southwestern pond turtle (*Actinemys pallida*). In 2022, we published a Species Status Assessment (SSA) compiling biological information and condition on both species. To assess future viability of the two species, we considered two scenarios representing the range of plausible environmental conditions through 2100. Evaluation of past, current, and future influences on species' requirements for long-term population viability revealed human modification/land conversion, predation, and drought as the most influential threats. Population viability was projected to decrease for northwestern and southwestern pond turtles under both scenarios. After a review of the best scientific and commercial information available, we found that listing the northwestern pond turtle and southwestern pond turtle is warranted and, in October 2023, we proposed to list both species as threatened under the Endangered Species Act with a Section 4(d) rule.

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Research and Conservation of Terrestrial Salamanders in Arid and Highly Seasonal Environments, Adventures with Camera Traps

Small, terrestrial salamanders show highly seasonal and highly localized activity patterns across much of California. This reality complicates both learning about the basic ecology of these species and designing conservation measures to understand and mitigate the impact of projects on their populations. Herpetologists continue to struggle with fundamental questions such as: Where should I look? When should I look? and, When can I know they aren't here? Answering these questions reaches the extreme for the Slender Salamanders of the genus *Batrachoseps*. While in-person surveys continue to uncover both new populations and new species, the person-hours, miles driven, and cost per animal are often impractical for conservation. Modification of camera trap deployment and advances in camera trap technology holds some promise to assist herpetologists in detecting these species but challenges remain. In studying a remote population of the California State Threatened Tehachapi Slender Salamander (*B. stebbinsi*) cover object surveys continue to detect this species at reasonably high densities but camera traps have yet to detect their presence. The Amphibian Task Force meeting offers an excellent opportunity to discuss a way forward, as additional species in this genus receive consideration for listing at the federal level.

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Desert Slender Salamander (*Batrachoseps aridus*) and Salamanders in the Desert (*Batrachoseps* sp.) Update, or What We Accomplished Since the 2020 APTF Meeting

The desert slender salamander (*Batrachoseps aridus*; DSS) is endangered under the Endangered Species Act by the USFWS and one of the first species listed under California's Endangered Species Act. It has now been over 25 years since any verified sighting of the species. The two known historic locations for this species were surveyed over the last 6 years, several times each, and during prime activity periods, to

determine the status of the habitat at the sites, and to attempt to detect any salamanders. Sampling was done with surrogate species for amphibian fungal disease detection. At neither site were salamanders detected during any surveys. We did find a possible third population discovered in the 1980's but failed to detect salamanders there in 2021. Our broad sampling for any salamanders in the desert starting in 2019 did detect multiple populations in San Diego and Imperial Counties, none of which appear to be the endangered DSS. We did detect one new population of apparent DSS in Riverside County in 2023 and we report it here for the first time as well as the potential rediscovery of the species. We will also discuss the novel desert animals from San Diego and Imperial Counties.

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Amphibian Update for Yosemite National Park – Insights on New Management Actions and Future Direction

In 2023, the federally threatened California red-legged frog (*Rana draytonii*) was reintroduced into two formerly occupied habitats in the park. At one site invasive American bullfrogs (*Lithobates catesbeianus*) were successfully removed but at the second site removal operations are still ongoing. We are taking this opportunity to learn if *R. draytonii* can be re-established concurrent with *L. catesbeianus* removal. Decision factors and future planned management actions will be discussed. The year 2023 also signified the functional extirpation of a meadow population of Sierra Nevada yellow-legged frog (*R. sierrae*). This drought-stricken population is now actively managed to maintain its presence on the landscape to preserve this genetic diversity. We will discuss the actions being taken. Finally, the return of the Yosemite toad (*Anaxyrus canorus*)? We will present data for several *A. canorus* populations that seem to suggest that populations in some areas of the park are stable and perhaps increasing.

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A New Herpetological Field Guide for California

California is home to ca. 209 species of amphibians and reptiles, including approximately 32 exotics, a few of which are invasive. Covering 425,000 hectares, spanning 9.5 degrees of both latitude and longitude, and containing the lowest and highest elevations in the continental U.S., such diversity is not unexpected. New discoveries in recent years, whether in the lab or field, have resulted in the recognition of many new species and point to the need for an updated, accurate, and comprehensive guide to the California herpetofauna. We began this project in January 2021 and turned in a final manuscript to the publisher in December 2023. The book contains 122 range maps, 165 color plates showcasing 943 photos of live specimens, 144 original color paintings of larval amphibians and sea turtles, and 51 line drawings. Importantly, this project involved >200 collaborators representing the gamut of California's herpetological community, from enthusiastic amateurs to professional herpetologists.

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Pond Characteristics that Influence Oviposition Site Selection for Two Pond-breeding Amphibians, Northern Red-legged Frog (*Rana aurora*) and Northwestern Salamander (*Ambystoma gracile*).

Ponds are crucial for all life stages of pond-breeding amphibians and often are used by multiple species for breeding. Two amphibians, Northern red-legged frog (*Rana aurora*) and Northwestern salamander

(*Ambystoma gracile*) often oviposit in the same pond but studies have focused only on one species or the other. I investigated the pond and oviposition site characteristics of thirty ponds in Humboldt County, CA, fifteen of which were used only by *R. aurora* and fifteen of which were used by both species for oviposition. I found that ponds used by both species have deeper water and less total vegetation cover. *Rana aurora* and *A. gracile* oviposited at different distances from the pond shoreline and surface, but *R. aurora* oviposited further from the shoreline when *A. gracile* was present compared to ponds without *A. gracile*. *Rana aurora* oviposited more often on submerged vegetation when *A. gracile* was present, and more often on emergent vegetation when *A. gracile* was absent. I found that the percentage of canopy cover affected water temperature around egg masses, which influences embryo growth and hatching success. My study emphasizes that managing ponds to account for oviposition site preferences for multiple amphibian species is key to amphibian conservation.

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Initial Demographic Estimates for Two Species of Narrowly Endemic Toads in Central Nevada: *Anaxyrus monfontanus* and *Anaxyrus nevadensis*

Hot Creek toads (*Anaxyrus monfontanus*) and Railroad Valley toads (*A. nevadensis*) are recently recognized species that arose from the western toad (*A. boreas*) species complex in central Nevada. Both of these species are narrow endemics that depend on isolated, spring-driven habitat and are subject to various conservation concerns. Little is known about these species, and we used Capture-Mark-Recapture methods to derive initial demographic estimates for discrete populations of each species (Hot Creek toads 2021 – 2023; Railroad Valley toads 2022 – 2023). We found that both species had relatively high monthly apparent survival (0.853 for Hot Creek toad and 0.893 for Railroad Valley toad) and that the estimated abundances increased throughout the study period. Capture probabilities varied by season but were highest in the spring for both species. These demographic estimates are a starting point for asking more questions about these unique toads of the Great Basin.

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Evolutionary Rescue and Reintroduction of Resistant Frogs Allows Recovery in the Presence of a Lethal Fungal Disease

Vast alteration of the biosphere by humans is causing a sixth mass extinction. Adaptation to modified environments often is the only means for species to persist, but the extent to which such "evolutionary rescue" can reverse biodiversity loss is largely unknown. Using results from genomic analyses, a 15-year reintroduction effort, and population modeling, we provide a compelling example of evolution reversing the declines of an imperiled amphibian and allowing the reestablishment of extirpated populations. The once-common mountain yellow-legged (MYL) frog is threatened with extinction by the human-facilitated

emergence of a lethal fungal pathogen (*Batrachochytrium dendrobatidis*; "Bd"). Although most MYL frog populations are extirpated following disease outbreaks, some persist and eventually begin to recover. We show that MYL frogs have undergone substantial evolutionary change following disease outbreaks, and that changes are associated with increased resistance/tolerance to Bd infection. Large-scale reintroduction of frogs from rescued populations resulted in the establishment of reproducing populations despite ongoing disease. In addition, results from viability modeling suggest that established populations have a low probability of extinction over 50 years. Collectively, these results provide a rare example of how evolutionary rescue and the reintroduction of resistant/tolerant individuals can allow the landscape-scale recovery of disease-impacted species.

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Build it and They Will Come – California Red-legged Frog Habitat Restoration Success in Northern Baja California

The California red-legged frog (*Rana draytonii*) is a large frog native to California and northern Baja California, Mexico that predominantly inhabits slower-moving permanent water sources such as streams, lakes, marshes, ponds, and ephemeral drainages in valley bottoms and foothills, as well as adjacent upland habitats. Range-wide population decline of the species has been significant and today the species is estimated to occupy less than 30% of its historical range. In Mexico, California red-legged frog populations are declining dramatically, primarily from anthropogenic stresses, including ground water mining, habitat conversion, and spread of exotic species. To improve habitat conditions for the species, a local non-profit conservation organization, along with international partners, created new pond and wetland habitat along an existing stream containing California red-legged frogs in 2018, and has performed annual maintenance since that time. Annual population surveys have shown a significant increase in population at the restoration site since 2018. This data shows that habitat restoration and maintenance may effectively increase local populations of California red-legged frog in Baja California; and funding from international partners to support conversation efforts like this one can likely be an effective use of funding to benefit threatened species over a relatively short period of time.

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Loss of an Invasive Fish Predator in an Amphibian Dominated Pond Ecosystem Causes Trophic Rearrangement

Top predators are known to be important drivers of trophic relationships in many ecosystems. Research has demonstrated that the introduction or loss of a top predator can initiate significant trophic rearrangements and alter food web dynamics. In the current situation, an invasive fish predator (Western Mosquitofish, *Gambusia affinis*) was environmentally eliminated by drought from a small farm pond in Contra Costa County CA in 2021. This pond contained three native species of amphibians (*Taricha torosa*, *Pseudacris sierra*, and *Anaxyrus boreas halophilus*). Interestingly, the year after the loss of *G. affinis* (2022), native California Red-legged Frogs (*Rana draytonii*) appeared in the pond. The presumptive food webs both prior and subsequent to mosquitofish loss were examined with stable isotopes during the spring and summer months of three years (2017, 2018 and 2022). Both vertebrates and macroinvertebrates were included in the

analysis. The results indicate community-wide alterations to the presumptive food webs. The impacts were largest in the late summer (August) when with the removal of the mosquitofish the larval newts gain almost an entire trophic level in their diet. These alterations may allow newt larvae to develop and leave the pond faster or in better physiological condition.

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Sals in the City: Surprising Response of the Wandering Salamander, *Aneides vagrans*, to Urbanization

Wandering Salamanders (*Aneides vagrans*), endemic to Northwestern California, are known to exist in coniferous forests, living in the crowns of some of the tallest coast redwoods (*Sequoia sempervirens*) in the world. What happens when the old-growth forests they inhabit are turned into cities and houses? A related species, the Arboreal Salamander (*Aneides lugubris*), is widely documented in urban centers with little natural habitat, particularly in the San Francisco Bay Area, but urban populations have not been described for other members of the genus. Utilizing several microhabitat variables centered around tree stumps, we explored which factors, if any, could explain the habitat use of *Aneides vagrans*, primarily in urban settings. We combined these results with personal and iNaturalist observations of the species from developed habitats in coastal Humboldt County to further understand the urban ecology of the species. Our findings indicate that not only are *A. vagrans* present in heavily urbanized areas, but that they appear to be thriving and reproducing in such habitats.

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Beyond the Karst: Diversification and Radiation of the Genus *Hydromantes* in Northern California

In recent years, populations of web-toed salamanders (*Hydromantes*) have been discovered in unexpected settings, causing us to question our concept of what constitutes suitable habitat. Among these are populations of the Mt. Lyell Salamander (*H. platycephalus*) living in xeric desert-edge canyons on the eastern slope of the Sierra Nevada, and more recently a cave-dwelling population found at low elevation in the Sierran foothills that has proved to be enigmatic. In 2018, Bingham et al. partitioned the Shasta Salamander (*H. shastae*) into 3 species based on mitochondrial DNA, allozyme loci, and modest morphological differences. Those authors acknowledged that the ranges of these northern species were not fully understood, and it was clear that large areas of the Klamath Mountains and other Northern California ranges had not been adequately explored for populations of *Hydromantes*. We began using a combination of satellite imagery and field experience to identify several areas that warranted surveys based on geology, elevation, and slope exposure. Over the past several years we have focused our survey efforts on increasingly rugged and remote areas in the Shasta-Klamath region. Though surveys are ongoing, preliminary results from our work will be shared along with future directions for research.

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An Update on CDFW Amphibian Efforts and Quantifying Drought Impacts on Select Amphibians in California

California's Mediterranean climate is expected to continue experiencing climate whiplash, going from periods of extreme wet to extreme dry with increasing frequency. The extreme dry periods vary across time and space, making the management of diverse habitats and species assemblages during and after drought periods challenging. Identifying quantitative and reproducible approaches to prioritizing which species may be most vulnerable, and which habitats or localities may be most at risk to both short- and long-term drought remains a significant management hurdle. We piloted an effort to quantify the risk to select listed frog species at scale by combining newly developed drought products, such as the Evaporative Demand Drought Index (EDDI), with occupancy records for species of conservation concern, generation time estimates, and range sizes. EDDI provides a measure of how anomalous the atmospheric evaporative demand (E0; also known as "the thirst of the atmosphere") is for a given location and time interval. When combined with species generation time and range size, it enables us to identify where drought exposure is greatest for the species, both in the near- and long-term, and in turn, facilitates science-based prioritization of management and monitoring efforts.

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Rogue Turtles with Exotic Leeches: The Northwestern Pond Turtle, *Actinemys marmorata*, as a Host for the Common North American Turtle Leech, *Placobdella parasitica*

There is conservation concern for the Northwestern Pond Turtle (*Actinemys marmorata*) throughout its range, and non-native parasitic leeches using *A. marmorata* as a host could impose an additional threat. Freshwater turtles native to eastern and central North America are common hosts of parasitic leeches and associated blood pathogens. The Common North American Turtle Leech (*Placobdella parasitica*) is widespread east of the Rocky Mountains where it parasitizes a wide range of turtle species. Occurrences of *P. parasitica* west of the Rocky Mountains are presumed to be translocated by turtle hosts from the east. We report *P. parasitica* using *A. marmorata* as a host in the Lower Rogue River, southwestern Oregon, where leeches appear to be well-established. Leech prevalence on turtles was significantly higher for adults than for juveniles but not significantly different between males and females. Body condition was not significantly different between adult turtles with or without leeches, although was slightly lower in turtles with leeches. The health impacts of leech introductions on the only native turtle in southwestern Oregon warrants further investigation to determine if *P. parasitica* represents an emerging threat by reducing fitness or serving as a vector to spread blood pathogens within populations of *A. marmorata*.

RICHMOND, JONATHAN*, ELIZABETH GALLEGOS, ADAM BACKLIN, and ROBERT FISHER. U.S. Geological Survey, San Diego CA.

A Bi-national Effort to Re-establish Populations of the California Red-Legged Frog (*Rana draytonii*) in Extirpated Parts of the Range in Southern California

Translocations are increasingly being used as a technique for mitigating declines or augmenting genetic diversity in protected species. However, protocols for species distributed across broad latitudinal ranges may require regionally specific considerations to increase success, given that environmental heterogeneity can impose different constraints on population re-establishment in different parts of the range. In this talk, we will describe the process of implementing genetically informed translocations of a threatened amphibian, California red-legged frog (*Rana draytonii*) from Baja California México to extirpated parts of the species range in southern California, where urban sprawl, invasive species, and other contemporary stressors add to the natural environmental challenges already present for amphibians in this warm, dry part of the southwest. We will briefly describe the binational collaboration that initiated and maintains the project, the fine-tuning of the entire translocation process, and the results of our efforts to date. While we have achieved considerable success in numerous aspects of the process, other key outcomes have yet to play out before we can declare the slam-dunk victory that we and the frogs are hoping for.

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Informing Management Strategy for the Relict Leopard Frog: An Attempt to Improve Augmentation Success through Pre-Exposure and Clearance of an Amphibian Pathogen

Translocation has become a common approach in amphibian conservation for establishing or augmenting populations; however, the strategy faces various challenges. One of these is the amphibian pathogen, *Batrachochytrium dendrobatidis* (Bd). Susceptible amphibians translocated to areas with Bd may have very low survival. One approach to mitigating disease susceptibility is to increase host resistance through priming of the immune system. The Relict Leopard Frog (*Rana onca*) is a species for which headstarting and translocation are current management strategies. Our aim was to determine the efficacy of using prior exposure to Bd followed by drug-mediated clearance (pre-exposure and clearance) to improve survival of headstarted *R. onca* used to augment a wild population in which Bd was present. We released two groups of headstarted frogs into the population: one pre-exposed and cleared, and another handled identically except exposed to a sham inoculum. We surveyed across 13 months post-release using a mark-recapture approach, and collected skin swabs to determine infection dynamics. We found that pre-exposure and clearance can significantly reduce Bd infection in the wild during winter months when the pathogen was prevalent in the environment. Findings from this study are relevant to conservation managers attempting to increase amphibian translocation success in a landscape where Bd is present.

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Prevalence of *Batrachochytrium* and Ranavirus in Southern Torrent Salamanders (*Rhyacotriton variegatus*) in Northern California

Amphibian population declines around the world have been attributed in part to the emergence of deadly pathogens. However, the severity of the effects of pathogens on amphibian communities can vary by species

and location. This study focuses on assessing the prevalence of *Batrachochytrium dendrobatidis* (*Bd*), *Batrachochytrium salamandrivorans* (*Bsal*), and ranaviruses in populations of southern torrent salamanders (*Rhyacotriton variegatus*). We also tested for association between environmental factors and the prevalence and viral load of the three pathogens in Northern California. A total of 309 skin swab samples were collected from salamanders in nine sites in Del Norte and Humboldt Counties. A quantitative PCR diagnostic assay was used to detect the presence of pathogen DNA in each sample. Approximately 16% of all *R. variegatus* swab samples contained ranavirus DNA, but neither *Bd* nor *Bsal* were detected. Ranavirus prevalence varied widely across sites (from 0-40%). The water temperature of the salamander's microhabitat significantly influenced an individual's ranavirus load. Additionally, ranavirus prevalence was significantly different across seasons. Ranavirus prevalence was highest in the winter, but viral load was highest in the spring. This study provides the first evidence of ranavirus infecting salamanders in the family Rhyacotritonidae.

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Is *Spea hammondii* One Species or Two?: Population and Landscape Genomics of a Vernal Pool Specialist

The spadefoot genus *Spea* has had a complex taxonomic history at the species, genus and family levels. However, for the last several decades, the current configuration of 4 species from western North America has been both stable and accepted by the community, albeit with the lingering possibility that the California/Baja endemic *Spea hammondii* may consist of two evolutionary lineages. Recent molecular data from across the species range clearly indicates that *S. hammondii* is composed of two allopatric, genetically very distinct lineages separated by the Tehachapi mountains. These two lineages are reciprocally monophyletic, occupy different climatic niches based on MaxEnt models, and are genetically diagnosable entities that can easily be considered separate species. Morphologically, the two are somewhat differentiated based on adult body size and relative spade size, although these differences are subtle. We have not yet analyzed call data, and would welcome any recordings that might contribute to determine whether and to what extent call variation may characterize these two lineages. Finally, both northern and southern populations of the western spadefoot have been proposed for listing under the federal Endangered Species Act, and we encourage all members of the APTF to provide comments to the USFWS by 5 February 2024.

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Lessons Learned from a Decade of Head-starting Endangered Frogs at the San Francisco Zoo

The San Francisco Zoo Conservation Department's head-starting program has evolved significantly in just a decade—from a single room to a dedicated, quarantine facility totaling over 2700 sq ft. The expansion of resources and the hard work of twenty-five Conservation zoo professionals contributed to the return of 5500 threatened, subadult frogs back to the wilderness of California. We discuss the challenges and successes of rearing eggs and tadpoles through metamorphosis, inoculating frogs with chytrid fungus, meeting the nutritional needs through all life stages, and more from our experiences raising and caring for thousands of these imperiled amphibians.

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Connecting the Canyons: Conservation Genomics of a Desert-Dwelling Lungless Salamander (*Batrachoseps campi*)

The Inyo Mountains salamander, *Batrachoseps campi*, is one of two described plethodontid salamanders confined entirely to desert habitat. These salamanders occupy discrete riparian areas in steep-walled canyons separated by mostly inhospitable terrain. The only previous genetic study on this species suggested that canyon populations are highly divergent from one another, with limited to no gene flow occurring between sites. Spurred by a 2012 petition for federal listing, our work re-examines the population structure and genetic divergence of *B. campi* using contemporary molecular approaches and an expanded set of populations. Specifically, 93 samples of *B. campi* from 17 of 24 known populations were sequenced using a reduced-representation genomic approach generating thousands of informative markers. Population assignment analyses indicate the presence of at least three genetic clusters with detectable admixture across populations. All clusters showed connection over the mountain crest, with the highest genetic diversity found in the southern part of the mountain range. Additionally, we failed to find detectable genetic structure at the finest scale examined, a 425 m stretch of continuous riparian habitat. The results of our work offer new perspectives and increased resolution of the evolutionary history and population dynamics of this species of conservation concern.

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The Nature and Extent of Algal Symbiosis in North American Ranids

The symbiotic relationship between the green alga *Oophila amblystomatis* and embryos of certain amphibian species is presumed to be mutualistic. However, the existence of a mutualism has only been experimentally tested in two closely related ambystomatid salamanders. These experiments show a positive correlation between algal density and embryonic growth, survival, hatching synchrony, and hatchling body size. *Oophila* has been documented within egg capsules of a growing number of amphibian species, including several ranid frog species. However, the nature and extent of this relationship remains unclear. We raised Northern red-legged frog (*Rana aurora*) and wood frog (*Rana sylvatica*) egg masses under three light treatments (24-hour light, 12:12 light:dark cycle, and 24-hour darkness) to test whether the symbiotic relationship between these ranid frogs and *Oophila amblystomatis* is a mutualistic one. We found that eggs raised in 24-hour darkness experienced decreased survival compared to lighted treatments, but hatchling body size, stage at hatching, or rate of development was not influenced by light treatment. We also extracted intracapsular fluid from Cascade frogs (*Rana cascadae*) and Foothill yellow-legged frog (*Rana boylii*) to genetically test for the presence of *Oophila*. Genetic analysis of these samples is currently ongoing.

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Perspectives on Northwestern Pond Turtle (*Actinemys marmorata*) Monitoring, Management and Habitat Restoration in Yosemite National Park

The restoration of Ackerson Meadow in the Yosemite region is the largest project of its kind to restore a Sierra Nevada meadow. The site, located at 4,600 feet in elevation, is occupied by Northwestern pond turtles (recently proposed for federal listing) and rests close to the species' eastward and elevational range boundary. We will report recent findings from this project that build knowledge of the Northwestern pond turtle's natural history in the montane zone and further the development of protections for the species during projects that impact occupied habitats. We will present radio-telemetry data on terrestrial habitat use for estivation and overwintering at the site, as well as highlights from the first phase of implementation of restoration actions regarding turtle clearance and approaches to minimize potential impacts.

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Movement Ecology of North Feather Clade Foothill Yellow-Legged Frogs

The foothill yellow-legged frog (*Rana boylii*) is a stream-dwelling amphibian native to California and Oregon at elevations below 4,500 feet. The North Feather clade is listed as threatened by the US Fish and Wildlife Service. The purpose of this study was to determine the extent of the population in local streams and to investigate movement ecology for management and species recovery actions. Visual encounter surveys were conducted 2016-2023 in the Spanish Creek metapopulation in Plumas County, California. We plotted frog capture locations in ArcMap to determine stream length used by frogs, distances moved, and direction of movements. One-hundred twenty individuals were included in our study. We found that *R. boylii* from four different streams bred in a single location. Females used longer reaches of stream than males. Individuals that were captured at least once during breeding season had longer within-year movements than individuals that were not captured during breeding season. Our results demonstrate that foothill yellow-legged frogs regularly move downstream to breed and return upstream, where they remain relatively stationary through summer and fall. To understand the extent of habitat use by *R. boylii*, surveys must be conducted both during and after the breeding season. Surveys conducted only during summer months will miss the majority of frog movements.

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A Pond Array for Investigating Predation on California Red-legged Frogs (*Rana draytonii*) by Rough-skinned Newts (*Taricha granulosa*)

In service of conserving amphibian populations, rigorous ecological experiments are still the gold standard for teasing out indirect or otherwise complicated but possibly crucial ecological interactions. One challenge of performing experiments in the wild on pond-breeding amphibians is the challenge of replicating the pond environment with minimum variation across ponds. Attempting to conduct studies across a sample of historically extant ponds are bedeviled by a host of confounding factors: variation in age of pond, vegetation, depth, temperature, predator population, etc. In order to conduct rigorous factorial tests of the interactions among newts (genus *Taricha*) and California red-legged frogs (*Rana draytonii*), we constructed an array of nine small ponds, approx. 7 m in diameter and 1 meter in depth, on a natural coastal terrace at Cotoni-Coast

Dairies National Monument north of the city of Santa Cruz, Santa Cruz County, California. Work included actual excavation of ponds, development of nearby springs and siting of two 5,000 water tanks, and a buried water delivery system to ensure water levels be maintained. We were able to create this array with a fairly small budget (< $200,000) within a short time frame (about 3 weeks). We discuss lessons learned and plans for initial experiments.

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Let's Dig In: Western Spadefoot (*Spea Hammondii*) Breeding Pool Construction in Coastal Orange County, CA

The USFWS's proposal to list western spadefoot as Threatened under the ESA prioritizes *Spea hammondii* habitat restoration and mitigation. Here we present on two vernal pool construction projects in Orange County, CA, covering pool materials, water retention, spadefoot translocation, and importation of water during dry conditions. In January 2005 Glenn Lukos Associates, Inc. constructed 22 unlined pools in the foothills of the Santa Ana Mountains for a mitigation project and translocated *S. hammondii* to these pools. In 2018 and 2022, USGS and partners compacted soil and added synthetic rubber liners at 5 of these mitigation pools in the hopes of improving water retention. Between December 2019 and February 2020, USGS and partners constructed 10 PVC lined pools and 2 clay pools in the San Joaquin Hills. The following rain season failed to fill the pools with sufficient water for *amphibian* breeding, so in April 2021 we imported water to 5 PVC lined pools to see if *S. hammondii* would breed in artificially filled pools. The winter of 2022-23 provided above average rainfall, allowing the first opportunity to test success. These projects have proven highly successful for increasing *S. hammondii* breeding and we will cover the major takeaways for future pool construction projects.

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Life in the Desert; Columbia Spotted Frogs (*Rana luteiventris*) in the Great Basin

The Columbia spotted frog (*Rana luteiventris*) has a range that reaches across western North America into the Great Basin watershed. Declines have been documented in Great Basin populations since 1939 due to multiple threats including habitat destruction, drought, invasive species, and low genetic diversity. These threats led to a listing as a "candidate species" under the Endangered Species Act in 1993. A 10-year Conservation Agreement and Strategy (CAS) plan for the state of Nevada was implemented in 2003 by multiple state and federal agencies, which aimed to reduce threats to *R. luteiventris* populations by restoring and maintaining habitat, and monitor population numbers. Several restoration ponds and channel improvement projects were implemented in the Reese River Management Unit in central Nevada. *R. luteiventris* was removed as a candidate species in 2015. We compared historical tissue samples taken of *R. luteiventris* prior to these habitat restoration efforts (1999-2000) and contemporary samples (2020-2021) to acquire data on active dispersal and gene flow among restored sites using multiple microsatellite loci. We found contemporary samples had higher heterozygosity than historical samples, and historical samples had higher relatedness. These results show genetic dispersal within and between sites, and the habitat restoration was successful in increasing movement.

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Atypical Breeding Habitat Use by *Spea hammondii* and *Rana boylii*.

During site selection and species surveys, biologists often prioritize areas considered "typical" for their target species, risking the potential to miss novel habitat usage or behavioral shifts. Several species, like the western spadefoot (*Spea hammondii*) and foothill yellow-legged frog (*Rana boylii*), have been experiencing the reduction of historical breeding habitat through land-use conversion and the changing of stream hydrologic regimes by dams, respectively. Faced with an uncertain future, amphibians may be adapting their behavior to use less “typical” habitat when quality conventional habitat is limited or no longer available, including the following two cases of western spadefoot and foothill yellow-legged frog breeding. These cases further highlight the need to survey a variety of macro- and microhabitat types, even the “atypical” ones.

POSTER PRESENTATIONS

BREHME, CHERYL, AND ROBERT FISHER. San Diego Field Station – San Diego Office, USGS Western Ecological Research Center, San Diego, CA.

Literature Review and Meta-analysis: Effectiveness of Amphibian and Reptile Road Crossing Systems

Many amphibian and reptile species are particularly susceptible to the negative impacts from roads as they slowly migrate among different habitats to meet their basic life history requirements, such as breeding, development, foraging, and overwintering. When roads intersect vital habitats for these species, it can result in reduced gene flow, lower population sizes and increased probability of population extirpation. Current practices to mitigate negative impacts on migrating amphibians and other small animals are typically to install one or several tunnels under the roadway with stand-alone barrier fencing to help lead animals to the passages. Most post-installation studies are focused on documenting reduction in road mortality or simple passage use. However, there is a need for more studies on crossing system effectiveness and relative permeability to target species (i.e. what proportion of individuals that encounter structures or fencing pass through to the other side?). We analyzed road crossing system permeability data of 41 studies from the United States, Austria, Australia, Canada, France, Netherlands, Sweden, Switzerland, and the United Kingdom. We present highlights of our literature review and results from a meta-analysis of factors that may influence the effectiveness of passage systems including passage dimensions, openness ratio, open vs. closed top, bottom substrate, and passage wetness on permeability to multiple amphibian and reptile species groups.

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A New Tiger Salamander King in Town: Documenting the Spread of Barred Tiger Salamanders (*Ambystoma mavortium*) within San Diego County

Invasive Barred Tiger Salamanders (*Ambystoma mavortium*; BTS) have been present within California for over 70 years, mainly relegated to the central and northern parts of the state, often where native *Ambystoma* live. While *Ambystoma mavortium* were mainly used as fishing bait or as pets, they have been a prohibited species in California for several decades. They have caused widespread issues once established due to hybridization with the endemic tiger salamander and driving native species declines. However, in Southern California (Ventura/Los Angeles County and south), these salamanders had been thought to not have been widely established due to the differences in habitat, with only one novel site in San Diego County having a long term established population. Recently we have found, that at least since 2017, there are multiple invasive populations now present in Central and Eastern San Diego County. While most of these populations are on private land, invasive *A. mavortium* have been found spreading throughout adjacent habitat and occur in several critical reserve areas for the proposed threatened Western Spadefoot (*Spea hammondii*). A screening for parasites and pathogens found these invasive salamanders to be very clean, lacking typical BTS parasites. Next steps for stemming this invasion are discussed.

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Divergence Time Estimation and Paleobiogeography of the Salamander Subgenus *Plethopsis*

The disparate global range of Plethodontid salamanders in the *Batrachoseps* subgenus *Plethopsis* is unique, and implicates perplexing historical biogeographic scenarios. Using uncorrelated relaxed molecular clock methods and fossil-calibrated divergence estimates from Shen et al. (2016), we present a time-scaled phylogeny for the genus *Batrachoseps* in order to test hypotheses concerning the diversification of the subgenus *Plethopsis*. Our estimated divergence time intervals detract support from the hypothesis that *Batrachoseps robustus* diverged as the flow of the Owens River changed course at ca. 3.2 Ma, as evidenced by sediment deposits at Searles Lake (Phillips, 2008). Instead, our estimates support that diversification of the known *Plethopsis* species began earlier, in the late Miocene to early Pliocene, as extensional activity formed the proto-Owens Valley and led to ensuing hydrological and climatic changes, driving vicariance between populations in the proto-southern Sierra Nevada and Inyo Mountains. Moreover, our estimates support a scenario wherein *B. campi* and *B. wrighti* diverged in the Pliocene as ancestral populations of *B. wrighti* expanded northward to Oregon via a corridor of relatively mesic habitat in the western Great Basin.

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Illegal Take of Northwestern Pond Turtle (*Actinemys marmorata*) Near the Northwestern Boundary of Sequoia National Park

Large or mass mortality events can cause extreme consequences to the affected population including loss of genetic diversity, demographic shifts, and even population crashes. These consequences can be especially detrimental to long-lived species and those with delayed sexual maturity such as the northwestern pond turtle (*Actinemys marmorata*). In July 2023, park staff were alerted about the illegal installation of a gill net observed in the North Fork Kaweah watershed. Twenty-four turtles were recorded as entangled within the net, and 23 turtle carcasses were retrieved by park staff. Our findings include a preliminary summary of morphometrics, sex, and age of the 23 recovered pond turtles, including individuals marked from long-term monitoring by the NPS. Future analysis of genetic samples will provide insight into the population structure and history of pond turtles within the watershed. Little is known about the status and ecology of western pond turtles throughout Sequoia and Kings Canyon National Parks, and results from this work will help managers develop conservation and management priorities for the species.