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ABSTRACTS

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Evaluating Wildlife Crossing Tunnels through Behavioral Analyses and Movement Rates of the California Tiger Salamander, *Ambystoma californiense*

In Sonoma County, California, a small population of the endangered California tiger salamander, *Ambystoma californiense*, undergoes an annual breeding migration across a well-used road. The trek increases the risk of vehicular mortality and local extirpation for this population. The Sonoma County Transportation and Public Works Department installed three steel tube wildlife crossing tunnels and directional fencing along a portion of the roadway. The design was tested through a series of experiments that evaluated the effectiveness of the tunnels in safely facilitating *A. californiense* under the road. Each evening prior to sunset, the moisture level inside one or two of the tunnels was increased by adding five to seven gallons of water via a soaker hose that ran the length of each tunnel. Captured *A. californiense* were placed in front of a tunnel entrance, perpendicular to the roadway to imitate a natural encounter. Researchers stepped back and recorded each individual's behavior and response to the tunnel entrance, and the time at which it entered or rejected the tunnel. Researchers or infrared cameras on the other side of the tunnel watched for the individual to exit and then recorded the time to determine the rate at which the salamander moved through the tunnel. Preliminary data suggests that *A. californiense* will use both wet and dry tunnels, but will travel more quickly through a wet tunnel. This would be advantageous for the species as predators have been identified using the tunnels as well.

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Thermoregulatory Behavior and Growth Characteristics of Western Pond Turtles on the Regulated Mainstem Trinity River and Unregulated South Fork Trinity River

The Western Pond Turtle (*Emys marmorata*) has declined throughout much of its range, resulting in Species of Special Concern status in California and Sensitive Species status in Oregon. Understanding population specific life-history traits is necessary to make management decisions that protect local populations. We monitored western pond turtles on the mainstem Trinity River near the Lewiston Dam and on the South Fork Trinity River near Willow Creek, California from 2003 to 2010 to better assist managers in making restoration designs and flow management decisions that benefit this long-lived species. From 2005 to 2007, we combined the use of radio-telemetry and temperature sensitive dataloggers on the external carapace of turtles to understand thermoregulatory behavior in relations to river temperatures on the two forks of the Trinity River. We also conducted three summers of mark-recapture sampling on each fork to obtain population estimates and other demographic parameters including age structure, sex-ratios and growth curves. The mainstem Trinity River has a colder thermal regime in the summer due to hypolimnetic releases from the dam, which resulted in differences in both thermoregulatory behaviors and growth compared to turtles on the South Fork. Here we examine the basking behaviors and growth curves of turtles from both forks under the age of ten. Turtles on the mainstem exhibit significantly longer basking time and stunted growth compared with those on the South Fork due to thermal pollution from hypolimnetic releases. We interpret these results as evidence for long term reduction in fitness of the mainstem population because stunted female turtles will have reduced reproductive output over their lifetimes. We also review thermal profiles of females during probable nesting forays and over-wintering.

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Status of Restoration and Research to Recover Mountain Yellow-legged Frogs in Sequoia and Kings Canyon National Parks

Both species of mountain yellow-legged frogs (MYLFs; *Rana muscosa* and *R. sierrae*) occur in Sequoia and Kings Canyon National Parks (SEKI), making it an important site in efforts to recover these declining species. As a result, much restoration and research on MYLFs have been conducted in SEKI. This paper will provide an update on the following actions to recover MYLFs in SEKI: 1) population monitoring to inform current status, 2) nonnative fish eradication and MYLF response, 3) recent research using anti-fungal and probiotic agents to mitigate effects of chytridiomycosis, and 4) plans for restoration and research in 2013 and beyond.

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California Department of Fish and Wildlife 2012 Amphibian-related Conservation Activities

A summary of various big-picture efforts nearing completion will be presented, including highlights of the draft final version of the revised "California Amphibian and Reptile Species of Special Concern" due to be published in 2013, along with previews of the upcoming publications "Strategic Plan for California's Native Amphibians and Reptiles," "California Amphibians and Reptiles of Future Concern" (climate change analysis), and "Addressing Amphibian and Reptile Regulatory Needs in California."

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Origins, Maturation and Prospectus of the Declining Amphibian Enterprise: Through One Perspective

Amphibians are ecologically important in ecosystems globally. I will review several themes and challenge some dogma. Many workers earmark the 1st World of Congress in England (1989) as the springboard for attention to amphibian declines. However, there were few talks there on the topic. Concern was growing much earlier (e.g., I was hired as the first herpetologist at Dept Interior in 1972~~)~~ with 16 experts on board by 1989). Groundwork was underway. A workshop on declining amphibians sponsored by the National Research Council (1990) in California launched a major response and concluded that declines of amphibians were a global problem. Amphibian losses in the U.S. are estimated to be at 33-60% and uniquely high. Still, published studies indicate that these are comparable to the plight of other freshwater groups. Losses of amphibian populations are attributed to a variety of known or suspected factors, including habitat loss and alteration, invasive species, and contaminants. Many biologists now study the extent and causes of amphibian declines, but no "silver bullet" explains losses. We should expect multiple factors and remedies reflective of diverse local conditions and stressors. We have improved our hypothesis testing, identification of factors causing declines and debate of the results. The road ahead is rosier than the past. Yet, much work on science and policy remains.

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Impacts of Landscape Features on the Genetic Structure of the California Giant Salamander (*Dicamptodon ensatus*)

The California Giant Salamander, *Dicamptodon ensatus*, has limited dispersal and is therefore dramatically affected by a variety of landscape features making it an ideal species for studying the interactions of landscape features and population structure in species with low vagility. *D. ensatus*, which is endemic to the northern California coastal region, is estimated to have speciated about 7 million years ago, making it one of the older lineages of salamanders in California. In order to assess the patterns of genetic divergence among sites from throughout their range on either side of the San Francisco Bay, we sequenced a 751 base pair portion of the mitochondrial control region from 175 samples taken from sites throughout their range. Results revealed two historic barriers that account for patterns of long-standing population distinction, one associated with the San Francisco Bay and a second barrier between Sonoma and Marin counties associated with ancient geologic features combined with unique modern climatic conditions. Thus, these data suggest the presence of three distinct evolutionary lineages within the species. In addition, we identified genetic structuring on a finer scale throughout the species' range which has important management implications. Finally, measures of genetic diversity varied by site, however, the isolation and loss of genetic diversity, associated with a unique morphologic condition, suggested cause for concern for one subpopulation in the Sierra Azul preserve in the Santa Clara county region.

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Health Assessments of Western Pond Turtles from Two Northern California Populations

Many Western Pond Turtles live in modified habitats and it is likely that habitat modification affects their physiological health. In 2011 and 2012 turtles were captured from two habitats in Northern California, a pristine nature reserve, and a wastewater treatment facility's oxidation ponds. Turtles were examined for external signs of disease and had blood tests performed, which are commonly used by veterinarians in evaluating health. This research presents the first known reference ranges for wild, free-ranging Western Pond turtles for common blood tests, including blood glucose levels, packed cell volumes, and twelve serum chemistries. We also compare population health attributes between the nature reserve and wastewater facility turtle populations, highlighting differences in blood serum chemistries and body damage. Lastly, we outline suggestions for others who may want to evaluate the health of individual Western Pond turtles or evaluate general health in populations using our methods. The reference intervals we present will be helpful to wildlife veterinarians in evaluating disease in this species and we hope that managers may use our study to assess the health of Western Pond turtle populations and plan conservation strategies in altered landscapes.

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"Hands-on, Minds-on" 3rd Grade Teaching Module: Life History and Population Ecology of Turtles

We present a teaching module aligned with the California Education and the Environment Initiative standards that teaches population ecology to 3rd grade students. We used ongoing Western Pond turtle research to inform a "hands-on, minds-on" module focused on population health, mark and recapture techniques, and the scientific method with an emphasis on applied math skills. Our teaching modules took place on the Big Chico Creek Ecological Reserve, where children learned about turtle trapping,

reading turtle marks, and long-term data collection. In addition, they engaged in discussions about the biology of *E. marmorata* and potential threats of invasive turtle species. Based upon hand-written thank you cards and assessment results, we conclude that children came away from these education sessions with a new appreciation for a native reptile, insight into why and how research is conducted, and an understanding of the life requirements of an aquatic turtle and the potential impacts of non-native fauna. Complete teaching materials will be provided for educators to download, when this material is published in an open source science teaching journal.

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The Sad Song of the Yosemite Toad: The Role of a Panzootic Fungus in an Enigmatic Decline

This is the first large scale study of the effects of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), on the Yosemite toad (*Bufo* [*Anaxyrus*] *canorus*). Between the years 2004 and 2012, skin swabs (n = 1,677) were collected from wild *canorus* individuals across the species range and tested for *Bd* presence and infection load by qPCR techniques. *Bd* was found within 23 of 27 (85%) of *canorus* basins or sampling areas (<5km^2) where 5 or more skin swabs were collected. *Bd* prevalence and infection load were higher on juveniles (prevalence = 22.7% and maximum infection load = 52,902 zoospores) than on adults (prevalence = 8.45% and maximum infection load = 373 zoospores). Seasonal variation was found in juvenile infections, which increased exponentially between the months of May and September. Experimental infections of five *canorus* toadlets via exposure to a sympatric reservoir species, *Psuedacris regilla,* suggest the susceptibility of juvenile *canorus* to chytridiomycosis at ecologically realistic doses of *Bd.* Four weeks of exposures resulted in peak infection on week 7 and mortality of the toadlet with the greatest exposure dose occurred on day 53. During a retrospective qPCR survey of 237 archived *canorus* museum specimens, *Bd* was first detected in 1961, matching the earliest record of *Bd* found on a North American amphibian. *Bd* prevalence and mean infection intensity detected on *canorus* specimens increased during the 1970's, 1980's and into the 1990's, providing evidence of chytridiomycosis as a plausible cause in the infamous historic decline of *canorus.*

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Amphibian Population Trends In Yosemite National Park: What Do We Know from Long-Term Monitoring?

In 2000, the U.S. Geological Survey implemented the Amphibian Monitoring and Research Initiative (ARMI) which is designed to provide data on amphibian population trends on federal lands throughout the U.S. We implemented the ARMI program at Yosemite National Park, California (USA) using two different approaches. One was a focused mark-recapture study of *Rana sierrae* at a single meadow complex, and the other was a statistically-based design that used double surveys at 175 sites across 14 watershed units to evaluate population trends in three anuran species (*R. sierrae, Pseudacris regilla, Bufo canorus*) throughout the 3,081 km^2 park. We tagged 757 individual *R. sierrae* from 2003 to 2011 and used mark-recapture data based on 2,431 captures to model population trends related to precipitation, sex, and year. The *R. sierrae* population fluctuated from 45 to 115 frogs over the nine years of our study, but there was no clear up or downward trend. We conclude that this *R. sierrae* population at Yosemite National Park is stable over a relatively long period of time, even in the presence of Bd. The watershed research was initiated in 2004 and continues as an ongoing program. We use double survey protocol at each site (pond, lake or meadow) to assess detectability for each species for each year. Detectability for

the three anurans is high, ranging from approximately 0.70 - 0.90. We have analyzed presence data along with 15 site and survey covariates to evaluate population trends for each of the three study species. There are different population trends for different species, and different trends in the two major river drainages within the park. While population trends for *Bufo* and *Rana* are somewhat inconsistent, *Pseudacris* populations are declining within each of the major drainages.

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Status of the Dixie Valley Toad: First Reports from Population Surveys and Sampling for Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis*

The Dixie Valley toad is a yet undescribed species with an extremely narrow distribution in northwestern Nevada, USA. Currently restricted to wetlands fed by four springs within Dixie Valley NV, this unique toad appears to be a relict member of the Western toad (*Anaxyrus* [*Bufo*] *boreas*) species group. Between 2009-2012, we surveyed populations of Dixie Valley toads and other nearby amphibians, including American bullfrogs (*Lithobates catesbeianus*), and a small population of Western toads (*A. boreas*). In 2011 and 2012, we also tested individuals for the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). None of the Dixie Valley toads or Western toads that we sampled tested positive for *Bd*. However, the prevalence of *Bd* infections among nearby populations of American bullfrogs increased dramatically from 18% in 2011 to 75% in 2012. The high incidence of the amphibian chytrid fungus among invasive *L. catesbeianus*, which are known vectors of potentially fatal disease, may represent a serious threat to this yet undescribed species. Furthermore, one of the largest geothermal reservoirs in Nevada underlies the Dixie Valley, and plans to increase the exploitation of these geothermal resources may negatively impact the severely limited essential habitat available for this toad. Therefore we recommend continuous rigorous monitoring to identify and enact essential conservation measures, along with increased efforts to update the species status for the Dixie Valley toad.

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It Takes a Basin to Raise a Frog: Results from a Decade of Monitoring Reproduction and Dispersal of the Cascades Frog (*Rana cascadae*).

This study examined the breeding demography and dispersal rates of a Cascades Frog (*Rana cascadae*) population occurring in the Klamath Mountains, California. Annual egg production was intensely monitored across a wide elevation and habitat gradient for ten years (2003 to 2012). The duration of breeding activity was explosive at individual sites but averaged 57 days (range: 43 to 76 days) annually across all sites. A total of 587 egg masses were counted in 42 water bodies with annual production ranging from 46 to 82 egg masses. Fifteen breeding patches were identified and ranged in complexity from single isolated water bodies to close aggregations of up to eight ponds. Not all breeding patches were used annually but ranged in use from eight to 14 patches per year. Overall, four out of 15 patches contained 65% of the egg masses. Furthermore, 11 out of 42 individual water bodies contained 70% of the egg masses. This variation in patch use coupled with the importance of a few individual ponds indicates source-sink recruitment dynamics are operating in this patchy population. Dispersal rates of marked frogs relocating away from natal patch complexes are among the highest ever recorded for amphibians averaging 51% for first-time breeders. In contrast, experienced breeders had high annual fidelity to breeding patches with only 7% dispersing to new breeding patches between years. Observed

interbasin dispersal over mountain passes equaled 1% of marked individuals indicating proximal basins in subalpine regions can lack strong demographic independence. Results from this study highlight the benefits of investing in long-term amphibian population monitoring at scales relevant to populations.

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Detection of Stream-breeding Amphibians Using Environmental DNA

Detection of aquatic vertebrates using environmental DNA (eDNA) in water samples is a promising new method for documenting the presence of native and invasive species. To test the efficacy of this method for detecting stream vertebrates, we designed molecular quantitative PCR assays for two low-density, lotic amphibians (Idaho Giant Salamanders, *Dicamptodon aterrimus*; Rocky Mountain Tailed Frogs, *Ascaphus montanus*) in north-central Idaho. We tested how sample collection methods affected detection probabilities using 1L water samples pumped through nitrate-cellulose filters. Preliminary results suggest that instream sampling lead to higher estimates of eDNA for tailed frogs than grab sampling. We also found that instream sampling lead to perfect detection in sampled streams, while detection probabilities for grab samples ranged from 0.83 – 1.0. We surveyed 13 streams using both eDNA and field methods; of these, both methods found tailed frogs at all sites, but eDNA methods found Idaho giant salamanders at two sites where they were not detected using field methods. We conducted field tests of the lower limits of detection for eDNA of Idaho giant salamanders, finding that the eDNA of this species could be detected after 6 hours 5 m downstream of 5 experimentally introduced (caged) salamanders. More work is required to test the limitations of eDNA applications; however, this technique has the potential to be a highly sensitive and cost-efficient tool for the detection and monitoring of both native and invasive species across aquatic systems.

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Use of the Antifungal Drug Itraconazole to Treat *Batrachochytrium dendrobatidis* on Wild Caught Juvenile Cascades frogs (*Rana cascadae*) from the Lassen Region of California

Populations of Cascades frogs (*Rana cascadae*) in the Lassen Region of California have declined, with only twelve known populations remaining. In the past five years we have documented low recruitment and survival of juvenile frogs, in part due to the amphibian disease, chytridiomycosis. In this experiment we tested the efficacy of treating wild-caught, newly metamorphosed, Cascades frogs using the anti-fungal drug itraconazole. Our goals were to determine if the drug cleared infections and if treatment would improve over-winter survival of frogs released at their sites of capture. In early September 2012, we captured 60 metamorphs, transported them to a temperature-controlled animal room at Humboldt State University, and randomly selected 30 for a 4-day, 10 min soak in 0.01% itraconazole solution. The other 30 frogs were soaked in RO water. Animals were swabbed before and after treatment and were marked according to treatment or control groups before being released. We completed two recapture surveys at two and five weeks post-treatment and swabbed all metamorphs encountered. Quantitative PCR analysis was used to determine *Batrachochytrium dendrobatidis* (*Bd*) zoospore levels for comparison between groups. Five weeks post-treatment 8 of 12 captured control frogs were positive for *Bd* (mean GE = 2717, SE = 911) and 1 of 8 treatment frogs were positive for *Bd* (GE = 244). During both follow up surveys, treatment frogs weighed significantly less than control frogs ($p<0.01$). This difference in weight could be a sub-lethal effect of the treatment.

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Habitat Factors Mediate Coexistence between *Rana cascadae* and Introduced Trout

Habitat heterogeneity and refuges have been recognized as important to predator prey coexistence in many ecological systems, however they are seldom considered in invasive species management. Non-native trout reduce the probability of occupancy of mountain lakes by the threatened amphibian, *Rana cascadae*, yet some lakes with trout do support breeding populations of the frogs. I measured extent of probable refuge habitat in lakes with introduced trout and cascades frogs to see whether refuge habitat is responsible for observed patterns of coexistence. I related habitat variables to probability of breeding by *R. cascadae* using generalized additive models. Factors most strongly related to fish-frog coexistence included a large extent of emergent vegetation, low bank slope, and presence of western toad (*Bufo boreas*) larvae. *Bufo boreas* larvae are unpalatable to fish and resemble small larvae of *R. cascadae*. They may provide protection from trout via Batesian mimicry. Higher numbers of nearby *R. cascadae* populations were correlated to coexistence to a lesser extent, indicating a small level of rescue-effect dispersal from nearby water bodies. Learning which types of lakes do and do not allow *R. cascadae* to coexist with fish may help managers decide which lakes to target for trout stocking or removal when formulating basin-wide management plans.

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The Grand Dame of the North Fork Feather River: Insights into Longevity from Photographic Identification of Individual Foothill Yellow-legged Frogs (*Rana boylii*) Using Chin Mottling Patterns

Since 2002, we have identified individual foothill yellow-legged frogs (*Rana boylii*) using mapping of chin mottling patterns by visually matching digital photographs. Photographs were collected from three separate field studies in California of adult and subadult frogs from Hurdygurdy Creek (Humboldt Co.; 2002-2004), North Fork Feather River (Butte Co.; 2004-2012), and Little and Big Carson creeks (Marin Co.; 2008-2012). Over 1,950 individuals have been identified to date from these studies and PIT-tags were inserted into a subset of 316 individuals. Eighty-four PIT-tagged individuals were recaptured and verified that no two individuals had identical markings and that these markings did not change with growth. Longevity estimates based on length frequency histograms and von Bertalanffy growth model analyses for this species indicate that both male and female *R. boylii* may live as long as 12 years. During 2012, a female first captured in 2004 and recaptured and photographed in 2005, 2007, 2008, 2009, and 2012 substantiated this estimate for female *R. boylii*. This method continues to be a reliable, non-invasive way to conduct mark-recapture studies on *R. boylii*, and has proven beneficial for determining movement, breeding patterns and estimates of longevity in this long-term study.

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Male Mate Choice and Species Recognition via Chemical Cues in Pacific Newts

Species recognition during mate choice is a critical component in maintaining reproductive isolation between species that are closely related. Pacific newts are comprised of four distinct yet closely related species. This particular study focuses on male mate choice behaviors among three of the four species living in sympatry in southern Mendocino county, California; *Taricha granulosa*, *Taricha torosa*, and

Taricha rivularis. Here, we examine mate choice decisions and species recognition of male *T. granulosa* via chemical cues alone. We conducted experiments in the field using a Y-maze apparatus. The maze allowed the flow of aqueous chemical cues towards the choosing male without revealing any visual cues. Mate choice was analyzed using a goodness of fit test and a generalized linear model to control for environmental variables. We found that males significantly preferred conspecific females when given the option of a water control. Males also significantly preferred the the cues of female conspecifics when given the option of female *T. granulosa* vs. male *T. granulosa*. Interestingly, *T. granulosa* males did not prefer the cues of female conspecifics over female *T. rivularis* with choices being almost random. However, *T. granulosa* males did prefer the cues of female conspecifics over female *T. torosa.* Our results suggest that male *T. granulosa* breeding in ponds can distinguish between female and male conspecifics via chemical cues, and that chemical cues may function to prevent heterospecific pairing between *T. granulosa* and *T. torosa*.

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Plasticity of Breeding in Foothill Yellow-legged Frogs (*Rana boylii*) Relating to the Spring Snowmelt Recession in the Sierra Nevada

In highly dynamic and stochastic riverine environments, organisms must constantly adapt to temporal and spatial changes. The lotic amphibian *Rana boylii* (foothill yellow-legged frog), has evolved to breed in synchronicity with seasonal spring snowmelt, generally timing egg deposition to occur during the end of spring runoff or the descending limb of the snowmelt hydrograph. For *R. boylii*, the major abiotic factors that can affect phenology (e.g., the timing of naturally recurring phenomena such as breeding) include day-length, temperature, and flow regime (snowmelt, rainfall, etc.). In the Mediterranean climate of California, the general pattern of wet winters and dry summers is predictable across years; however, individual water year types vary across a broad spectrum of hydroclimatic conditions, from very dry to very wet, and are associated with differences in the magnitude and timing of precipitation and correlated shifts in water temperature. In streams and rivers these differences from year to year can be large and may be exacerbated by local conditions related to hydroelectric power generation. We present preliminary research showing large differences in breeding timing in *R. boylii* between a wet water year, with some of the latest oviposition documented in the Sierra Nevada, and a subsequent dry water year. Based on additional research data, *R. boylii* appears to exhibit high plasticity in the timing (in days) of breeding, but still remains strongly correlated with the spring snowmelt recession. This research helps expand current knowledge about breeding phenology for this species, and may inform future management planning and climate change monitoring efforts by more accurately defining the potential breeding period in the Sierra Nevada.

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The Demography of Sources and Sinks in an Amphibian Metapopulation

The discrete habitats occupied by subpopulations of a metapopulation often vary in character such that survival and production vary through time. We examined this hypothesis by analyzing the demographics of 12 subpopulations within a metapopulation of Columbia spotted frogs (*Rana luteiventris*) in central Idaho. Preliminary results suggest that adult survival was high 0.81 (95% CI: 0.68 – 0.90) and did not vary among subpopuations. Reproduction was high, with successful breeding occurring about 75% of the time over the last 17 years in all but three subpopulations. Despite fairly stable reproduction, recruitment of juveniles and recruitment of adults varied widely among some subpopulations and in some years, suggesting that factors influencing metapopulation dynamics vary spatially and temporally. Preliminary

analyses indicate that only two subpopulations functioned consistently as sources (lambda > 1), whereas the others either functioned as sinks (lambda < 1) or fluctuated between source and sink. Understanding the demography of subpopulations can help explain the persistence of species in heterogeneous landscapes and aid conservation and management decisions regarding habitat protection.

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The Impact of a Fungal Pathogen on the Cascades frog: Does Variation in Virulence Matter?

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has been implicated in epidemics and extinctions of amphibians worldwide. The Cascades frog (*Rana cascadae*) has been shown to be susceptible to *Bd* in the laboratory, and has experienced dramatic declines at the southern end of its range (in northern California). Interestingly, *R. cascadae* is still widely distributed in the California's Klamath Mountains in spite of the fact that *Bd* is widespread. We hypothesized that different patterns of *R. cascadae* population persistence in the Klamath Mountains and southern Cascades may be due differences in *Bd* virulence or frog susceptibility (or both) between these two mountain ranges. To test these hypotheses we conducted a laboratory experiment in which *R. cascadae* from the Klamath Ranges and the southern Cascades were exposed to *Bd* from each mountain range. We also monitored *R. cascadae* abundance and *Bd* infection at the *Bd* collection sites for multiple years. Our experiment showed that a *Bd* strain isolated from the Klamath Ranges was much more virulent than a *Bd* strain isolated from the Southern Cascades, but there were no discernible differences in susceptibility between frogs collected from different sites. The site from which Bd was isolated in the Klamath Ranges experienced high rates of infection and a dramatic decline in the abundance of juvenile frogs which may be due, at least in part, to the presence of a particularly virulent *Bd* strain. These findings suggest that local processes may play a key role in determining the impact of *Bd* on declining amphibians.

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Pond-and-Plug Meadow Restoration Creates Habitat for Native and Invasive Species

The primary meadow restoration method now being used to repair highly degraded mountain meadow systems in California is "pond-and-plug". This method aims to restore hydrologic processes that allow the existence and persistence of a shallow water table and can result in meadow habitats with greater vegetative productivity, increased floodplain inundation, increased ability to sequester carbon, and greater habitat stability. However, pond-and-plug restoration requires major land disturbance and results in novel systems. Alluvial material is excavated from the degraded channel and adjacent floodplain and is used to plug the incised channel in the meadow habitat. This process, repeated down the length of the channel through the project area, results in a series of borrow pits and dams that can cover several acres. In many cases, the created ponds become permanent, deep water habitats that can easily be invaded by detrimental non-natives such as American bullfrogs (*Lithobates catesbeianus*) and signal crayfish (*Pacifastacus leniusculus*). Most pond-and-plug restoration projects occur in the Sierra Nevada between 1400 m and 2100 m (4600-6900 ft) elevation and, thus, may become pathways for non-native species to move up from the foothills and invade sensitive high elevation habitats. For example, the meadow restoration ponds at Carmen Creek (1450 m elevation) on the Tahoe National Forest support high densities of bullfrogs and crayfish. As large-scale pond-and-plug projects become more common, both pre- and post-project assessments should include consideration of the costs of invasion by non-native aquatic species.

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Population Genetics of Decaying Range Edges in the California Red-legged Frog (*Rana draytonii*).

The U. S. Geological Survey is accumulating population genetic and phylogeographic data on the threatened California Red-legged Frog (*R. draytonii*) to investigate the effects of demographic declines on genetic diversity and population structuring throughout the species' range. Efforts to date have focused mainly on populations forming the range edge in southern California, where extensive declines have occurred over the past 40+ years. We find significant decreases in gene flow and genetic diversity from interior to edge locations, and that the isolation and limited diversity of edge populations leaves them highly susceptible to local extinction. We have recently begun to assess the genetic structuring of populations in the northern Sierra Nevada to test whether imprints of demographic decline mirror those at the southern range edge, and to dispel a myth that *R. draytonii* were introduced to the Sierra Nevada to provide gold miners with a tasty food source. This talk will present preliminary results of this work, as well as other range-wide phylogeographic patterns. We will also discuss how our data are being used to inform translocation and re-establishment efforts for *R. draytonii* in San Diego County.

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Uncovering the Origin of Pathogenic Chytrid Fungus in Japan

The globally emerging amphibian disease, chytridiomycosis or chytrid, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (hereafter "Bd") has devastated amphibian populations on multiple continents. Amphibian population crashes, due to chytridiomycosis have resulted in critically low regional population level drops and in some cases species extinctions. However, many species are not susceptible to the disease. There has been much debate on the origin and mechanism of the spread of Bd. To understand and contain the propagation and expansion of a disease it is essential to understand its origins, because areas of endemism should reveal mechanisms for host survival. Previous studies for origination of Bd show strong evidence for an endemic lineage in Japan. Amphibians in Japan have not shown the types of mortality rates and crashing population events as in other areas of the globe, suggesting a host pathogen history of coexistence. In this study we used a retrospective analysis on 670 archived amphibian museum specimens collected across the archipelago of Japan from 1900 to 2011. We used a PCR assay developed in our lab to test museum specimens and found that Bd was not extensively prevalent across a spatial and temporal scale. Detection of low Bd prevalence in the past suggests the disease may be an emerging pathogen even in Japan.

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Roundup Exposure Decreases Risk of *Batrachochytrium dendrobatidis* Infection in Pacific Treefrog (*Pseudacris regilla*) Larvae

Some researchers describe the association between the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) and amphibian population declines as a classic case of a novel pathogen causing extreme effects in naïve host populations. Nevertheless, the risk, severity, and outcome of *Bd* infection is influenced by many environmental factors, and thus we ignore them at our peril. We tested the influence of the herbicide Roundup and a water mold tentatively identified to the genus *Achlya* on risk of *Bd* infection. Roundup has direct negative effects on *Bd* as well as amphibians, so its effects on amphibian-*Bd* interactions are difficult to predict. Similarly, water molds produce zoospores that could compete with *Bd* zoospores for space on amphibians, but water mold infection could weaken resistance of amphibians to *Bd*. We employed a factorial laboratory experiment that exposed Pacific treefrog (*Pseudacris regilla*) larvae to three levels of Roundup (0, 1, and 2 ppm of active ingredient), two water mold treatments (present and absent), and two *Bd* treatments (present and absent). We did not find any effects of water mold. However, there was a negative, linear relationship between Roundup concentration and proportion of larvae infected with *Bd*, suggesting that direct negative effects of Roundup on the fungus are more important than direct negative effects of Roundup on hosts in determining the prevalence of *Bd* infection. Although our study did not detect competition between water mold and *Bd*, further exploration of interactions between *Bd* and other microorganisms could discover new ways to control *Bd* in wild amphibian populations.

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Delayed Life History Effects, Multi-Level Selection, and Evolutionary Tradeoffs in the California Tiger Salamander

Delayed life history effects (DLHEs) occur when fitness in one life stage affects fitness in subsequent life stages. Given their biphasic lifecycle, pond-breeding amphibians provide a natural system for studying DLHEs. In this study, we use numerous mark-recapture techniques (visual implant alphanumeric tags, visual implant elastomer, and photographic pattern recognition) and a large drift fence array (221 fences monitored over a six-year period) to document selection in a population of the endangered California tiger salamander (*Ambystoma californiense*). We find that DLHEs are prominent across all life stage transitions and that there is variation in whether selection acts at the individual or cohort level. We also demonstrate that there is large variation in average cohort fitness (> one order of magnitude), which is the variation that DLHEs act upon. A literature review reveals that this high level of intercohort variation is not restricted to *A. californiense*, but also occurs in numerous other pond-breeding amphibians, and that appropriately documenting the magnitude of intercohort variation requires long-term studies (~two population turnovers). Given the profound effect that DLHEs can have on population dynamics, understanding intercohort variation in average metamorph quality and the action of selection at the individual or cohort level is critical for developing realistic models of population dynamics. When developing models of population dynamics, greater attention should be paid to variation in average fitness rather than focusing exclusively on variation in total numbers. Finally, we empirically document an

evolutionary tradeoff between mass at metamorphosis and date of emergence. This tradeoff may play a role in maintaining the variation in mass (fitness) at metamorphosis.

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Chytridiomycosis Disease Ecology across *Batrachoseps attenuatus* Populations in California

Batrachochytrium dendrobatidis (Bd) is a virulent fungal pathogen that causes the disease chytridiomycosis among amphibians and is implicated in the extirpation of amphibian populations and the extinction of amphibian species throughout the world. The pathogen has flagellated zoospores that swim through water or along amphibians' moist skin to infect their hosts. Though chytridiomycosis is widely considered to be an aquatic disease, it has recently been detected in completely terrestrial salamanders, such as the California slender salamander, *Batrachoseps attenuatus*. Nearly 1300 *B. attenuatus* museum specimens that had been collected over 70 years were tested to reveal temporal and spatial variation in the presence and infection intensity of Bd infection in different populations. We compared contemporary infection levels to historical infection levels in all Bd positive populations in order to determine the persistence of infection over time. We also estimated within-site spatial patterns of Bd infection in contemporary populations. Because *B. attenuatus* is highly gregarious in its nesting behavior, we propose that social behavior may provide an opportunity for transmission of Bd.

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Evolutionary Dynamics and Management Challenges of Hybrid California Tiger Salamanders

Sixty years ago, tens of thousands of non-native barred tiger salamanders (*Amybystoma tigrinum*) were introduced into the Salinas Valley of central California, where they began hybridizing with native California Tiger Salamanders (*A. californiense*: CTS). Fifty years later, the CTS was listed under the US Endangered Species Act. Comprehensive population genetic data from our lab indicates that about a quarter of the range of the endangered CTS is now an admixture of native and non-native genes, and that two classes of non-native genes exist on the landscape. In Central coast regions near the original introduction sites, hybrid swarm populations have replaced pure native CTS; these populations often contain > 70% non-native alleles, and have life history features associated with the non-native species. In addition, about 5% of the genome is superinvasive, consisting of highly favored non-native alleles that have spread another 100 km beyond the hybrid invasion front. We know little about the ecology of superinvasive populations. A key question is how to manage this invasion in the context of an endangered species. Should hybrids be protected, particularly those with only superinvasive alleles? Should we manage for genetic purity, for ecological authenticity, or simply for the greatest possible fraction of native genes? Given that it appears virtually impossible to remove the non-native genes, what is the most appropriate conservation target for this system? More generally, how should we manage hybrids that threaten the ecological and evolutionary integrity of endangered native taxa and communities?

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***Batrachochytrium dendrobatidis* Prevalence in Northern Red-legged Frogs (*Rana aurora*)—10 Years Later**

Nieto (2004) found that the amphibian chytrid *Batrachochytrium dendrobatidis* (*Bd*) existed in larval *Rana aurora* at a low (6.4%) overall prevalence throughout the coast of northern California in the summers of 2001 and 2002. The primary goal of this research was to assess the current prevalence of *Bd* in the same populations of *R. aurora*. In summer 2011, the same thirteen populations, ranging from Eureka to Crescent City, were sampled. A total of 81 tadpoles (including 16 with abnormal mouthparts) and 42 frogs were non-lethally swabbed for qPCR analysis. Larvae were also processed for histological examination. Based on qPCR analysis, estimated prevalence of *Bd* in the summer of 2011 was 4.8% in larvae and 14.3% in adults. Based on histological analysis, prevalence in tadpoles was 3.3%. The prevalence of *Bd* appears to be remarkably consistent, with no significant differences between studies or life stages. As in Nieto's (2004) study, oral abnormalities were a significant predictor of *Bd* infection in larval *R. aurora*. For *Bd* identification, molecular and microscopic diagnoses yielded nearly identical results. These results suggest that *Bd* is endemic in these *R. aurora* populations.

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How Should We Manage Amphibian Biodiversity in California?

California has a rich and diverse assemblage of native amphibians. These species face many daunting management challenges and are subject to a complex regulatory and legal environment. Additionally, more than half of them (53%) are recognized as threatened, endangered, or designated with special status due to imperilment. Given these concerns, there is an urgent need to evaluate the current management framework for amphibian taxa in California and to develop a more comprehensive management strategy to guide future conservation decisions. Here, we report on progress to develop a cohesive vision for the management of amphibian biodiversity in California. The document is intended to address the current and upcoming management needs for this taxon to provide guidance to the California Department of Fish and Game. We also present recommendations and needs that were highlighted at the California Reptile and Amphibian Conservation and Management Forum held in Placerville, CA in 2011 following last year's CA/NV APTF meeting. The management strategy addresses such issues as consumptive use of native amphibians, data use and management, capacity building, conservation education, public outreach, citizen science, improved coordination with federal partners, and methods for prioritizing amphibian conservation areas based on distributions and habitat quality. The management strategy also highlights the major threats that affect these species in California, provides an ecoregional overview of these threats, and highlights information needs or recommended management actions.

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Amphibian Conservation and a *Growing Issue* on the North Coast

Since 2008, the Department of Fish and Game's Coastal Conservation Planning (CCP) group has documented in coastal northern California the distribution of the northern red-legged frog (*Rana aurora*) and foothill yellow-legged frog (*R. boylii*), both California Species of Special Concern. Data derived from visual encounter surveys are intended to populate the California Natural Diversity Database for use by the public, planners, consultants, and managers especially in the context of discretionary projects subject to the California Environmental Quality Act. The goal of our work is to keep native frogs

"common" by documenting their occurrence and addressing conservation threats to the extent possible. CCP has increased internal and external awareness of amphibian declines and conservation through education and outreach, partnerships, presentations and focused recommendations and guidance on land use planning and development, including permit conditions. We will report on these topics as well as *Growing Issues* related to Proposition 215 "medical" marijuana cultivation that have implications on the conservation of amphibian and aquatic resources in Northern California.

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Woodland Salamanders as Metrics of Forest Ecosystem Recovery: A Case Study from California's Redwoods

Woodland (Plethodontid) salamanders occur in huge numbers in healthy forests in North America where abundances of many species vary along successional gradients. Their high numbers and trophic role as apex predators on decomposer arthropods influence nutrient and carbon pathways at the leaf litter/soil interface. Extreme niche conservatism and low vagility confer additional advantages to using these salamanders as metrics of forest ecosystem integrity (resistance-resilience). Mill Creek is a 103 km^2 commercially logged redwood forest watershed acquired as parkland where original primary forest is to be restored. This study evaluated woodland salamanders as metrics of seral recovery at Mill Creek. Surface counts and body condition were examined in four sets of stands, two early seral, one mature, and one of primary forest (never harvested old-growth). Later seral sites were closer to the coast where fog increased available moisture, while younger sites were further inland at higher elevations where fog was reduced. We used ANCOVA with a PCA-derived composite landscape covariate to distinguish the effects of coastal proximity and advancing succession. Both effects increased counts of California Slender Salamanders (*Batrachoseps attenuatus*); advancing succession alone increased counts of Ensatina (*Ensatina eschscholtzii*). Body condition means and variances in these two species were lower in older stands; in the Del Norte salamander (*Plethodon elongatus*) only means were lower. Coastal proximity increased the mean body condition of *E. eschscholtzii*, but did the opposite in *P. elongatus*. Modeling of counts and body condition along environmental gradients associated with succession indicated that increased structural complexity in late seral forest supported larger populations, with greater competition within (intraspecific) and among (interspecific) species, in a relatively more rigorous selective regime; suggesting greater population fitness in two species that appears directly related to advanced succession.

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California Amphibian and Reptile Species of Future Concern

Climate change poses unique challenges to wildlife managers. There is uncertainty in both how the climate will change and how those changes will filter through the complex web of interactions that determine habitat suitability for individual species. For amphibians in particular, we often lack critical natural history information that would help determine which taxa will be most sensitive. As part of the California Department of Fish and Game's ongoing efforts to assess the vulnerability of different taxonomic groups to climate change, we used ecological niche models to predict current and future ranges for all 153 reptile and amphibian species in the state. During this process we compiled a data set of over 120,000 unique localities, used model selection to appropriately parameterize models and select species-specific climate variables, and downscaled future climate data specifically for this project from the forthcoming 2014 version of the Intergovernmental Panel on Climate Change Fifth Assessment Report. We are currently in the process of developing metrics to assess vulnerability from our models of future species distribution across a range of approaches to modeling climate and different possible future carbon

dioxide levels. Our goals for this project include providing guidance to the Department on which taxa are most vulnerable, suggesting monitoring strategies to track predicted responses to climate change over time, and contributing new tools to the rapidly developing field of ecological niche modeling.

* Indicates speaker in multi-authored presentation