California/Nevada Amphibian Population Task Force
2012 Meeting

PRESENTATION ABSTRACTS

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ABSTRACTS

* Indicates speaker in multi-authored presentation

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Evaluation of Methods for Detecting *Batrachochytrium dendrobatidis* from Formalin-preserved Amphibian Specimens using qPCR

The fungal pathogen *Batrachochytrium dendrobatidis* (Bd), the causative agent of chytridiomycosis, is a significant contributor to worldwide amphibian declines and extinctions. While non-invasive methods to detect Bd from formalin-preserved specimens have emerged in recent years, success and failure rates of Bd detection from formalin-preserved specimens under typical museum conditions have not been quantified. We collected and swabbed American bullfrogs (*Lithobates catesbeiana*) to obtain known (live, pre-preservation) Bd infection status. We then formalin-fixed and stored the specimens under conventional museum conditions. We employed multiple sampling and qPCR methods to measure the effectiveness of using qPCR to detect Bd. We compared the qPCR results of the known Bd infected individuals with subsequent sampling techniques after preservation. We present and discuss our data in terms of feasibility and effectiveness in Bd detection. A standardized, non-invasive sampling method to detect Bd in historical specimens can reduce ambiguity in results across researchers and clarify the past role of Bd in amphibian communities.

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Can the Sierra Madre Yellow-legged Frog be Saved in Southern California? A Case Study.

Since the early 1970s, the Sierra Madre yellow-legged frog (*Rana muscosa*) has declined precipitously in southern California. A working group was formed in 2000 to develop a framework for conservation and recovery for the species. Two priority restoration actions were identified and implemented. They included non-native trout removal and limiting recreation use through temporary forest closures at sites occupied by the frog. With 12 years of annual monitoring at the site before and after restoration, we report on the frog's response to these restoration efforts at a single Bd positive site in the San Gabriel Mountains. Frogs responded to removal of non-native trout by re-occupying newly fishless habitat. This response had a three year lag from when fish were removed to when frogs reoccupied. The frogs responded to reduced recreation immediately and showed an 875% increase of the mean number of adult frogs detected/survey over five years.

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Ecological Role of the Salamander *Ensatina eschscholtzii*: Direct Impacts on the Arthropod Assemblage and Indirect Influence on the Carbon Cycle in Mixed Hardwood/Conifer Forest in Northwestern California

Terrestrial salamanders are the most abundant vertebrate predator in northwestern California forests and fulfill a critical ecological, and arguably keystone, role converting invertebrate to vertebrate biomass based on their sheer numbers. The most common species of terrestrial salamander in northwestern California is the Ensatina salamander (*Ensatina eschsccholtzii*) (hereafter Ensatina). In an experimental design we tested top down effects of Ensatina on leaf liter invertebrates and the potential for such top down effects to decrease the rate of leaf litter decomposition and thus foster carbon sequestration. The study ran during the rainy season (November through April) over two years (2007-2009) in the Mattole River watershed. Results from the first year indicate a strong top-down effect and a 13% decrease in litter decomposition compared to controls. This is attributed to Ensatina's selective removal of large and competitive detrivores (beetle and fly larva, millipedes) enabling smaller detrivores to become more numerous. Thus the Ensatina promotes healthy invertebrate populations via optimal foraging theory and simultaneously shifts the density of key functional groups (shredders) which promotes carbon sequestration in Northern California forests. Results from year 2 indicate that these effects are ameliorated by weather conditions and that the capacity of carbon sequestration and direct salamander impacts may be influenced by water year.

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Terbinafine Hydrochloride as a Treatment for *Batrachochytrium dendrobatidis* Infection

Amphibian chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), has been implicated in the decline and extinction of amphibian species worldwide as well as sometimes catastrophic losses among animals in captivity. Conservation of threatened amphibians, including captive breeding efforts and maintenance of zoo, research and private collections, requires effective control of pathogens. Several chemical compounds, including Formalite III®, itraconazole, and chloramphenicol, have been used to treat amphibians infected with *Bd*, with varying levels of success. Here, we report successful clearance of *Bd* in five species of post-metamorphic anurans and one caudate species, using terbinafine hydrochloride, available over-the-counter as Lamisil AT™. Treatments consisting of 5-minute soaks in fresh 0.01% or 0.005% terbinafine HCl for either five consecutive days or for six treatments spread across 10 days successfully cleared *Bd* from 100% of 81 test subjects in eight trials. Our results indicate that terbinafine HCl has a high therapeutic index as a treatment for *Bd* infection in living post-metamorphic amphibians.

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Status of the Mountain Yellow-legged Frog, Yosemite Toad, and Pacific Chorus Frog in the Sierra Nevada, CA

From 2002-2009, the Forest Service implemented a long-term, multi-scale monitoring program on national forest lands in the Sierra Nevada for the mountain yellow-legged frog (*Rana sierrae, Rana muscosa*), Yosemite toad (*Anaxyrus* [*Bufo*] *canorus*), and Pacific chorus frog (*Pseudacris regilla*). Small watersheds, 2-4 km^2, were surveyed across the ranges of the mountain yellow-legged frog and Yosemite toad. From 2006-2009, abundances of Yosemite toad adult males and egg masses were quantified in six meadows in two watersheds. Monitoring results corroborate other assessments that the mountain yellow-legged frog has declined in distribution and abundance across its Sierran range. Breeding was found in 0.04 (se=0.007) of watersheds rangewide, 0.48 (se=0.041) of recently occupied (1990-2001), and 0.03 (se=0.028) of historically occupied watersheds (prior to 1990). Few watersheds contained large populations. Yosemite toads have declined from levels observed historically, but are still fairly widespread relative to recent records. Breeding was found in 0.22 (se = 0.01) of watersheds range wide, 0.81 (se = 0.03) of recently occupied (1990-2001), and 0.12 (se = 0.03) of historically occupied watersheds (prior to 1990). Abundances of adult males and egg masses in the two intensive watersheds were small. Pacific chorus frog breeding was found in an estimated 0.25 (se = 0.01) of watersheds range wide and in 0.95 (se = 0.02) of watersheds where the species likely occurred historically. This program, which uses a probabilistic design, provides statistically rigorous distribution and abundance information to support conservation and management decisions.

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Sierra Nevada Mountain Yellow-legged Frogs Conservation Strategy: An Integrated Interagency Restoration Plan (California Department of Fish and Game, National Park Service, U.S. Fish and Wildlife Service, and U.S. Forest Service)

Following on the Conservation Assessment for Mountain Yellow-Legged Frog (*R. sierrae* and Sierran populations of *R. muscosa*), an interagency collaborative process has engaged to complete and implement a Conservation Strategy to guide restoration actions across the Sierra Nevada. The U.S. Fish and Wildlife Service, U.S. Forest Service, National Park Service, and California Department of Fish and Game are crafting a science-based framework to serve as the technical foundation for coordinated restoration and mitigation activities addressing the conservation needs of these two species. This effort will include: a coordinated monitoring and data sharing agreement; guidelines for translocation activity and potential use of captive propagation/head starting; management consideration to deal with Bd spread in currently unaffected populations; guidelines for mitigating project activities in the presence of frog populations; and prioritized restoration areas selected based primarily on ecological need, but also taking fiscal, social and political realities into consideration. This Conservation Strategy is intended to culminate in an implementation MOU directing the adaptive management of these sensitive populations.

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Footnotes to Eastern Sierra California Fish and Game SNYLF Restoration Projects

Region 6 staff of California Department of Fish and Game continues efforts to restore fishless habitat for Sierra Nevada yellow-legged frogs in the eastern Sierra. Ongoing projects partially funded by the now-completed Section 6 grant E-2-F-38 were finished within the grant period including fish removal and securing of five-plus waters in the Gable lakes Basin, three waters in the San Joaquin Basin, and three waters in the Treasure lakes Basin in the Bishop Creek drainage. Additionally, work in 2011 resulted in seven waters secured for frogs in the Independence Basin and three in the headwaters of the West Walker Basin. An amendment to the funding grant added Ruth Lake in the West Walker Basin, which is an ongoing (non-funded) project. Rather than focusing on the restoration statistics for these waters, which seem to be common throughout most fish removals in the Sierra Nevada, i.e. the numbers of fish removed and the subsequent increase in the number of frogs utilizing the waters, this talk will document the difficulties in achieving restoration (typically bureaucratic rather than physical); share lessons learned (for example, utilizing gear in non-traditional methods); and emphasize the future of successful restoration in perpetually difficult times: collaboration. Funding for restoration by CDFG continues to be through federal grants: Section 6, State Wildlife Grant, and Sport Fish Restoration Act.

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Overview of a decade of amphibian research and monitoring in southern California; with focus on the Santa Monica Mountains National Recreation Area

Over 20% of the federally listed T & E amphibian species occur in southern California. The status of many of these and other amphibian species was poorly known since the 1960-70's, prior to major range declines. To fill some of these knowledge gaps, we initiated terrestrial surveys using pit-fall traps for amphibians in southern California in 1995; then augmented these with widespread aquatic surveys beginning in 2000 with the USGS Amphibian Research and Monitoring Initiative (ARMI) Program. To date we have conducted 1,000's of surveys across 100's of miles of creeks/rivers and in 100's of ponds/lakes. During this work we have followed the ARMI model with Apex, Mid-level, and Baseline study/research sites. We are/have discussed our ranid frog Apex and Camp Pendleton Mid-level sites at Amphibian Populations Task Force (APTF) meetings, but have never discussed our Santa Monica Mountains NRA Mid-level studies at APTF. Here I review briefly the overall program, and more specifically the work in the Santa Monica Mountains, which now includes over a decade of monitoring at some sites for the entire amphibian community.

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Re-examination of Approaches to Manipulating Invasive Reed Canarygrass (*Phalaris arundinacea*) to Enhance Oregon Spotted Frog (*Rana pretiosa*) Oviposition Habitat in Western Washington State, USA

We followed up on an experiment showing that mowing is a promising approach to enhance Oregon Spotted Frog (*Rana pretiosa*) oviposition habitat. We applied two experimental approaches (mowing and mowing with burning) in 15 m × 30 m plots, a scale 64× that used in the original experiments. We used equal numbers of treatment and reference (control) plots in each experiment, one mowing four replicates and one mowing and burning two replicates. Treatments were applied in fall 2009 and reapplied in fall 2010. Within both experiments (all plots), *R. pretiosa* laid eggs exclusively in treatment plots. As in the original experiments, the few egg masses laid outside of treatment or reference plots were in habitat similar to that of treatment plots. Based on stem density, recovery of the tall emergent structure of Reed Canarygrass (*Phalaris arundinacea*) following either treatment approach occurred within the season after the original manipulation. Slight reduction in thatch depth suggests that Reed Canarygrass incurs an energetic cost as a result of treatment application; small sample size leads us to be cautious in this interpretation. Our results confirm that mow or mow and burn treatments can influence where *R. pretiosa* deposit eggs, which presents a clear opportunity to potentially enhance oviposition habitat. However, we suggest that this approach be applied experimentally with caution because scale effects, effects on non-egg *R. pretiosa* life stages, and co-occurring species remain poorly understood. Lastly, simple mowing clearly lacks longevity, but the mow and burn option deserves further exploration under better experimental circumstances.

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A Synopsis on the Vegas Valley Leopard Frog: History, Recent Findings, and Conservation Implications

The taxonomy of the Vegas Valley leopard frog (*Rana fisheri = Lithobates fisheri*) has long been debated. Even as populations of this frog (*sensu stricto*) in southern Nevada went extinct, the significance of this event was tempered by the nearby persistence of frogs thought by some to be the same species, the Relict leopard frog (*Rana onca = Lithobates onca*). More recently, a general consensus has been reached that these two frogs are distinct species. Evidence emerged that *R. fisheri* was morphologically similar to the Chiricahua leopard frog (*Rana chiricahuensis = Lithobates chiricahuensis*), a federally listed species of which the nearest populations were 400 km distant. This latter perspective was recently supported by a phylogenetic study that placed century-old museum specimens of *R. fisheri* within an extant lineage of *R. chiricahuensis*, effectively resurrecting *R. fisheri*. Phylogeographic patterns presented in that study, indicated that *R. chiricahuensis* may consist of two major lineages – one composed of the *R. fisheri* specimens from Las Vegas Valley and most *R. chiricahuensis* inhabiting the Mogollon Rim in central Arizona, and the other comprised of all other *R. chiricahuensis* including those in southeastern Arizona, New Mexico and Mexico – a perspective consistent with an earlier genetic assessment of *R.*

chiricahuensis (*sensu stricto*). The inference was that only the northwestern lineage (and populations) of *R. chiricahuensis* were attributable to *R. fisheri*, and that the other lineage might best be maintained as *R. chiricahuensis*. We caution, however, that this perspective would have implications beyond taxonomy, impacting conservation actions along the Mogollon Rim where both lineages are present. More importantly, preliminary assessments from an on-going rangewide genetic study indicate that the patterns might not be that simple, with broader admixture of lineages on the Mogollon Rim.

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Modeling the Past to Understand the Present: Historical Distribution and Patterns of Decline in the Endangered Mountain Yellow-legged Frog (*Rana muscos*a, *Rana sierrae*)

The mountain yellow-legged frog (*Rana muscosa, Rana sierrae*) has disappeared from the majority of its range, and is now being considered for listing under both the U.S. and California Endangered Species Acts. Developing effective recovery actions will require an accurate description for both species of (1) their likely native ranges, (2) historical probabilities of occurrence across those ranges, and (3) the patterns of decline. We used species distribution models (Maxent) to describe the likely historical distribution and probability of occurrence for both species across the Sierra Nevada. We then used the modeled distribution in combination with an extensive data set of current localities to quantify the patterns of decline. The modeled range of both species was in general agreement with that previously described using museum collections, but for areas that lacked historical occupancy data included or excluded some areas based on predicted habitat quality. The modeled probabilities of occurrence indicated that the highest probabilities were associated with high elevation areas with abundant lake habitat, but also suggested relatively high occurrence probabilities in some areas characterized by meadow/stream habitats that contained few or no lakes. For all watersheds predicted to have contained frogs historically, *R. sierrae* are now absent from 44% and *R. muscosa* are absent from 59%. These findings have important utility in the design of future recovery actions undertaken on behalf of the mountain yellow-legged frog across the Sierra Nevada.

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SAVE THE FROGS! – Creating a Frog-Friendly California

SAVE THE FROGS! (www.savethefrogs.com) is a Santa Cruz-based 501(c)(3) public charity whose mission is to protect amphibian populations and to promote a society that respects and appreciates nature and wildlife. Since arriving in California in 2010, we have: (1) given over 120 free presentations on amphibian conservation at California schools, universities, community groups, government agencies and other nonprofits, reaching 5,899 attendees. (2) We are nearing completion making Santa Cruz the first county in the nation to ban the importation, sale, release, and possession of American Bullfrogs and we are petitioning Governor Jerry Brown to do likewise. (3) 100 supporters attended our Save The Frogs Day Rally at San Francisco's City Hall to call on the city to protect the Sharp Park Wetlands and the endangered California Red-Legged Frogs and San Francisco Garter Snakes that live there. Supervisor John Avalos attended, pledged his support and soon thereafter introduced legislation to initiate transferring the management of the land to the National Park Service. After receiving 4,179 letters from SAVE THE FROGS! supporters, the San Francisco Board of Supervisors

voted 6-5 in support of the legislation. (4) We persuaded two California restaurants to remove the wild-caught frogs from their menus. (5) We have held 6 public events at Antonelli Pond in Santa Cruz, at which several hundred people have helped restore habitat for California Red-Legged Frogs. We call on all scientists to hold educational events on the 4th Annual Save The Frogs Day (April 28, 2012).

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Diet of the Cascades Frog (*Rana cascadae*) in the Trinity Alps Wilderness of Northern California: Are they really generalists?

Frogs in the family Ranidae are considered generalist predators that consume prey as it is encountered in the environment. However, few studies have attempted to quantify available prey and compare these taxa with what is actually being taken by the frogs so a thorough understanding of their foraging ecology is lacking. This study examined prey availability and diet of *R. cascadae* in a northern California montane basin in the summer of 2007. Based on the analysis of stomach contents from 275 frogs, *Rana cascadae* consumed 3052 prey items from 102 families of insects confirming that across all age classes, both genders, and three seasons this species appears to be a generalist predator. However, differences in diet composition were detected between seasons, life-stages, and genders. Adult females consumed more Acridid grasshoppers in the summer than adult males or juveniles. Adults consumed a greater volume of prey than juveniles, but of more interest, adult females consumed more than adult males. All life-stages and both genders showed evidence of prey selection for Tipulid flies. Adult male and juvenile frogs, but not females, also showed selection for insect larvae in the summer. Shifts in prey use with changes in ontogeny were also documented with frogs consuming more large prey items and fewer small prey items as they grew.

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Status of the California State Listing of the Mountain Yellow-Legged Frog (*Rana sierrae* and *Rana muscosa*) as Threatened / Endangered

On January 27, 2010, the Fish and Game Commission (Commission) received a petition from the Center for Biological Diversity to list all populations of mountain yellow-legged frog as "Endangered" under California Endangered Species Act (CESA). After evaluating the petition, the Commission published a Notice of Findings on October 1, 2010 accepting for consideration the petition to list MYLF under CESA. Between November 2010 and November 2011, pursuant to Fish and Game Code 2074.6, the Department of Fish and Game (Department) prepared a report detailing the status of *R. sierrae* and *R. muscosa* and the Department's recommendations to the Commission regarding listing. For the purposes of the report, the Department, in collaboration with US Forest Service, US Geological Survey and University of California researchers, compiled a comprehensive set of data on the mountain yellow-legged frog, which was used to estimate historical and recent declines of mountain yellow-legged frog across their native range. On December 1, 2011, the Department submitted a "REPORT TO THE FISH AND GAME COMMISSION - A STATUS REVIEW OF THE MOUNTAIN YELLOW-LEGGED FROG (*Rana sierrae* and *Rana muscosa*)" wherein the Department recommends to the Commission that designation of *R. sierrae* in California as threatened and *R. muscosa* as endangered is warranted. Currently, the Commission is accepting public comments before the listing hearing, scheduled for February 2, 2012, in Sacramento.

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Mitochondrial Evolution and Phylogeography of the Endangered Arroyo Toad (*Anaxyrus californicus*).

The Arroyo Toad, *Anaxyrus californicus*, is an endangered bufonid found in rivers draining to the Pacific Ocean in California, USA, and Baja California, México, as well as a few drainages flowing into the Mojave Desert. This study analyzed mitochondrial DNA sequence variation across the range of the species. The ND1 gene and Dloop regions of mtDNA were sequenced (859 and 451 bp, respectively), and aligned sequences were analyzed using maximum likelihood, maximum parsimony, and Bayesian inference. Despite the conservation status of *A. californicus* as endangered in the United States, mitochondrial gene relationships have never before been constructed for this species. Herein, DNA sequence data are compared across its range to determine gene relationships and infer evolution. Two well supported clades are resolved for northern and southern populations, excepting a few northern haplotypes being identified within the range of the southern group. Phylogeography, congruence with other regional studies, and biogeographic comparisons are explored, and provide further evidence for the complex biodiversity of the southern California and northern Baja California region. These findings are important to better understand relationships among extant populations of the endangered Arroyo Toad.

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Using Traditional Fisheries Technology to Monitor a Highly Aquatic Frog (*Rana luteiventris*) in Central Nevada

To facilitate management of Columbia spotted frog (*Rana luteiventris*) populations in Nevada, a conservation agreement and strategy (CAS) was signed in 2003 by multiple agencies. For the Toiyabe population in central Nevada, mark-recapture monitoring has taken place annually since 2004 using passive integrated transponder (PIT) tags. We deployed two antenna arrays, traditionally used in fisheries management, to help us understand frog activity, movement patterns, and survival outside of our annual one week mark-recapture efforts. Initial results from 2011 indicate that this technology may be a useful tool to monitor amphibians.

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Evidence of Chytridiomycosis Resistance in Pacific Chorus Frog (*Pseudacris regilla*) Suggests Role as Reservoir in Disease Spread.

Chytridiomycosis, a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), is driving amphibian declines and extinctions in protected areas globally. The introduction of invasive reservoir species has been implicated in the spread of *Bd* but does not explain the appearance of the

pathogen in remote protected areas. In the high elevation (>1500 m) Sierra Nevada of California, the native Pacific chorus frog, *Pseudacris regilla*, appears unaffected by chytridiomycosis while sympatric species experience catastrophic declines. We investigated whether *P. regilla* is a reservoir of *Bd* by comparing habitat occupancy before and after a major *Bd* outbreak and measuring infection in *P. regilla* in the field, testing susceptibility of *P. regilla* to *Bd* in the laboratory, examining tissues with histology to determine patterns of infection, and using an innovative soak technique to determine individual output of *Bd* zoospores in water. *Pseudacris regilla* persists at 100% of sites where a sympatric species has been extirpated from 72% in synchrony with a wave of *Bd*. In the laboratory, *P. regilla* carried loads of *Bd* as much as an order of magnitude higher than loads found lethal to sympatric species. Histology shows heavy *Bd* infection in patchy areas next to normal skin, a possible mechanism for resistance. The soak technique was 77.8% effective at detecting *Bd* in water and showed an average output of 68 zoospores per minute. The results of this study suggest *P. regilla* should act as a *Bd* reservoir and represent experimental evidence of a new resistance mechanism in a reservoir species.

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Conservation Genetics at the Margins of a Species' Boundary: A Case Study on the California Red-legged Frog (*Rana draytonii*) in Southern California

Populations at the margins of a species distribution are often evolutionarily unstable, given that range limits can naturally reflect maladaptation to environments beyond the current boundary, increased competition with other species, restricted gene flow, or interactions between these and other variables. When other non-natural factors are added to the equation (e.g., habitat destruction, fires, and novel diseases), the prospects for population persistence at the range boundary becomes increasingly more difficult, largely because the temporal scale over which these processes operate do not allow sufficient time for organisms to adaptively respond. This bodes poorly for marginal populations belonging to species of conservation concern, where survivorship even under natural conditions can be tenuous. We examined the population genetics of the California Red-legged Frog (*Rana draytonii*) at the southern-most extension of the species' range in California, where peripheral populations have undergone severe declines since the 1960s. Our study was based on microsatellites and mtDNA sequences, and targeted all of the last known populations of *R. draytonii* in Santa Barbara, Ventura, and Los Angeles Counties. We investigated the effects of habitat degradation, anthropogenic-induced fires, and the presence of chytrid fungus on the diversity, gene exchange and the spatial genetic structuring of populations. Because representatives from several populations are currently under consideration for translocation, we used the genetic data to provide an evolutionarily-based recommendation about the most suitable sources, where maximizing genetic diversity in the founder population and preserving the historical phylogeographic structure of the species were the main objectives.

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Testing Mark-Recapture Techniques for Wild Mountain Yellow-Legged Frog Tadpoles (*Rana muscosa*)

Most anuran species have a dual life history which includes an aquatic tadpole stage and a terrestrial adult stage. Both of these life history periods are critical for a species' survival. However, it is often difficult to

answer basic ecological questions about the tadpole life-stage because it is hard to identify unique individuals using traditional marking techniques. A photo mark-recapture survey was conducted on tadpoles of the critically endangered mountain yellow-legged frog (MYLF), *Rana muscosa*. This survey aimed to provide detectability and survival estimates of a robust tadpole population in the San Jacinto Mountains. Tadpole spot patterns were found to be unique enough for re-identification, and patterns were retained over the duration of our twelve week stream survey. Over the course of four sampling events, there were 723 total captures, 52 of which were recaptures, with a total of 671 individual tadpoles captured. This work demonstrates that photo mark-recapture is possible in MYLF tadpoles, and may prove to be an important tool in understanding the population dynamics of amphibians.

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Calibrating our Progress Towards Recovery of Amphibian Populations: An Area-based Approach and Occupancy Modeling

Like many amphibian species worldwide, the Chiricahua leopard frog (*Rana chiricahuensis)* experienced a dramatic, rangewide decline during the past three decades and was listed under the Endangered Species Act (ESA) as threatened in 2002. A species recovery plan was finalized in 2007 that included four recovery criteria that, when reached, will have: 1) established sufficient populations and metapopulations, 2) managed the necessary aquatic breeding habitats, 3) managed important dispersal corridors, and 4) reduced threats so that the Chiricahua leopard frog no longer needs the protection of the ESA. Although great progress has been made since federal listing, progress on recovery criterion 1 has been hampered by 1) the dearth of suitably configured landscapes that could "host" candidate metapopulations, and 2) the difficulty of establishing and monitoring stable and viable metapopulations given the limited human and financial resources available. I develop a conceptual area-based approach to calibrate progress toward recovery that is applicable to the Chiricahua leopard frog that utilizes occupancy modeling to gauge progress in establishing, managing, and monitoring viable metapopulations. This approach is easier to design and implement, makes fewer assumptions, and is less biased than the current "strict metapopulation" approach and is applicable to other patchily-distributed amphibians.

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California's Amphibian and Reptile Species of Special Concern

The Amphibian and Reptile Species of Special Concern (ARSSC) document plays an important role in management and conservation planning for California's native herpetofauna. We have updated the 1994 ARSSC document in collaboration with the California Department of Fish and Game (CDFG). First we identified 218 taxa present in California, then selected 74 taxa to evaluate for ARSSC status. We evaluated taxa based on metrics designed to capture the geography of declines, changes in population biology over time, key ecological attributes associated with risk, and estimates of future impacts. We solicited feedback on our initial assessments over a 60 day public comment period, during which our website was viewed by over 800 people. After incorporating public feedback, 46 taxa met our criteria for designation as ARSSC including 8 salamanders and 14 frogs. Compared to the previous treatment, 17 amphibians retained ARSSC status, 6 are new, and 7 are no longer considered ARSSC. Frogs and toads ranked highest in conservation concern, with turtles scoring nearly as high. Taxa that rely on aquatic habitat to complete their life cycles ranked higher than terrestrial taxa. The mountains surrounding the Central Valley and the northern coastal California ecoregions have the highest numbers of amphibian

ARSSC, with lower numbers in the southern ecoregions of the state. The final ARSSC document including peer-reviewed species accounts and range maps is currently under CDFG review.

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Populational Variation of Evasive Behavior in the Oregon Spotted Frog (*Rana pretiosa*).

Based on casual observations that captive reared post-metamorphic Oregon Spotted Frogs (*Rana pretiosa*) from one population appeared to display a more vigorous response to predator approach than another geographically independent population, we developed a method to quantify their latency-to-response. We found that captive-reared Oregon Spotted Frogs from a population east of the Cascade Mountains in Washington State (Conboy Lake National Wildlife Refuge) respond significantly faster than their captive-reared counterparts from lowland western Washington (Upper Black River), and that the speed of response increases with frog size. This differential led us to compare captive-reared frogs to wild ones from the same source population. Non-intuitively, using frogs from Conboy Lake, we found that wild frogs respond less rapidly and less frequently to the same pseudo-predator stimulus than their captive reared counterparts. This finding leads us to consider the possibility that captive-rearing may be creating frogs that are over-responsive to predators, which may have negative implications for animals targeted for release. This work is part of a broader study that seeks to determine whether intrinsic aspects of the anti-predatory behavior of native ranid frogs may make them vulnerable to the American Bullfrog (*Lithobates catesbeianus*) predation.

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Update: Columbia Spotted Frog (*Rana luteiventris*) Habitat Restoration and Population Monitoring in Central Nevada

Between 2000 and 2003, drastic population decreases were seen within the Toiyabe population of Columbia spotted frog (*Rana luteiventris*) in central Nevada. In an attempt to increase population size, the Humboldt-Toiyabe National Forest created additional perennial habitat in Warner and Indian Valleys, NV. During the fall of 2004, nineteen new ponds were created. Population monitoring in 2006 and 2007 showed an increased usage of the new ponds, as well as, an overall population increase. As a result of pond success, the Humboldt-Toiyabe created an additional fifteen ponds in 2010. Monitoring in 2011 again showed use of the newly created ponds with increased population growth. To further enhance the available habitat, in 2010 and 2011 watershed improvements were implemented to maintain meadow hydrology. These improvements included stabilization of headcuts and "plug and ponding" in entrenched stream areas. The population growth in Warner and Indian Valleys since the start of restoration in 2004 suggests that the habitat improvements are positively affecting the Columbia spotted frog populations.

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Population Responses of the Foothill Yellow-legged Frog (*Rana boylii*), the Western Pond Turtle (*Actinemys marmorata*), and the Non-native Bullfrog (*Rana catesbeiana*) to the Managed Flow Regime and Altered Riverine Environments of the Trinity River, Northwest California

Research on the herpetofauna of the Trinity River of northwest California has been on-going at PSW since the 1990's. This long-term project has enabled us to document changes in the distributions, behaviors, and demographic parameters of several species that might go un-noticed in research conducted over a shorter time span. We review evidence of repeated cohort collapse and a shifting pattern of development and metamorphosis in the foothill yellow-legged frog (*Rana boylii*). On the managed fork of the Trinity, the western pond turtle (*Actinemys marmorata*) exhibits thermoregulatory behavior and growth characteristics that differ from turtles on the undammed south fork, and suggest a possible long-term impact on population fitness. The bullfrog (*Rana catesbeiana*) has invaded and expanded below the Trinity Dam; where it breeds in relict mine tailing ponds and other permanent lentic habitats that occur some forty miles downriver of the dam. The distribution and abundance of this non-native predator is negatively associated with the mean number of native herpetofaunal species along this reach. I discuss the relationships of the herpetofauna with artificial flow regulation and habitat modifications, and identify management options for reversing some of these negative trends.

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Amphibian Exposure to Aquatic Herbicides

Conflict between native amphibians and aquatic weed management in the Pacific Northwest (PNW) is infrequently recognized because most native stillwater-breeding amphibian species move into upland habitats during the summer, when application of herbicides to control weeds in aquatic habitats typically occurs. However, for aquatic species present in wetland habitats through the summer, such as the Washington State Endangered Oregon Spotted Frog (*Rana pretiosa*) and the larvae of selected salamanders, aquatic weed management may pose a risk. Acute toxicity of herbicides used to control aquatic weeds tends to be low, but the direct effects of herbicide tank mixes (active ingredient, surfactant, and dye) on Oregon Spotted Frogs has remained unexamined. To address this gap, we exposed juvenile Oregon Spotted Frogs to mixtures of the herbicide Imazapyr (Polaris AQ, 28.7% active ingredient), Agri-Dex surfactant, and Hi-Light dye in a 96-hour static-renewal test. The Imazapyr-Agridex combination was chosen because of its low toxicity to fishes and its proven effectiveness in aquatic weed control. Concentrations were those associated with low (3.5 liters/hectare) and high (7.0 liters/hectare) volume applications of Polaris AQ to control Reed Canarygrass (*Phalaris arundinacea*), and a clean-water control. Following exposure, frogs were reared for two months in clean water to identify potential latent effects on growth. Endpoints evaluated included feeding behavior during the exposure and grow-out, growth, and liver condition index. We recorded no mortalities. We also found no significant differences for any endpoint between the herbicide-exposed and clean-water control frogs.