California/Nevada Amphibian Populations Task Force

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Photograph by Brian Todd

ABSTRACTS

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ORAL PRESENTATIONS

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Predicting *Batrachochytrium Dendrobatidis* Infection in Foothill Yellow-Legged Frogs (*Rana boylii*) in the Alameda Creek Watershed

The fungal pathogen *Batrachochytrium dendrobatidis* (Bd) has been identified as the primary factor in many amphibian declines around the world, yet its effect on lowland populations of California anurans, such as the foothill yellow-legged frog (*Rana boylii*) is poorly understood. *Rana boylii* has declined from more than half of its former range, but has not responded lethally to Bd infections in the laboratory. In the fall of 2013 we observed dead and dying juvenile *R. boylii* in the Bay Area's Alameda Creek, attributed to an outbreak of chytridiomycosis through high fungal loads observed on dying animals. Bd is known from the watershed as early as the 1960s, but the outbreak coincided with extremely low stream flows that concentrated frogs in drying pools and expanded the distribution of non-native bullfrogs (*Lithobates catesbeianus*). We aimed to determine whether variations in flow regime, drought, temperature, or invasive species influence Bd prevalence or load in *R. boylii* in the Alameda Creek watershed. We present the results of two years of post-outbreak sampling for Bd in this species. We used a mixed effects modeling approach to determine which environmental and biological factors best predict the intensity and prevalence of Bd infection in *R. boylii* at the Alameda Creek site.

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Reduced Survival of California Red-legged Frog Larvae from Populations Co-occurring with Introduced Bullfrogs

Invasive intraguild predators can have particularly strong adverse effects on native species, due to the combined pressures of predation and competition. Bullfrogs (*Lithobates catesbeianus*) are known to have these effects on native anurans, including the California Red-legged Frog (*Rana draytonii*). This study expanded on our previous work that showed that larvae of *R. draytonii* that had coexisted with invasive bullfrogs displayed a behavioral response to bullfrog chemical cues that evolutionarily naïve individuals did not show. To test whether this response was truly adaptive, we raised *R. draytonii* larvae in mesocosms with overwintered bullfrog larvae and examined the role that evolutionary experience and habitat complexity played in predicting *R. draytonii* survival and body condition. Surprisingly, evolutionarily experienced *R. draytonii* had lower survival and body condition, regardless of treatment, indicating that the presence of bullfrogs may have negative effects on *R. draytonii* populations even in areas where the two species coexist.

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Yosemite Toad Ecology: Insights from Long-Term Monitoring on Abundances, Demography, Movement, Habitat Usage, and the Effects of Drought

The Yosemite toad (*Anaxyrus canorus* [=*Bufo canorus*]), federally listed as threatened under the ESA, has been monitored since 2006 in multiple breeding meadows clustered within one watershed on the Sierra Nevada National Forest. A collaborative effort by the USFS Sierra Nevada Monitoring Program, Sierra Nevada National Forest, and Pacific Southwest Research Station has conducted capture-mark-recapture of breeding adults to monitor *A. canorus* demography, radio-tracking to monitor adult movement and habitat use, and groundwater and surface water measurements to characterize meadow breeding habitats. *Anaxyrus canorus* breeds in wet meadows at snowmelt, after which adults move to nearby terrestrial habitats. Populations of *A. canorus* were small with less than an estimated 20 adult males per meadow, abundances in several meadows appeared to be declining, and numbers in one meadow may be increasing. Egg deposition occurred in very shallow (median = 4 cm) warm water and egg masses commonly desiccated or froze. Survival of tadpoles to metamorphosis was related to surface water duration. Adults moved an average of 270 m from breeding areas and appeared to prefer open areas. Occupied terrestrial sites had less canopy cover and fewer woody species than unoccupied sites. Adults were philopatric to both breeding meadows and upland terrestrial areas. Preliminary analysis suggests the persistence of surface water may be relatively uncorrelated with water table depths. During the recent extended drought, breeding habitat was greatly reduced or completely absent and few adults were observed. Quantitative and qualitative results from this long term monitoring provide critical information for the management and conservation of *A. canorus*.

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Jump Back to the Santa Monica Mountains: Project Update for the California Red-legged Frog (*Rana draytonii*) Translocation.

Historical data suggests that federally Threatened California red-legged frogs (*Rana draytonii*) were in all of the major streams of Santa Monica Mountains National Recreation Area (SAMO). Currently, there is only one population of about 100 adult California red-legged frogs near the park, in the Upper Las Virgenes Canyon Open Space Preserve (ULV). The ULV population is genetically unique and distinct from other populations in Ventura and Los Angeles Counties, therefore it will be important to populate streams with local individuals. In 2011, we initiated a project to re-introduce California red-legged frogs into suitable stream habitats in SAMO via translocation of partial egg masses. Our goal is to establish new populations of California red-legged frogs in the Santa Monica Mountains, both to increase the overall population size and to create redundant populations which would prevent the extirpation of the species from the area if a catastrophic event occurred in ULV. We will discuss the results of the 2014 and 2015 translocations to two streams within SAMO, as well as the future plans for the project.

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Modifying Canopy Shading in the Riparian Zone during Timber Harvest: Preliminary Results from Coastal Giant Salamander (*Dicamptodon tenebrosus)* Monitoring

Timber management approaches establishing continuous dense mature riparian buffers along watercourses with the intent of providing cold water temperatures, high levels of large wood, and sediment filtration may overlook the importance of overall productivity in aquatic ecosystems. Here we introduce preliminary findings resulting from a pilot project in northwestern California evaluating the response of local instream productivity to riparian canopy thinning using a mark-recapture study of aquatic larval salamanders. Growth and movement of larval coastal giant salamanders (*Dicamptodon tenebrosus*) have been monitored over the past year within a 520 m reach of continuous stream habitat. Mark-recapture sampling was conducted utilizing visible implant elastomer and PIT tags in late summer and early winter prior to and shortly following canopy thinning. Recapture efforts have continued bimonthly following treatment to examine the effects of the changes in the riparian structure on salamander growth and movement. Overall, an average of 306 larvae have been located per survey, although only 19% of marked animals have been recaptured. The majority of recaptured larvae have demonstrated high fidelity to within-site location, although downstream movements as great as 488 meters have been documented. Examination of the effects of the riparian treatment on growth and movement has thus far been inconclusive. Further preliminary results on salamander growth, movement, and density will be presented surrounding this study as well in the context of general *D. tenebrosus* ecology.

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Giant Gartersnakes (*Thamnophis gigas*) Selectively Forage on Native Anurans, Despite High Abundance of Introduced Prey

The introduction of exotic species can alter trophic dynamics of native species. Threatened giant gartersnakes (*Thamnophis gigas*) forage on a prey community that is comprised almost entirely of exotic species. We examined prey selection of giant gartersnakes by analyzing trap by-catch and regurgitated gut contents of snakes in four basins in the Sacramento Valley of California to determine whether these snakes actively select their prey or if they are simply consuming what is abundant. Although non-native anuran and fish species dominate the available prey community, giant gartersnakes most strongly selected native Sierran treefrogs (*Pseudacris sierra*) metamorphs. If all prey types were equally available, giant gartersnakes would have selected Sierran treefrogs with a probability of 0.596 (0.0254 - 0.855). Giant gartersnakes also were more likely to select introduced American bullfrog (*Lithobates catesbeianus*) adults over all tested introduced fishes. Managing prey communities to increase native amphibian abundance within the giant gartersnake's range might benefit populations of this rare snake.

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Eradication of Invasive American Bullfrogs (*Lithobates catesbeianus*) in Yosemite Valley and the Planned Establishment of a Core California Red-legged Frog (*Rana draytonii*) Population

The American bullfrog has plagued Yosemite Valley for more than 50 years possibly contributing to the decline and extirpation of several native amphibian species. In 2015, Yosemite National Park successfully eradicated bullfrogs from Yosemite Valley and has opened a niche for other ranid species. Unfortunately, our only other known ranid to occupy the Valley, the foothill yellow-legged frog (*Rana boylii*), has been extirpated and attempts to identify a source donor population have not been successful. In addition, remaining habitat in Yosemite Valley is believed to be marginal at best for foothill yellow-legged frogs due to past human disturbance and reintroductions of this species may not be successful. The California red-legged frog (*Rana draytonii*) has been historically documented in Yosemite National Park, but not in Yosemite Valley. However, due to past human hydrological modifications, current restoration actions, and now the removal of bullfrogs, Yosemite Valley now has a suitable niche including breeding habitat to support the California red-legged frog. Through a combination of captive rearing and direct introductions, Yosemite National Park is attempting to establish a core California red-legged frog population in a portion of its former southern foothill range where it no longer occurs. If successful, the results of an established population in a protected National Park may allow for the establishment of additional California red-legged frogs in a critical portion of its historic range.

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Intra-Annual Trends in Reproductive Rates and Breeding Habitat Use of the Yosemite Toad (*Anaxyrus canorous*) in Yosemite National Park: Evidence of Drought Related Regional Reproductive Failure

Yosemite National Park is one of the oldest and largest pieces of preserved land in the United States. Yet, like most of the world, Yosemite's amphibian populations are declining. Since 2004, three of the park's six native pond-breeding amphibians were federally listed as critically endangered, endangered, or vulnerable. Unshielded from climate change, since about 1900 global warming has been associated with a > 50% reduction in the size of Sierra glaciers, increased temperatures by an average of 0.88 C, and decreased annual snowpack below 8,500 feet elevation. The reproductive strategy of Yosemite toads, i.e., explosive breeding following snow melts and rapidly maturing larvae, may make them susceptible to climate change impacts. In this study, we investigated inter-annual variation among meadows in breeding occupancy of Yosemite toads, as well as cohort fate among used meadows, during an extreme drought year (2015). Specifically, we used a paired random sampling design to survey a targeted sub-sample of the park's meadows 2-3 times throughout the season (with ~2 weeks between surveys). In our first survey, we found a lower proportion of breeding detections (34% occupancy) than found among the same meadows in the prior seven years (67% occupancy). However, we also detected high rates of inter-annual variability in breeding habitat use as breeding was detected in the second, but not the first survey in 31% of meadows. Saliently, we found almost complete reproductive failure at the low end of the species' elevational range due to meadows drying faster than the time needed for larvae to metamorphose.

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An Open Population Approach to Estimating Breeding Female Amphibian Abundance from Replicated Egg Mass Counts

Reliable inference about amphibian abundance requires accounting for surveyors' inability to detect every individual in a population. So-called imperfect detectability is not only an issue for mobile larval and adult life stages, but also for sessile eggs. Egg mass counts are a popular index of breeding female amphibian abundance, but surveys often do not account for imperfect detectability, and those that do usually incorporate multiple observers. We applied multi-state Jolly-Seber models to repeated egg mass counts by a single observer within a breeding season to estimate annual breeding female abundance of California Red-legged Frogs (*Rana draytonii*) at a pond in Point Reyes National Seashore. The method successfully estimated detection probabilities, entry probabilities, survival probabilities, the number of egg masses present at each survey, and the total number of egg masses laid in a breeding season, using Gosner stage data and flags to mark existing egg masses. Single-survey detection probabilities were generally > 0.85, with abundance estimates only very slightly greater than the total number of unique egg masses counted. The method is applicable to amphibian species that have extended breeding seasons and have females that only lay one egg mass per year, is less costly than using double-observer methods, and is likely applicable to many existing datasets. Limitations and possible model extensions will be discussed.

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Assessing the Relict Leopard Frog to Inform the Upcoming ESA Listing Decision

By the late 20th century, *Rana onca* (= *Lithobates onca*) was restricted to eight historical sites in two areas of southern Nevada, with only about 1100 frogs in existence. Speculations on the causes of this decline have focused on loss or degradation of habitat, introduction of nonnative predators, and recently epizootic disease. In 2001, a voluntary conservation team formed to conduct actions and develop plans to conserve *R. onca*. The species, however, was petitioned for listing under the Endangered Species Act in 2002, with the U.S. Fish and Wildlife Service (USFWS) determining that listing was warranted but precluded by higher priorities. By 2005, the conservation team had developed a formal conservation agreement and strategy (CAS). Management actions have predominately focused on monitoring populations, establishment of new populations, and modest efforts at maintaining or creating habitat. Recently, the USFWS entered into a multidistrict litigation settlement to conduct listing actions for more than 250 species, including *R. onca*. Under this litigation, the USFWS is currently conducting a Species Status Assessment of *R. onca* in order to inform a listing decision in 2016. We summarize more than a decade of management actions conducted under the CAS, and describe stressors and threats. Since systematic management began, remnant historical populations have been maintained, although augmentation has been considered necessary at some of these sites. A major negative impact was the decline of the largest historical population resulting from storm caused debris flows in 2006; this population has not yet recovered. Translocations have established *R. onca* at numerous experimental sites, with some of these sites now containing the largest populations. Overall, management actions have substantially increased

the number of sites occupied by *R. onca*, and monitoring indicates an increasing trend in relative abundance and a modest overall increase in actual population size.

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A Questionable Role for Amphibian Chytrid Fungus in the Decline of the Relict Leopard Frog

The decline of *Rana onca* (= *Lithobates onca*) has left remnant historical populations in geothermally influenced springs in two distinct areas of southern Nevada. Habitat loss or degradation and introduction of nonnative predators were associated with the decline. The limited distribution of *R. onca* within thermal water, however, led to speculation that epizootic disease may be an important factor. We conducted field sampling to detect the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) within the historical range of *R. onca*. We detected *Bd* within one area occupied by *R. onca*, but not in the other. We conducted laboratory experiments to assess the susceptibility of *R. onca* to infection from *Bd* isolates in the absence of confounding environmental factors. We used two isolates that were associated with severe anuran declines in California. We maintained two sample groups of *R. onca* during experiments, representing frogs derived from eggs collected in areas where populations differed in current exposure to *Bd*. Our experiments demonstrated that *R. onca* was susceptible to *Bd* infection under temperatures favoring pathogen growth. Survivorship, however, was not affected by *Bd* regardless of source population, with infected frogs appearing asymptomatic and most (64%) clearing infections. In the field, we observed several *R. onca* with *Bd* infections that survived for at least 8–13 months after initial testing, with one frog clearing their infection. Our data suggest that *R. onca* has inherent resistance to *Bd* and chytridiomycosis or has evolved such resistance. We speculate on the possibility of attenuation of the *Bd* isolates we used and also caution that the resistance we observed under laboratory conditions may not emulate situations in the wild.

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The Ecology of the Sierra Nevada Yellow-legged Frog (*Rana sierrae*) on the Lassen and Plumas National Forests

In the northern Sierra Nevada, the federally endangered *Rana sierrae* is found in few locations with small abundances. Developing conservation options is difficult due to the limited knowledge of *R. sierrae* ecology in this region, particularly in stream habitats. Addressing this information gap was identified as a high priority in the recently completed Mountain Yellow-legged Frog Conservation Strategy. The USDA Forest Service has initiated a study investigating population abundance, habitat requirements, and movement patterns in stream and lake habitats on the Plumas and Lassen National Forests. Visual encounter surveys were conducted June-October, 2015 at 10 lakes and 13 streams and 85 adult frogs were marked with PIT tags. The highest abundances were found in lake habitats where an average of 11.2$\pm$14.5 adults were found per lake compared with an average of 4.1$\pm$2.9 adults found per stream reach. Evidence of reproduction was observed in 2 of the 5 occupied lakes, and 2 of the 7 occupied stream reaches, though only a few tadpoles were found at each location. In streams, *R. sierrae* was found more often in pool habitats including 100% of frog observations in lake outlets and 61% of frog observations in streams. Frogs moved overland between lakes about 200 m on two occasions. On one stream, recaptured

frogs moved as little as 10 m and as great as 1645 m from their original point of capture. Data from this study will provide the basic ecology needed to provide direction for development of conservation actions on the Lassen and Plumas National Forests.

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Invasive Species Removal Increases Threatened California Red-Legged Frog Abundance and Recruitment: Insights into Post-Invasion Restoration

Nonnative invasive species often have detrimental effects on native ecosystems and species. Eradicating introduced species, however, can disrupt established ecosystem processes, such as predator-prey interactions. Field trials of invasive species removal, followed by time-series data on the abundance of the native species, inform responses of target species for conservation after control measures are applied. The threatened California red-legged frog (*Rana draytonii*) and the Sierran chorus frog (*Pseudacris sierra*) suffer predation and competition pressures from invasive fish and American bullfrogs (*Lithobates catesbeianus*). We examined the effects of eradicating the exotics on the abundance and recruitment of *P. sierra* and *R. draytonii*. We conducted dependent double-observer surveys during and after the invasive species were removed between 2014 and 2015. Our results showed that the estimated abundance of adult *R. draytonii* per survey increased from 0.18 (95% highest posterior density interval, 0 – 0.89) in 2014 to 6.98 (4.82 – 8.82) in 2015. Recruitment of the native anurans occurred only after the fish and larval *L. catesbeianus* were extirpated. Over a similar period, *R. draytonii* abundance at reference ponds decreased, and the recruitment of the two native anurans occurred both years. The estimated abundance of *L. catesbeianus* steeply declined after the first-year removal. Continued removal can directly facilitate the restoration of *R. draytonii* and *P. sierra* populations, while additional monitoring is necessary to determine whether this trend is sustained over the long term.

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Ecology of the California Red-legged Frog (*Rana draytonii*) in Coastal Dune Systems

More than 60% of the coastal dunes at Point Reyes National Seashore have been invaded by non-native plants such as European beachgrass (*Ammophila arenaria*) and iceplant (*Carpobrotus edulis*), threatening one of the largest remaining expanses of native dune plant communities in California. The National Park Service has begun restoration of this native dune ecosystem by removing these invasive plants, but little was known about how California red-legged frogs (*Rana draytonii*) used this habitat. We surveyed 25 sites in 20 seasonal drainages that run through dunes with independent double surveys, and found California red-legged frogs in 90% of the drainages. For a more detailed understanding of habitat use, we attached radio transmitters to 22 frogs in 3 of these drainages between April – September 2015, and we collected habitat data on paired use and random points. Frogs used locations that had less of the invasive, non-native plants than paired random locations (European beachgrass mean used 3% vs. random 16%; iceplant mean used 8% vs. random 18%). Mean distance moved between initial capture and last known location was 78 m (range 0 – 620 m). All frogs remained in the drainage where we initially caught them, even though the habitat dried considerably throughout the study. The results of this study will inform the Park Service how to protect the California red-legged frogs while they proceed with dune restoration, and sheds light on the frog's use of an under-studied habitat that exists elsewhere in its range.

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Disease Intervention and Captive-Rearing of Mountain Yellow-legged Frog Populations in Sequoia and Kings Canyon National Parks

Both federally endangered species in the mountain yellow-legged frog complex (*Rana muscosa* and *Rana sierrae*) occur in Sequoia and Kings Canyon National Parks (SEKI). A substantial body of research has shown nonnative trout and the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) are the primary threats to mountain yellow-legged frog populations. Although trout eradication has been successful in recovering several frog populations, frogs remain vulnerable to *Bd* outbreaks. During the summer of 2015, we conducted two emergency intervention efforts to help avert extirpation of mountain yellow-legged frog populations: 1) reducing *Bd* infection intensities with anti-fungal drugs during a *Bd*-caused mass die-off event, and 2) collection for captive-rearing of early life-stage frogs from two populations that continue to decline following past *Bd* outbreaks. In the field *Bd*-treatment experiment, adult frogs collected from two adjacent lakes were held in pens for a 10-day period, during which frogs were treated with the anti-fungal drug itraconazole. One week later, another round of adult frog treatments were completed in a nearby lake. During the course of both treatment periods, *Bd* loads declined dramatically. For captive-rearing efforts, late-stage tadpoles and recent metamorphs were collected, flown out of the backcountry via helicopter, and delivered to captive-rearing facilities by staff from the Oakland and San Francisco Zoos. Collected individuals experienced low mortality during the transfer and subsequent anti-*Bd* treatments. We plan to release mature frogs back to the collection locations when frogs reach adult size. Ongoing research continues to refine these methods, which are anticipated to help populations persist, despite the presence of *Bd.* SEKI is finalizing a long-term, landscape scale restoration plan, which seeks to use the complementary techniques of trout removal, anti-*Bd* treatments, translocations, and reintroductions to recover these endangered amphibians.

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Spatial Transferability of Yosemite Toad (*Anaxyrous canorus*) Habitat Models across National Park Lands in the Sierra Nevada

Having clear understandings of the spatial distribution and relationship between species and their habitat is essential for any conservation effort. One of the most common ways to address these topics is through the creation of species distribution and habitat suitability models. Meaningful evaluation of these models requires testing on spatially independent datasets, especially if the desired outcome is the ability to make species–habitat generalizations that can inform management decisions. We tested the performance of a Yosemite toad occupancy model trained in Yosemite National Park and transferred to a spatially independent location in Kings Canyon National Park. Model performance on holdout data from Yosemite (previously published) was extremely high (84% predicted occupancy), whereas model performance in Kings Canyon was low (46% predicted occupancy). Assessment of the environmental data showed Yosemite and Kings Canyon having non-overlapping environmental envelops for available habitat between the parks. We suggest that a simpler model trained on variables deemed important through niche analyses can improve both our ability to extrapolate to other regions within the species range and make ecologically meaningful inferences about species habitat. By doing so we can create habitat models that better inform the conservation and management of the species.

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Using the Endangered Species Act to Protect Rare Amphibians and Reptiles in California and Nevada

The Center for Biological Diversity works to secure a future for all species, great and small, especially those hovering on the brink of extinction. By petitioning the U.S. Fish and Wildlife Service to provide Endangered Species Act protection for imperiled amphibians and reptiles - and following up with lawsuits when necessary - the Center is working to obtain federal safeguards and protected habitat for herps in California and Nevada and across the country. Through hiring its second full-time attorney and biologist dedicated to conserving amphibians and reptiles, the Center is expanding its campaign to address the amphibian and reptile extinction crisis. In this presentation, Jenny discusses the Center's work to protect turtles, snakes, frogs, lizards and salamanders in California and Nevada. These efforts include filing the largest-ever Endangered Species Act petition focused on amphibians and reptiles, securing habitat protections and recovery plans for listed species, advocating for salamander import restrictions to prevent the introduction of Bsal into the United States, and litigating to secure restrictions on pesticide use in endangered species habitats.

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Historical Isolation and Future Adaptation: Using Evolutionary Genomics to Conserve the Yosemite Toad

Amphibian conservation in the 21st century is beset by many anthropogenic impacts including habitat alteration, chemical toxicants, pandemic disease, and climate shifts. The Yosemite Toad (*Anaxyrus canorus*) is a species of meadow-specializing amphibian endemic to the high-elevation and federally protected Sierra Nevada of California, yet approximately 50% of its populations have been recently extirpated. Although limited evidence exists for species-wide declines due to pesticide use or disease, climate change is projected to have a major hydrological impact on its core meadow habitat. In this study, we use double-digest RADseq to produce 1000s of unlinked nuclear SNPs along with numerous remotely-sensed habitat attributes in a landscape genetics framework to answer four primary questions: (1) At what spatial scale(s) should Management Units be delineated? (2) Which environmental, topographic, and climatic attributes most facilitate meadow connectivity? (3) How do node-based (meadow) versus link-based (landscape) habitat attributes contribute to genetic connectivity among toad populations? (4) Do toad meadows exhibit source-sink genetic structure, and how will this shift with climate change? We apply a novel gravity modeling approach along with a graph-theoretical network analysis that has wide application to modeling microevolutionary dynamics in patch-separated amphibian populations. Our results reveal an ancient genetic rift and contact zone within Yosemite, and separate environmental drivers of gene flow among four phylogeographic groups of toads. We find that meadows with higher moisture regimes produce larger tadpoles and toads with higher site fidelity. Our climate models predict that southern Yosemite will be least impacted by climate change, while western and northern Yosemite will become fragmented and decentralized within the meadow network. Our results will have significant utility for prioritizing the future management and conservation of this iconic California native.

MOUNTAIN YELLOW-LEGGED FROG AND YOSEMITE TOAD WORKING TEAMS.
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Current Status of *Rana sierrae, Rana muscosa,* and *Anaxyrus canorus* Conservation

On April 29, 2014, the Sierra Nevada yellow-legged frog (*Rana sierrae*) and the southern mountain yellow-legged frog (*Rana muscosa*) were listed as federally endangered under the Endangered Species Act, and the Yosemite toad (*Anaxyrus canorus* [=*Bufo canorus*]) was listed as threatened. Conservation efforts for these species began as early as the late 1990s and early 2000s when collaborative multi-agency working teams were formed to develop Conservation Assessments intended to guide conservation strategy and recovery planning for the species. Team members include US Fish and Wildlife, USDA Forest Service, and National Park Service biologists, and species experts. Conservation Assessments for these species are now published and the Conservation Strategy for *R. sierrae* and *R. muscosa* is close to completion. The Conservation Strategy for *R. sierrae* and *R. muscosa* identifies potential actions to restore populations (e.g., fish removal to increase amounts of available habitat, strategic re-introductions of frogs, disease mitigation), management practices for routine land-use activities that facilitate recovery, and inventory, monitoring, and research priorities. It includes an action plan organized at three spatial scales, the species' ranges including the five genetic clades, national forest and national park administrative units, and frog conservation areas where specific actions are identified. The Strategy is intended to provide guidance and prioritization for conservation actions while retaining implementation flexibility. The Conservation Strategy for *A. canorus* is in the early stages of development.

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Yosemite Toad (*Anaxyrus canorus* [= *Bufo canorus*]) Breeding Occupancy of Meadows in Yosemite and Kings Canyon National Parks, CA

The Yosemite toad (*Anaxyrus canorus*) is listed as threatened under the U.S. Endangered Species Act due in part to evidence that its site occupancy has declined by about one half from historical levels. To better understand patterns of Yosemite toad breeding occurrence, we conducted park wide surveys in Yosemite and Kings Canyon National Parks from 2009-2014. We then evaluated how breeding occupancy patterns compared with those conducted a decade earlier and how our survey results compared with historical museum records dating back to the 1900's. We found current breeding by *A. canorus* was detected at 213 of 1336 meadows (16%) at Yosemite, and 27 of 508 meadows (5%) at Kings Canyon. These sites constitute approximately one-third of all breeding sites across the species' range. Current surveys at Yosemite resulted in significantly higher occupancy rates (15%) among the 446 sites recently surveyed a decade earlier (10%). Breeding was detected at 54% (25) of the 46 meadows where breeding was recently detected, and at 10% (40) of the 400 meadows where breeding was recently undetected. Thus, by including sites where breeding was recently undetected, the current number of meadows with detections was increased by more than half (40 of the 65 current detected sites). Current surveys detected breeding at 4 of 20 historical sites (20%). Current detection rates were much higher for sites that had multiple years of historical collections (3 of 6 sites, 50%) than sites with only one year (1 of 14 sites, 7%). Current occupancy rates at Kings Canyon (5%) were not significantly different from recent rates (7%) at the 247 sites surveyed a decade prior. Similar to the results from Yosemite, over half (7 of 12) of the total breeding detections occurred at sites where breeding was undetected a decade earlier. The only historical record was from a single site in 1942 and breeding was detected in the current surveys. These results indicate that during the past decade breeding occupancy on National Park Service lands has either increased or remained the same, a result that was possible only because previously undetected sites were included in the current survey.

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Population Genetic Structure of the Endangered Sierra Nevada Yellow-legged Frog (*Rana sierrae*) in Yosemite National Park Based on Multi-locus Nuclear Markers

The mountain yellow-legged species complex (*Rana sierrae* and *Rana muscosa*) has declined precipitously in distribution and abundance during the past century. A clear picture of the genetic structure of remaining populations is critically needed to inform future restoration actions. In this study, we conducted a population genetics study focused solely on the Sierra Nevada yellow-legged frog (*R. sierrae*) in Yosemite National Park. We collected samples from 23 sites across the park using non-invasive swab sampling, and obtained genetic data from mitochondrial and nuclear DNA via sequencing. Data were analyzed to describe the distribution of genetic variation in a geographic context. Our mitochondrial DNA-based results confirm the results from a previous study that two haplotype groups occur in Yosemite, one in the Tuolumne River watershed and one in the Merced River watershed. Results from our nuclear DNA-based analysis provided a more nuanced perspective. Specifically, based on nuclear DNA the overall genetic structure of *R. sierrae* across Yosemite is one characterized not by prominent breaks between watersheds but instead one primarily of isolation-by-distance, in which genetic differentiation is correlated with geographic distance between sites. Additional genetic structure associated with geographic features was also evident. These results provide a much more detailed description of the genetic structure of *R. sierrae* populations across Yosemite than was available previously, and will provide critical information for the design of recovery actions for this endangered frog, including translocations and reintroductions.

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The Effect of Climate and Habitat Use Patterns on Chytrid Infection Dynamics in Remnant Cascade Frog (*Rana cascadae*) Populations

Understanding the effects of endemic chytridiomycosis, the disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has become important for threat assessment and conservation of many at-risk species. This task is challenging because the population-level impacts of chytridiomycosis are thought to depend on environmental context. We conducted an in-depth study of the impact of Bd on remnant populations of the Cascades frog (*Rana cascadae*) in northern California in order to understand the effect of environmental drivers on Bd infection and frog population dynamics. We used mixed-effects models to explore the relationship between climate/habitat variables and Bd infection, and mark-recapture models to investigate frog population trajectory and the effect of infection on individual survival. Bd was present in all years at all of the sites where Cascades frogs persist in the southern Cascade Range. Estimated population sizes at our largest three locations have decreased substantially since 2008 with Bd at least partially responsible for decreased survival. In particular, recruitment to adult life stages has been greatly affected by Bd. Our analysis of the environmental drivers of the probability of Bd infection indicate that climatic variables influence infection -- the probability of Bd infection decreased with increasing water temperature at the site of capture and increasing mean daily maximum air temperature in the 4 weeks prior to capture. These results suggest that warmer weather has the potential to ameliorate the effect of Bd on declining remnant Cascades frog populations.

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Translocation of California Red-legged Frog to Restored Critical Habitat, Solano County: Methods, Challenges, and Preliminary Results

California Red-legged Frog (*Rana draytonii*: CRLF) is a federally threatened species and considered a California Species of Special Concern. Historically, efforts have focused on preservation and restoration of occupied habitats. However, little has been done with amphibian translocations to unoccupied habitat as a recovery tool for listed species. WRA is implementing a phased restoration of 4 of 12 ponds located on Ridge Top Ranch Conservation Bank - a 744-acre working cattle ranch. Surveys in 2010 concluded CRLF to be absent and additional due-diligence found invasive predators and abiotic impediments to be absent as well. Beginning in October 2010, two pilot ponds were fenced, planted with select vegetation, and irrigation installed. Two additional ponds were fenced and seeded in autumn 2012. In consultation with US Fish and Wildlife Service, WRA translocated egg-masses to the restored ponds in 2012 and 2013. Preliminary data suggest the efforts will ultimately be successful despite drought-related challenges. Population monitoring over the next five years, including mark-recapture data, will provide information on demography, dispersal, colonization, and habitat usage to further inform the recovery the species.

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Effects of Season on Ability to Detect Coastal Tailed Frogs (*Ascaphus Truei*) Using an eDNA Approach

Environmental DNA (eDNA) sampling techniques have been developed to increase the sensitivity of detection of amphibian species in aquatic environments. Compared to traditional sampling methods, eDNA techniques are more cost effective, reduce stress on the animals, require less time in the field, and have higher detection rates. Various environmental factors may affect eDNA concentrations, and consequently influence the sensitivity of detection of aquatic amphibians. This research assessed the effect of season on the efficacy of eDNA sampling for Coastal Tailed Frogs (*Ascaphus truei*). Coastal Tailed Frogs are small, cryptic amphibians that can be found in cold, fast-moving streams. Their sensitivity to environmental conditions makes them a valuable tool for evaluating stream health. At three streams in Northern California, we performed relative abundance surveys using a "rubble rousing" technique and collected eDNA water samples every 100 m throughout a reach of approximately 2 km. The number of samples taken fluctuated seasonally due to varying water flows. In addition, we collected data on pH, water temperature, flow rate, and turbidity of streams. Each water sample was tested for *A. truei* DNA with two species-specific primers and analyzed using quantitative PCR. The eDNA sampling and relative abundance surveys had high detection rates of *A. truei* during the months of July, September, and early October for all streams (>90%). During mid-winter months the detection rate for eDNA sampling fell below 70%, which we attribute to a large increase in water flow for all streams. The relative abundance surveys maintained a detection rate of 90%. Stream pH, water temperature, and turbidity remained fairly constant for each stream during the three sampling seasons. Our results show that eDNA techniques are efficient for monitoring aquatic amphibians during the spring and summer, but they are not recommended for use during winter months.

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Ecological Consequences of Disease-driven Decline of Mountain Yellow-legged Frogs (*Rana muscosa* and *Rana sierrae*)

The endangered mountain yellow-legged frogs (*Rana muscosa* and *Rana sierrae*) have declined in abundance and distribution, most recently due to disease-driven local extinctions caused by the amphibian chytrid fungus. Local extinctions of these frogs have the potential to affect other taxa in the lake community, as frogs can be abundant, highly connected consumers that occupy a high trophic position in the lake food web. To explore community responses to mountain yellow-legged frog declines, we conducted several studies in fishless Sierra Nevada lakes. Tadpoles reduced algal abundance in mesocosm experiments, but had no effect on algal abundance in within-lake grazing experiments. Through aggregation behavior and excretion, tadpoles can create spatial-temporal heterogeneity in ammonia, which has potential to benefit nitrogen consumers like diatoms. However, within-lake experiments indicated that tadpole aggregations do not strongly affect diatom community diversity and composition. Lastly, we observed benthic macroinvertebrate communities in 22 lakes in a five-year natural experiment. Macroinvertebrate communities in these lakes were similar regardless of the presence or absence of frogs and tadpoles; no taxa exhibited release from competition or predation, and there were no invasions and few secondary extinctions. Overall, declines of mountain yellow-legged frogs had weak top-down effects on lake communities, which may result from resource limitation and food web structures typical of high elevation lakes. However, these same characteristics suggest that frogs' aquatic and terrestrial predators may respond strongly to mountain yellow-legged frog declines.

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A Novel Conceptual Model Predicts Disease Risk in Amphibians

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has caused the greatest known wildlife pandemic, infecting over 500 amphibian species. Over a decade of research has shed light on how host amphibians, *Bd*, and environment interact to cause declines. But predicting which species are susceptible to chytridiomycosis and where has remained elusive. Here, we introduce a novel conceptual model that explains differential susceptibility of ectothermic hosts to a generalist pathogen. Our model predicts that, given a conducive environment, infection risk will decrease as an amphibian host's thermal tolerance exceeds that of the *Bd* pathogen. We tested this prediction using a global database of *Bd* infection prevalence representing 11,435 assays from 53 species across five continents. Host amphibians with higher thermal tolerances had lower *Bd* infection prevalence than did hosts with lower thermal tolerances. The relationship between host thermal tolerance and infection prevalence was generalizable across multiple amphibian families and spatial scales, and depended on environmental context, but host thermal tolerance was always the single best predictor of infection risk. Our study is the first to identify how variation among host species in their thermal tolerances underlies differential disease susceptibility to describe which species are likely to succumb to chytridiomycosis and where.

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An Overview of Recent Foothill Yellow-Legged Frog (*Rana boylii*) Research in Northern California

The Foothill Yellow-legged Frog (*Rana boylii*) is a species of concern in Oregon and California and was petitioned for federal listing in 2012. The USFWS found that there was substantial evidence for further investigation and a detailed review of the species and its conservation status is currently being conducted. We recently completed a multi-agency conservation assessment for *R. boylii* in California. The assessment will be released as a General Technical Report in the upcoming months. While water development and diversions have been well-documented as a primary cause of population declines, the assessment identified information gaps that include a better understanding of how the breeding ecology of the species relates to modified flow regimes and temperatures. Here, we summarize current and on-going research on foothill yellow-legged frogs in Northern California by Pacific Southwest Research Station scientists and collaborators. In a study within the Trinity River watershed, we found that oviposition, hatching success, and metamorphosis occurred later, and metamorphs were smaller and leaner along the regulated and colder mainstem relative to six unregulated tributaries. In two additional studies, water temperature appeared to influence the onset and patterns of breeding activities. Later breeding activity delays metamorphosis and may have future consequences such as lower over-wintering survival of post-metamorphic frogs and smaller size at maturity. Management of reservoir-controlled rivers should account for the influence of the thermal regime on the onset of breeding and patterns in oviposition and calling activities, and the development of vulnerable embryonic and larval life stages to improve outcomes for declining frog populations.

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An Evaluation of Wet Meadow Hydrogeomorphology for Sensitive Amphibian Species Habitat

Meadow restoration is an important tool for recovering wet meadow habitats within degraded systems, yet how to specifically design restoration to benefit conditions for highly at-risk native Sierra Nevada amphibians has not been investigated. In particular, information on their habitat requirements and the hydrogeomorphic processes that sustain those conditions is lacking. As part of a collaborative study characterizing the habitat suitability requirements for two sensitive species, the Yosemite toad and Cascades frog, we are evaluating the hydrogeomorphic processes that create and maintain wet meadow habitat. Current and potential meadow conditions are constrained by the primary hydrogeomorphic processes within the meadow system. There are two primary hydrologic sources in montane wet meadows: inputs from surface water and inputs from groundwater. In most locations feedbacks occur between surface and sub-surface hydrologic sources seasonally, but many meadows will be dominated by one primary source. Using a variety of traditional and new techniques, we are measuring seasonal and annual hydrological characteristics and hydrogeomorphic processes at a subset of three occupied meadows in each focal species' range over the next three years. Methods include traditional groundwater monitoring via wells, soil stratigraphy analysis, aerial and near-infrared imagery analysis, timelapse photography of meadow conditions, surface water monitoring, water quality analyses, topographic mapping, hydrogeomorphic typing, and surface thermal monitoring. Information gleaned from these techniques, in conjunction with developed habitat suitability models, may better define the physical processes necessary to maintain appropriate habitat conditions so that meadow restoration for focal amphibians may be self-sustaining.

POSTERS

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The Sierra Madre Yellow-legged Frog (*Rana muscosa*), Moving Forward

The U.S. Geological Survey has conducted research to aid in the conservation and recovery of the Sierra Madre yellow-legged frog (*Rana muscosa*) in southern California for the past 15 years. This research, based on monitoring the remaining populations, has documented declines, developed a better understanding of decline mechanisms, evaluated disease, developed restoration tools and techniques, and refined captive breeding and release protocols. We are now using this knowledge to implement restoration actions across the historic footprint of the species with a broad group of relevant government agencies, NGOs, and other stakeholders. Currently we are developing predictive models based on 15 years of field data to help make informed management decisions on future restoration actions and conservation goals.

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Using Genetic Tools to Investigate Distribution and Connectivity of Two Sierra Nevada Amphibians, *Rana sierrae* and *Rana boylii*

Numerous amphibian populations throughout the world are declining and genetic tools can be useful for informing conservation and management actions for these threatened species. In the United States, the Sierra Nevada yellow-legged frog (*Rana sierrae*) and foothill yellow-legged frog (*Rana boylii)* have experienced large drops in population size. *Rana sierrae* is currently listed as endangered under the U.S. Endangered Species Act and *R. boylii* is under consideration for listing. One issue of management concern for these species is that records for the distribution of these two yellow-legged frogs are sometimes unclear as to which species was present. We are developing species specific environmental DNA (eDNA) assays to test these questionable sites in Plumas National Forest, California. In this project, eDNA consists of genetic material captured by filtering water that can be used to detect rare and elusive species by signaling presence of the species of interest. We will also be looking for evidence of hybridization between these species, as biologists conducting surveys in the area have found individuals with phenotypes intermediate to the two species. We will use microsatellite loci to investigate potential hybridization along this overlapping portion of their ranges, as breeding between these two frogs would be an added complication in conservation efforts. To further aid in the conservation of endangered *R. sierrae*, we will investigate gene flow and population structure to inform possible translocation efforts. Lake populations of *R. sierrae* are more numerous than its stream dwelling counterparts and we will investigate whether genetic differentiation counter-indicates translocation between the two aquatic systems.

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Foothill Yellow-legged Frog and Local Gravel Mining: Creative Coexistence

One of the healthiest known populations of the foothill yellow-legged frog (*Rana boylii,* FYLF), a California Species of Special Concern, is located in Greenhorn Creek in the western foothills of the Sierra Nevada in Nevada County, California. The special thing about this particular population of FYLF is that it exists alongside a local family owned and operated gravel mining operation, Hansen Brothers Enterprises (Hansen Bros.). Like the frogs, Hansen Bros. has been using this creek for their livelihood for over 60 years. This Project illustrates how the forward thinking and stewardship of a local company, along with the flexibility and creativity of a government agency, can lead to proactive and adaptive management allowing for the peaceful coexistence of two typically combatting areas of industry and conservation science.

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An Issue of Life or Death: The Use of Environmental DNA to Detect Viable Individuals in Wilderness Restoration and Management

The analysis of environmental DNA (eDNA) is a promising tool to aid in conservation projects concerning the eradication and management of aquatic invasive species. The method of analyzing aquatic eDNA samples to detect species is very sensitive but currently does not distinguish between live and dead animals. Invasive fish removal projects in the historically fishless lakes of Yosemite (YOSE) and Sequoia Kings Canyon (SEKI) National Parks provide a unique opportunity for eDNA experimentation and methodological validation. Both parks have restored lakes that only contain dead fish, because during fish removal fish carcasses are thrown back into lakes to facilitate nutrient cycling. Testing restored and non-restored sites will validate that eDNA methods can differentiate between DNA that comes from viable versus non-viable sources. To determine the best method for detecting eDNA from live fish, we sampled water from fish tanks containing live and dead goldfish using filters with different pore sizes. Preliminary results suggest dead fish DNA is degraded and smaller sized than live fish DNA. We found that 1.2µl polycarbonate track etch membrane (PCTE) filters still detected DNA from live fish and were less likely to detect DNA from dead fish than filters with smaller pore sizes. Throughout the 2015 summer, we collected water samples from all of YOSE and SEKI restoration sites using 1.2µl PCTE filters. Further analysis is required before conclusions can be made; however, these techniques could increase efficiency of ongoing fish removal efforts by the National Park Service and other agencies facing similar problems with invasive species.

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Coastal Tailed Frog (*Ascaphus truei*) Nest Sites and Microhabitat Associations in the Redwood Timberlands of Northern California

Endemic to the Pacific Northwest, Coastal Tailed Frogs (*Ascaphus truei*) are known to inhabit cold, fast-flowing forested streams. As a result of this they tend to deposit their eggs in well sheltered areas, usually on the undersides of cobble or boulder, making nest sites difficult to find. Due to the cryptic nesting habits of *A. truei*, there is a lack of information regarding oviposition sites, especially in northern California. To our knowledge, only seven nest sites have been documented in northern California. Here

we present information on oviposition microhabitat for 13 new *A. truei* nest sites discovered in the coastal region of northern California.

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Assessing the Need for Endangered Species Act Protection of the Foothill Yellow-Legged Frog (*Rana boylii*): What Do Breeding Censuses Indicate?

In response to a USFWS request for information regarding *Rana boylii*, we compiled data for this riverine frog which has disappeared from more than half its range. Because *R. boylii* females lay a discrete and readily countable clutch of eggs, a standard practice among researchers, agencies, and utility companies is to assess abundance by making annual spawning censuses. We used data from the last 25 years collected at 49 sites in California which met our criteria (experienced surveyors, multiple visits, extensive searches). Southern Sierra and Coast Range populations are apparently so sparse that we found no quantitative monitoring data. Comparisons among locations revealed that population size is significantly reduced where thermal and flow regimes are modified by dams and fluvial habitat is fragmented by reservoirs. To determine whether populations show directional changes through time, we used generalized least squares models for 20 time-series with ≥ 4 consecutive observation years. While declines occurred in two free-flowing rivers and increased in another in recent drought years, the best-fit models (using maximum-likelihood estimation) did not generally identify significant trends in abundance across geographic locations or flow modification. The absence of consistent trends, however, should not be misconstrued as stability. Volatility, as measured by median coefficient of variation, was 66.9% in regulated vs. 41.6% in unregulated rivers. When volatility is combined with small population size, trends may not be detectable until populations decline below critical thresholds and cannot recover from extirpation risks common to managed rivers such as extremes in streamflow, disease, and non-native predators.

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Lexicon of Love: Vocalizations in Multiple Populations of *Rana boylii*

Vocal communication in frogs has many purposes including securing territory, warding off intruders or competitors, and finding mates. *Rana boylii*, the focus of this study, primarily call under water due to their small vocal sacs. Consequently, full characterization of their call spectrum has proven challenging. Little is known about the vocalizations of this species, apart from one preliminary study conducted in a single population. This previous study distinguished five distinct calls made by *R. boylii*, but did not characterize variation among populations. I examined variation in call types within and among populations of *R. boylii* during the mating season and compared them for differences across three geographically isolated populations. I recorded underwater vocalizations using a hydrophone and analyzed dominant frequency, duration, and note/pulse duration using the bioacoustics software Raven Pro. At least one previously undescribed call has been identified from one site, and characterization of differences among populations in predominant calls is ongoing. Knowledge of mating communication habits is essential to inform potential future translocation efforts for this rapidly declining frog.

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Environmental DNA Sampling as an Alternative to Traditional Sampling Methods to Determine Occupancy of Stream-associated Amphibians in Headwater Streams

Appropriately managing landscapes requires accurate occupancy information for species of interest. With environmental DNA (eDNA) techniques, biologists can use easy-to-obtain water samples and habitat covariates to model species' occupancy across a landscape. From 2000-2003, Welsh and colleagues surveyed 60 south fork Trinity River headwater tributaries to determine occupancy of tailed frogs (*Ascaphus truei*) and giant salamanders (*Dicamptodon tenebrosus*). Here, we resurveyed these sites using traditional rubble rousing methods in 2013 (n=22) and eDNA sampling methods in 2014 (n=58) in order to contrast the two methods. Restricting the analysis to the 22 sites visited in 2013, traditional and eDNA methods achieved similar per observation detection probability estimates (eDNA observation: 2 L water sample; traditional observation: 5 1-meter belts) when assuming site occupancy is unchanged between the two years (0.68 vs 0.62 for *A. truei*; 0.77 vs 0.85 for *D. tenebrosus*). For the 58 eDNA sampled streams, our occupancy models suggest *D. tenebrosus* detection was not dependent on environmental covariates, while detecting *A. truei* depended on stream temperature and time between sampling and filtering water samples (both of which accelerate eDNA degradation). Removal of a thermal regime covariate from the occupancy side of the model caused *A. truei*'s detection probability to be underestimated and occupancy to be drastically overestimated, demonstrating the importance of carefully designed models; unmodeled heterogeneity can bias detection and occupancy estimates. Modern techniques are shifting some of the workload from the field to molecular laboratories and computer analyses but it is still critical to incorporate ecological knowledge when implementing new methods.

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Getting Them Past the Bottleneck: Report on Two Salamander Rescue Rearing Pilot Projects

Climate change is predicted to increase the frequency and severity of droughts in California. In some instances human intervention may be necessary to allow for successful reproduction in key breeding populations of sensitive amphibians. During the historic drought of 2012-2014, we rescued eggs and larva of two species of salamander, the endangered Santa Cruz long-toed salamander, *Ambystoma macrodactylum croceus*, and the California newt, *Taricha torosa*, from ponds that were threatened by pre-metamorphic drying. Approximately 400 larval long-toed salamanders were reared in 11 100-gal feeding troughs. 306 metamorphs were successfully released and returned to their natal pond. Over 150 larval newts were raised in a cooperative project with a local K-12 school and repatriated. In the case of the newt study, tank effects suggest that undocumented differences in rearing regime can have minor effects on outcomes. Overall, the two studies confirm the feasibility of rescue rearing as a potential management tool.

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Feral *Xenopus laevis*: A Source or Sink for Bd?

African clawed frogs (*Xenopus laevis*) have established feral populations in southern California but it is unclear how they are affecting native amphibian populations. As asymptomatic carriers of *Batrachochytrium dendrobatidis* (Bd), these invaders have the potential to act as reservoirs and spread Bd to native amphibians. To explore whether or not *X. laevis* acts as a source for Bd infection, we analyzed Bd infections in feral populations in southern California and found that they have low infection prevalence (3%) as well as low load (<1 ZE). In addition, the study explored the potential for the filter feeding larval *X. laevis* to consume Bd in its mobile zoospore stage. We discovered that larval *X. laevis* will consume Bd zoospore, which may further limit the potential for *X. laevis* to act as a reservoir for Bd.

* Indicates presenter in multi-authored presentation