California/Nevada Amphibian Population Task Force
2011 Meeting

PRESENTATION ABSTRACTS

Yosemite National Park, CA
January 6-7, 2011

In Memory of Jeff Maurer, an Outdoor and Amphibian Enthusiast Extraordinaire, 1963 - 2009

California-Nevada Amphibian Populations Task Force
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ABSTRACTS

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Inter-annual Variability in Observations of California Tiger Salamander Breeding in Central California Stock Ponds

The biphasic California tiger salamander often colonizes and utilizes artificial water bodies for its aquatic breeding phase. We looked at data collected over a 9-year period during mitigation monitoring of cattle stock ponds and created wetlands in eastern Contra Costa County. Among 73 ponds where California tiger salamanders were known to breed during this time, we found that breeding occurred sporadically and unpredictably. At only a single breeding site did breeding occur every year. Among all sites, breeding was detected an average of 2.9 times per pond in a 9-year period. Years when breeding was not detected ranged from 0 years (breeding detected every year), to 6 years where no breeding was observed (n = 3 ponds). Eighteen aquatic breeding sites (25%) had gaps between observed breeding events of 3 years or more. Seventeen aquatic breeding sites (23%) had detected breeding only once in the 9-year period. We contend that the extremely high level of inter-annual variability of detected breeding may result in underestimated rates of site occupancy, may place some populations in jeopardy, and may potentially slow recovery of this threatened species.

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The Sierra Madre Yellow-legged Frog: A Decade of Monitoring, Conservation, and Restoration

Since the early 1970s, the mountain yellow-legged frog (*Rana muscosa*) has declined precipitously in southern California. With less than 200 adult frogs in the wild and occupying less than 1% of historic range, the mountain yellow-legged frog may be the most endangered vertebrate in southern California today. Over the past decade, many individuals, NGOs, and government agencies have collaborated as a working group to conserve this species. The goals of this working group are to stabilize declines and

begin the recovery process for this frog. In working to achieve these goals, we have shared data and resources, streamlined state and federal permitting, and developed and implemented new techniques and protocols. Over the last few years we have seen success with the restoration efforts employed. These efforts include invasive species removal, captive breeding, translocation, and strategic Forest Service area closures. With the appropriate pieces in place, we feel confident that this short-term success will continue and accomplish real recovery for the mountain yellow-legged frog.

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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 (*Emmys marmorata*) is a California State Species of Special Concern and listed as a Sensitive Species by the Oregon Department of Fish and Wildlife, as the species has been declining throughout its range. Understanding population specific life-history traits is necessary to make proper management decisions for those local populations. We have been monitoring western pond turtles on the mainstem Trinity River near the Lewiston Dam and on the South Fork Trinity River near Willow Creek, California since 2003 to better assist managers in consideration of both restoration designs and possible flow management that may benefit this long-loved species. From 2005 to 2007, we combined the use of radio-telemetry and thermometers on the external carapace of turtles to better understand their thermoregulatory behavior in relations to river temperatures on these two forks of the Trinity River. We also conducted a three summer field study with mark-recapture on each river to obtain population estimates and to glean information on demographic parameters such as 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 and these results in both a change of that population's thermoregulatory behaviors and in their growth in this colder aquatic environment compared to the turtles on the South Fork Trinity River. We present here both the basking behaviors and growth of turtles under the age of ten and contend that these two aspects of western pond turtles are linked together.

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Factors Influencing Lotic Breeding Amphibians in the East Bay Regional Park District

The East Bay Regional Park District (District) consists of 65 parks, recreation areas, wilderness lands, shorelines, preserves, and land banks, encompassing over 108,000 acres in Alameda and Contra Costa Counties. Five amphibian species breed in the District's streams: California Newt (*Taricha torosa*), Western Toad (*Bufo boreas*), Pacific Treefrog (*Hyla regilla*), California red-legged Frog (*Rana draytonii*) and Foothill Yellow-legged Frog (*Rana boylii)*. We evaluated aquatic habitat suitability for amphibians using abiotic and biotic variables in 42 streams. For 26 distinct stream reaches occupied by *R. draytonii*, we found sub-adults and adults inhabiting streams with gradients ranging from 0.4 – 21.0% slopes. Stream gradient appeared not to be a limiting factor for distribution of *R. draytonii*, but flow velocity and exposure to extremely high stream flows did appear to determine oviposition site selection. From 1996 to 2010 we also conducted focused breeding censuses in the largest watershed, Alameda Creek, to contrast the populations among a reach with natural flow variation, a reach experiencing flow diversion, and a

reach with artificial flow conditions regulated by Calaveras Dam. The time series data for *R. boylii*, *R. draytonii*, and the other taxa, support the hypothesis that variability of flows, specifically the ratio of maximum to minimum flow during the breeding season, correlates strongly with negative population trajectories and high mortality of eggs and larvae. These results suggest that although 80% of the District area is protected as natural parkland, the resident amphibians may remain vulnerable to anthropogenic stressors such as flow regulation and climate change.

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Recent Amphibian-Related Conservation Actions in Sequoia and Kings Canyon National Parks

This paper provides an overview of recent management and research projects designed to conserve three species of threatened amphibians in Sequoia and Kings Canyon National Parks (SEKI), including two species of mountain yellow-legged frogs (MYLF) and the Yosemite toad (YOTO). In 2001 SEKI began removing nonnative fish from naturally fishless lakes and associated streams to assess the feasibility of restoring aquatic habitat for native species, with an emphasis on conserving MYLFs. By 2010 SEKI had removed approximately 40,000 fish, including full eradication from eight lakes and near-eradication from three lakes. In nine of these lakes, in which MYLFs remained disease-free three years after trout removal, average population density increased significantly. Several of these populations are now among the largest in the entire range of MYLFs. Due to these results, SEKI is proposing to expand this work to additional high elevation basins across these parks, including using alternative methods to remove fish from larger waters and achieve eradications more quickly. Results of recent surveys for MYLF populations across much of SEKI will be presented to report the current status of these two species in these parks. Results of recent surveys for YOTO populations in the northern portion of SEKI will also be presented to indicate the current status of YOTO at the southern terminus of its range.

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Update on Revised *Amphibian and Reptile Species of Special Concern in California* and Other CDFG Amphibian-related Conservation Efforts

The amphibian and reptile Species of Special Concern (SSC) document, first completed for the Department of Fish and Game by Mark Jennings and Marc Hayes in 1994, is currently under revision via a Department contract with the University of California at Davis. The draft document is scheduled for review by a Technical Advisory Committee, herpetologists, agency staff and others during January 2011, with publication anticipated in late 2011 or early 2012. The document includes accounts for 45 taxa containing brief sections on identification, taxonomic relationships, life history, habitat requirements, distribution, abundance trends, threats, status, management recommendations, implications of climate change, as well as monitoring, management and survey needs. Also included are range and distribution maps for all taxa. This publication will join the 2008 bird SSC update, along with concurrent revisions of

the fish and mammal SSC publications. Updates on other pertinent Department activities will also be provided.

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Development of Regional Habitat Suitability Criteria for Foothill Yellow-Legged Frog Oviposition and Tadpole Rearing in Northern Sierra Nevada Rivers

In hydropower relicensing, a variety of tools are available to determine impacts of flow prescriptions on sensitive aquatic species. Instream flow modeling is one tool that is used to assess habitat suitability for foothill yellow-legged frogs (*Rana boylii)*. Using data on microhabitat conditions from *R. boylii* oviposition and tadpole rearing locations at eight study sites in the northern Sierra Nevada, we developed regional Habitat Suitability Criteria (HSC). Both univariate (percentile-based and interval-based Habitat Suitability Indices [HSI]) and multivariate (logistic regression) techniques were evaluated. Predictive performance and transferability of the HSC were compared by applying the models to validation data gathered from other rivers in the Sierra Nevada, as well as with a 2-dimensional hydrodynamic model. Conditions under which predictive performance was poor were evaluated to discern the limitations of each technique. Univariate models performed well on rivers that had similar geomorphology to the study rivers. Small rivers and creeks with shallow depths and finer substrates required locally-derived HSC. The percentile-based HSI is recommended as a regional habitat suitability criteria when the goal is to assess categorical levels of suitability. The interval-based HSI would be appropriate if further information on population outcomes (e.g., population trajectory, survival rates) could be quantitatively linked to fine scale gradients of suitability in hydraulic conditions. The univariate HSI are easily applied in 2-dimensional hydrodynamic models, which can provide information on oviposition and tadpole rearing conditions under various flow regimes. Managers can use HSC to make flow recommendations beneficial to *R. boylii* during the hydropower relicensing process.

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Terbinafine Hydrochloride in Ethanol Effectively Clears *Batrachochytrium dendrobatidis* in Amphibians

Amphibian chytridiomycosis, caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), has been implicated in the decline and extinction of amphibian species worldwide, in addition to catastrophic losses of animals in captivity. Conservation of threatened amphibians, including captive breeding and maintenance of animals in zoos, research facilities, 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 (HCl) in alcohol, which is available over-the-counter as Lamisil AT™ (Novartis Pharmaceuticals Inc., New York, NY). Treatments consisting of 5-minute soaks in fresh 0.01% or 0.005% terbinafine HCl in alcohol 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 in alcohol has a high therapeutic index as a treatment for *Bd* infection in living post-metamorphic amphibians.

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Pesticides, Air Flow, and Population Declines of Sierra Nevada Alpine Frogs

Airborne pesticides from the Central Valley of California have been implicated as a cause for population declines of several amphibian species, with the strongest evidence for the mountain yellow-legged frog complex (*Rana muscosa* and *R. sierrae*) in the Sierra Nevada. In this presentation I summarize results of three recently published or in-press articles authored by many co-authors and myself that are pertinent to the pesticide-decline hypothesis for these species. We measured pesticide concentrations in multiple media at multiple times at up to 28 sites at high elevation in the southern Sierra Nevada and evaluated the pesticide-decline hypothesis in three ways: (1) we described the temporal variation in concentrations in lake water and compared these values to established critical levels; (2) we tested the hypothesis that pesticide concentrations decrease with distance from the Valley, a pattern that could explain the east-west pattern in population declines; and (3) we tested the hypothesis that pesticide concentrations are correlated with frog population status (i.e., fraction of suitable sites occupied within 2 km of a site). Media represented were air, lake water, sediment, and tadpoles of a surrogate species (*Pseudacris sierra*); we also measured acetyl cholinesterase activity in *P. sierra* tadpoles. Results do not support the hypothesis for a pesticide effect on frog populations. Concentrations of up to nine pesticide compounds (both currently and historically used forms) were extremely low, on the order of 1 part-per-trillion in lake water and 1 to 10 parts-per-billion in tadpole tissue and sediment, well below critical levels. Evidence for a distance effect in concentrations or cholinesterase activity was limited. Virtually no association was found between frog population status and any chemical metric. In contrast, a more parsimonious explanation for the dramatic and continuing population declines of these frogs is chytridiomycosis.

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A Programmatic Safe Harbor Agreement for the Recovery of Two Threatened Amphibians in Alameda County, California.

Safe Harbor Agreements (SHA) were developed under the Endangered Species Act to provide protection and incentives for private or non-Federal landowners who voluntarily restore habitat for federally listed threatened and endangered species. The Alameda County SHA for the California tiger salamander (*Ambystoma californiense*) and California red-legged frog (*Rana draytonii*) is the second in California that is "programmatic" – which covers a large area with multiple landowners. This programmatic SHA was developed within the context of a "Wildlife Friendly Pond Restoration Program" to encourage and support ranchers who want to repair, restore, and manage their stock ponds and surrounding uplands for habitat and provide: compelling cost-share levels by leveraging funding from federal and state programs;

species specific habitat restoration guidelines; and programmatic environmental permitting to reduce regulatory hurdles. The Alameda County SHA is intended to: promote ecosystem restoration through the voluntary restoration, enhancement, and management of essential habitat for threatened amphibians in the county; provide regulatory assurances to landowners who participate and their neighbors; and accomplish habitat restoration and threatened species recovery without negatively affecting ranching activities. We have enrolled seven ranches, (4,191 hectares) with 15 restored ponds. We restored suitable breeding habitat in 9 failed ponds, maintained and improved conditions at 6 failing ponds, and enhanced management at all sites. We will discuss the collaborative efforts leading up to the final plan, implementation of pond restoration, and current progress and expansion of the SHA within the county.

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Contaminants and the Decline of Cascades Frogs

Our study had three specific aims. First was to determine if Cascades frogs and Pacific chorus frogs (*Pseudacris regilla*) living at the same sites accumulate contaminant residues at similar levels. Second was to use chorus frogs as a proxy for Cascades frogs to determine if contaminant residue levels in chorus frogs are significantly higher in areas where Cascades frogs have disappeared than in areas where Cascades frogs are still present. Third, to determine if sediment contaminant levels were higher in areas where Cascades frogs have disappeared than in areas where Cascades frogs are still present.

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Exploring the Evidence of a Historical Chytrid Epidemic in the Yosemite Toad by PCR Analysis of Museum Specimens.

Presented here is a preliminary examination of the historical prevalence of the amphibian Chytrid fungus, *Batrachochytrium dendrobatidis* (Bd) in preserved museum specimens of the Yosemite toad (*Bufo canorus*). The use of previously unemployed qPCR techniques allows for the testing of *many* individuals without the destructive and time consuming rigors histological examination. Skin swabs were collected from 130 museum specimens (dated 1950-2005) and were tested for the presence of Bd with a method recently validated in Dr. Vance Vredenburg's lab by Tina Cheng. As compared to histological examination, Cheng reported zero false positives and few false negatives with the use of Prepman DNA extraction and qPCR for Bd-detection in specimens of *Batrachoseps attenuatus.* When grouped by decade, the detected prevalence of Bd in Yosemite toads ranged from 0% (pre 1960) up to 82% in the 1990's, and again dropped off after the year 2000. The results appear consistent with an SIR disease epidemic model but further sampling is needed, particularly for animals collected in the 1980's (n=3). Further sampling may provide strong evidence that Bd has played a significant role in the decline of the Yosemite toad over recent decades, and that a Bd epidemic occurred during the time of observed die-off events (e.g., Kagarise Sherman and Morton). This work is an ongoing part of a master's thesis project designed to quantify the effects the fungal pathogen, Bd on the threatened Yosemite toad. Expected beneficial impacts of this research include enabling the more effective conservation management of a declining amphibian.

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Do Geothermal Ecosystems Provide Amphibians with Refuge from Chytridiomycosis?

Dramatic declines and extinctions of amphibian populations throughout the world have been associated with chytridiomycosis, an infectious disease caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*). Laboratory experiments have demonstrated that temperature plays an important role in chytridiomycosis disease-host dynamics, and that *Bd* ceases growth at temperatures above 28°C. Here we describe natural micro-climatic conditions that correlate with a significant reduction in *Bd* prevalence in amphibian hosts. We sampled 221 anurans, including 201 Lowland Leopard frogs (*Rana yavapaiensis*), from 12 sites in Arizona, 7 of which were influenced by geothermal water sources. There was a strong inverse correlation between the water temperature where each frog was captured and *Bd* prevalence. In locations where the disease is known to be present, estimated prevalence of *Bd* dropped from 70-90% at 15°C, to 0-20% in water > 30°C. Within sites, we also observed a strong inverse correlation between *Bd* status of frogs and water temperature. These results are intriguing in light of the current imperiled status of the Relict Leopard frog (*Rana onca*), a close relative to *R. yavapaiensis*. All remaining naturally occurring populations of *R. onca* are now restricted to geothermal influenced springs, and resultant streams, with source temperatures ≥ 30°C. In recent sampling, we found *Bd* positive frogs at one site, and while the sample sizes were low, 2 of the 4 positive frogs were found in the lowest water temperatures recorded for sampled individuals. Our findings suggest that geothermal waters may provide amphibian populations, including *R. onca,* with critical refugia from *Bd* and chytridiomycosis. Some geothermal ecosystems appear to be habitats where amphibian populations can persist in the wild despite the presence of *Bd*, and therefore may provide certain species critical time to develop resistance to chytridiomycosis.

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Are Native Amphibians and Exotic Prey Promoting the Use of Historically Fishless California High-mountain Habitats by River Otters, *Lontra canadensis*?

"My love for the Sierra is like no otter" Jeff Maurer, March 2009

In California river otters are thought to inhabit primarily low elevation water bodies. However, we compiled river otter occurrence records that indicate their presence throughout the high-elevation portions of the Klamath, southern Cascades, and Sierra Nevada mountain ranges. Based on 122 records, river otters were observed in water bodies ranging from 1,100 to 3,288 meters in elevation, with records dating from 1900 to 2010. Many observation records were very recent, with 46% occurring between 1999 and 2010. Over 93% of the water bodies with records of otters contained non-native fishes and 22% contained non-native crayfish. Of the records occurring at water bodies without non-native prey all supported native amphibian and reptile prey. Based on 18 detailed foraging accounts, non-native fishes and crayfish represented 89% of the total prey items while native anurans and invertebrates represented

22%. While we are unsure whether or not river otters occurred in high elevation water bodies prior to the introduction of fish and crayfish, our findings suggest they could have expanded their range into these habitats following the introduction of non-native prey. Given that river otters are known to prey on herpetofauna, sensitive montane amphibian species including the Cascades frog (*Rana cascadae*) and the Sierra Nevada yellow-legged frog (*Rana sierrae*) may be subject to increased predation via facilitation by non-native prey. More research is needed to understand the influence non-native prey has on the distribution and abundance river otters in high elevation areas.

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A Species Distribution Model for Guiding Oregon Spotted Frog (*Rana pretiosa*) Surveys Near the Southern Extent of Its Geographic Range

The Oregon Spotted Frog (*Rana pretiosa*), endemic to the Pacific Northwest, was once considered widespread in complex, warm water wetlands. Over 70% of historic populations are thought to be extirpated with range-wide habitat loss exceeding 90%. Using Maxent, we developed a series of Species Distribution Models (SDMs) to identify suitable habitat and predict the distribution of *Rana pretiosa* toward the southern extent of its geographic range. These SDMs were generated from two sets of spatial data, a set of occurrence points and a suite of environmental variables. Occurrence data included all verified populations within the study area. Environmental variables, used to characterize habitat associated with recognized populations, included variables derived from climatic, topographic, land cover and soil datasets. Three unique SDMs were averaged to produce a single distribution map that predicts and ranks suitable habitat across the species' southern range. We used the averaged output from the SDMs, along with aerial, topographic and National Wetlands Inventory imagery, to identify optimal survey sites within the Klamath and Pit River hydrographic basins. We surveyed 18 sites repeatedly and investigated another 44 sites. We attempted to focus our efforts on private land, as most preceding surveys in this region were conducted on public land. While we did not detect *Rana pretiosa* in California, we documented two individuals at a previously unrecognized site in Klamath County, Oregon. This detection is significant because it represents the species' most northern point of occurrence in the Wood River and because only eight extant populations are currently recognized within the Klamath River hydrographic basin.

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Assessment of the Vulnerability of *Rana pretiosa* to *Batrachochytrium dendrobatidis*

The Oregon spotted frog (*Rana pretiosa*) is at risk across its geographic range. Discovery of the chytridriomycete fungus, *Batrachochytrium dendrobatidis* (*Bd*), in declining populations of *R. pretiosa* raised the possibility that this etiological agent might be a contributor to these declines. This led us to experimentally examine the sensitivity of *R. pretiosa* to *Bd*. Juvenile *R. pretiosa* (4-6 g) exposed to two strains of *Bd* were followed over a 90-day post-exposure period. Though all individuals in the exposed groups became infected, no frog in either group died or showed behavioral or morphological manifestations of disease. Moreover, by the end of the exposure period, nearly all frogs had cleared their infections, and skin sloughing hypothesized as accompanying infection clearing appeared minimal.

However, frogs in both exposed groups gained significantly less mass than frogs in the control group. This experiment, and the recent discovery of *Bd* in non-declining populations of *R. pretiosa*, suggests that the species is *Bd* resistant. We remain cautious in this conclusion, as tested animals were obtained from a population in decline; so tested frogs may be descendants of adults surviving a catastrophic epizootic. Lastly, minimal skin sloughing argues for investigating other mechanisms, such as antimicrobial peptide activity, as the basis for the clearing of *Bd* infections in *R. pretiosa*.

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Chytridiomycosis: An Emerging Infectious Disease

Amphibians worldwide are facing major declines, over 1/3 of all known species are threatened with extinction. Various factors are involved such as pollution, loss of habitat and chemical contaminants. Recent studies have shown that a deadly fungus, *Batrachochytrium dendrobatidis* (Bd), which causes the disease chytridiomycosis, plays a key role in amphibian declines. This disease has affected over 200 species, driving many to extinction. However, not all species of amphibians are susceptible. For example in the Sierra Nevada mountains of California, the Pacific chorus frog (*Pseudacris regilla)* remains abundant despite the rapid collapse of overlapping mountain yellow-legged frog (*Rana sierrae*). To test susceptibility in the lab, 11 juvenile Pacific chorus frogs were infected and monitored over a 10 week period. The infection levels were tested weekly using a real-time PCR assay for Bd. Six frogs became lightly infected with Bd, but after 8 weeks (the length of time susceptible species succumb to Bd) all frogs survived. To increase transmission the frogs were housed together for three months. Infection levels significantly increased in 2 frogs causing 1 to die. These results suggest that the Pacific chorus frog is only susceptible to Bd when Bd transmission is high. Symbiotic bacteria, known to protect some species, may be allowing the Pacific chorus frog to survive. I propose to follow up this study by creating a real-time PCR assay for a known bacterium that protects some species of amphibians from Bd.

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***Batrachochytrium dendrobatidis* in Arizona: Detection by Water Filtration and the Influence of Water Chemistry on Bd Dynamics**

The fungal pathogen *Batrachochytrium dendrobatidis* (Bd) has been linked to amphibian declines worldwide. There is an urgent need to understand the ecology of this pathogen and develop efficient techniques to monitor Bd in the field. We monitored Bd prevalence in 20 populations of boreal chorus frogs (*Pseudacris maculata*) from ponds in Arizona using two monitoring techniques: water filtration and amphibian skin swabs. We also monitored concentrations of the major nutrients, nitrogen (N) and phosphorus (P), and salinity of pond water to examine correlations between water chemistry and Bd prevalence. Initial data indicate that water chemistry, especially salinity and N:P ratios, may influence Bd transmission. N:P ratios in pond water were positively correlated with Bd load and prevalence. Pond water salinity was negatively correlated with Bd incidence. Water filtration was an effective technique to monitor Bd in small ephemeral ponds with high Bd prevalence. Of the ponds that tested Bd positive by skin swabs, 95% (16 of 17) were also found to be positive after three repeated samplings using water filters. These results will aid in site selection for repatriation efforts for frogs susceptible to Bd.

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Changing the Outcome of Amphibian Chytrid Fungus Epizootics Using Field Treatments: Does it Work?

During the past several decades the amphibian chytrid fungus (*Batrachochytrium dendrobatidis* – "Bd") has spread across much California's Sierra Nevada mountains with devastating impacts on amphibians. Mountain yellow-legged frogs (*Rana sierrae*, *Rana muscosa*) are highly susceptible to chytridiomycosis (the disease caused by Bd) and populations are typically extirpated following Bd arrival and subsequent epizootics. However, some populations survive the Bd epizootic and persist despite ongoing chytridiomycosis. Recently-developed models suggest that the probability of frog population persistence might be increased by decreasing the density of Bd zoospores. In 2009-2010 we conducted a field experiment in which we treated *R. sierrae* with the antifungal drug Itraconazole to reduce both infection intensities on frogs and zoospore densities in the associated aquatic habitats. Frogs were treated at three sites and subsequent frog-Bd dynamics at these sites were compared to those at three untreated control sites. All six populations had experienced Bd-caused die-offs during the previous 1-2 years. Prior to treatment, infection intensities were high in all treatment and control populations. Treatment dramatically reduced infection intensities and infection intensities remained low through the summer and fall. In late-summer, all three treatment populations produced a large cohort of juvenile *R. sierrae*, a cohort not seen at the control lakes. However, by Summer 2010 infection intensities had returned to high levels and resulted in complete mortality of juveniles. These results highlight the difficulty of developing effective Bd mitigation strategies.

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SAVE THE FROGS! -- Protecting Amphibian Populations in California and Beyond

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 our arrival in California in February 2010, we have accomplished a significant amount to protect the state's amphibian populations, including: (1) giving over 35 free public presentations on amphibian conservation at schools, universities, nonprofits, museums and community groups; (2) coordinating the 2nd Annual Save The Frogs Day (April 30th, 2010), which was legally recognized by Santa Cruz Mayor Mike Rotkin (who attended my Save The Frogs Day talk at Monarch Community School and is now an active supporter of our efforts). The 2nd Annual Save The Frogs Day was the largest day of amphibian education and conservation action in the planet's history, with 104 events in 21 countries; (3) We persuaded San Francisco's Restaurant Gary Danko to remove the wild-caught Pig Frogs (*Rana grylio*) from their menu, making them the first restaurant in the world to remove frog legs from their menus for environmental reasons, and setting an important precedent in the culinary world; (4) We helped make California the first state in the country to stop issuing permits for the importation of non-native frogs (and turtles) for use as food: 1,194 of our supporters sent letters to the CA Fish & Game Commission urging them to take this action, and I testified at the Commission's re-consideration hearing at which they voted 3-2 in our favor; (5) We helped get the California Tiger Salamanders listed as threatened under the state Endangered Species Act, when 434 of our supporters sent letters to the CA Fish & Game Commission in the 36 hours before the successful 3-2 vote; (6) We ran a campaign to get non-native fish removed from 60 lakes in Sequoia & Kings Canyon National Parks: 707 of our supporters sent the Park Service letters in support of the action; ours comprised over 95% of the comments the Service

received (a decision has yet to take place); (7) We have recently initiated a community-powered habitat restoration project at Antonelli Pond in Santa Cruz, home to a population of endangered California Red-Legged Frogs; (8) We are currently working to ban the importation and sale of American bullfrogs into and within the state of California. Save The Frogs welcomes your input on how to mobilize our society and create significant amphibian conservation outcomes in California and beyond.

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The Perils of Unpalatable Periphyton!

The invasive diatom *Didymosphenia geminata*, a single-celled alga that produces excessive mucopolysaccharide stalks, is becoming increasingly prevalent in North American rivers. When hydrologic and thermal regimes are altered by dams in the Sierra Nevada, *Didymosphenia* as well as native mucilaginous taxa (e.g. *Gomphoneis, Gomphonema*) can dominate near-shore environments where Foothill Yellow-legged Frogs (*Rana boylii*) rear. In the laboratory, we investigated the response of grazing tadpoles to mucus-producing diatoms on rocks collected from rivers where flows fluctuate daily with short term power generation; one with *Didymosphenia* and one without. Rocks from an unregulated coastal stream with high food-quality periphyton served as controls. Food sources were crossed with temperature treatments that mimicked cool regulated river conditions, or matched tadpole thermal preference. Both factors had significant effects on food consumption and tadpole growth. Under cool conditions, tadpoles gleaned little food and were only able to maintain body weight on high food quality algae, despite there being similar or greater biomass per unit area on the regulated river rocks. At warmer temperatures, tadpoles ingested *Didymosphenia* dominated periphyton at a rate similar to tadpoles consuming control periphyton, but did not grow, with a 72 hr relative weight gain of 4.3 ± 5.4% vs. 30.7 ± 3.4% for controls. For the site dominated by native stalked mucilaginous diatoms, tadpole weight loss was 21.0 ± 9.2 % (cold) and 16.6 ± 5.6 % (warm). These results indicate the importance of a holistic approach to river management that integrates consideration of abiotic and biotic factors to conserve amphibians.

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Observations on the Decline of an Isolated Columbia Spotted Frog Population in Northeast Nevada

Three Districts of Humboldt-Toiyabe National Forest in northeast Nevada support Columbia spotted frog (Great Basin Population), a Candidate species. Long-term monitoring sites utilizing PIT tags, one per District, were established in 2004 and 2005 to better understand local characteristics of this frog. One site - Pole Creek - has displayed a sharp population decline. This observation appears to be largely due to multiple consecutive years of low or no juvenile recruitment. Presented are several factors which may be contributing to the decline, including weather events, habitat change, disease, and site isolation. Management implications beyond the Pole Creek site are also discussed.

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Landscape Epideomiology of *Batrachochytrium dendrobatidis* in Central California

Amphibian chytridiomycosis (caused by *Batrachochytrium dendrobatis*; Bd) was first identified in 1998 and has since been implicated in numerous amphibian declines worldwide. Most researchers have since investigated broad-scale geographic and taxonomic occurrences of the pathogen in tropical lotic or cool montane systems. In this study, we analyzed how environmental factors, land use practices, and landscape structure may affect the dynamics of the pathogen's distribution in a landscape dominated by lentic systems within a region of Mediterranean climate. We quantified the occurrence of Bd, testing the six resident amphibian species that occur in 54 isolated perennial and ephemeral ponds in central California between May and June annually from 2004 to 2007. The geographic distribution of Bd within the landscape varied markedly between years. Inter-annual variation in climate affected the pond landscape structure indicating that climate conditions indirectly influence the distribution of the pathogen Fourteen ponds, 12 perennial and 2 ephemeral, were positive for Bd > 3 yr of the study and were treated as Bd hotpots for comparative purposes. Occurrences of Bd within the landscape were spatially autocorrelated and ponds within 1000-1500 m of Bd hotspots were more likely to test positive. Local land use, (presence/absence of grazing or recreational activity and developed lands), did not influence Bd status of a pond, indicating that the most likely means of Bd transmission between ponds may be waterfowl and/or amphibians.

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Landscape Genetics of Foothill Yellow-Legged Frogs (*Rana boylii*) in Regulated and Unregulated Rivers: Assessing Riverscape Connectivity and Genetic Fragmentation

The stream breeding *Rana boylii* is experiencing range wide population declines. Among multiple stressors, hydroelectric projects may have the greatest potential impact on *R. boylii* because of flow regulation and structures such as dams, reservoirs, and powerhouses. River regulation can fragment the landscape and reduce connectivity within and among *R. boylii* populations, which ultimately may limit gene flow and reduce genetic diversity. We tested the hypothesis that *R. boylii* populations in watersheds regulated by hydroelectric generation have lower genetic diversity and riverscape connectivity compared with unregulated watersheds (without dams or hydroelectric generation). Six different rivers in the Sierra Nevada were compared; pairing similar-sized hydroelectric-regulated and unregulated rivers in adjacent watersheds. Genetic structure within and among *R. boylii* populations was characterized using mitochondrial DNA (mtDNA) from 62 frogs to estimate gene flow, and random amplified polymorphic DNA (RAPD) from 98 frogs across 199 loci to estimate genetic diversity. A quantitative geo-spatial network analysis utilized frog distribution data from multiple sources to test distances between *R. boylii* study localities and the nearest tributary confluence. Results indicated differences between regulated and unregulated streams in study populations at a landscape and genetic level. Regulated study rivers showed lower genetic diversity and divergence within subpopulations compared with unregulated study rivers, and *R. boylii* adult and breeding localities were significantly closer to tributary confluences in regulated study rivers compared with unregulated rivers; valuable information for conservation management of this species.

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Evaluating the Impact of a Fungal Pathogen on the Cascades Frog (*Rana cascadae*) and other Native Amphibians in the Mountains of Northern California

The fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) is associated with declines and extinctions of amphibians worldwide. In the western United States, frogs and toads in montane habitats have proven to be particularly susceptible. We focus on the effects of *Bd* on the Cascades Frog (*Rana cascadae*) and other native amphibians in the mountains of northern California. In 2008 and 2009, we sampled amphibians for *Bd* at over 100 sites throughout the range of *R. cascadae* in northern California. *Bd* was widespread throughout the region, occurring in amphibians at 67% of all sites surveyed. The pathogen was detected in 26% of post-metamorphic *R. cascadae* and 1.5% larvae. In addition, the pathogen was detected in three other common amphibians – Western Toad (*Bufo boreas*), Pacific Chorus Frog (*Pseudacris regilla*), and Rough-skinned Newt (*Taricha torosa*). The prevalence of *Bd* in post-metamorphic *R. cascadae* changed through the course of the season, but the direction of the change was dependent on life stage. Prevalence tended to be similar for all size classes at the beginning of the season, but it increased over the course of the season in subadults, while tending to decrease in adults. Subadult *R. cascadae* also had higher pathogen loads than adults. In order to gather more detailed information on the seasonal dynamics of *Bd* infection, we sampled a number of *R. cascadae* populations 3 – 7 times throughout the 2009 and 2010 summer seasons. We individually marked a subset of the animals in each population, allowing us to follow infection dynamics at both the individual and the population level during this time period. We suggest that younger, smaller individuals are less effective at clearing the pathogen, and are therefore more susceptible to the disease that it causes.

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Status of Remnant Populations of Cascades Frogs (*Rana cascadae*) in the Southern Cascades of California

The southern Cascades of California, especially in the Lassen Region, have experienced severe declines of the Cascades frog (*Rana cascadae*) with less than ten known populations remaining. In 2008, we initiated a project to study remaining populations with the goals of (1) estimating their size and viability, (2) determining reasons for poor recruitment, and (3) recommending and implementing actions to conserve and improve the remaining populations. We conducted capture-mark-recapture surveys and used the data to estimate population sizes and age-specific survival at five sites. Population estimates ranged from < 10 frogs at Colby Creek to over 150 frogs at Carter Meadow. Numbers of juvenile frogs were small at all sites surveyed. 2009 survival estimates ranged from over 80% at Nelson Creek to 40% at Old Cow Creek. We believe reasons for low and decreasing population sizes include low survival of egg masses and larvae due to desiccation and high water temperatures, and low survival of juvenile frogs due to chytridiomycosis. We swabbed all captured frogs for *Batrachochytrium dendrobatidis* (the pathogen responsible for chytridiomycosis). We then related prevalence and loads to population

demographics, presence of other stressors, and habitat variables. In addition, in 2009 and 2010, we initiated specific on-the-ground restoration measures at Colby Creek and Carter Meadow to improve breeding conditions by deepening pools to increase hydroperiod and/or decrease summer water temperatures.

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Toward Immunogenetic Studies of Amphibian Chytridiomycosis: Linking Innate and Acquired Immunity

Chytridiomycosis is a leading cause of amphibian declines worldwide, yet the critical question of why some species can tolerate chytrid infections without developing disease symptoms remains largely unanswered. Considerable evidence links environmental conditions and interspecific variability of the innate immune system to differential infection responses, but other sources of individual, population, or species-typical variation may also be important. In this talk, I review evidence supporting a role for acquired immune defenses against chytridiomycosis, and advocate for targeted investigation of genes controlling acquired responses, as well as those that functionally bridge the innate and acquired immune systems. Identifying links between immunogenetic variability and different infection responses will assist in answering key questions about chytridiomycosis susceptibility and host-pathogen coevolution, and will draw much needed attention to the importance of considering evolutionary processes in amphibian conservation management and practice.

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Influence of Multiple Pathogens on Amphibians: Individual and Combined Effects of *Ribeiroia* and *Batrachochytrium dendrobatidis* on Pacific Treefrogs

In nature, individual hosts often encounter multiple pathogens simultaneously, which can lead to additive, antagonistic, or synergistic effects on hosts. These effects could greatly affect host populations by influencing infection prevalence or severity. However, ecologists and managers often overlook the influence of pathogen combinations on threatened species. This is especially true in amphibian conservation, even though multiple pathogens coexist within amphibian populations and several pathogens have been implicated in amphibian population declines and extinctions. We investigated interactive effects of the trematode *Ribeiroia* sp. and the fungus *Batrachochytrium dendrobatidis* (BD) in one amphibian host, *Pseudacris regilla* (Pacific treefrog). We used *P. regilla* larvae in a 2×2 factorial laboratory experiment with two treatments, exposure and control (no pathogen added), for each pathogen. We continued the experiment through *P. regilla* metamorphosis and observed each metamorphosed individual for four weeks after complete resorption of its tail. The relatively low dose of trematodes in the *Ribeiroia* exposure treatment (twelve cercaria per larva) was great enough to increase the number of deformities in *P. regilla*. Consistent with previous studies on frogs, all malformations in *Ribeiroia*-exposed individuals were hindlimb deformities. BD exposure increased the percentage of BD-infected

individuals from zero to 31%. We detected no effects on survival, growth, or developmental rate, and found no evidence of interactive effects of BD and *Ribeiroia*. However, such interactive effects might occur under ecological conditions not tested here or with different hosts/pathogen combinations.

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An Artificial Hibernation Technique to Promote Breeding in a Captive Population of Southern California Mountain Yellow-Legged Frog (*Rana muscosa*)

The southern California mountain yellow-legged frog (*Rana muscosa*) is a critically endangered species whose adult population in the wild is estimated to be less than 150 individuals. The San Diego Zoo Institute for Conservation Research has housed a captive population of *Rana muscosa* since 2006 in collaboration with our partners at the USGS, California Department of Fish and Game, US Forest Service, and US Fish and Wildlife Service. One of the goals of the southern California mountain yellow-legged frog conservation program is to develop captive a captive breeding protocol for the species. During the first breeding season in 2009 only a single clutch of eggs was produced. Because *Rana muscosa* is a high elevation species that undergoes a winter hibernation, we decided to test the effect of an artificial hibernation on captive reproductive success during the 2010 breeding season. We used an experimental approach to determine the benefit of hibernation on reproduction. Half of our captive frogs were hibernated at 4 degrees Celsius for a period of 60 days. All other frogs were maintained at 13 degrees Celsius during the same period. Following the hibernation period frogs were paired together for breeding according to their hibernation treatment with a total of 4 treatments. These treatments included: both frogs hibernated, neither frog hibernated, only male hibernated, and only female hibernated. Reproductive interest was measured by recording three different breeding behaviors for males and four behaviors for females. Reproductive behavior was compared using a Kruskal-Wallis test and our results demonstrate that hibernation had a significant effect on both male and female reproductive behaviors among the treatments.

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Evaluating Potential Habitat and Temperature Limiting Factors for Foothill Yellow-Legged Frogs (*Rana boylii*) in the McCloud River Downstream of McCloud Dam

It has been well-documented that fluctuations in spring and summer flows during foothill yellow-legged frog (*Rana boylii*) oviposition and tadpole-rearing seasons can affect the species by altering the water depth and velocity in its breeding habitat. The water temperature regime, which provides cues that prompt the initiation of breeding by adult frogs, is also known to affect egg mass and tadpole development. As part of the relicensing of PG&E's McCloud-Pit Hydroelectric Project, we examined habitat and temperature data to evaluate potential limiting factors for *R. boylii* in the lower McCloud River. Using empirical measurements, we applied the "expert habitat mapping" methodology to assess the quality and quantity of suitable habitat for *R. boylii* eggs at three sites in the lower McCloud River under three different flow scenarios. We applied habitat suitability criteria developed using habitat datasets from 15 rivers in the Sierra Nevada and Coast ranges (Lind and Yarnell 2008). We also collected and analyzed stream temperature data to examine patterns in temperatures along the length (longitudinal gradient) of the river and to assess differences between well-mixed (mid-channel) and edgewater locations at breeding sites. This investigation provided an opportunity to increase the understanding of

this species' limited distribution in the lower McCloud River while helping to inform watershed managers of conservation strategy options when developing flow regimes for regulated watersheds.

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Community Effects of Mountain Yellow-legged Frog Tadpoles

The ecological consequences of worldwide amphibian declines and extinctions are not fully understood. Frogs and tadpoles may influence community structure and dynamics via competition and predation. In the Sierra Nevada, mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) have been extirpated from most of their historical range, and remaining populations are severely threatened. To describe the roles of *R. muscosa* and *R. sierrae* tadpoles in alpine lake communities, I performed two experiments. In 2009, a field enclosure experiment investigated the effects of tadpole and mayfly nymph density on epiphyton growth. In 2010, a mesocosm experiment investigated the effect of tadpole and mayfly nymph presence-absence on epiphyton growth. The 2009 field enclosure experiment revealed no effect of tadpoles as consumers, though intraspecific effects among tadpoles were observed. In a mixed effects model, mayfly density was negatively associated with epiphyton growth at one of two sites. Site and enclosure predicted variation in epiphyton growth at both sites. Preliminary analysis of the 2010 mesocosm experiment suggests that tadpole presence reduced epiphyton growth. Results from these experiments suggest that magnitude of consumptive and competitive effects of tadpoles are site and scale dependent, and variation due to abiotic processes may overwhelm the potential of tadpoles to have detectable effects on communities. It may be premature to make statements about ecological effects of amphibian extinctions based on taxonomy, rather than based on species identity and ecosystem type. Ongoing studies of the ability of tadpoles to affect nutrient fluctuations and producer diversity, and of the resistance and resilience of alpine lake communities to mountain yellow-legged frog extinctions may be discussed.

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Sex-biased Predation on Newts of the Genus *Taricha* by a Novel Predator and its Relationship with Tetrodotoxin Toxicity

Newts of the genus *Taricha* have long been studied in regards to their skin toxin, Tetrodotoxin (TTX). It has been shown that the TTX levels across populations of *Taricha* are highly variable, and this has been mostly attributed to the interaction between *Taricha* and their only documented predators, garter snakes of the genus *Thamnophis*. Here we show that predators other than *Thamnophis* prey extensively on some newt populations. Ledson Marsh in Annadel State Park, Santa Rosa, CA is a breeding ground for both the California newt (*Taricha torosa*) and the rough-skinned newt (*Taricha granulosa*). Predation on these newts was tracked from 1998-2009 and was most often in the form of evisceration and significantly male-biased. As TTX seems to have been developed as an antipredator defense in *Taricha*, we used Fluorometric High Phase Liquid Chromatography analysis to quantify TTX levels in the skin of ten male

and ten female newts of each species to determine the influence that TTX levels may have on sex-biased predation in this population. We found *Taricha* females were not significantly more toxic than males. Also, we found that *T. torosa* were significantly more toxic than *T. granulosa*, which is in contrast with other newt toxicity studies.

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Frogs, Fish and Forestry: The Need for a Holistic View of Network Processes to Conserve Native Stream Biodiversity in Forest Catchments

I review research conducted on entire stream catchments in Northwest California, from headwater swales to river mouths, indicating how headwater processes, both natural and anthropogenic, influence downstream fish-baring reaches. The implications of these relationships for salmonids and other elements of native biological diversity are explored. Comparing riparian protections from the U. S. federal Northwest Forest Plan with those of the three Pacific Northwest states, I discuss fluvial and geomorphologic process domains of stream networks and how they relate to these guidelines. Focusing in particular on headwater (1st to 3rd order) channels, evidence for the effectiveness of current riparian management to maintain viable populations of native amphibians is reviewed. Using evidence from multiple studies of amphibian environmental relationships, including several from the redwood region, I document the ineffectiveness of current riparian protections to prevent increasing water temperatures, the introduction of fine sediments, and the loss of large woody debris by detailing the responses of headwater amphibians to these adversely altered attribute states. Combining the concepts of the stream continuum and the dendritic network, I examine linkages between the status of biota in headwater reaches and elements like salmonids that depend on downstream conditions. This research indicates that to recover and maintain sensitive species at upper and lower extremes and throughout stream networks will require recognizing and applying the concept of hydrologic connectivity. Embracing this concept is essential to promote adequate management of stream networks to protect all the parts and the interconnecting processes needed to maintain catchment-wide ecological integrity. These results have implications for stream networks worldwide.

*Indicates speaker in multi-authored presentation.