California/Nevada Amphibian Populations Task Force 2023 Meeting

Sebastopol Community Cultural Center
Sebastopol, CA
January 12–13, 2023

ABSTRACTS

ORAL PRESENTATIONS

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Ovipositioning Phenology of the California Red-legged Frog (*Rana draytonii*) in the Sierra Nevada of California

Timing of ovipositioning is typically a function of environmental conditions for amphibian species. The timing of this natural history function is often predictable, particularly for bi-phasic species that occur in temperate environments. For declining species, aspects of the reproductive natural history are used to facilitate detection of the occupation of a habitat. We found that the California red-legged frog (*Rana draytonii)* breeding season is consistent among two disparate populations-coastal and Sierra Nevada populations. In the Sierra Nevada population, frogs breed through the winter and early spring months with an average peak in the observations of egg masses occurring in mid-March, which is approximately one month prior to the onset of egg mass surveys set by agency survey protocols. We recommend conducting egg mass surveys in the Sierras from mid-February to mid-April in order to detect breeding for this species.

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The Southern Mountain Yellow-legged Frog (*Rana muscosa*): Update on Trends, Current Research and Recovery Actions

The southern mountain yellow-legged frog (*Rana muscosa*) is a critically endangered species and is continuing to decline across its range. Here we present an update on the present status, current research, and recovery efforts from 2019-2022. Research includes studies on defining relevant conservation targets, longevity, revisiting conservation units, and understanding disease dynamics for this species. We also provide a summary of recovery actions including post-fire emergency rescue, translocations, and captive breeding. Overall, we have a better toolset now to deal with this species recovery, but the continuing drought has severely limited our options for reintroduction sites.

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Recovery Actions for the Endangered Mountain Yellow-legged Frog Complex in the Southern Sierra Nevada

Sequoia and Kings Canyon National Parks (SEKI) protects both species of the endangered mountain yellow-legged frog complex (*Rana muscosa* and *R. sierrae*). The parkwide and adjacent regional population, however, is vulnerable to being extirpated, primarily due to nonnative trout and disease (chytridiomycosis; *Bd*). While removing trout populations to restore critical habitat is relatively straightforward, it can be challenging to achieve eradication due to the size and complexity of lake basins and the strong migration ability of common taxa (*Oncorhynchus mykiss* spp.). Additionally, nearly all of the extant frog populations in this region are still naïve to *Bd* or still adapting to develop enough disease resistance to become persistent. For approximately 25 years collaborative teams have endeavored to restore lake ecosystems and recover these frog species in and near SEKI using research, applied and adaptive management, and an evolving suite of methods. This presentation describes actions conducted in 2020-2022 by staff from SEKI, UCSB, Oakland and San Francisco Zoos, and CDFW. Although results show both progress and continuing challenges, recent developments point toward incremental positive change at the landscape scale.

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Microbiomes, Disease Research and Population Management – How Collaboration is Key to Western Pond Turtle Conservation Across the Range

Climate change and drought, new disease pathogens, and increasing human disturbance are setting a stage for further decline in populations of turtles around the country. However, an expanding collaboration of stakeholders and some innovative partnerships are paving a new chapter in conservation for California's freshwater aquatic turtle species. From Washington to Baja California, the Western Pond Turtle is the subject of research questions and conservation efforts including several projects focusing on Bay Area turtles.

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Can Hydroperiod Management Reverse Invasive Hybridization in the California Tiger Salamander?

Invasive species present one of the greatest threats to the conservation of biodiversity. When invasives hybridize with endangered native taxa, they severely complicate recovery actions. Across a large swath of central California, a hybrid swarm consisting of admixed endangered California tiger salamanders ("CTS", *Ambystoma californiense*) and introduced barred tiger salamander (*Ambystoma mavortium*) has replaced native populations, threatening CTS with genomic extinction. Here we employ a large-scale, genomically-informed field ecological experiment to test whether habitat restoration can reinstate natural selection favoring native salamander genotypes. We constructed 14 large, semi-natural ponds and manipulated their

hydroperiods to evaluate larval survival and mass at metamorphosis. Consistent with earlier work, we found overwhelming evidence of hybrid superiority which persisted across all hydroperiod treatments. In contrast to previous mesocosm-based studies, native CTS never exhibited greater fitness than hybrids, suggesting that hydroperiod management alone will not shift selection to favor native genotypes. However, shortening pond hydroperiod may represent a cost-effective strategy to limit the overall productivity of ponds with non-native genotypes, complimenting additional strategies such as targeted hybrid removal. At a broader level, our experimental approach leverages extensive ecological knowledge, modern genomic tools, and a naturalistic, *in situ* replicated design to critically evaluate and expand the potential toolkit that managers can use to address this, and other recalcitrant biological invasions.

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***Batrachochytrium dendrobatidis* Risk in Yosemite Toads (*Anaxyrus canorus*) Varies across Seasons and Life Stages**

Yosemite toads (*Anaxyrus canorus*) are endemic to high-elevation sites in the Sierra Nevada, California. Toad populations in Yosemite National Park suffered sharp declines for unclear reasons in the late 1970's, from which they have yet to recover. This project set out to systematically evaluate the threat that the amphibian fungal pathogen *Batrachochytrium dendrobatidis (Bd)* posed to the vulnerable populations in the Tioga Pass area of Yosemite, and the data are informing a recovery plan and planned re-introductions using captive-reared toads. Here, I will present the results of the *Bd* surveillance in toads, which provide evidence that significant *Bd* transmission occurs in juvenile life stages during hibernation. The data raise several questions: What mechanisms (e.g. behavior, immunology, environment) are driving transmission in hibernating juveniles? How does climate-driven shortening of winter seasons affect host vulnerability to infection during hibernation? I will also discuss plans to address these questions in future research.

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Five-year Status Review for the Santa Barbara County Distinct Population Segment of the California Tiger Salamander, *Ambystoma californiense*

Five-year status reviews give the U.S. Fish and Wildlife Service the opportunity to periodically review the best available scientific information about a listed species and assess its progress toward recovery. Inhabiting rangelands along the central coast of California, the Santa Barbara County Distinct Population Segment of the California Tiger Salamander is managed across six metapopulation areas. A final Recovery Plan was published in 2016, which outlined 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 actions to advance recovery of the species. After

reviewing the best available scientific information, we concluded that the California tiger salamander remains an endangered species in Santa Barbara County, with increased threat from climate-induced drought and inbreeding depression. Five-year status reviews assist the Service and its partners in identifying conservation needs and enhancing prioritization of conservation efforts for listed species.

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Two Decades of Foothill Yellow-legged Frog (*Rana boylii)* Population Monitoring near Mount Tamalpais in Marin County, California

For the past 19 years (2004 to 2022), Kleinfelder/Garcia and Associates (GANDA) have conducted annual egg mass counts and mark-recapture of *Rana boylii* at Little Carson Creek, and Big Carson Creek in Marin County, California, for the Marin Municipal Water District (MMWD). A new population was discovered nearby in San Anselmo Creek in 2017, which has been monitored since 2018 for Marin County Open Space District (MOSD). Though populations at other historic sites in these watersheds have disappeared, populations at Little and Big Carson Creeks have been dynamic, but stable over the past two decades. We will provide a summary of this long-term monitoring effort in addition to how that information is informing a reintroduction of the species at a historically occupied site at Cataract Creek on Mt. Tamalpais.

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Comparative Microhabitat Use of Two California Native Ranids, California Red-legged Frog and Foothill Yellow-legged Frog in a Riparian Woodland

Amphibians are the most threatened group of vertebrates in the world, due to human-caused impacts of climate change and habitat alteration on amphibian accessibility to moisture and appropriate temperatures for thermoregulation. Two imperiled species, the California red-legged frog (*Rana draytonii*) and the foothill yellow-legged frog (*Rana boylii*), overlap in some ranges and may differ in how they use shared microhabitats. Understanding how these species partition their use of microhabitat features is critical for species management. I conducted a nocturnal microclimate-based study in Sonoma County, CA along Copeland Creek, taking frog body temperatures and microclimate variables from June to October 2021. Using mixed models, I evaluated the relationship between body size, body temperature, and microhabitat features. I found that body size was a prominent driver in body temperature and microhabitat use. As body size increased in California red-legged frogs, distance to water and elevation from water line increased, and relative humidity and body temperature decreased. Thermal images showed that although individuals were basking in locations warmer than the frog itself, there was no evidence that basking location was determined by the fine-scale thermal landscape. However, water balance characteristics of larger frogs appeared to allow them to spend time further from water with a reduced risk of desiccation. Studies of microhabitat use are critical for understanding how frogs living in sympatry use their habitat, as changing microhabitat conditions affecting

temperature and moisture levels will likely play a critical role in species management in the face of changing climates.

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Annual Amphibian Update for California Red-Legged Frog, American Bullfrog, and Yosemite Toad in Yosemite National Park

The federally threatened California red-legged frog (*Rana daytonii*) was first introduced to Yosemite Valley in 2016. In 2019, captive reared *R. draytonii* at the San Francisco Zoo successfully bred in Yosemite Valley for the first time. A status update will be given for this population in the Valley. An update to the ongoing effort to remove the invasive American bullfrog (*Lithobates catesbeianus*) from historically occupied *R. draytonii* wilderness sites (Swamp Lake & Gravel Pit Lake) will also be provided. We will also discuss the long-term strategy for reintroducing *R. draytonii* at these two locations where bullfrogs likely caused their extirpation. An update on a reintroduction pilot for the federally threatened Yosemite toad project and other related toad work will also be given.

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In Hot Water: Dixie Valley Toad Distribution and Behavior in Dixie Meadows, Nevada

Dixie Valley Toads (*Anaxyrus williamsi*) are a recently-listed endangered species endemic to a small system of cold and hot springs in the Dixie Valley, Nevada, USA. Understanding how the toads use their desert spring environment is essential for their conservation. We studied the distribution and behavior of Dixie Valley Toads as it relates to surface water availability and temperature in Dixie Meadows. We found that Dixie Valley Toad larvae were more likely to occur in survey plots far from spring heads with a high coverage of surface water, low emergent vegetation cover, and water temperatures between 20–28°C. Adult toads were more likely to occur in plots with greater coverage of surface water and water depth > 10 cm. Plots with more emergent vegetation cover and surface water were more likely to be colonized by adult toads. Radio-tracked adult toads were very closely associated with water in spring and fall, and selected habitats in spring and autumn that were closer to water and warmer than nearby available locations. Dixie Valley Toads selected brumation sites in or near water, often near springs. The reliance of Dixie Valley Toads on water within a specific range of temperatures suggests that alteration to historical flows and water temperatures are likely to affect the toads.

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Comparing Skin and Gut Microbiome Diversity Across the Phylogeny of a Salamander Ring Species, *Ensatina eschscholtzii*

Vertebrates such as amphibians are known to host diverse communities of microorganisms (termed the microbiome) that affect the host's immune system, behavior, and physiology. For example, research has revealed that amphibian skin microbiomes likely play an important role in mitigating the impact of fungal pathogens through the presence of anti-fungal bacteria. While skin microbial communities have now been profiled for many species of amphibians, the gut or intestinal microbiome remains relatively unexplored. Work in other vertebrates has shown that the composition of the gut microbiome often matches a host group's complex evolutionary history. Therefore, while amphibian skin microbiomes may depend more on habitat than host genetics, gut microbiomes are more likely to be conserved over evolutionary time, correlating with host phylogeny. This study compares the skin and gut microbiomes across most subspecies of the salamander ring species *Ensatina eschscholtzii* (whose phylogeography has been well characterized). Prior research suggested that the skin microbiome of *Ensatina* salamanders varies according to habitat, rather than phylogeny. By re-sampling the very same locations from this previous study, we discovered that the gut microbiome is entirely distinct from the skin microbiome, with the gut microbial community being much more diverse. However, we also found that neither the gut, nor the skin microbiome were influenced by phylogeny.

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An Intensive Capture-Mark-Recapture Study of Cascades Frogs, *Rana cascadae*, on Managed Private Timberlands in the Southern Cascades

Cascades frogs occur in Northern California and are a candidate species under the California Endangered Species Act due to declining populations from a variety of threats. Historically the Klamath Mountains have held large populations of Cascades frogs. However, in the Southern Cascades range, few extant populations exist, and population numbers are generally low. We monitored one of the few remaining robust populations in the Southern Cascades, which occurs at the southern extreme and lower elevational limits of its range. We intensively monitored the population using capture-mark-recapture techniques for 4 consecutive years. From 2019-2022, the population experienced variable climatic conditions, including a historically wet winter in 2018-2019 and record-setting drought in 2021. During this time, we have been able to assess population structure and seasonal movements. This intensive data collection has allowed us to develop conservation actions to help maintain the population, and further adds to the overall knowledge of the species.

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Opportunities and Challenges for Re-Introduction of Foothill Yellow-Legged Frogs (*Rana Boylii*) at their Southwestern Range Limit, Part II: In Situ Rearing and Translocation

We evaluated the efficacy of in situ rearing to introduce foothill yellow-legged frogs (FYLF) to San Miguel Creek (SM) and enhance recruitment in Los Burros Creek (LB) at Fort Hunter Liggett in Monterey County, CA. In April 2022, we transferred 233 eggs from 3 egg masses laid in LB to 23 cages spread across 4 locations, 2 per creek. We supplemented food and monitored tadpoles until their release. Releases occurred before metamorphosis when equalizing densities among cages, when front limbs emerged, or at the end of the experiment, in July. We compared survival and growth of caged and unenclosed tadpoles among sites, and considered effects of water temperature, clutch, and pathogen prevalence. Even with unforeseen complications including a bear destroying all cages at one site, infection by multiple trematode and crustacean parasite taxa, severe drought, and blooms of algal species inedible by tadpoles, 88 individuals reached metamorphosis by time of release. There were no significant differences in body condition between locations for tadpoles (P=0.85) and metamorphs (P=0.31). Average survival between visits was 90% and 98%, with overall survival of 53% and 64% at SM and LB, respectively, excluding destroyed cages and early releases. This pilot provides a foundation for continued efforts in bolstering FYLF populations.

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Environmental DNA as an effective tool for detecting northwestern pond turtles (*Actinemys marmorata*) in freshwater streams.

Environmental DNA (eDNA) analysis is becoming a widely used and powerful tool for resource monitoring and management. Understanding the limitations of eDNA detection is critical when informing management decisions. However, eDNA detection probabilities require species- and habitat-specific study designs. We applied eDNA survey techniques to an ongoing long-term monitoring program for northwestern pond turtles (*Actinemys marmorata*) in a freshwater stream system, Willow Creek, located in California's central Sierra foothills. In 2022, we conducted paired visual encounter and snorkel surveys as well as eDNA sample collection. We used a multi-scale occupancy model to determine eDNA detection probability at three nested levels of sampling: primary sample site within the study area, secondary sampling units collected from each primary sampling site, and technical replicates of each secondary sampling unit. We detected northwestern pond turtle DNA in 100% of the sites using eDNA techniques compared to 37% of the sites using traditional visual encounter and snorkel surveys. These results bolster

the credibility of using eDNA for detection of northwestern pond turtles and provides additional validation for management decisions informed by presence/absence results.

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Anecdotal Data Associated with 116 California Tiger Salamander (*Ambystoma californiense)* Observations During a Construction Project in Livermore, California

Pacific Gas and Electric Company (PG&E) owns and operates electric and natural gas lines throughout California. PG&E identified the need to replace 5 miles of vintage pipeline located in Livermore, Alameda County, CA by the end of 2018. The project alignment included a landscape of grazed non-native annual grassland, ruderal areas, and dryland farmed fields subject to tilling and other agricultural practices. Numerous occurrences of California tiger salamander (*Ambystoma californiense*, CTS) were located within 1.3 miles of the project alignment, small mammal burrows were present throughout ruderal and grassland areas, and known CTS breeding habitat was present in mitigation ponds on adjacent conservation lands. CTS was identified as a species with a high potential to occur on the project site. After completion of CEQA review, PG&E finally received project discretionary permits in August 2018. PG&E immediately began pre-construction activities required by the permits. Pipeline replacement within occupied CTS habitat during the wet season required PG&E to navigate numerous challenges. A total of 116 CTS were observed during work, and CDFW did not approve marking of individuals. Therefore, we provide anecdotal information for the CTS encountered during burrow excavation activities, construction monitoring, evening surveys and focused night surveys during the wet season.

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Opportunities and Challenges for Reintroduction of Foothill Yellow-Legged Frogs (*Rana boylii*) at their Southwestern Range Limit, Part I: Population and Site Assessment

Among the six clades of *Rana boylii*, California's south coast clade is the most imperiled and urgently needs conservation action. We investigated opportunities to expand distribution beyond the current locations in Monterey County by: (1) assessing extant population size and chytrid status; (2) searching for additional donor populations; and (3) evaluating physical habitat, thermal suitability, and biotic integrity at potential receiving streams. We conducted visual and environmental DNA surveys at 9 sites with, or 18 near (5.2±0.7 km), historical records in the California Natural Diversity Database. We detected *R. boylii* only at the known extant sites in the

Santa Lucia Range; one coastal, one interior. These sites are 8 km apart in adjacent watersheds but have water temperatures at opposite extremes of the species' thermal niche. Egg mass density at the warm inland creek was low (4.6 clutches/km) compared to the cool coastal stream network (16.7/km). Of the unoccupied sites, half are precluded from reintroduction because we detected non-native predators (e.g., bullfrogs, crayfish). For the half with native fauna, five had sufficient streamflow or remnant pools that would provide refuge for frogs during droughts. Translocation projects are underway at two of these sites plus tadpole head-starting at the inland source stream.

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Potential Benefits of Beaver to Wetland and Wet Meadow Restoration and Cascades Frog Recovery, *Rana cascadae*

The North American Beaver (*Castor canadensis*) is considered a "keystone species". Beaver dams and associated ponds can increase surface and groundwater storage, improve water quality, repair eroded channels, reconnect streams to their floodplains, sequester carbon and create and maintain wetland and riparian habitats. Beaver create habitat complexity and diversity in otherwise simplified stream systems and can prolong critical summer stream flow or provide perennial flow to degraded streams that would otherwise run dry. Beaver dams, canals, burrows and food caches can greatly expand off-channel, wetland and wet meadow habitats providing many benefits to select frogs, fish, birds, mammals and other wildlife. Recent research at Child's Meadow in Lassen County by US Forest Service researcher Karen Pope has shown that presence of beaver dams increased density and productivity of the imperiled Cascades frog (*Rana cascadae*). Kate will discuss potential benefits of beaver to wetland and wet meadow restoration and share updates on California beaver restoration policies and opportunities. Karen will share findings from her research.

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Ten Years of Evolutionary Research on the Yosemite Toad (*Anaxyrus canorus*): What Can the Past and Present Tell Us About Their Future?

Yosemite toads (*Anaxyrus canorus*) are extremely vulnerable to ongoing climate change. Adults exclusively breed in the transient and exceptionally shallow ponds of mountain meadows making them highly dependent upon seasonal snowpack and associated groundwater and runoff levels. Tadpoles regularly face high desiccation mortality exacerbated by opportunistic parasites and predators. Adults and subadults are sensitive to temperature-induced reduction in body fat levels that can influence their overwintering survival and fecundity. Over the past decade, we have used genomic, climate, and landscape data to elucidate how this iconic species has previously adapted alongside climate experienced during the Pleistocene and present-day, to predict the future fate

of the species. Pleistocene ice sheets have fractured their distribution into several "pure" and "fused" lineages, while modern-day populations are further divided into "hub" and "satellite" populations. In two recent publications, we forecasted how 21st century climate change will encourage range shifts upward in elevation, while simultaneously favoring the adaptive success of certain higher elevation lineages. Our simulations predict a 29% demographic reduction over 90 years of climate scenario RCP 8.5, consistent with previous estimates. Our novel landscape genomic approach offers practical conservation suggestions, such as identifying climate refugia, protecting migrational corridors, and combining low-diversity lineages with similar adaptations into one conservation unit.

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Restoration of Amphibian Habitat: Geographic Differences and Lessons Learned

Natural habitat has been lost or severely fragmented in much of the United States. In many regions a few generalist amphibians remain common and widespread while sensitive species have been extirpated or isolated in scattered preserves separated by expanses of agricultural or urban land. In such places, habitat restoration has been very effective in enhancing populations of some common amphibian species. California's north coast is different because very large areas of open space remain intact to varying degrees, and landscape level ecological processes still function. Urban and agricultural land is less extensive than in other regions. Disturbances such as logging and wildfire can be severe but are temporary. Native amphibian species are present across the landscape and while numbers are likely reduced, most remain common in appropriate habitat. Habitat restoration in this landscape is important; rather than attempting to recover lost populations, it is often possible to prevent those losses from occurring or to allow natural recolonization of amphibians into restored habitat. Additional information is needed, including identification of best remaining example reference sites, improving understanding of restoration techniques, and monitoring the response of amphibians to habitat restoration and enhancement.

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Walking on the Wet Side: Microhabitat Selection in *Batrachoseps relictus*

The genus *Batrachoseps* comprises a species-rich group of 22 small, mostly elongated, terrestrial salamanders restricted to the Pacific Coast. As a group, they are characterized by a strongly conserved morphology, low vagility, and generalized habitat requirements. However, recent field studies suggest that some species may have relatively specialized ecological requirements. One of these, the Relictual Slender Salamander (*Batrachoseps relictus*), stands out by having adopted an aquatic lifestyle. Focused surveys of aquatic habitat types have led to the discovery of seven additional populations in the last four years, including three in 2022. No populations of *B. relictus* have been discovered outside of aquatic microsites. Individual populations occupy discrete patches of habitat (< 0.1 ha) in perennial or seasonal springs and seepages. These

microsites also are used for communal nesting. Reliance on discrete, specialized, patchily distributed habitat, in combination with small population size and restricted gene flow across the small range, make this species vulnerable to local extinction via climate change or stochastic events such as catastrophic wildfires. These factors are relevant given the recent proposal by the U.S. Fish and Wildlife Service to list *B. relictus* as Endangered.

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Attempted Population Reduction of the American Bullfrog, *Rana catesbeiana* Shaw 1802 (Amphibia, Anura, Ranidae) at the Resighini Rancheria, Klamath, Del Norte County, California

We used hand-held GPS receivers, baited minnow traps, visual encounter survey transects (VES), air rifles, dip-nets, and Fyke nets, to map and collect American Bullfrogs at wetland sites over the *ca.* 455-acre Resighini Rancheria, in Klamath, Del Norte County, California, from 2018 through 2022. We collected over 1,600 individual bullfrogs, mostly 1st- and 2nd-year larvae, from nine (N=9) wetland sites and observed the species at an additional four (N=4) sites on Resighini Rancheria land holdings. An additional *ca*. 350 bullfrogs were also collected from two (N=2) wetland sites in Humboldt County in 2020, 2021 and 2022. We were able to identify seven (N=7) distinct life-stages, or age-classes, of the bullfrogs detected. We dissected metamorph, juvenile, sub-adult, and adult frogs obtained, to record prey items. We also studied the phenological cycle of bullfrog occurrence and activity. It was determined that the non-native, invasive bullfrog populations impact many species of pollinating insects, including: hornets, ants, wasps, bumble bees, dragonflies, gnats, craneflies, beetles, and honey bees; as well as native and non-native fish and amphibians. A truncated discussion of how our data compare to previous studies on the predatory impacts of non-native, invasive populations of bullfrogs on wetland eco-systems is presented.

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Thermal Ecology of the Relict Leopard Frog

All historical remnant populations of *Rana onca* occupy hot springs in the eastern Mojave Desert; however, the populations at hot springs do not appear to be thriving. Meanwhile, large populations have been established through translocation at cold-water sites. We aim to compare frogs at hot and cold springs to determine whether hot temperatures at hot springs during critical seasons (winter and summer) are generally beneficial or detrimental. While still in early stages, we have initiated a series of experiments and field monitoring to better understand the thermal ecology of *R. onca*. Using a thermal gradient, we measured temperature preference of juvenile frogs and initiated seasonal measurements of wild-caught adult frogs. In the wild, we are using data loggers implanted into abdominal cavities of adult frogs to collect continuous body temperature data. We have successfully recaptured implanted frogs following a summer season to retrieve data. Loggers have also been placed within frog habitat to reference ambient air and

water temperatures. In order to assess feeding at high and low temperatures, we ran feeding trials with groups of juvenile frogs. In the field, we have begun measuring body condition of adult frogs to assess seasonal changes in energy reserves. The findings of this research are intended to inform management efforts to evaluate, enhance, and expand high quality habitat for this species, particularly at hot springs.

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Cascades Frog (*Rana cascadae*) Egg Mass Survival Pre and Post Process-Based Restoration of a Montane Meadow in the Southern Cascades

Round Valley Meadow is a mid-elevation montane meadow located on private industrial timberlands at the southernmost end of the Cascades mountain range. Cascades frog (*Rana cascadae*) occupy this 6-hectare perennially wet meadow. In the fall of 2021, process-based restoration work was performed, resulting in ninety-six small beaver-dam-analog (BDA) structures being installed. In the spring of 2021 prior to restoration work, 100 egg masses were located, with 12% desiccating prior to hatching and an additional 22% of egg masses deemed unsuccessful due to the dewatering of habitat that the hatched tadpoles were occupying prior to metamorphosis. Post restoration surveys in 2022 resulted in 81 egg masses being located, with 1% desiccating prior to hatching and 6% deemed unsuccessful due to dewatering. Of these eighty-one egg masses, 26% were located within the lentic habitat created by the restoration BDA structures. The survival of egg masses at Round Valley Meadow depends on the meadow's ability to maintain sufficient surface water depth and flow during the spring and summer months. Process-based restoration not only increases water storage capacity in these montane meadows but can also create adequate breeding habitat for Cascades frog.

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Fraying Edges and Widening Gaps: How Patterns of Population Decline Translate to the Rangewide Genetic Structure of the California Red-legged Frog *Rana draytonii*

Environmental gradients, habitat loss and extirpation can create gaps within a species' distribution and lead to receding range edges. These processes can dramatically impact dispersal between populations and the partitioning of genetic variation across the landscape, particularly for species with linear-shaped distributions like the threatened California red-legged frog *Rana draytonii*. This talk describes how we use population genetic and genomic data to better understand how range gaps and receding range edges in *R. draytonii* have influenced patterns of genetic diversity and phylogeographic structure across the species' range, and how we are using this information to inform recovery actions in extirpated parts of the range in southern

California. Our findings support a center-edge effect, where interior populations in the central coastal California have higher genetic diversity, are less differentiated, and show greater conformity to a pattern of genetic isolation-by-distance compared to smaller and more geographically isolated populations at the southern terminus of the range, where the effects of genetic drift are pervasive. We also highlight results that challenge longstanding narratives about the species' historical biogeography and degree of genetic interaction with the northern red-legged frog *Rana aurora* in coastal Mendocino County.

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Identifying Drivers of Population Dynamics for Foothill Yellow-legged Frogs (*Rana boylii*) Using Time Series of Egg Mass Counts

Alteration of natural flow regimes by dams, water diversions, and climate change have been implicated in amphibian declines. Identifying drivers of amphibian declines requires long time series given high natural variability in abundance. Multiple population viability analysis (MPVA) models integrate abundance data from different populations to estimate how environmental factors influence population growth. Flow alteration has been linked to declines and extirpations in the Foothill Yellow-legged Frog (*Rana boylii*). No study has analyzed abundance data from populations throughout the range of *R. boylii* in an MPVA model. We compiled time series of egg mass counts from *R. boylii* populations in 36 streams and fit an MPVA model to quantify how streamflow metrics and stream temperature affect population growth. Population growth was positively related to stream temperature and was higher in the

years following a wet year with high total annual streamflow. Density-dependence was negatively related to streamflow seasonality, and density-dependence was weakest for intermediate rates of change in streamflow during the spring. Our results highlight how altered streamflow can further increase the risk of decline for *R. boylii* populations. Managing stream conditions to better match natural flow and thermal regimes would benefit the conservation of *R. boylii* populations.

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Assessing Environmental Noise and Hearing Abilities among Populations of Pacific Chorus Frogs (*Hyliola regilla*) along an Urban-Rural Gradient

Anthropogenic noise, or human-generated noise, has increased to unprecedented levels and is considered a hazardous form of pollution. This pollutant alters the acoustic environment and has been observed to negatively impact species that communicate acoustically. Many studies have examined how acoustic communication signals change to counteract the detrimental effects of anthropogenic noise. However, we know little about the physical properties of the noise and whether hearing abilities also change in response to human-generated noise. Using populations of Pacific chorus frogs (*Hyliola regilla*) across an urban-rural gradient in the San Francisco Bay area, I am examining variation in (1) the acoustic environment and (2) the ability of the auditory system to detect signals in noise. Initial analyses of the acoustic environment display temporal variation in sound intensity across frequencies of 250Hz to 4.0kHz. Between populations, my results show minor differences in hearing abilities in noise. This is one of the first studies to provide a detailed characterization of this novel pollutant and its potential effects on hearing abilities. As humans continue to expand beyond their current boundaries, it is crucial to understand how sensory systems respond to noise, to ensure that researchers can begin to enact plans for the conservation of species that may be detrimentally influenced.

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Conservation Genomics of California Amphibians and Reptiles: the California Conservation Genomics Project and Beyond

In late 2019 the state of California funded the California Conservation Genomics Project (CCGP), bringing a new level of genomic data and analyses to conservation issues across the state. The project includes 18 genera of amphibians and reptiles, sampled across their geographic and ecological range in California, that we are sequencing at the whole-genome level, bringing an unprecedented level of genetic information to the analysis of phylogeographic structure, patterns of high and low genomic diversity, and differentiation of genes that are important in climate adaptation now and in the future. In some cases, including the western pond turtle, western spadefoot, and glossy snake, these data can be combined with other data streams, primarily RADseq reduced representation sequencing, allowing us to determine whether the

gains in precision and accuracy from whole genome resequencing are worth the costs. We present a brief overview of progress to date on the CCGP, a calendar for completing this phase of the project, and some ideas on future directions.

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The Northern Leopard Frog (*Rana pipiens*) Still Occurs in California

The Northern Leopard frog (*Rana pipiens*) is a wide-ranging nearctic species that barely enters California east of the Sierra Nevada Crest. By the 1970s the frog was apparently extirpated from many historical locations in the American West, and in California had not been observed since 1994. Here we report on the presence of a presumed native population in the Walker River watershed, Mono County, California. Opportunistic surveys combined with auditory monitoring and other techniques are being employed to better characterize the population. Surveys in 2020, 2021, and 2022 documented very few adult frogs and juveniles. Breeding was documented in 2020, and one first-year frog (metamorph) was documented in 2021. Auditory monitoring in 2021 and 2022 resulted in 1.33 TB of sound files, and preliminary analysis has shown males calling in April 2021. A preliminary eDNA assessment failed to document the species at historical localities. Plans for habitat enhancement are now being considered at the known location. Of importance, this outlier population may harbor rare alleles or adaptations not present in more easterly locales.

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An Emerging Extinction Vortex in the Santa Barbara County Distinct Population Segment of the California Tiger Salamander, *Ambystoma californiense*

An extinction vortex is characterized by reduced population sizes leading to inbreeding and genetic drift, subsequent reduction in fitness and reproductive output, which feed into further population loss. Using landscape genetic tools, we have documented several hallmarks of an ongoing extinction vortex in the Santa Barbara County Distinct Population Segment of the California tiger salamander (*Ambystoma californiense,* SBCTS). First, we found evidence of reduced effective population sizes as compared to other tiger salamander populations in the Central Distinct Population Segment. Second, we examined the relationship of regional habitat configuration to genetic diversity and found that genetic diversity of a breeding pond was positively correlated with the number of neighboring ponds and the proportion of those ponds that were naturally occurring. Third, using controlled common garden experiments, we compared metrics of fitness and recruitment to a "healthy" Central Distinct Population Segment population of tiger salamanders, and found both reduced offspring survival and size at metamorphosis in

SBCTS populations. Taken together, our data suggest that the SBCTS is a species on the brink of collapse. Finally, we discuss potential management solutions, including our ongoing genetic rescue effort.

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Environmental DNA Detection of the California Tiger Salamander (*Ambystoma californiense*) on the Santa Rosa Plain

Environmental DNA (eDNA) is a monitoring technique that has been used more frequently in recent years as it is useful in the detection of rare species. We investigate its utility in the detection of larvae in a distinct population segment of the California Tiger Salamander (*Ambystoma californiense*) at Alton Mitigation Bank in Santa Rosa, CA. Our study compared the presence and absence detection of *A. californiense* using eDNA sampling and traditional dip net sampling, determined the relationship between eDNA concentration and larval capture rates, and investigated the possibility of residual eDNA signals remaining from the previous season. Presence and absence of *A. californiense* with both sampling methods matched, apart from three instances, indicating highly comparable utility. The probability of detection using eDNA based on dipnet data was 0.92. eDNA concentrations were positively correlated with the larval capture rates acquired by dipnetting. Finally, residual eDNA from the previous season was not detected in water and soil samples after the first major rain event of the year. eDNA is a novel monitoring technology that can expand the surveillance of *A. californiense* on the Santa Rosa Plain and could be used effectively to supplement traditional sampling methods for population monitoring.

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A Pond Array for Studying the Interactions of Native Pacific Newts (Genus *Taricha*) with California Red-legged Frogs (*Rana draytonii*) (PRESENTATION CANCELLED)

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 (<$200,000.00) within a short time frame (about 3 weeks). We discuss lessons learned and plans for initial experiments.

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Social Group Size Influences Pathogen Transmission in Salamanders

Individuals within animal societies are expected to mitigate the costs and enhance the benefits associated with group living. For example, sociality can facilitate the sharing of beneficial microbes among individuals, but can also increase transmission of pathogens, representing a major cost of group living. We examine the costs of sociality in the California slender salamander (*Batrachoseps attenuatus),* a terrestrial salamander which naturally forms close social aggregations. We investigate whether innate sociality (e.g., skin-to-skin contact) increases an individual's transmission risk of *Batrachochytrium dendrobatidis* (*Bd),* a fungal pathogen that emerged throughout the salamander's range over the last 50 years and has decimated hundreds of amphibian species globally. We found that in captivity, *B. attenuatus* exhibit random mixing within social groups, resulting in high contact rates and high potential for *Bd* transmission. Our experimental infection trials resulted in 50% mortality after one month in moist conditions. In order to test how group size affects pathogen transmission, we manipulated social group size and found a marked effect on the spread *Bd* among individuals; a single, uninfected individual contracted *Bd* much more rapidly in larger groups of infected individuals. Surprisingly, this did not translate into a more rapid death rate or higher pathogen infection loads. Our results show that the innate behavior of group formation represents a per-individual risk of socially acquired directly transmissible pathogens and is magnified in larger social groups. This study highlights one important cost of sociality in terrestrial salamanders and underscores the general susceptibility of social animals to novel invasive pathogens

POSTER PRESENTATIONS

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Land Use Impacts on Movement Patterns of California Tiger Salamanders (*Ambystoma californiense*) in a Vernal Pool Complex

Researching species in their native habitats is key to implementing effective conservation management for endangered species, particularly for fauna adapted to complex ecosystems such as vernal pools. Moving between terrestrial habitat to vernal pools, is a critical life history component for the endangered Sonoma County California Tiger Salamander (CTS- *Ambystoma californiense*). To better understand movements within a group of vernal pools surrounded by varying land uses, we employed a two-year pitfall trapping study. Pitfall traps and drift fences were installed around four pools on a preserve in Santa Rosa, CA. We investigated orientation of individual adult salamanders leaving their breeding pools, influences of surrounding land use on movement, and levels of among-pool movement. We found that individuals moved non-

randomly, maintaining a relatively consistent direction toward original entry points when exiting. Furthermore, the distance between entry and exits was significantly affected by surrounding land use; individual entries associated with rural residences tended to stray further, relative to entries associated with preserve and cattle grazed preserve. Additionally, initial salamander weight significantly affected the average distance between entries and exits. Within a breeding season, 10.8% of individuals visited a second pool; however, most individuals visited only one pool. Our results indicate that CTS move non-randomly, and that movement is influenced by surrounding land uses. Moreover, despite the high density of pools, among-pool visitation was low, indicating that the level of expansion into new breeding pools likely occurs slowly over time.

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In situ Treatment of Juvenile Frogs for Disease Can Reverse Population Declines

Effective management of wildlife populations threatened by disease requires accurate predictions about the consequences of intervention. However, generating such predictions is challenging, especially for organisms with complex life histories that are also threatened by climate change, such as montane amphibians. Cascades frogs (*Rana cascadae*) in northern California have experienced dramatic declines associated with the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), and remnant populations are also threatened by changing climate conditions. We evaluated the population-level impacts of treating Cascades frog metamorphs with the antifungal chemical itraconazole using a field experiment and population simulations. We explored the influence of larval habitat on these treatment effects by including metamorphs from different larval habitat types. We found that frogs treated with itraconazole were more than four times more likely to survive their first winter than untreated controls and had reduced *Bd* infection intensity compared to other surviving frogs from the same cohort in the following year. We also found an effect of larval habitat type on *Bd* infection in recently metamorphosed frogs, with the lowest levels of infection occurring in frogs emerging from larval habitats that tend to be intermediate in temperature and drying rate. Applying the differential apparent overwinter survival of treated and untreated metamorphs to population projections suggests that intermittent antifungal treatment of metamorphs has the potential to restore population viability. Our results indicate that in situ treatment of individual hosts may be a useful component of a comprehensive management strategy to reduce the risk of pathogen-mediated population declines and extirpations.

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Assessing Variation in Acoustic Signals and Auditory Processing in Pacific Chorus Frogs (*Hyliola regilla*) along an Urban-Rural Gradient

Environmental noise is an invisible yet ubiquitous feature of every habitat, but today anthropogenic noise caused by human activity is altering natural acoustic environments. This novel environmental stressor has led to concern over the effects it may have on animal behavior,

especially for acoustic communication. Acoustic communication involves senders who produce vocal signals that must be discerned by receivers, but anthropogenic noise may limit the transmission and perception of these signals. Our current understanding about the impacts of human noise on animal communication is limited to effects on sender signaling behavior, and there are no corresponding studies examining receiver's signal processing mechanisms. This study tests the hypothesis that signals and signal-processing mechanisms will be shaped in ways that facilitate efficient communication in noisy urban environments. In six populations of Pacific chorus frogs (*Hyliola regilla*) along an urban-rural gradient, we examine variation in acoustic communication signals and auditory sensitivity. Studies on both signaler and receiver responses to human noise pollution are essential to improve our understanding of its effects on animal behavior. These types of studies can inform the development of mitigation efforts as human expansion will only continue.

* Indicates presenter in multi-authored presentation