California/Nevada Amphibian Populations Task Force

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ABSTRACTS

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Using Environmental DNA to Detect Aquatic Species: Case Study of a Cryptic Amphibian

Genetic monitoring methods provide a means to obtain population metrics from cryptic (visually-evasive), rare, and hard to study aquatic organisms. A reconnaissance tool that is easy to deploy, cost effective to use over a large survey area, and unambiguously identifies target species is environmental DNA (eDNA). The presence of cryptic species is ascertained by using molecular genetic assays to detect within water samples DNA that has been shed into the aquatic environment. The eDNA protocols are generally used to expand capabilities for investigating the presence, distribution, or containment of species. Under controlled laboratory conditions eDNA methods can reliably detect concentrations down to 10 copies of DNA (i.e., quantities equivalent to less than a single cell), but field techniques influence detection probability. An investigation is presented regarding the initial test of an eDNA protocol to detect the presence of Oregon Spotted Frog (*Rana pretiosa*), a medium-sized cryptic aquatic frog endemic to the Pacific Northwest that is a candidate species for listing under the Endangered Species Act. The species distribution is believed to be substantially reduced from its historic range, but a species inventory is hampered by difficulties in detecting the species in the field, likely resulting in unrecognized populations. In the initial phase of this project, molecular probes were developed and eDNA methods were investigated as a less intensive and invasive means to survey species distribution relative to available habitat.

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Status of the Mountain Yellow-legged Frog Complex in Sequoia and Kings Canyon National Parks

Both species in the Sierra Nevada mountain yellow-legged frog complex (MYLF; *Rana muscosa* and *Rana 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 monitoring and restoration of MYLFs have been conducted in SEKI. This presentation will describe 1) the current status of MYLF populations in SEKI, 2) results to date of nonnative fish eradication efforts and subsequent MYLF response, 3) needs for future research to facilitate recovery of MYLFs, and 4) the status of an environmental impact statement that is proposing additional restoration actions.

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First Report of Infections by the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* in Wild Populations of the Amargosa Toad *Anaxyrus nelsoni*

The Amargosa toad (*Anaxyrus nelsoni = Bufo nelsoni*) is a species of conservation concern with a restricted distribution within the Oasis Valley in Nevada. The amphibian chytrid fungus,

Batrachochytrium dendrobatidis (*Bd*), which causes the potentially fatal disease chytridiomycosis, has been broadly linked to declines and extinctions of amphibian populations. The disease has been implicated in the death of a captive Amargosa toad, and was also detected in 2005 in samples of American bullfrog (*Lithobates catesbeiana*) from one site in the Oasis Valley. Therefore, we tested 310 Amargosa toads and 62 closely associated bullfrogs for *Bd* using qPCR during spring and summer periods over two years. We found high prevalence of *Bd* infections over both seasonal periods and across years. Overall, 48.7% of the Amargosa toads and 61.3% of bullfrogs sampled were infected. The infection levels were generally low; the mean number of *Bd* copies (zoospore equivalents) was 7814 from infected Amargosa toads and 4200 from infected bullfrogs, although potentially lethal infections were also detected (as high as 202,000 *Bd* copies in Amargosa toads). These findings are important because *Bd* has been shown to cause mortalities and population declines in closely related toad species. These data also have implications regarding how Amargosa toads should be handled during population monitoring surveys to ensure that *Bd* is not spread between individuals.

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Amargosa Toad, *Anaxyrus nelsoni* Survey Results and Conservation Activities in the Oasis Valley, Nye County, Nevada

Brief updates on the population status and management of Amargosa toad, *Anaxyrus nelsoni* are presented. A night time mark-recapture survey for Amargosa toad habitat in Oasis Valley was conducted in May and June, 2013. Population estimate data were analyzed using Program MARK to account for heterogeneity in capture probabilities. This was the 16th consecutive annual survey combining the efforts of state and federal agency personnel, university staff, volunteers, and local residents of Beatty, NV. Surveys support the cooperative efforts of the Amargosa Toad Working Group tasked with implementation of the Conservation Agreement Strategy. The U.S. Fish and Wildlife Service's 12-month review (2010) found that the Amargosa toad was not warranted for listing. The Conservation Agreement Strategy is currently under review and revision. Habitat restoration is ongoing in the Amargosa River to remove invasive vegetation, non-native species, and un-natural water diversions. Restoration was completed and tadpoles were re-introduced at Bryan Spring.

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Evidence for Contemporary Evolution in Tadpoles to Optimize Behavioral Response to Introduced Fish

Introduced predators are one of the leading causes of amphibian declines worldwide. However, if amphibian larvae are not immediately extirpated by invaders, they may undergo rapid evolution driven by this new selection pressure. We studied anti-predator behaviors in fish-naive and fish-invaded populations of Cascade frog tadpoles (*Rana cascadae*) to test the hypothesis that fish-invaded populations have evolved greater reductions in activity and increased refuge use. We

raised tadpoles from eggs collected from the field in fish-free aquaria and performed behavioral assays with and without fish chemical cues. Fish chemical cues induced strong decreases in activity and increases in refuge use. However, the effect of fish chemical cues on behavior was less strong in fish-invaded than in fish-naïve populations. Data patterns suggest that responses differed because populations coexisting with fishes were less active and in refuges more than naïve populations in the absence of cues, but maintained activity levels and refuge use better when cues were present. There may be selection pressure for reduced anti-predator behaviors when fish are present because of high fitness consequences of activity reduction. Extended refuge habitats may cause realized predation risk to be lower than perceived predation risk. Additionally, the risk-allocation hypothesis predicts weakened anti-predator behavior when periods of high risk are frequent. Weakening of the anti-predator response in fish-invaded populations may be a signal of contemporary evolution toward optimization of time spent foraging versus time spent avoiding predation. This may mean there is hope for long-term coexistence of native species and invasive predators, but whether these adaptations come with unexpected fitness consequences remains to be seen.

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Acoustic Monitoring of Breeding Amphibians at Yosemite National Park and Point Reyes National Seashore

The calling behavior of frogs and toads at breeding sites lends itself to acoustic monitoring of these amphibian populations. We are using Automated Recording Devices (ARD) at two National Park units in California, Yosemite National Park and Point Reyes National Seashore, to monitor the breeding efforts of amphibians by recording their calls. We are monitoring both common species (Pacific chorus frogs, *Pseudacris regilla*, occurs in both parks) and imperiled species (Yosemite toad, *Anaxyrus canorus*, Yosemite; California red-legged frog, *Rana draytonii*, Point Reyes) to investigate whether breeding phenology will shift with changing climatic conditions. The use of ARD is also providing a more complete picture of the diel calling patterns of these species, and how some species are partitioning their acoustical environment by frequency and time in order to breed successfully while surrounded by noisy neighbors. Information gathered through acoustic monitoring is very valuable for conserving rare amphibians and ensuring that common species remain common.

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Recovering Mountain Yellow-legged Frog Populations in the Sierra Nevada: What Works and What Doesn't?

Mountain yellow-legged frogs (*Rana muscosa*, *Rana sierrae*) were once the most common amphibian in the Sierra Nevada, but during the past century they have disappeared from >90% of their historical range due primarily to impacts from introduced fish and the amphibian chytrid

fungus. As a consequence of their increasingly imperiled state, both species were recently proposed for listing under the U.S. Endangered Species Act. Recovery of these species depends critically on the development of effective methods to mitigate threats, and techniques for mitigating disease impacts are particularly poorly understood. I will provide an overview of recent studies aimed at developing methods for recovering mountain yellow-legged frog populations, and assess the effectiveness of methods that have been evaluated to date. These methods include fish removal, frog translocation, frog reintroduction, captive rearing and breeding, and disease mitigation using anti-fungal drugs, probiotics, and immunization. Several of these methods show considerable promise in facilitating the recovery of mountain yellow-legged frog populations, but additional research is essential to refine mitigation measures and ensure their effective implementation.

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Special Status Amphibians in California's State Vehicular Recreation Areas

California Department of Parks and Recreation operates 8 State Vehicular Recreation Areas (SVRAs). Some are located where amphibians face local extirpation. In the watershed of Corral Hollow Creek (Alameda Co.), Carnegie SVRA contains two federally threatened species (California Red-legged Frog, *Rana draytonii*; California Tiger Salamander, *Ambystoma californiense*) and two California State Species of Special Concern (Western Spadefoot Toad, *Spea hammondii*; Foothill Yellow-legged Frog, *R. boylii)*. Although the Off-Highway Motor Vehicle Act of 1988 requires annual monitoring to determine compliance with "a wildlife habitat protection program to sustain a viable species composition", built-in trigger points to activate closure and restoration (e.g. a 10% decline in population abundance) are mis-matched with sampling techniques (e.g. dip netting for larvae). State Parks' documents do not explicitly report the impact of OHV use on amphibians and data gaps preclude the use of sophisticated time series analyses to detect trends. However, simple comparisons of occupancy of ponds and streams by amphibians suggest detrimental effects of OHV use on species distribution. For example, at Carnegie SVRA, I compiled tabular presence / absence data and found that mean ± 1 SE California red-legged frog occupancy in riding areas was 8.09 ± 3.05% (n = 8 yr) compared to 31.8 ± 9.2% (n=10 yr) on a neighboring parcel of land currently without riding (T=5.4, df=12, $p<<0.01$). California tiger salamander occupancy with OHV riding was 5.4 ± 3.8%, compared to 31.8 ± 9.2% without riding (T=-2.7, df=12, $p=0.01$). The 3,400 acre control parcel, Tesla Park, is currently slated for OHV development.

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Persistence of California Red-legged Frogs in Central California despite Evidence of Long-term *Batrachochytrium dendrobatidis* Infection and the Presence of an Invasive Congener

Chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), has been a major contributing factor to worldwide declines in amphibian diversity. Interspecific and

intraspecific variation in the susceptibility and severity of *Bd* infection have been documented; however, few studies have examined long-term persistence of amphibian populations despite *Bd* infection. We examined persistence of populations of California red-legged frog, *Rana draytonii*, and the non-native American bullfrog (*Lithobates catesbeianus*) on Vandenberg Air Force Base (VAFB) in central California by comparing survey data from 1995, 2008, and 2013. In addition, standardized surveys conducted at a subset of sites allowed us to compare an index of population density between 1995, 2008, and 2013. Using a standardized swabbing protocol and quantitative polymerase chain reaction (qPCR) methods, we assessed *Bd* infection characteristics of each population in 2008 and 2013. To assess *Bd* infection of populations surveyed in 1995, we applied a newly developed swabbing protocol and extraction method utilizing qPCR to examine preserved museum specimens collected in 1995 at or near the same locations surveyed in 1995. Despite widespread *Bd* infection in 1995, 2008, and 2013, there were no significant differences in density index between these years for *R. draytonii* or *L. catesbeianus*. All populations of *R. draytonii* accessible for resurvey persisted between 1995 and 2013, and *L. catesbeianus* populations persisted at three of four sites over the same period. We conclude that, although *Bd* and *L. catesbeianus* have been present on VAFB for at least 18 years, neither factors have affected the distribution or the density of *R. draytonii* populations during that period.

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How the Partners for Fish and Wildlife Program Can Assist With Amphibian Conservation

The U.S. Fish and Wildlife Service's Partners for Fish and Wildlife Program (PFW) provides technical and financial assistance to private landowners and Tribes who want to voluntarily improve fish, wildlife, and plant habitat on their lands. The program's focus is on improvement of wetland, riparian, sagebrush/grasslands, desert scrub, wet-meadow, forest, and aquatic habitats to benefit migratory birds, and threatened, endangered and other sensitive or declining species. In Nevada, the PFW program has focused on habitat restoration projects and public education about the endemic Amargosa toad (*Anaxyrus nelsoni*) to help keep it off the Endangered Species list. Local conservation efforts with private landowners, local governments, non-profit organizations, and government agencies have reduced threats and improved the rangewide status so that listing was found not to be warranted. Working with landowners and other partners, the PFW program has helped to restore 5 springs, 0.5 miles of stream habitat, and approximately 72 acres of toad foraging and breeding habitat for a species that only occurs over a 10-mile stretch of river and associated springs. In California, priorities have been habitat restoration projects to help recover the federally endangered California red-legged frog (*Rana draytonii*), California tiger salamander (*Ambystoma californiense*) (endangered in Santa Barbara and Sonoma Counties) (threatened in Central CA DPS), and proposed threatened Oregon Spotted frog (*Rana pretiosa*).

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Hikers, Bikers, Horses, Dogs and Frogs: Embracing Public Use and Managing for Foothill Yellow-legged Frog (*Rana boylii)* Populations within the Mount Tamalpais Watershed of Marin County, California.

Marin Municipal Water District (MMWD) in Marin County, California provides a major source of domestic water for its customers living within the Mt. Tamalpais Watershed. In addition to being a valuable natural resource, the watershed is held in trust as a natural wildland, scenic open space, and daytime recreational use area for the residents of Marin County and the San Francisco Bay Area. MMWD is also committed to conservation of special status plant and animal species, including the foothill yellow-legged frog (FYLF). From 2004 to 2013, Garcia and Associates (GANDA) have monitored FYLF at Little Carson Creek and at Big Carson Creek and two tributaries where FYLF breeding sites are subject to heavy recreational use. Though populations at other historic sites in the watershed have disappeared, FYLF populations in these small creeks have been dynamic, but stable over the past decade. We will provide a summary of survey results and an overview of MMWD's approach to protecting the species as it continues to provide recreational opportunities to the public and considers possible reintroduction into historic breeding areas.

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Historic Prevalence of *Batrachochytrium dendrobatidis* in the Sierra Nevada of California

Amphibian populations in the Sierra Nevada exhibit various responses to infection by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*), ranging from persistence at high-prevalence, low-intensity infections to population die-offs at high intensities. We used molecular techniques to assay the presence of *Bd* in over 1200 preserved amphibian specimens representing seven anuran species collected between 1900 and 2005 in the Sierra Nevada. We found that *Bd* prevalence rapidly increased in the early 1970s, and quickly increased in geographic range, suggesting either rapid spread or multiple introduction points. By the 1990s *Bd* was present in every county surveyed. Early detection in central and northern counties are consistent with the hypothesis that current patterns of population persistence are due to previous exposure to *Bd* and subsequent selection for resistance.

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Status, Management and Recovery of *Rana sierrae* and *R. muscosa* in the Sierra Nevada – an Update on the CDFW High Mountain Lakes Project

The California Department of Fish and Wildlife (CDFW) has been actively engaged in *R. sierrae/muscosa* management and conservation since the mid-1990s. In 2001, CDFW formed the High Mountain Lakes project to conduct broad scale inventories of fish and frogs throughout the Sierra Nevada. Since then, approximately 90% of mapped Sierra Nevada lakes outside of

national parks have been surveyed by CDFW. After twelve years the project has surveyed well over 10,000 water bodies, determined the current distribution of *R. sierrae* and *R. muscosa* populations outside the national park boundaries; accurately described fish distributions across the mountain range; and described the distribution of the amphibian chytrid fungus (Bd). Those data have been used to inform management and conservation actions including: fish removal and translocations to benefit *R. sierrae*; realignment of CDFW's aerial stocking activities in the Sierra Nevada; and the listing processes for both CESA and ESA. This discussion will focus on *R. sierrae* and *R. muscosa* status, conservation and restoration within the Sierra Nevada by clade including actions conducted to date by CDFW and partner agencies, as well as thoughts regarding future actions necessary for the conservation of the species.

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Infection Dynamics in an Endangered Relict Population of Cascades Frogs: Impacts of Life History, Microhabitat Use, and Climate on the Prevalence of *Batrachochytrium dendrobatidis*

The Cascades frog (*Rana cascadae*) has become rare in the southern Cascade Range in northern California. After six years of studying the remaining populations, we have determined that chytridiomycosis is a primary contributor to population declines. *Batrachochytrium dendrobatidis* (*Bd*) occurs in the 11 known remaining populations but its effects vary among populations, years, and individuals. We assessed climatic and frog-scale characteristics to determine the range of factors that influence infection dynamics within populations. Our best composite model (based on AIC) revealed that air temperature patterns within 2 weeks of capture (mean of maximum, max temperature drop), age and size of frogs, and microhabitat use (water pH, water temperature, onshore vegetation) influence the probability of infection. Based on recapture data, adult frogs move among habitats and change from *Bd*+ to *Bd*- or vice versa in a predictable manner. Juvenile frogs are least likely to survive the disease. Implications of these results will be discussed.

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Calibrating Indices of Relative Abundance to Estimate Population Size of the Relict Leopard Frog

The relict leopard frog (*Rana onca = Lithobates onca*) is a species of conservation concern currently managed under a voluntary conservation agreement and strategy. Monitoring of populations is an important management component and occupied sites have been systematically monitored since 2004 using visual encounter surveys. This monitoring approach was not intended to provide estimates of actual abundance, but instead was used to assess status and general trends over time. Application of a more intensive sampling method, such as mark-recapture, allows the possibility for calibrating estimates of actual abundance with relative

abundance derived from visual encounter surveys, a technique previously described as "double sampling". We developed ratio estimates at four representative sites where mark-recapture and visual encounter surveys were conducted concomitantly. The average ratio estimates derived at each of the four sites were then applied selectively to calibrate results from visual encounter surveys at other sites that shared similarities in habitat conditions and where mark-recapture had not occurred. Using this approach, an overall estimate of population size was derived for 2012. The only previous estimate of population size for *R. onca* dates to 2001 when approximately 1,100 adult frogs were estimated using a similar approach. The current estimate indicates a likely modest increase in overall population size.

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Rana muscosa Reintroduction Efforts in Southern California

We reintroduced over 300 juvenile Mountain Yellow-Legged Frogs (*Rana muscosa*) into stream habitat in southern California. This was our first reintroduction for this life stage in our ongoing recovery efforts for the species. Using an experimental approach we tested the benefits of a pre-release acclimation at the field site. Comparing the survival and settlement patterns between acclimated and hard-released frogs we did not observe any benefit to holding frogs in acclimation prior to release. This research was carried out in collaboration with the USGS, US Fish and Wildlife Service, US Forest Service, University of California Natural Reserve System, LA Zoo, and San Francisco State University.

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The Ash Meadows Fish Conservation Facility

Low fish counts at Devils Hole and a concomitant lack of back up populations, due in part to inadequate facilities, prompted the U.S. Fish and Wildlife Service and partners to secure Southern Nevada Public Land Management Act funds to build a state of the art facility. Construction of the Ash Meadows Fish Conservation Facility (AMFCF) was completed in June 2013. The primary objective of the AMFCF is to aid in the conservation of imperiled aquatic species, especially the critically endangered Devils Hole pupfish *Cyprinodon diabolis*. Since construction of the AMFCF was completed in June 2013, Facility staff has focused on *C. diabolis* recovery efforts, including attempts in August and November 2013 to collect pupfish eggs from Devils Hole to propagate at the AMFCF. The Facility currently has 21 Devils Hole pupfish, all of which were hatched from eggs collected from Devils Hole in November 2013.

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Highway One Revisited: Twenty years of Road Surveys for California Red-legged Frogs in Coastal Santa Cruz and San Mateo Counties

In 1994-1995 we collected over 300 road-killed California red-legged frogs, Rana draytonii, from an 80 km reach of Highway One, a coastal road that transects across numerous watershed outlets between the city of Santa Cruz and Half Moon Bay, California. We revisited the study site in 2010-2013, using the same methods. We found red-legged frogs to be represented in nearly every major locality in both time periods, with similar distribution and abundance. California red-legged frogs appear to be sustaining a strong metapopulation on the coastal side of the San Francisco Peninsula.

* Indicates speaker in multi-authored presentation