

# INTERAGENCY CONSERVATION STRATEGY FOR MOUNTAIN YELLOW-LEGGED FROGS IN THE SIERRA NEVADA

# (Rana sierrae and Rana muscosa)

California Department of Fish and Wildlife, National Park Service, U.S. Fish and Wildlife Service, U.S. Forest Service



Rana muscosa adult; © Joel Sartore

Version 1.0, November 2018

#### Acknowledgements

This Conservation Strategy was developed through the collaborative effort of a large number of dedicated individuals from federal and state agencies, academia, zoos, and similar groups. We would especially like to acknowledge the following persons for their substantial involvement and contributions to this document.

Sarah Allen, Stephanie Barnes, Danny Boiano, Betsy Bolster, David Bradford, Cathy Brown, Jessie Bushell, Isaac Chellman, Diana Craig, Carlos Davidson, Steven Detwiler, Dawne Emery, Gary Fellers, Cay Goude, David Graber, Rob Grasso, Barnie Gyant, Jim Harvey, Pat Kleeman, Roland Knapp, Jan Knight, Stafford Lehr, Mitch Lockhart, Diane Macfarlane, Heather McKenny, Chad Mellison, Curtis Milliron, Sarah Mussulman, Laura Patterson, Jill Seymour, Phil Strand, Luisa Studen, Cynthia Tait, Rachel Van Horne, Vance Vredenburg, Katie Wilkinson, Lucas Wilkinson.

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# Use of This Document

This Conservation Strategy for mountain yellow-legged frogs in the Sierra Nevada was prepared by an interagency technical team comprised of members from the U.S. Fish and Wildlife Service (USFWS), U.S. Forest Service (USFS), National Park Service (NPS), and California Department of Fish and Wildlife (CDFW), under the guidance and direction of a Steering Committee comprised of management staff from the same agencies. This Strategy covers the Sierra Nevada range of the Federally endangered northern Distinct Population Segment of the mountain yellow-legged frog (*Rana muscosa*) and the entire range of the endangered Sierra Nevada yellow-legged frog (*Rana sierrae*) (USFWS 2014a). Both species are referred to collectively as "mountain yellow-legged frogs." The Federally endangered southern Distinct Population Segment of the mountain-yellow legged frog is the subject of a separate conservation strategy (USFS 2002) and was not included in this current effort. *R. muscosa* is listed as endangered by the State of California, while *R. sierrae* is listed as threatened.

This Strategy is intended to serve as technical guidance to staff and managers of the member agencies. The Strategy provides a framework for the coordinated planning of ongoing and future restoration activities aimed at conservation of mountain yellow-legged frog populations in the Sierra Nevada of California. It is based on sound scientific principles focusing on the conservation needs of both species and potential conservation actions available to maintain viable populations of the species across their native ranges. The Strategy maintains flexibility for various agencies to plan, prioritize, and implement the conservation actions. It provides a blueprint with options that individual agencies can select from as funding and other resources become available.

In certain cases, conservation actions may be constrained by current ecological, logistical or socio- political conditions and frog population status, and restoration options may be limited. Final actions, commitments, and resource allocations will be determined by individual agencies. The Strategy does not propose management actions on privately owned land, nor does it suggest management direction for privately owned lands.

It is anticipated that this document and its associated support documents (Attachments 1 through 5) will be updated periodically to incorporate results from future inventory, monitoring, and research when these activities indicate that changes or amendments to the Strategy are necessary.

Subsection 4(f) of the Endangered Species Act (ESA) requires the USFWS to develop and implement recovery plans for the conservation of endangered and threatened species. Similar to the process used to develop this Conservation Strategy, the recovery planning process involves the identification of actions that are necessary to halt or reverse the species' decline. The information and priority conservation actions identified in this Strategy will inform development of a future recovery plan for *R. sierrae* and *R. muscosa*, and implementation of this Strategy will guide conservation and recovery efforts until a recovery plan is in place.

### I. Introduction

The vision for this Conservation Strategy is:

To ensure viable, self-sustaining populations of mountain yellow-legged frogs (Rana muscosa) and Sierra Nevada yellow-legged frogs (Rana sierrae) with meta-populations well-distributed across their historical ranges in the Sierra Nevada (Figure 1) so as to maintain the genetic and ecological diversity characteristic of both species.

This vision statement is based on our current understanding of the taxonomy and genetic structure of mountain yellow-legged frogs. Specifically, the mountain yellow-legged frog is a species complex made up of two closely-related taxa: the mountain yellow-legged frog (*R. muscosa*) that is found in the San Gabriel, San Bernardino, and San Jacinto Mountains in southern California and in the southern Sierra Nevada, and the Sierra Nevada yellow-legged frog (*R. sierrae*) found in the central and northern Sierra Nevada (Vredenburg *et al.* 2007). There is no overlap in the ranges of the two species.

This taxonomy has been adopted by the American Society of Ichthyologists and Herpetologists, the Herpetologists' League, the Society for the Study of Amphibians and Reptiles (Crother *et al.* 2017), and by the USFWS (USFWS 2014a). In addition to the deep genetic split in the south-central Sierra Nevada that divides *R. muscosa* from *R. sierrae*, both species also contain considerable genetic variability that is geographically structured (Vredenburg *et al.* 2007). The distinct population units (clades) located in the Sierra Nevada (three for *R. sierrae*, two for *R. muscosa*) (Figures 2 and 3) form the basis of the conservation planning detailed in this Strategy.

The Sierra Nevada Mountain Yellow-Legged Frog Conservation Assessment (Brown *et al.* 2014) contains a detailed narrative that summarizes the classification and description, biology and ecology, and threats affecting extant populations of *R. sierrae* and Sierran populations of *R. muscosa*. The reader is referred to that document for background information on these two species. The Strategy begins where the Assessment left off. It begins with a summary of the conservation guidance made in the Assessment, describes the scientific basis and rationale underlying the key conservation approaches, and outlines specific proposed actions necessary to restore *R. muscosa* and *R. sierrae* in the Sierra Nevada. In large part, these approaches and actions focus on mitigating the impacts of the two most important threats to mountain yellow-legged frogs in the Sierra Nevada: introduced fish and the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; "Bd"). In addition, the Strategy as "Frog Conservation Areas" or FCAs) identified for potential conservation actions (Attachment 1); a Conservation Action Plan (CAP) implementation tracking table (Attachment 2); a translocation/ reintroduction protocol (Attachment 3); guidance on decontamination to minimize the spread of pathogens (Attachment 4); and information regarding the approach that will be followed for captive rearing/breeding and salvage of individuals at imminent lethal risk (Attachment 5).

Identifying the historic range of mountain yellow-legged frogs is difficult because available specimens and survey data were primarily collected after the species had already disappeared from the majority of their range (CDFG 2011). Following the approach used by CDFW for their 2011 *Status Review of the Mountain Yellow-legged Frog* (CDFG 2011), this Strategy used a MaxEnt species distribution model to produce a quantitative description of the species' ranges and probabilities of occurrence. The resulting range boundaries (Figures 1, 2, and 3) encompass the areas with  $\geq 0.4$  probability of historical occurrence. The intent of these boundaries is not to delineate the maximal historic ranges of the species but to identify the likely historical ranges for the purposes of guiding development of the Strategy.

Most of the historical range of the mountain yellow-legged frog was naturally fishless due to steep stream gradients that prevented the upstream movement of fish from occupied downstream habitats when glaciers melted at the end of the Pleistocene epoch approximately 10,000 years ago (Moyle *et al.* 1996). Historically, mountain yellow-legged frogs occupied large portions of this extensive fishless aquatic habitat (Grinnell and

Storer 1924). However, the interest in developing recreational fisheries in these fishless lakes and streams led to extensive stocking of several species of trout starting in the late 1800s, which was facilitated by aerial stocking beginning in the early 1950s. Although stocking was successful in developing recreational fisheries, these fisheries also had unintended and widespread impacts on native species including mountain yellow-legged frogs (see Knapp and Matthews 2000, Finlay and Vredenburg 2007).

Bd is an amphibian-specific pathogen that causes the disease chytridiomycosis, and was first described in 1998 (Berger *et al.* 1998, Longcore *et al.* 1999). Since then extensive research has demonstrated that during the past several decades this pathogen has caused the decline and/or extinction of hundreds of amphibian species worldwide (Skerratt *et al.* 2007). The mountain yellow-legged frog is highly susceptible to Bd and has suffered serious declines as Bd has spread across the Sierra Nevada (Vredenburg *et al.* 2010).

Although most mountain yellow-legged frog populations are extirpated following the mass die-offs that result from the arrival of Bd in a naïve population, some persist despite ongoing chytridiomycosis (Briggs *et al.* 2010). These remaining populations are generally small, isolated, and vulnerable to extinction (Brown *et al.* 2013, USFWS 2014a).

This Strategy describes conservation approaches to be used to conserve mountain yellow-legged frogs in the Sierra Nevada that lay the foundation for the CAP. The CAP describes and prioritizes specific conservation actions for R. *muscosa* and R. *sierrae* across their respective ranges. These actions are described separately for each of the five clades (population units) in the Sierra Nevada and are designed to conserve and expand the remaining populations of mountain yellow-legged frogs in each clade.

Conservation approaches identified in this Strategy include the strategic removal of fish to increase the amount of available high quality habitat, strategic reintroductions (e.g., translocations) of frogs, and disease intervention. Fish removal has been demonstrated to be effective in restoring mountain yellow-legged frog populations resulting in increased abundances and expansions into newly created fish-free habitats (Vredenburg 2004, Knapp *et al.* 2007). As mountain yellow-legged frogs have been entirely extirpated from large portions of some of the clades, future reintroductions will be necessary to achieve the vision of having meta-populations well distributed across their historical ranges. Our understanding of the effectiveness of translocations in allowing the reestablishment of frog populations is incomplete, particularly in the presence of Bd, and successful implementation of this approach will require experiments, including disease mitigation measures, and a framework that allows "learning by doing". Further, the ability to conduct these translocations is dependent on the availability of frogs from appropriate source populations and captive breeding may be needed in some situations. Opportunities also may exist to "learn by doing" to increase knowledge of appropriate "best management practices" (BMPs) for mountain yellow-legged frogs and their habitats.

This Strategy proposes to use an adaptive management approach. Adaptive management provides a framework for acquiring and using information from the implementation of conservation and management actions, and is increasingly used in situations where complete information on which to base resource management decisions is lacking. Adaptive management is defined by the National Research Council (2004) as: "...flexible decision making that can be adjusted in the face of uncertainties as outcomes from management actions and other events become better understood. Careful monitoring of these outcomes both advances scientific understanding and helps adjust policies or operations as part of an iterative learning process."

This adaptive approach was chosen for this Strategy because, although the impacts of potential stressors and the mitigation of some of these impacts (e.g., introduced trout) are unusually well- understood in mountain yellow-legged frogs, current knowledge of how to respond to impacts of other factors (e.g., disease) remains insufficient to allow the prescription of some clearly defined set of conservation actions over a long time interval with a high probability of success. The intent of using this adaptive framework is to deal with

inherent uncertainties regarding how best to manage mountain yellow-legged frogs and their habitat in a way that allows up-to-date information to be used to best balance short-term risks against long-term conservation gains. Experimentation is a critical aspect of this process. Thus, conservation actions that have substantial uncertainty in effectiveness will be implemented initially as experiments, and the results will be incorporated into subsequent decisions regarding prioritization and implementation of future actions.

The application of the adaptive management framework for the implementation of conservation actions is described in the final section of this Strategy, and highlights the inventory, monitoring, and research needed to fill critical knowledge gaps. The Strategy also contains recommendations regarding data management and sharing, and describes an interagency body comprised of staff, experts, and decision-makers that will oversee and manage the implementation of this Strategy.



*Figure 1*. Estimated historical range of the Sierra Nevada yellow-legged frog (*Rana sierrae*) and the mountain yellow-legged frog (*Rana muscosa*) in the Sierra Nevada.



Figure 2. Estimated historical range of the Sierra Nevada yellow-legged frog (Rana sierrae).



*Figure 3.* Estimated historical range of the northern DPS of mountain yellow-legged frog (*Rana sierrae*).

# II. Guidance from the Conservation Assessment

As part of the routine USFS practice for developing Conservation Strategies, a document preceding the final Strategy is first completed that collates the available state of the science, and complements and guides the final conservation approach adopted. The Mountain Yellow-Legged Frog Conservation Assessment (Brown *et al.* 2014) identified topics to be addressed within this Conservation Strategy. It indicated that the Strategy should provide mechanisms to manage both species throughout their entire ranges, for all life history stages, and for all required habitats (e.g., lakes, streams, ponds). It suggested the Strategy should seek to stabilize populations and increase abundances by protecting existing populations and increasing the connectivity among them, while managing both species at multiple scales.

Specifically, at least three scales were suggested:

- The coarsest scale involves management at the species level. At this scale, it is recognized that the "mountain yellow-legged frog" is made up of two species, *R. sierrae* and *R. muscosa*, and that in the Sierra Nevada significant genetic structure exists within the two species (3 major clades within *R. sierrae* and 2 major clades within *R. muscosa*; Figures 2, and 3).
- An intermediate scale involves management of the two species by the different administrative units and participating agencies, including individual National Forests and National Parks. This scale recognizes the scale at which decisions occur and resources are allocated to implement conservation actions.
- The finest scale is management at the level of basins (watershed units). At this scale, each basin generally is assumed to contain a single frog population, often distributed across a collection of lake, pond, stream, and meadow habitats. It is at this basin scale that most conservation actions will be conducted. The Assessment suggested that basins be prioritized for restoration based on diverse criteria including habitat suitability, complexity, and uniqueness, disease status, fishery status, source frog populations, and other features that may be important for comprehensive conservation and management of the species.

The Assessment suggested three broad categories of conservation actions: 1) actions directed towards restoration; 2) mitigation efforts to avoid and minimize effects to frogs from program activities undertaken by land management and resource agencies; and 3) research to address areas of uncertainty and information gaps.

- <u>Direct Restoration</u> Actions aimed at direct restoration focused on habitat restoration and reducing habitat fragmentation. The Assessment highlighted that species conservation approaches should not only protect existing populations, but also provide mechanisms for re-establishing mountain yellow-legged frogs in nearby areas. One primary means to achieve this restoration is increasing the extent of fishless habitat within the ranges of the two species. Fish-caused impacts essentially occur throughout the entire geographic and altitudinal range of mountain yellow-legged frogs and fish removal has been shown to be highly effective as a restoration tool. Thus, actions taken to restore habitats to their original fishless condition have the potential for far-reaching positive effects. Fish removal could take the form of altered stocking practices or fish eradication.
- <u>Mitigation</u> The Assessment considered common land management activities within the jurisdiction of the implementing agencies and feasible to mitigate via active conservation measures. These include (1) fire management, (2) habitat restoration, (3) livestock grazing, (4) locally-applied pesticides, (5) mining, (6) recreational activities, (7) research activities, (8)

roads, (9) vegetation and fuels management, and (10) water development and diversion projects. The Assessment also recommended management designed to avoid further habitat fragmentation and population isolation, avoid disease transmission, and minimize environmental stressors that might interact with pathogens to exacerbate their effects.

- <u>Research</u> The Assessment identified high priority research questions, including:
  - o Effects of fish stocking on water quality and pathogens,
  - Role of recreational activities in pathogen dispersal, impacts to frog habitat, and direct impacts on frogs,
  - o Influence of meadow habitat condition on frog occupancy and abundance,
  - o Effects of vegetation and fuels management on frogs,
  - o Effectiveness of translocations in reestablishing populations,
  - Studies of mountain yellow-legged frog populations in stream/meadow habitats and in lower elevation areas,
  - Epidemiology of Bd to identify vectors and interactions between disease and other stressors, and
  - Risk posed by airborne contaminants to frogs, and possible interactions with other risk factors.

Several potential risk factors were deemed either of insufficient importance as conservation threats, and/or could not feasibly be addressed within the jurisdiction of the implementing agencies. These included acid deposition, UV-B radiation, and climate change. However, the Assessment concluded there might be a role for agencies in guiding scientific inquiry to further understand these issues or the larger management approaches to these threats. In addition, recent research suggests that airborne contaminants may not be as important a risk to mountain yellow-legged frogs as previously believed (Bradford *et al.* 2010, USFWS 2014a). Thus, these four risk factors are not addressed in this Strategy. Finally, the Strategy broadens the research topic to include inventory, monitoring, and research under the umbrella of the overall adaptive management framework.

# III. Conservation Objectives

# A. Population Goals

This Conservation Strategy identifies actions that will restore numbers of populations across the landscape, and abundances within metapopulations to levels representative of the species' historical genetic, ecological, and geographic diversity. It aims to do so in restored habitat that is resilient to natural and human-induced perturbations and stochastic events. This achievement will provide sufficient population redundancy to allow for recovery from catastrophic events. Conservation actions are developed and applied by clade, to protect genetic diversity. FCAs (Figures 4 through 11; see also Attachment 1) are identified and mapped over the range of the two species to provide geographic diversity, and the inclusion of lake, stream, and meadow habitats within each clade-specific restoration unit ensures ecological diversity and species resiliency.

Because of the presence of Bd, population abundances in the future may not often reach the large numbers that occurred historically. In particular, survival through to post-metamorphic lifestages is greatly reduced in a chytrid positive landscape. The remaining small populations are inherently less resilient (Shaffer 1981), which increases the importance of redundant populations and highly connected, restored habitat areas. Thus, until more is learned about the demography of populations persisting with Bd, this Strategy places a large emphasis on actions that increase the amount of suitable (fishless) habitat near currently occupied areas, to enhance connectivity and dispersal and facilitate re-colonization of currently vacant habitats; and also to augment existing small populations.

Restoration opportunities vary among the clades. There are few remnant populations in Clades 1 and 5, and restoration opportunities in these largely stream-based populations are limited. More restoration options are available in Clades 2, 3, and 4, and these opportunities are reflected in the Strategy with numerous on-site restoration actions. This Strategy has stressed feasibility and prioritized immediate opportunities to achieve restoration goals. It is important to shore up respective clades while opportunities exist, as restoration of highly endangered clades can be very costly and exceedingly difficult to implement.

Restoration and enhancement of populations within Clade 1 of *R. sierrae* and Clade 5 of *R. muscosa* is a very high conservation priority, as these two clades are the most critically endangered. It is anticipated for the foreseeable future that restoration of populations in Clades 1 and 5 will involve some sort of captive rearing ("headstarting") and perhaps breeding. However, it would be prudent to assure success with these methods in a Bd-positive landscape using applied experimentation in the less endangered clades before embarking on field collections that may have effects on the source populations ('salvage' opportunities aside). These efforts are part of this Strategy to be achieved through current research and *in-situ* restoration projects.

Identifying specific population benchmarks denoting recovery objectives (and associated performance metrics that can be tracked towards this end) requires further expert derivation and scientific vetting to establish appropriate thresholds. Such measures must be derived for the two respective species across their range, but go beyond the current Strategy. This effort will be conducted in a future recovery plan for the mountain yellow-legged frogs, to be completed by USFWS per their obligations under the ESA.

# B. Habitat Goals

This Conservation Strategy seeks to restore aquatic habitats across the range of the species to enhance and expand stable populations across the landscape, and thereby reduce habitat fragmentation to naturally facilitate restored metapopulation function. Mountain yellow-legged frogs are highly aquatic. Therefore, the habitat goals focus on aquatic habitats.

Restoration goals recognize that the species are found in most such habitat types, including lakes, wet meadows, and streams; although the most commonly used habitats vary across the two species' ranges. In the central, high-altitude areas of their Sierra Nevada range where the species are currently most abundant, populations are most commonly found in lakes. In contrast, for the northern and very southern regions of their Sierra Nevada range, populations are most commonly associated with streams. The ecology of the species in lakes has been relatively well-studied, whereas little is known about habitat associations in stream and meadow environments. Thus, this Strategy extrapolates information about the species' lake ecology to streams and meadows. The Strategy recommends further study and research to address this current knowledge gap.

In many locations, frogs use a complex of habitats for breeding, foraging and summer use, and overwintering. Because of the multi-year tadpole stage, breeding habitat requirements are more restrictive—requiring perennial water that does not dry during the summer and is sufficiently deep to prevent freezing in winter. Adults and subadults move to additional habitats during the summer, which are more varied and include nonpermanent water. Overwintering habitats (which may be the same as breeding sites) provide refugia that protect hibernating life stages from freezing.

Connectivity among the complex of habitats is important, both to allow frogs access to foraging sites during the summer, and to facilitate dispersal and recolonization among populations. Frogs will travel along streams and overland. To successfully achieve these dispersal and migratory behaviors, the absence of fish is of primary importance.

Based on these requirements, high quality habitats are defined as:

- 1. Fishless.
- 2. Perennial breeding sites that maintain water during the entire tadpole growth phase (a minimum of 2 years, but sometimes longer). During periods of drought, these breeding sites may not hold water long enough for individuals to complete metamorphosis, but they may still be functional breeding habitat if they provide sufficient habitat in most years to foster recruitment within the reproductive lifespan of individual adult frogs.
- 3. Additional foraging habitats within movement distances of breeding sites and can be lakes, streams, springs, or wet areas in meadows.
- 4. Basking areas commonly in the form of open sandy or gravel banks, rocks projecting above or just beneath the surface of the water, or rocky shorelines.
- 5. Cover from predators commonly in the form of substrate (e.g., silt, cobble), undercut banks, shoreline vegetation, downfall logs or branches, or rocks.
- 6. Overwintering refugia where thermal properties of the microhabitat protect hibernating life stages from winter freezing. Current research suggests these include perennial aquatic habitats such as lakes and ponds typically deeper than 3 m, streams with water flows sufficient to prevent freezing, or underwater crevices or holes within granite, in and near shore.
- 7. Sufficient connectivity provided by site proximity, fishless streams, or other corridors to allow for movement between aquatic habitats used for breeding, foraging, and overwintering, and for dispersal.
- 8. Upland areas adjacent to or surrounding breeding and nonbreeding aquatic habitat to provide area for feeding and movement by mountain yellow-legged frogs.
- 9. Habitat complexes sufficient to provide for breeding, foraging, and overwintering requirements.

# IV. Conservation Strategy

The primary approach used in this Strategy is to identify habitats suitable for mountain yellow-legged frog reintroduction and recolonization, including habitat that would be suitable for frogs if not for fish presence. When present in these targeted areas, fish removal will be one option considered to improve habitat for frogs. Ideally, frogs would naturally recolonize sites near currently occupied populations. Active translocations would introduce frogs to more isolated sites. For translocations, frogs may be either wild or captive-bred, and efforts will follow appropriate State and Federal permitting for the capture, handling, movement and reintroduction of threatened or endangered species. The active restoration or enhancement of degraded mountain yellow-legged frog habitat to expand extant populations or provide the opportunity for successful establishment of translocated populations is an essential component of this Strategy, and cannot be substituted by a captive breeding program alone.

The recommended approaches to conserve and restore mountain yellow-legged frog populations are grouped into three general categories: (1) actions to directly restore frog populations, (2) management practices to facilitate population recovery, and (3) inventory, monitoring, and research to test assumptions, fill information gaps, and suggest new management approaches or restoration approaches.

# A. Actions to Restore Frog Populations

### Fish Removal

The impact of introduced trout on mountain yellow-legged frogs (and the recovery of frog populations following trout removal) is well documented (See Box 1). Fish removal is a primary tool used in this Strategy to restore high-quality habitat and allow for expansion of existing populations, to reconnect currently occupied habitats, and to create high quality fishless habitats for translocations.

Entire trout populations can be removed from lakes, either passively by halting stocking and allowing nonreproducing populations to die out (Armstrong and Knapp 2004), or by active eradication. Active fish eradication from lakes can be accomplished with minimal environmental impact through intensive gill netting and electrofishing (Knapp and Matthews 1998, Knapp *et al.* 2007, Vredenburg 2004). For this technique to be effective, the site must be isolated from downstream fish populations by barriers (e.g., waterfalls).

When effective barriers are present, fish can be removed from lakes using gill nets and simultaneously from associated inlet and outlet stream reaches using electrofishing. However, electrofishing in the very low salinity waters typical of the Sierra Nevada is not particularly effective and fish eradication from long stream stretches may be very time consuming, or impossible. In addition, although trout populations have been eradicated from lakes as large as 12 ha (30 acres) in surface area, there may be an upper limit in lake size above which the probability of successful fish eradication is reduced.

Piscicides, which are chemicals that are toxic to fish, have been used to eliminate fish populations. One such chemical, rotenone, is registered by the California Department of Pesticide Regulation for applications targeting fish in California waters. Rotenone targets all gill-breathing organisms and so it has effects on organisms other than fish. While this compound has not yet been used to remove fish from lakes in the Sierra Nevada specifically to restore habitat for mountain yellow-legged frogs, such use was recently approved by Sequoia-Kings Canyon National Park (NPS 2015, NPS 2016). Use of piscicides such as rotenone may be required when the goal is to remove fish from stream habitats or to remove all fish from an entire lake basin or watershed where the area is too expansive to treat using only mechanical methods such as gill nets and electrofishing. The cooperating agencies participating in this Strategy intend to utilize piscicides on a case-by-case basis, and follow all pertinent permitting procedures, within the suite of tools available to achieve restoration goals.

Following restoration of impacted sites via fish removal, monitoring is still required to evaluate our success and adapt our approaches, if necessary. Importantly, research and long-term frog population monitoring is needed at fish removal sites in Bd-positive areas (See *Box 1*).

#### Box 1. Success of Fish Removal in Restoring Populations

The majority of the lakes, ponds, and streams within the native ranges of both R. *muscosa* and R. *sierrae* in the Sierra Nevada were naturally fishless. Starting in the late 1800s, fishes were widely introduced (Knapp 1996). Introduced fish species were primarily trout stocked to create recreational fisheries (rainbow, golden, brook, and brown trout), but at lower elevations also included other sport fish (e.g., brown bullhead) and small cyprinids introduced as baitfish (golden shiners, tui chubs).

Both trout and mountain yellow-legged frogs require permanent water bodies to complete their life cycle. These frogs have a tadpole stage that overwinters 1-3 years before metamorphosing. Consequently, successful reproduction is restricted to water bodies that don't dry up during the summer or freeze completely during the winter (Knapp and Matthews 2000). In addition, post-metamorphic frogs overwinter underwater and therefore also require permanent water. The presence of introduced trout renders otherwise suitable permanent water bodies less suitable for frogs. Based on surveys conducted at thousands of water bodies in the southern and central Sierra Nevada, the probability of mountain yellow-legged frog occurrence is significantly reduced by nonnative trout (Bradford 1989, Bradford *et al.* 1998, Knapp and Matthews 2000, Knapp *et al.* 2005).

The recovery of frogs following trout removal is well documented. When mountain yellow-legged frogs are present in the vicinity of fish removal sites, populations rapidly recover to levels typical of fishless sites soon after fish are eradicated (Knapp 2005, Knapp *et al.* 2007, Vredenburg 2004). The impacts of fish on frogs are due largely to predation by trout, but competition between trout and frogs for food also may occur (Finlay and Vredenburg 2007). Trout introductions have severely fragmented remaining frog metapopulations (Bradford *et al.* 1993).

To date, trout have been actively eradicated from at least 70 lakes, all using gill nets. Fish removal projects in the southern part of the range were conducted in areas not yet reached by Bd, whereas those relatively few conducted to date in the northern part of the range were in Bd-positive areas. In the disease-free areas, fish eradication has nearly always been successful in allowing a dramatic increase in frog numbers and spatial distribution (Vredenburg 2004, Knapp *et al.* 2007, CDFG 2011, NPS 2015, NPS 2016). Preliminary results suggest that recovery of Bd-positive frog populations following fish removal may be slower and less predictable than at Bd-negative sites. However, the removal of one (fish) of the two important stressors impacting frog populations will benefit at least some of the affected frog populations over the long term. Research and long-term frog population monitoring at fish eradication sites in Bd-positive areas will help to clarify the extent of this benefit.

#### **Disease Mitigation**

Mountain yellow-legged frogs are extremely susceptible to Bd, which is now present throughout almost all of the species' range. When chytridiomycosis reaches Bd-naïve frog populations, outbreaks commonly result in the extirpation of the host population (Vredenburg *et al.* 2010). Frog populations that survive the initial Bd-caused population crash and are persisting despite ongoing chytridiomycosis continue to suffer negative effects from the disease. These effects include very high mortality of metamorphs, recruitment failure, and elevated adult mortality (Briggs *et al.* 2010). As a consequence, persistent frog populations are often small and vulnerable to extirpation.

Our understanding of how to mitigate the effects of chytridiomycosis in amphibians is still in an early stage, and much remains to be learned before effective field treatments can be broadly applied (Woodhams *et al.* 2011, Woodhams *et al.* 2012). However, during the last few years, important progress has been made in mitigating the effects of chytridiomycosis in mountain yellow-legged frogs and this suggests directions for future research efforts (see *Box 2a*).

#### Box 2a: Disease Mitigation Research—Intervention and Adaptive Immunity

Research on techniques to mitigate the effects of chytridiomycosis in amphibians is still in the early stages (Woodhams *et al.* 2011, Woodhams *et al.* 2012). In general, approaches are designed to reduce Bd loads on frogs to (1) reduce the density of infective zoospores thus reducing pathogen pressure, and (2) provide time for frogs to mount an effective adaptive immune response to Bd.

Results from a recent laboratory experiment indicate that *R. sierrae* are able to mount an effective adaptive immune response against *Bd* (Toothman and Briggs, unpubl. data), and adaptive immune responses against Bd have been documented in other amphibians (Ramsey *et al.* 2010, Murphy *et al.* 2011). A number of field treatment experiments using anti-fungal drugs have been conducted to date for mountain yellow-legged frogs, all in Kings Canyon National Park and using both *R. muscosa* (2006, 2009) and *R. sierrae* (2009, 2010, 2015). Frog treatments conducted in 2006 used malachite green (Parker *et al.* 2002) and those in 2009, 2010, and 2015 used itraconazole (Garner *et al.* 2008, Knapp *et al.* in review). Results suggest that treatment effectiveness is life stage-specific. Treatment of adults, metamorphs, and tadpoles all resulted in dramatic decreases in *Bd* infection intensity. *Bd* loads remained low in treated adults, resulting in relatively high survival rates (Vredenburg, unpubl. data). However, in metamorphs and tadpoles *Bd* loads increased again and resulted in high subsequent mortality (Knapp and Vredenburg, unpubl. data). The different treatment outcomes between adults and metamorphs/tadpoles suggest that the mechanism driving these results was adaptive immunity and not reduced zoospore density.

Itraconazole treatment appears to benefit adult frogs by allowing time for them to mount an adaptive immune response against Bd. However, it remains unclear whether increasing adult survival will be sufficient to maintain treated populations over the long-term, because high metamorph mortality in the presence of Bd may create ongoing recruitment failure that ultimately causes population extirpation. In frogs, adaptive immunity may be poorly developed in tadpoles and metamorphs. In mountain yellow-legged frogs, it isn't yet clear when after metamorphosis the adaptive immune system becomes functional but it is likely to require several months (Toothman, unpubl. data). This suggests that when no adults are left at a site but tadpoles remain abundant (as is usually the case immediately following the Bd-caused frog population crash) a viable treatment strategy might be to bring metamorphs into captivity, clear them of Bd, rear them past the age at which the adaptive immune system is fully developed, infect them with Bd, clear them, and release them back into their original habitat. In theory, these frogs should have a well-developed immunity against Bd. This strategy has been employed with success to date in the Eldorado National Forest, and currently is being attempted in Sequoia-Kings Canyon National Park.

Efforts to mitigate the effects of chytridiomycosis have focused on two different approaches: (1) treatment of animals with antifungal drugs to reduce their Bd loads and increase survival, and (2) augmentation of the frog's skin microbial community with beneficial bacteria (bioaugmentation) to slow infection upon exposure to Bd (see Box 2b). Reducing Bd loads on frogs could produce long- term benefits to frogs via two potential mechanisms. First, it may reduce the density of infective zoospores, thereby reducing pathogen pressure and increasing frog survival. Second, reducing Bd loads could provide additional time for frogs to mount an effective adaptive immune response (see Box 2a). Such immune responses are triggered by exposure to a pathogen and allow the vertebrate immune system to recognize and remember specific pathogens, thereby generating immunity. Initial research suggests that both anti-fungal drug treatment/immune system activation and bioaugmentation confer at least some benefits to frogs affected with chytridiomycosis. Recently, a study in the Desolation Wilderness involved exposing Bd-naïve captive-reared frogs to Bd to stimulate an immune response. Frogs are then cleared of infection (with itraconazole - see Bax 2a) prior to their reintroduction into the field. Another study, in Kings Canyon National Park, involved treating adult frogs with itraconazole during an epizootic outbreak of Bd in a larger, previously disease-naïve R. sierrae population. Frogs were retained in outdoor pens during a 10-day period, during which frogs were treated with itraconazole to reduce Bd loads and increase the probability of population persistence.

#### Box 2b: Disease Mitigation Research--Bioaugmentation

A potentially important component of the amphibian innate immune system is the microbial community living commensally on their skin (Woodhams *et al.* 2007). The goal of bioaugmentation treatments is to increase the density of bacteria that have strong anti-fungal properties. *Janthinobacterium lividum* is found on the skin of some temperate zone amphibians (Lauer *et al.* 2009), including mountain yellow-legged frogs (Woodhams *et al.* 2007). *J. lividum* produces the metabolite violacein that can inhibit the growth of Bd at even relatively low concentrations (Brucker *et al.* 2008). In laboratory experiments, augmentation of *J. lividum* onto the skin of red-backed salamanders and mountain yellow-legged frogs prevented Bd-caused mortality (Becker *et al.* 2009, Harris *et al.* 2009).

In a field trial conducted in Kings Canyon National Park in 2010, adult R. *sierrae* were treated with *J. lividum* at the beginning of a Bd outbreak. Over the course of two summers following treatment (2010, 2011), treated frogs had lower Bd infection intensities and substantially higher survival than did untreated control frogs (Vredenburg, unpubl. data). *J. lividum* concentrations on treated frogs declined quickly following treatment and as such, it remains unclear whether the effect of *J. lividum* was due to a long-term protective effect or whether it provided short-term protection during which time the frogs mounted an effective adaptive immune response against Bd. To determine whether *J. lividum* treatment of metamorphs would produce similar beneficial effects, in 2012 R. *sierrae* metamorphs at a single pond at which Bd had arrived two years prior were first treated with itraconazole to reduce their infection intensities and then treated with *J. lividum*. Bd loads and frog survival were compared between treated frogs and control frogs that were not given any treatment. Following release of all experimental animals, Bd loads were lower and frog survival was higher in the treated frogs versus the control frogs, but eventually, treated frogs developed high Bd loads and succumbed to chytridiomycosis. Therefore, any short-term benefit that may have been conferred by *J. lividum* was not maintained over the long term.

Bioaugmentation treatments also show promise for adult frogs, but additional research is needed to identify the best bacteria to use in these treatments. The ideal bacterium would have strong anti-Bd properties, would colonize and persist on frogs for the long-term, and would have no negative effects on frog health. It remains uncertain whether *J. lividum* meets all of these criteria.

Given current knowledge, this Strategy does not yet incorporate widespread use of disease intervention methods. However, it does encourage continued research and development of techniques that eventually may be broadly applied (see *Adaptive Management*). Further, it does emphasize the importance of measures that reduce the risk of Bd spread, an important aspect of disease mitigation that can currently be implemented. Bd has shown a remarkable capacity to spread, and now nearly all mountain yellow-legged frog populations in the Sierra Nevada are Bd-positive (USFWS 2014a). However, a few R. *muscosa* and R. *sierrae* metapopulations in Sequoia and Kings Canyon National Parks and the adjacent John Muir Wilderness remain Bd-negative.

Given that significant progress in mitigating the effects of Bd is possible in coming years, delaying the introduction of Bd to these basins as much as possible is important. In addition, Bd strains differ markedly in their virulence (Berger *et al.* 2005), including the many strains infecting mountain yellow-legged frogs in the Sierra Nevada (Morgan *et al.* 2007, Knapp *et al.* 2016). Therefore, it is also important that more virulent strains not be introduced into frog populations that are currently persisting with less virulent strains

#### Box 3: Translocations and the Role of Bd

Development of successful mountain yellow-legged frog translocation techniques is in the initial stages. The Bdstatus of translocated animals and translocation sites appears to strongly influence success. In Bd-negative areas, transfer of even relatively small numbers of uninfected frogs resulted in the establishment of reproducing populations (Knapp, unpubl. data). This high translocation success under Bd-negative conditions is likely a consequence of naturally high survival of adults, juveniles, tadpoles, and eggs (Pope and Matthews 2001), especially when densities are low. However, translocation of Bd-naïve frogs into sites where mountain yellowlegged frogs had previously been extirpated by Bd outbreaks eventually resulted in extirpation when the translocated frogs became infected with Bd (Vredenburg and Knapp, unpubl. data). The failure of these translocations is likely related to the high susceptibility of Bd-naïve frogs to chytridiomycosis; however, the role of Bd in driving the success or failure of translocations using frogs from persistent populations remains uncertain.

Researchers working in Sequoia and Kings Canyon National Parks are experimenting with translocations using immunization and reintroduction. Frogs are captured, infected with Bd in captivity, cleared again before release back to the wild. These experiments have been conducted on source populations that are both Bd-positive and Bd-naïve. Future frog translocations to re- establish populations previously extirpated by Bd will most likely involve the transfer of Bd-positive frogs from persisting populations to Bd-positive sites. The reduced susceptibility to Bd of frogs from persistent populations relative to those from Bd-negative populations (Knapp *et al.* 2016), due at least in part to an adaptive immune response against Bd, should allow relatively high success of such translocations.

If Bd infection is a critical factor in translocation success (and it likely will be), future studies are needed to determine whether Bd mitigation measures (anti-Bd drug treatments, bioaugmentations) could be used to increase the probability of translocation success.

The most effective method of preventing the anthropogenic spread of Bd into Bd-negative areas and the spread of Bd strains among Bd-positive areas is for people who are moving between sites (especially researchers) to disinfect all of their gear between each visited water body. Bleach and quaternary ammonia are highly effective disinfectants against Bd (Johnson *et al.* 2003), and are also effective against bacteria, viruses, and other fungi. Others have used complete drying as a means of disinfection. Recently, Sequoia and Kings Canyon National Parks updated their decontamination protocol after further literature search to determine recommended concentrations of bleach and quaternary ammonium (Quat). This protocol appears in Attachment 4, for use by agency biologists and researchers in order to minimize the risk of Bd spread. The USFWS is requiring the use of this protocol for all surveys and research activities through its ESA \$10(a)(1)(A) permits.

#### Reintroduction

Given that mountain yellow-legged frogs have disappeared from more than 90% of their historical ranges in the Sierra Nevada, simply enhancing remaining frog populations will be insufficient to restore *R. muscosa* and *R. sierrae* metapopulations so that extant metapopulations are well distributed across a geographic extent sufficient to confer species level resilience. Thus, frog reintroductions will be an important mechanism to re-establish populations in areas where they have been extirpated, and to ensure adequate distribution of populations across sufficient geographic expanse, and in ecologically diverse settings.

Reintroduction is an important tool in wildlife conservation, and has been used to re-establish populations of a wide diversity of species, including amphibians (Germano and Bishop 2009). In this document, the terms "reintroduction," "translocation," and "captive breeding/rearing" all refer to the transfer of frogs with the goal of re-establishing them inside their native range (Armstrong and Seddon 2008).

Reintroductions may occur with wild frogs translocated from nearby populations, or captive-bred frogs that are released into the wild. Reintroductions may occur in currently unoccupied or occupied habitats to augment existing populations. Within this Strategy, and the larger reintroduction program, rigorous care will be taken to honor "pedigree" (genetic origin within clade, watershed, and even specific water body, where appropriate).

This section addresses reintroductions, including translocations and captive breeding/rearing. It reviews the pertinent issues associated with these approaches, and those issues common to both. Because these processes are ongoing and learning is still informing our decisions, actions will be coordinated in real time by experts and the implementing agencies. Accompanying the Strategy are a protocol for translocation and reintroduction (Attachment 3) and guidance on captive rearing (Attachment 5), which will be updated periodically as needed

#### Translocations

Research on mountain yellow-legged frog translocations and reintroductions is in the early stages. So far, several translocation projects have been conducted in the Sierra Nevada, and success appears to be strongly influenced by the Bd-status of the translocated animals and/or translocation sites (see *Box 3*). Translocations that have been conducted in Bd-negative environments appear to have been successful, whereas those in environments with Bd have had variable success.

Translocations are a major tool proposed in this Strategy. Translocations refer to the transfer of wild-caught frogs from one area to another, inside their native range (Armstrong and Seddon 2008). When available, translocations of wild animals are the preferred method for reintroductions, with captive breeding and subsequent reintroduction considered a last resort (USFWS and NOAA 2000). Most of the issues surrounding translocations (e.g., Bd, frog genetics, identifying suitable source populations) also apply to captive breeding, and are discussed below.

#### Chytridiomycosis and Reintroductions

Because Bd is now widespread among remaining mountain yellow-legged frog populations in the Sierra Nevada, the most likely scenario for future translocations will involve the transfer of Bd-positive frogs from persisting populations to Bd-positive sites (see Bax 3). This near ubiquity of Bd across the mountain yellow-legged frog's Sierran range poses challenges for translocations. In most cases, adults would be the best life stage to translocate because they have the potential to reproduce immediately and have relatively high annual survival. A relatively small number of translocated adults could, in theory, quickly produce a self-sustaining frog population.

However, remaining persistent frog populations typically have relatively small numbers of adults, which may be too small to allow any adults to be removed for translocations. Although tadpoles and metamorphs are sometimes abundant in these persistent populations, due to Bd infection their survivorship in the weeks after metamorphosis typically is extremely low. Therefore, without any Bd mitigation efforts, translocation of infected tadpoles and/or metamorphs is less likely to be successful.

Therefore, an alternative approach with a reasonable chance of success involves the following process for late-stage tadpoles and metamorphs: (1) bring into captivity; (2) clear them of Bd; (3) rear them past the age at which the adaptive immune system is fully developed; (4) re-infect and clear them again to further stimulate an immune response; and (5) release the frogs (now adults) at the translocation site (see *Box 2a*). Further research addressing the influence of Bd on translocation success and potential mitigation measures is currently underway (see *Box 3*).

#### Captive Breeding and Rearing

In 2000, the USFWS and National Marine Fisheries Service (NMFS) published a policy (hereafter, "Policy") that addresses the role of captive propagation in the conservation and recovery of species listed as endangered or threatened under the ESA (USFWS and NOAA 2000). The Policy provides guidance and establishes consistency for use of captive propagation as a component of a listed species recovery strategy. The Policy was intended to help ensure smooth transitions between various phases of conservation efforts such as propagation, reintroduction, and monitoring, and to foster efficient use of available funds.

The Policy supports the captive propagation of listed species when recommended in an approved recovery plan, or when necessary to prevent extinction of a species. Appropriate uses of captive propagation include: supporting related research, maintaining refugia populations, providing individuals for reintroduction or augmentation of existing populations, and conserving species or populations at risk of imminent extinction or extirpation. In the time interim to issuance of the recovery plan (which will specifically call for captive propagation), initiation of these activities will require the USFWS Regional Director's approval.

Research on, and the implementation of, captive breeding and rearing has been initiated for populations of the Southern DPS of *R. muscosa*, all of which are small and isolated. In response to continued declines and extirpations of populations in this DPS, the USFWS officially approved an experimental captive breeding, reintroduction, and monitoring program (USFWS 2007, see Attachment 5). The San Diego Institute for Conservation Research (ICR) and Los Angeles Zoos currently are participating in this experimental program. Thus far, this program has developed captive populations using frogs collected in emergency salvages, allowed for the breeding of individuals in captivity, conducted concurrent research on the biological requirements of captive propagation and rearing, and allowed the first reintroductions of captive-reared and captive-bred *R. muscosa* back into the wild. Additionally, in 2013, under the authority of a temporary Memorandum of Understanding (MOU) with CDFW, the San Francisco Zoo began captive rearing *R. sierrae*. The Oakland Zoo has also started a program, and both zoos are currently rearing frogs from collected egg masses, tadpoles, or juveniles ("headstarting") for reintroduction to their native habitat. Results from these programs are preliminary, and work will continue further develop the captive breeding and release program.

Because most remaining populations of *R. muscosa* and *R. sierrae* are comprised of very few individuals, and many are isolated from each other, there often are few available source populations to use for translocations. This is particularly the case for Clades 1 and 5, and the northern part of Clade 2. Therefore, these imperiled populations may require headstarting more vulnerable lifestages collected from the wild (i.e., egg masses/tadpoles/metamorphs) until they develop into later lifestages with a higher chance of survival (i.e., juveniles and adults), and/or captive breeding altogether, to provide a source of frogs for reintroductions, and to preserve genetic diversity throughout the species' ranges.

This Strategy proposes to initiate a captive breeding program for the most endangered clades of mountain yellow-legged frogs in the Sierra Nevada with the objectives of (1) providing frogs for reintroductions or population augmentations, (2) preserving genetic diversity of R. *sierrae* and R. *muscosa*, and (3) preventing extinction of the species by maintaining captive populations.

Additionally, this Strategy includes captive rearing of vulnerable early lifestages for eventual release (see preceding section). Initially, this Strategy proposes to develop captive breeding and reintroduction techniques using frogs from more stable populations, prior to removing frogs from more vulnerable areas. This approach will be adjusted as more information becomes available. Based on current information, Clades 1 and 5 and the northern part of Clade 2 are the most imperiled. Therefore, populations in these areas are eventual target populations for captive rearing and breeding. Provided facilities are available, headstarting will also be used for purposes of population augmentation, as well as a learning opportunity to refine methodology.

Procedures and protocols for captive breeding of Sierra Nevada mountain yellow-legged frogs will be developed in collaboration with the southern California R. *muscosa* restoration efforts. The majority of institutions involved in programs assisting the conservation of endangered and threatened species in the United States are members of the Association of Zoo and Aquarium (AZA). The AZA has developed numerous strategies, protocols, and standards that address concerns associated with captive animal populations involved in conservation-based breeding programs. USFWS and NOAA (2000) encourages captive breeding programs to follow, as may be practical, the protocols and standards of the AZA and other appropriate organizations for the captive propagation of animal species.

The San Diego Zoo ICR produced a husbandry manual for *R. muscosa*, on which the San Francisco Zoo has based their husbandry protocols (Bushell, pers. comm.). This manual includes guidelines on enclosures, equipment, caged furniture and plants, substrate, lighting and temperature; and stage- specific considerations, including stocking densities, diet, air quality, water quality, health evaluations, hibernation and breeding. Lessons learned from the continued captive breeding, rearing, and release activities at the San Diego Zoo ICR and Los Angeles Zoo, and recently, San Francisco and Oakland zoos, will assist with development of species-specific protocols to improve reproductive success and reduce mortality in captive *R. sierrae* and *R. muscosa*. This information is critical in order to successfully re-establish frogs in the wild.

#### Source Populations and Salvage

For translocations and broodstock for captive breeding, decisions on the level of appropriate numbers and lifestages of animals collected from the wild will need to be made and will be dependent on the status of the population and the reason for translocating animals or removing them from the wild (e.g., permanent removal for broodstock in a captive breeding program or temporary captivity to undergo disease treatment). In many cases, source populations likely will be extremely small, particularly for captive propagation since it is considered a last resort. Since survival in the wild of eggs, tadpoles, and metamorphs is relatively low, if these lifestages are present, it often will make the most sense to harvest a proportion of them before removing juveniles and adults, particularly when there are very few individuals (but see *Chytridomycosis and Reintroductions* above). On the other hand, adults can potentially reproduce right away, and one study has found evidence of a density-dependent response to harvesting, where reproduction in this large population increased following removal of adults used for translocations (R. Knapp, pers. comm.). However, this density-dependent response under consideration for conservation actions do not contain numerous adults. Therefore, such a response may not occur in remnant populations.

Once a population has been identified for translocation or captive propagation, emergency salvage opportunities should be considered. Although *R. sierrae* and *R. muscosa* typically breed in perennial waters, they sometimes occur in shallow waters and intermittent streams that occasionally dry (Lacan *et al.* 2008), providing opportunities to salvage animals that would otherwise die. Salvaged tadpoles from drying pools in southern California formed the broodstock for the captive colonies at the San Diego Zoo ICR and Los Angeles Zoo. For captive breeding, catastrophic events such as wildfires or floods, while rarer in the Sierra Nevada than in southern California, also may contribute to decisions on when and where to collect animals. When a population appears to be moving toward extirpation due to multiple years of no detectable recruitment, one option is to move all of the animals into captive breeding; (for example, these circumstances occurred in a population in the San Bernardino Mountains). Very small isolated populations with highly limited observed breeding are typical in Clade 1 and in stream populations in northern Clade 2, and these populations may be moving toward extirpation. The ecology of these populations is not well-known, and further study should occur to provide a basis for management decisions.

#### Genetics

USFWS and NOAA (2000) note that if captive breeding is identified as an appropriate strategy for the recovery of a listed species, it must be conducted in a manner that will, to the maximum extent possible, preserve the genetic and ecological distinctiveness of the listed species and minimize risks to existing wild populations. If adequate information is available, they strongly recommend development of genetic management documents. However, they also recognize that, in many instances, there is insufficient biological knowledge of the species to develop these documents and that the requirement for these documents may unnecessarily burden conservation and recovery efforts.

For captive breeding, adequate numbers of source frogs for use as broodstock will likely not be available for areas most in need of reintroductions. When developing the genetics management component of a captive breeding and reintroduction plan, it is important to recognize that failure to establish populations at reintroduction sites could be the result of releasing inbred individuals or individuals with "inappropriate provenance (i.e., genetically adapted to conditions different from those at the release site)" (Armstrong and Seddon 2008). Inbreeding depression in captive-bred individuals is not only a potential risk to the success of establishment efforts, but, if captive-bred frogs are used to augment extant populations, inbreeding depression could negatively affect the fitness of any offspring produced from mating with wild individuals (Araki and Schmid 2010). Therefore, decisions will need to be made on the number of individuals and locations within a source population will be necessary to ensure adequate genetic diversity. In some situations, breeding animals from different populations may need to be considered as an option, although the Policy discourages intercrossing and requires special approval if it's not part of an adopted recovery plan.

#### Regulatory Permitting and Reporting for Captive Breeding and Rearing

The facilities and principal investigators involved in captive breeding and rearing must obtain an MOU from CDFW prior to initiating these activities per the California Endangered Species Act. Further, as *R. sierrae* and the northern DPS of *R. muscosa* are listed under the Endangered Species Act, in the absence of an approved recovery plan that specifically names captive breeding as a conservation strategy, initiation of these activities requires the USFWS Regional Director's approval. The facilities and principal investigators involved in the captive breeding and rearing activities will need to obtain recovery permits under Section 10 of the Endangered Species Act.

CDFW reporting requirements will be specified in the MOU and are typically concordant with requirements in USFWS prepares annual reports to the Director, and in order to compile this information typically requires that permittees submit reports no later than January 31st of each year.

#### Habitat Restoration

In some situations, physical habitat restoration may be necessary in occupied areas to improve and maintain habitat quality. Habitat restoration includes stream bank stabilization, stream-crossing improvement, meadow restoration (e.g., headcut repair, water table stabilization), riparian vegetation enhancements, and lakeshore habitat improvement. Physical habitat restoration also would improve water quality and watershed conditions of these occupied habitats.

# **B.** Management Practices to Facilitate Recovery

A conservation action available to participating agencies is implementation of mitigation measures so that ongoing land management activities reduce or eliminate the negative impacts associated with threats to individuals and their habitat. As a matter of policy, the U.S. Department of Interior employs an approach to mitigation that first favors avoidance, followed by minimization of risks, and finally (when impacts are unavoidable following the first two options), compensation (USDOI 2015). Often, Section 7 consultation

under the ESA results in a combination of these approaches. Mitigation may be achieved through application of BMPs, which avoid or minimize the exposure of listed individuals to mortality risks. Such BMPs may be incorporated as standard procedure during the various activities in which the participating agencies routinely engage. For example, the BMPs and Standards and Guidelines already in place for USFS under the Sierra Nevada Forest Plan Amendment Aquatic Management Strategy (USFS 2004) have been carried over within the Programmatic Biological Opinion (USFWS 2014b, USFWS 2017) for the Sierra Amphibians in USFS Region 5, with a monitoring component included.

Compensatory mitigation involves substitute resources or environments, but may take the form of offsetting habitat enhancements. As available, such measures will be implemented in concert with the active restoration actions outlined within this Conservation Strategy to meet the conservation needs of the mountain yellow-legged frogs.

# C. Adaptive Management: Inventory, Monitoring, Research and Collaboration

This Strategy uses an adaptive management approach intended to facilitate adjustment of the CAP as new information becomes available. Uncertainties, information needs, and knowledge gaps have been identified throughout this document. This section provides the framework and guidance for collecting and sharing data to address these information gaps.

Efforts towards achieving the restoration goals within this Strategy will also include inventory, ongoing monitoring, research, and iterative planning and coordination of activities and actions. These efforts may be integrated; for example, some of the project-based monitoring will address research needs, and some restoration projects may be implemented as experiments.

Implementation will be conducted at the discretion of participating agencies. Interagency coordination will be facilitated using an expanded interagency technical team that will periodically convene to update the working group on ongoing activities, results, and findings from restoration work. It is anticipated that such a larger team will comprise an expanded group of experts and personnel beyond the core of the current technical team. In addition, sub-teams will be convened for specific tasks, as needed, to focus on narrowly defined components of the overall Strategy (e.g., a rapid response team for implementing salvage and translocation decisions that includes State and Federal permitting staff).

To effectively adjust the Strategy based on new information, results and data collected must be efficiently compiled, evaluated, and made accessible, especially across agencies with overlapping mission (e.g., USFS and CDFW). Thus, this section also contains recommendations on data management and regular evaluations of the Strategy's success.

### Inventory

Inventory needs include: site, watershed, and larger scale surveys and assessments to fill specific information gaps needed to develop restoration plans, and generally include surveys for frogs, fish, and habitat, and evaluation of restoration options. Nearly all lake and pond habitats for mountain yellow-legged frogs within the Sierra Nevada were visited during Sierra Lake Inventory Program (SLIP) and CDFW High Mountain Lake (HML) inventory surveys between 1995 and 2010. However, many locations in which mountain yellow-legged frogs were not observed during the inventory have only been surveyed a single time. Additionally, there are some gaps and additional habitat types that need further inventory (e.g., streams). These can be segregated into two broad classes of inventory: 1) Site-specific inventories to better evaluate current watershed population status, and 2) large-scale inventories to locate populations in currently unsurveyed areas.

#### Watershed and Site-scale Inventories to Assess Options for Conservation Actions

The CAP identifies multiple FCAs that need further surveys to evaluate frog and fish populations (extent, distribution, and status), and habitat conditions to help identify restoration options (see also Attachments 1 and 2)

# Large-scale Inventories to Locate Extant Frog Populations

Stream and Meadow Inventories: Relatively little survey effort has focused on stream habitats, which provide the majority of habitat in frog Clade 1, northern part of Clade 2, and Clade 5. Due to factors such as dense vegetation, many streams are particularly difficult to survey effectively, and the task of inventorying all streams, as has been completed for lakes, is logistically challenging. Meadows within the Yosemite toad range, particularly those located in grazing allotments on National Forest lands (Stanislaus, Sierra, and Inyo National Forests) and in Yosemite, Sequoia, and Kings Canyon National Parks, have been fairly extensively surveyed since the late 1990s, though there are gaps and the majority may have only been surveyed once. However, meadow habitats outside these areas generally have not been systematically surveyed for mountain yellow-legged frogs.

These unsurveyed stream and meadow habitats should be the focus of a strategic inventory effort to be accomplished from the time of this conservation strategy's development through, at minimum, 2025, or as otherwise dictated by a future Recovery Plan), especially in highly imperiled clades. A strategic approach needs to be developed that identifies high priority places to survey, and effective methods for detecting frogs (e.g., multiple surveys, double-observer techniques). Possible criteria for prioritizing surveys include historical frog localities, absence of fish, meadow-stream complexes, and the Maxent-generated probability of historical occupancy by mountain yellow-legged frogs (Figure 1). Specific high priority regions for stream and meadow surveys are identified in the CAP Table (Attachment 2), which will be updated annually as needed in order to reflect ongoing and new survey efforts.

<u>Additional Geographic Areas</u>: In general, not all subalpine and alpine areas within CDFW Region 4 (Sierra, Stanislaus, and Sequoia National Forests) have been fully surveyed. The CAP Table (Attachment 2) lists specific inventory needs for each administrative unit and will be updated annually as needed to reflect ongoing and new survey efforts.

# Monitoring

Ongoing monitoring is needed at both project- and range-wide scales to evaluate changes in frog populations over time following implementation of specific restoration projects, as well as to gauge the effectiveness of the Strategy as a whole. This section discusses the basic monitoring components needed.

#### Site-specific Monitoring

Site-specific monitoring is needed to detect and mitigate disease outbreaks, and to improve our knowledge regarding the effectiveness of specific conservation actions. At least initially, individual actions should be conducted as experiments with intensive monitoring to gauge action effectiveness.

As information is acquired, future actions may need less intensive monitoring. Specific project-scale monitoring activities to implement within the Strategy are enumerated in the CAP Table (Attachment 2), which will be updated annually as needed.

#### Bd Monitoring in Bd-negative Populations

Monitoring is needed to detect pending epizootics early enough to employ treatments intended to improve frog survival. This Strategy recommends that all Bd-negative populations be swabbed at least once per season (ideally 2-3 times per season) and that swabs be analyzed immediately.

#### Population Response to Fish Removal in Bd-negative Areas

When fish are removed in areas where Bd is absent, Bd-negative populations are expected to increase in abundance and/or Bd-negative mountain yellow-legged frogs are expected to recolonize from nearby occupied sites. Because strong positive effects of fish removal on Bd-negative mountain yellow-legged frog populations are well documented and highly predictable, limited monitoring of future fish removal projects will suffice. This Strategy recommends that visual encounter surveys (VES) be conducted at least once per year starting in the year prior to the initiation of fish removal, during fish removal (estimated 2-5 years if using gill nets and/or electrofishing), and for at least 4 years following complete fish eradication. Bd monitoring also would occur as described above.

#### Population Response to Fish Removal in Bd-positive Areas

When fish are removed in areas where Bd is present, the goal is for existing Bd-positive populations to increase in abundance and/or for Bd-positive mountain yellow-legged frogs to recolonize from nearby occupied sites. The effectiveness of fish removal in recovering Bd-positive frog populations has been assessed in only a few locations, and much remains to be learned. Thus, relatively intensive monitoring of future fish removals in Bd-positive areas is important.

This Strategy proposes that VES be conducted three times per year starting in the year prior to initiation of fish removal, during fish removal (2-5 years if using gill nets/electrofishing), and for at least 4 years following complete fish eradication. Within a year, surveys conducted early, mid, and late season would ensure that population estimates include the various activity periods that might affect numbers of frogs present at a given time. Collection of Bd swabs for qPCR analysis is recommended to allow evaluation of population recovery in relation to Bd loads.

#### Effectiveness of Frog Translocation to Reestablish Populations

Because mountain yellow-legged frogs are extirpated from >90% of their historical range, translocation of frogs (most likely collected from persistent Bd-positive populations) and reintroduction of captive-reared frogs will likely play a role in recovering frog populations. Information on the effectiveness of translocation and reintroduction methods to reestablish frog populations is needed.

Because relatively small numbers of frogs are typically translocated, assessment of translocation outcomes will likely require capture-mark-recapture (CMR) methods. This Strategy proposes to mark adults with PIT tags and juveniles with toe clips, and conduct CMR for a minimum of 4 years after translocation and more years may be required. PIT tags have few negative effects on adult frogs, eliminate the ambiguity inherent in other marking methods, and are relatively affordable.

Simultaneous CMR work at source populations would provide important information on population size and response to frog removals. At large source populations, visual encounter surveys instead of CMR monitoring may be sufficient and more efficient; CMR is very time-intensive (and thus expensive) in large populations. This Strategy proposes that monitoring at source populations be initiated at least one year prior to the translocation and continue as long as monitoring is conducted at translocated populations. This Strategy proposes the collection of Bd swabs for qPCR analysis in source and translocated populations to evaluate the impact of Bd on translocation success.

#### Effects of Habitat Manipulation in Vicinity of Mountain Yellow-legged Frog Populations

Habitat manipulations (e.g., timber harvest, prescribed fire) occur in the vicinity of mountain yellow-legged frog populations, but their effects on the species are unknown. Because habitat manipulations will likely occur in areas with small populations and effects may be subtle, this monitoring will require capture-mark-recapture (CMR) methods. This Strategy proposes that this monitoring take place opportunistically, taking advantage of projects that are implemented in mountain yellow-legged frog habitats. This Strategy proposes to mark adults with PIT tags and juveniles with toe clips, and that CMR be initiated several years prior to the habitat manipulation and continue for at least 4 years post-manipulation.

#### Large-scale Monitoring to Describe Population Trends

Knowing the population trends of *R. muscosa* and *R. sierrae* across their ranges is crucial for evaluating the general success of the Strategy, evaluating whether levels of restoration efforts need adjustment, and understanding the overall status of the species. Monitoring at large scales is challenging and there are many trade-offs associated with available approaches and statistical designs. Monitoring can be costly and is a common item that is dropped when budgets are reduced. It is critical to address these information needs with sufficient statistical rigor so that the important questions are adequately answered. It also is critical that results are properly interpreted and applied.

This Strategy recognizes the critical importance of monitoring as well as the logistical constraints discussed above. It proposes developing minimum, intermediate, and comprehensive designs that differ in level of rigor and comprehensiveness. An alternative approach could be to divide monitoring objectives into separate components. A minimum core component would always be implemented, with additional components implemented when funding is available. Designing such an approach would require careful thought to ensure that all data collected are useful. Ideally, the large-scale monitoring would build upon previous efforts conducted by participating agencies (CDFW, USFS, NPS), all which have conducted large-scale inventories or monitoring using different designs.

#### Track Recently-extant Mountain Yellow-legged Frog Populations (1995-current)

The purpose of this monitoring is to track losses of populations present as of 1995, naturally- recovering populations, and to provide an updated inventory of currently extant populations for use in adjusting the CAP. At a minimum, this Strategy proposes to census, all currently extant (at the time of Strategy finalization) *R. muscosa* and *R. sierrae* populations known in the Sierra Nevada once every five years. 'Currently extant' is defined to be sites where frogs were found during the period 1995-2015. This minimal approach will allow assessment of population trends only in known populations; thus, the only range-wide occupancy trends possible to discern are stable or declining ones. It will not allow assessment of population trends in all habitats across the species' Sierran ranges. In addition, newly found populations cannot be used for assessment of trends, so addition of sites to the census would need to be done using a statistically valid method. A different design would be required to accomplish the latter objectives. Some of the project-scale monitoring may provide limited information on expansion into new habitats.

Resurveys of currently occupied sites would be a considerable commitment of resources, and would require the involvement of personnel from participating agencies (NPS, USFS, CDFW) and researchers. For example, nearly all of the recently occupied sites in Yosemite National Park (~350) were surveyed by four people during the summer of 2012.

In all surveys, this Strategy proposes that field crews conduct VES using a standardized protocol and ideally, with field computers (e.g., smartphones) that allow rapid upload of data into a relational database (see below). Collection of frog skin swabs for use in Bd assays also should be considered, but is not essential except in Bd-

negative areas or areas where frog populations have never been assessed for Bd presence (see above).

#### Detect Population expansion by Surveying Unoccupied Habitat

Ideally, large-scale monitoring would include the ability to detect population expansions (i.e., frogs colonizing new sites, a primary goal of this Strategy) and not just declines. Detecting expansion would require surveys of habitats that are not currently occupied. Because it is not feasible to survey all unoccupied habitats, some type of sampling design would be needed. Depending on the design, this could add a small or large increase in survey effort to the census described above. For example, sampling could be designed to measure expansion into sites close to existing occupied areas or to measure expansion over larger geographic areas (see below). Some of the project-scale monitoring may provide limited information on expansion into new habitats.

#### Population Trends Across the Sierra Nevada Range of R. sierrae and R. muscosa

The most comprehensive large-scale monitoring approach would evaluate population trends across the species' ranges in the Sierra Nevada. This would provide the ability to detect population trends in either direction (increases and decreases). In addition to surveying currently occupied habitats, this would require surveys in habitats that are not currently occupied, and sampling that is spatially distributed across the species' range.

There are multiple design options that increase efficiency and affordability of monitoring programs. Still, implementing a monitoring approach that accomplishes this objective within today's budget constraints would be challenging, and for such an approach to be successful, it would need to be sustained over sufficient time to determine whether the Strategy is achieving its population goals (i.e., for at least several mountain yellow-legged frog generations).

### **Research Needs**

Research needs address specific knowledge gaps that hinder our ability to develop effective conservation actions. Currently, information is lacking regarding: (1) the underlying genetic makeup of mountain yellow-legged frog populations; (2) precise distributions of respective clades and genetic distances; (3) population dynamics and long-term benefits of restoration actions in light of the widespread presence of chytridiomycosis; and (4) a firm understanding of the best methodologies for translocations and reintroductions. Better survey coverage and understanding of stream-based mountain yellow-legged frog population ecology is also needed.

#### Genetic Analyses

The current genetic structure of *R. muscosa* and *R. sierrae* in the Sierra Nevada is based on Vredenburg *et al.* (2007), a study based on a single mitochondrial marker. As such, additional genetic analyses based on nuclear markers to substantiate and better describe the five-clade boundaries in the Sierra Nevada are needed. Future research should use both mitochondrial and nuclear markers. Recent research conducted to describe landscape-scale genetic structure of *R. sierrae* in Yosemite National Park and *R. sierrae*/*R. muscosa* in Sequoia-Kings Canyon National Parks indicate that skin swabs collected for Bd assays contain sufficient frog DNA for these analyses (Poorten et al. 2017). DNA extracts are currently available from hundreds of populations in the Sierra Nevada, including many that are now extirpated, and are currently being used to describe the rangewide population genetics structure of both species.

#### Increasing the Survival of Frogs during Bd Outbreaks

Bd outbreaks in Bd-naïve frog populations typically cause population extirpation. However, recent field experiments indicate that treatment of frogs during Bd outbreaks with anti-fungal drugs (e.g., itraconazole) or perhaps probiotic bacteria (e.g., *Janthinobacterium lividum*) can increase the survival of adults and allow the development of persistent frog populations (see *Box 2b*). To date, treatment conducted on adults has resulted in increased survival, and treatment conducted on juveniles and tadpoles has not resulted in increased survival. To complement the large-scale treatment experiment conducted with adults in 2015, additional field experiments conducted during Bd-caused epizootics that utilize hundreds of frogs would answer key questions regarding the long- term survival of treated frogs and subsequent rates of recruitment and population growth. Such an experiment, in which hundreds of adult *R. sierrae* were treated in the field with itraconazole during a Bd outbreak in 2015, is currently underway. The population is being examined annually using VES, CMR, and Bd swabbing methods to monitor post-treatment effects.

#### Translocations and Reintroductions of Wild and Captive-bred Frogs

Both translocations and reintroductions are likely to play a critical role in the restoration of mountain yellowlegged frogs in the Sierra Nevada, but the success of these efforts remains uncertain, largely because of the presence of Bd, and many questions remain (see *Bax 3*). Because Bd is now widespread in the Sierra Nevada, most translocations will likely involve the transfer of frogs into Bd-positive sites. Studies of transfers of Bdpositive frogs from persisting populations are underway, and preliminary results suggest successful establishment may be possible in some situations. However, transfers of Bd-naïve frogs, either wild or captive-bred, will rarely, if ever, be successful at establishing new populations in Bd-positive habitats without some sort of active disease mitigation. In these situations, translocations would likely be considerably more effective if used in combination with treatments that reduce frog susceptibility to chytridiomycosis (see *Baxes* 2a-2b).

Because relatively little is known about the effectiveness of mountain yellow-legged frog translocations and reintroductions, including how to mitigate the effects of chytridiomycosis, all such activities should be conducted as carefully monitored experiments in which all frogs are PIT tagged and their survival is quantified using CMR methods. In addition, little is known about the effects of removing frogs from source populations. Thus, translocations need to be carefully planned to minimize risks associated with the removal of frogs from source populations, and typically will require that the size of source populations be quantified, both to determine how many frogs and what life stage can safely be removed, and to monitor the effects of frog removals.

#### Frog Ecology in Stream and Meadow Habitats

In many of the areas where mountain yellow-legged frogs are most at risk, the species' primarily inhabit streams and or stream/meadow complexes. Although the ecology of these species is relatively well studied in lake-dominated habitats, there is little information on their ecology in stream and meadow environments. This knowledge is crucial for developing successful restoration actions and identifying potential translocation and reintroduction sites in these regions. Questions related to stream/meadow populations include: 1) What interactions exist between frogs and nonnative fish and other exotic predators (e.g., crayfish)?; 2) What are frog habitat requirements for breeding, feeding, and overwintering?; 3) What are the factors allowing frog persistence in streams that partially dry during summer?; 4) To what extent and how successful are frogs at using beaver-created habitat?; and 5) What are frog movement patterns?

### Effects of Meadow Restoration

Several opportunities exist to learn how meadow restoration projects affect mountain yellow-legged frog populations. Population monitoring of frogs in these areas would provide invaluable information on how to

design and implement future restoration projects to maximize benefits to mountain yellow-legged frogs and minimize potential impacts.

# Coordination, Data Management, and Information Sharing

The effective implementation of this Strategy will require coordination by all involved parties (Federal and State agencies, university and other researchers). This Strategy proposes that a committee be formed to provide guidance and oversight of Strategy implementation. This implementation committee would meet at least twice annually to review progress to date and to prioritize and coordinate future work. For example, topics of a winter meeting could include reviewing accomplishments of the previous field season and those of a spring meeting could include prioritizing and coordinating future work. As needed, coordination calls for salvage and translocation events, captive breeding and rearing activities, or responses to catastrophic events (e.g., disease outbreak or wildfire) may be convened.

Implementation of the Strategy will occur within an adaptive management framework in which the CAP and Strategy are revised as needed based on newly acquired information. To effectively accomplish this goal, data collected during inventory, monitoring, and research must be quickly processed, analyzed, and made available to all involved parties. Given that data are likely to be collected and used by different federal and state agencies and researchers, the use of a standardized data collection and management system would facilitate rapid data processing and access. Although there are some hurdles to overcome, data collection onto handheld field computers (e.g., smartphones) and remote upload to a common server would aid in the standardization and efficiency of data collection, processing, and quality control.

Examples of decisions and hurdles to work out include:

- 1. What common data to collect in the various survey efforts,
- 2. What data to store in common and how to deal with the remainder,
- 3. Responsibilities and availability of technical expertise to develop one shared database system,
- 4. Responsibilities and availability of technical expertise to manage the shared database and the use of field computers, and
- 5. Where to house the server that would hold the data. Handheld field computers are currently used by several mountain yellow-legged frog inventory, monitoring, and research groups and could be expanded to include all involved entities. In addition, most of these groups have relatively comprehensive and sophisticated data management systems that could be integrated. This Strategy proposes that these data management topics be addressed by the team that convenes to discuss monitoring within a year of Strategy implementation.

### **Reporting and Interactive Collaboration**

Many of the restoration goals within this Strategy will require funding to implement. Implementing agencies are encouraged to collaborate to reduce the burden on any one party and ensure that objectives are being addressed collaboratively. This Strategy and its associated attachments are living documents, and the overall effort will need to adapt to funding constraints, natural events, and through further research, implementation of the Strategy, and monitoring of results. Every five years, the core technical team will write a status update report to the Steering Committee documenting the status and results of the various implemented actions.

# V. Conservation Action Plan

The CAP identifies and prioritizes specific conservation actions for *R. muscosa* and *R. sierrae*. It is based on available knowledge of the current distribution of the species and their habitat, known threats, available conservation approaches, and priority information gaps. The Plan is designed to achieve the population goals identified above, and includes: (1) strategic creation of fishless habitats, primarily by fish removal, (2) strategic research on translocation methods, the effects of Bd presence, and disease mitigation, (3) ongoing implementation of BMPs associated with routine management and land-use activities, and (4) other actions appropriate for specific circumstances. In some situations, further information needs are identified to evaluate available conservation options.

The Conservation Assessment recommends a three-scale approach that recognizes ecological, administrative, and logistical practicalities. The scale of the species' range in the Sierra Nevada is the broadest scale. Conservation actions at this level were identified and prioritized to represent genetic, geographic, and ecological diversity. To represent genetic diversity, conservation actions are identified and organized by clade. The intermediate scale includes the National Forest and National Park administrative units in the Sierra Nevada. Administrative units are the scale where funding and other resource allocation decisions are made. The smallest scale is the FCA, which is the unit for which actual site-specific management plans are developed.

For each of the five clades (three for R. *sierrae*, two for R. *muscosa*), FCAs were identified based on the presence of frogs and available habitat. Available habitats were identified using the habitat quality criteria outlined under the habitat goals (see above). FCAs were delineated to include occupied sites, important habitats, potential restoration sites, and sufficient connectivity. Watershed boundaries were followed when appropriate. The distribution of FCAs was examined at several scales (rangewide, clade, administrative unit) to evaluate adequate coverage. However, in practice, so few frog populations remain that most currently occupied areas were included. Some FCAs are currently unoccupied but were included as potential translocation sites.

For each FCA, information was compiled on current and historical frog occupancy, habitat quality, fish presence, spatial representation of the population relative to other occupied areas within the clade, other important considerations, and the status of previous, current, or proposed restoration actions (see Attachment 1). This information aided in the identification and prioritization of restoration opportunities, and provides guidance on conservation actions for each FCA.

Potential conservation actions were identified in each FCA (Attachments 1 and 2). The proposed actions do not always include active restoration (e.g., fish removal). For example, special protection may be accorded areas that currently have large intact populations and habitat complexes. In some areas, fish are expected to die off naturally with no intervention needed (passive fish removal), and the proposed action is to monitor these areas over time to confirm that fish are no longer present. Some areas are currently unoccupied by frogs and were identified as potential translocation sites. In some cases, further assessment and/or research to develop effective actions are identified.

The Plan was developed by prioritizing potential restoration actions using multiple criteria including status and uniqueness of the frog population, feasibility of the action (e.g., fish removal, translocation), recreational use, and other important considerations.

The prioritization is organized by administrative unit (i.e., National Park, National Forest) and is designed to provide guidance on options and priorities as resources to implement the Strategy become available while maintaining flexibility to allow each agency and administrative unit to choose appropriate projects within their jurisdictions. Thus, the intent of this Plan is to facilitate the implementation of the Strategy by allowing the agencies the ability to choose from a set of options within their jurisdictions, budgets, and other constraints.

The FCAs are considered an adaptive tool for identifying and managing high priority activities for mountain yellow-legged frog conservation and recovery. When appropriate (e.g., in response to new scientific information), new FCAs may be added and the geographical extent of existing FCAs modified. Under some circumstances, existing FCAs may be removed if new knowledge indicates that the area no longer suitable as an FCA. Attachments 1 and 2 of the Strategy will be updated annually, as needed, to reflect any such changes. The Plan is summarized below, organized by clade and then administrative unit. Priority actions and additional details are provided in Attachment 2. Attachment 2 is considered a living document that will be updated as needed during implementation committee meetings (see *Coordination, Data Management, and Information Sharing*) to document Strategy implementation and the identification of new proposed priority actions.

# A. Clade 1 (Rana sierrae)

Clade 1 is in the northwest portion of R. *sierrae*'s range in the Feather River drainage (Figure 4). The Clade 1 range encompasses portions of the Lassen and Plumas National Forests. Clade 1 individuals commonly inhabit streams, possibly because lakes are scarce. This region contains some of the lowest elevation R. *sierrae* populations currently known (e.g., approx. 5,400 ft in the Deane's Valley FCA), presumably a function of the more northern latitude. The predominantly forested environments in this clade provide relatively unique R. *sierrae* habitat when compares with many other areas the species occupies. R. *boylii* also occurs in this region, possibly in the same streams, and there is uncertainty about species identification in some locations.

Little is known about the ecology of the species in this region, including its historical distribution and abundance, where it breeds, and how it uses stream habitats. Currently, frogs are known from only a few locations and populations are extremely small. Non-native trout are present in most area streams and lakes and, generally, fish removal in many areas of Clade 1 may not be feasible with current methods. Compared with other parts of the species' range, R. *sierrae* populations in this clade overlap more with common land use activities such as vegetation and fuels management, and threats such as wildfire. Although the impacts of these activities and threats on the species are unknown, loss of any individuals could impact populations because of the very small sizes of extant frog populations.

As of the time of the Strategy's finalization, all Clade 1 FCAs (Figure 4) contain very small R. *sierrae* populations. There are few lake-based populations in Clade 1 (e.g., Gold Lake, Mount Pleasant, and Oliver Lake FCAs) with the remainder inhabiting streams. Restoration opportunities are limited in Clade 1 given current methods and the scarce, small populations.

### Lassen National Forest

At the time of the Strategy's finalization, only one FCA, Oliver Lake, was identified on the Lassen National Forest, and the population in this FCA may already be extirpated. Sierra Nevada yellow-legged frogs were most recently found by the U.S. Forest Service in 2005, and no frogs were found in surveys conducted in 2008 and 2013 by CDFW. Surveys of this area are needed to determine whether the species is still present. In addition, initial surveys of the High Lakes area to the north conducted in 2003 and 2013 found limited restoration opportunities. Additional data collection is necessary to more fully evaluate options. Captive breeding and/or rearing will likely be needed to restore populations to this Forest (see below under *Plumas National Forest*).



Figure 4. Sierra Nevada Yellow-legged Frog Conservation Areas: Clade 1.

# **Plumas National Forest**

Frogs occur in streams of all sizes in this region, ranging from small tributaries to larger creeks, many of which are difficult to survey effectively. The task of inventorying all streams (similar to what has been done for lakes) is logistically challenging and not likely the best use of limited resources. Rather, a strategic approach is warranted that identifies high priority survey locations. Survey goals are to find currently unknown frog populations, identify potential habitats for reintroduction, and evaluate fish removal options. As a first step, Spanish Creek in the Gold Lake FCA was identified as a priority location to survey for additional information on frog population status, habitat quality, and fish removal options.

Second, fish removal at Gold Lake in the Gold Lake FCA is nearly complete. Otherwise, there are limited opportunities for removing fish from *R. sierrae* habitats in Clade 1. There are relatively few lakes on the Plumas National Forest and *R. sierrae* more typically use stream habitats in this region, where fish removal is often much more difficult.

Third, the introduction of captive-bred frogs may be needed to restore populations in Clade 1. Captive breeding may be required because there are currently no potential donor populations in this clade large enough to sustain the removal of frogs. However, there are challenges to successfully implementing reintroductions, including: (1) development of successful captive breeding and rearing techniques, and (2) identification of appropriate locations for reintroductions. A greater understanding of how *R. sierrae* use stream environments will facilitate successful reintroductions. Information gaps include knowledge on breeding, rearing, adult, and overwintering habitat requirements in streams. It also will be necessary to identify appropriate fishless streams and those where fish can be successfully removed. Developing fish removal options for this clade is a relatively high priority because of the high risk of extirpation of frogs in this area.

# B. Clade 2 (Rana sierrae)

Clade 2 is geographically the most expansive, and its frogs use the broadest range of environments of any of the five identified clades (Figures 5-7). *R. sierrae* in this clade occur on portions of the Plumas, Tahoe, Eldorado, Lake Tahoe Basin Management Unit (LTBMU), Stanislaus, Inyo, and Humboldt-Toiyabe National Forests, and the northern half of Yosemite National Park. Environments in Clade 2 range from more forested landscapes in the northern part of the clade (where the frog occurs primarily in streams) to high elevation alpine landscapes (where the frog occurs primarily in lakes: Tahoe National Forest and south).

Clade 2 encompasses areas where the species is the most (northern Clade 2) and least (southern Clade 2) at risk. Other than Bd, the dominant threats in Clade 2 are introduced fish and the prevalence of small isolated populations. Only a few existing populations remain in the northern half of the clade, and abundances in these populations are extremely low. In the northern part of the clade, more overlap occurs with common land use activities such as vegetation and fuels management, and threats such as wildfire. Although the impacts of these activities on the species are unknown, because of the very small population sizes, loss of any individuals could affect population persistence. Conversely, Clade 2 also contains the Emigrant Wilderness and northern part of Yosemite National Park where a high concentration of small populations appear to be persisting despite the presence of Bd, and a few large populations still remain.

Clades 2 FCAs encompass most of the known occupied habitats (Figures 5-7). Some Clade 2 FCAs were identified in the region of overlap between Clades 2 and 3 (unknown lineage based on the mtDNA marker). Included are areas identified for potential translocations. The majority of the FCAs are focused on lakes, but stream-based FCAs were identified where conservation opportunities exist.

In the northern portion of this clade, similar to Clade 1, active conservation options are limited because the frogs occur predominantly in stream habitats where fish removal is difficult. In lake habitats, conservation

actions currently consist primarily of fish removal. Frog translocations are underway in this clade, and FCAs include areas identified for future reintroductions. In general, conservation actions have been identified and prioritized strategically to increase the amount and connectivity of available high quality fishless habitat, with the goal of increased occupancy.

# **Plumas National Forest**

Currently, Clade 2 frogs are known from the northern Plumas National Forest in streams surrounding Antelope Lake and in the south near the Tahoe National Forest border. There are only a few other known historical localities.

Further information is needed to determine conservation options in these areas. Thus, the immediate conservation actions proposed are to assess Lone Rock Creek (Antelope FCA) for restoration options. Because this area contains privately owned land inholdings, large-scale frog management and monitoring would require written permission from landowners.

# **Tahoe National Forest**

Historical R. *sierrae* localities are known from much of the Tahoe National Forest. FCAs on this Forest, which include most currently occupied areas, include two of four areas clustered at the boundary with the Plumas National Forest, several in the South Fork Yuba River watershed, two in the Truckee River drainage, and one to the south in the Granite Chief Wilderness.

Clade 2 populations on the Tahoe National Forest are primarily lake-based, though there are three notable stream populations at Rattlesnake Creek, an unnamed creek near Fordyce Lake, and Independence Creek. Most of the populations are very small. Perhaps the largest remaining R. *sierrae* population in the northern part of the species range is found in a wet meadow beaver complex near Independence Creek.

Conservation actions on this forest primarily include fish removal and collection of further information to evaluate additional options. A fish removal project is in progress in the French Lake FCA. Additional surveys are proposed in several areas with stream-based populations, and identifying other survey gaps is warranted. Rattlesnake Creek and Independence Creek provide potential study areas for investigating the ecology of stream-dwelling populations. A large meadow restoration project being implemented in Perazzo Meadow FCA may provide an opportunity to evaluate how frogs respond to this type of restoration. *R. sierrae* have been occasionally found in this meadow. Exploration of captive breeding and translocation is warranted because of vacant suitable habitat and very small sizes of existing populations.

# Eldorado National Forest and Lake Tahoe Basin Management Unit

Historical *R. sierrae* localities occurred throughout much of the higher elevations on the Eldorado National Forest, and also include some lower elevation (5,000-6,500 ft), primarily stream habitats. Frogs were historically present in multiple locations on the LTBMU, but now may be extirpated in the Basin. There is considerable high quality habitat in the alpine regions of these National Forests. FCAs on the Eldorado encompass a large portion of the currently occupied areas and are clustered in the Mokelumne Wilderness, Desolation Wilderness, and adjacent areas on the LTBMU. Existing populations are predominantly on the Eldorado National Forest and are primarily lake-based, though there are a few that inhabit streams. Most populations are small in comparison with the few known historical abundances (Bradford 1991, Pope 1999), but still have more frogs and more consistent reproduction than more northern populations in this clade. Three of the FCAs, Desolation Valley, Highland Lake, and Jeff Davis Creek, have large populations.



Figure 5. Sierra Nevada Yellow-legged Frog Conservation Areas: Northern Clade 2.



Figure 6. Sierra Nevada Yellow-legged Frog Conservation Areas: Middle Clade 2.



Figure 7. Sierra Nevada Yellow-legged Frog Conservation Areas: Southern Clade 2.

Conservation actions on these forests primarily include fish removal and frog translocation. Multiple restoration projects on these forests have been completed or are currently occurring, including active and passive removal of fish from lakes in the Highland Lake, , Gertrude Lake, Island Lake, Clyde Lake, Desolation Valley, Ladeux Meadow Creek, Jeff Davis Creek, Lucille Lake, and Cagwin Lake FCAs. Potential reintroduction sites occur in other areas where fish are not currently present including the Deadwood Canyon, most of Devil's Hole Lake, and Mokelumne Peak FCAs. Fish removal has been identified as a goal for additional sites within the Eldorado FCAs, but is not feasible given the limitations of current methods. Translocation studies are underway for the Lucille Lake and Cagwin Lake FCAs on the LTBMU using frogs from nearby Desolation Valley and vicinity (Eldorado National Forest). Monitoring the source populations in Desolation Valley and vicinity. In the Mokelumne Wilderness, additional surveys are needed to determine frog and fish status, restoration options, and to develop a translocation plan for the area. A meadow restoration project was implemented in Indian Valley in the Jeff Davis Creek FCA, and monitoring the *R. sierrae* population in this FCA provides an opportunity to learn how the species responds to meadow restoration.

### **Stanislaus National Forest**

Historical localities are known from the northern part of the Stanislaus National Forest in the headwaters of the North Fork Mokelumne River and North Fork Stanislaus River, and in the southern part of the forest in and near the Emigrant Wilderness. Frogs are still present in two areas in the north, near Wheeler Lake and Stanislaus Meadow. In the southern portion of the forest, the Emigrant Wilderness appears to have a relatively high concentration of R. sierrae with frogs still scattered across much of the wilderness. Multiple small populations appear to be persisting, and a few large populations (likely reflecting historical abundances) still exist (Bradford 1991, Pope 1999, Vredenburg et al. 2010). The Emigrant Wilderness contains a large quantity of diverse habitats that include complexes of lakes of different sizes and depths, smaller ponds, wet meadows, and streams. In this region, there are opportunities to implement restoration projects with the potential for a large benefit to the species. There are opportunities to increase the amount and connectivity of high quality habitat by strategic fish removal. Because frogs are still present in multiple locations, dispersing frogs may recolonize unoccupied habitats or augment nearby smaller populations. Although Bd often may prevent the re-establishment of large populations (even in high quality habitats), increasing the number and connectivity of occupied areas should increase the redundancy and resiliency of R. sierrae populations in this region and the likelihood of overall persistence of the species as a whole (see *Population Goals*). FCAs encompass the two northern populations and most of currently occupied habitats in the Emigrant Wilderness.

The two populations within the northern portion of the Stanislaus National Forest are stream-based and are small. No active restoration has occurred to date, but passive fish removal has occurred in Wheeler Lake. At Stanislaus Meadow FCA, identification of possible future restoration activities is warranted. Stanislaus Meadow also is a potential study site to investigate the ecology of *R. sierrae* in streams and meadows.

Conservation actions on the Emigrant Wilderness focus on fish removal. Because fish will likely die out in many lakes in the absence of stocking, passive fish removal is the first and most efficient approach. This Conservation Strategy proposes to monitor the success of passive fish removal within the first five years of Strategy implementation. At that point, further planning and assessment will occur to determine appropriate next steps. A few lakes in the Emigrant Wilderness have been targeted for active fish removal, and require additional assessment. Two high priority sites include Snow Lake in the Summit Meadow FCA and Starvation Lake in the Spring Creek FCA.

### Humboldt - Toiyabe National Forest

Historical R. *sierrae* sightings on the Humboldt-Toiyabe occurred just north of Lake Tahoe and south along the eastside of the Sierra Nevada from the Mokelumne Wilderness to just west of Twin Lakes at the border

with Yosemite National Park. Most populations in the Humboldt-Toiyabe are currently very small. There is only one large population, which is found in Rainbow Meadow FCA. Populations are primarily lake-based though *R. sierrae* also use meadow habitat in some areas (e.g., Rainbow Meadow).

Conservation actions on the Humboldt-Toiyabe focus on fish removal and translocation. Some restoration has been implemented in the Sisters Lakes and Rainbow Meadow FCAs where fish have been removed from several lakes. In the Sisters Lakes FCA, fish removal from Stella Lake would benefit the species. Passive fish removal is planned for Tamarack Lake within the Jeff Davis Creek FCA. Finally, additional work is needed to determine other translocation options including identifying potential translocation sites, their fish removal potential, and possible source populations.

# **Yosemite National Park**

In Yosemite National Park, Clade 2 extends from the Tuolumne River watershed (in the northern half of the park) to just north of the Merced River to the west and south to the Ritter Range on the Inyo National Forest in the east. Historically, R. *sierrae* occurrences were scattered throughout the Clade 2 portion of Yosemite National Park. Currently, the highest concentration of remaining populations is in the northern part of the Tuolumne drainage where frogs are present in multiple locations. Frogs are now largely absent from the central part of the park where there is less lentic habitat (southern part of Clade 2). Only a few large R. *sierrae* populations remain scattered throughout the Clade 2 portion of the park. FCAs encompass most of the currently occupied habitats, and several unoccupied areas were identified as potential translocation sites. In the northern FCAs in the Clade 2 portion of Yosemite National Park, frogs still occupy the majority of available lentic habitats, but at low abundances. More typically, in other areas of the Clade 2 region of the Park, frogs inhabit only a portion of the available habitat. Relative to the other parts of the species' range, a large number of the FCAs are currently fishless, and in general, Yosemite National Park contains large amounts of high quality habitat.

Yosemite National Park has identified general goals for the various FCAs. However, implementation of goals for some FCAs may not yet be possible given current methods and the scarcity of donor populations for translocations. Conservation actions focus on fish removal and translocation, and several projects have already been implemented. Fish have been removed from several lakes (e.g., Ardeth Lake, Bartlett Creek, and Mattie Lake FCAs). Translocation projects have been conducted in several areas, including the Dog Lake, Hook Lake, and Spiller Lake FCAs. Top priority projects are removing fish from Roosevelt Lake, Dog Lake, and Budd Lake, continuing translocation of frogs to Lower Skelton Lake (Dog Lake FCA) and Miller Lake (Hook Lake FCA), and translocating frogs to Tiny McCabe Lake (McCabe Lakes FCA).

# **Inyo National Forest**

Most of the Inyo National Forest is discussed in the Clade 3 section. However, the Hall FCA, just east of Yosemite National Park, was identified as a potential area for fish removal and translocations. This area contains high quality lentic habitat for *R. sierrae* and numerous historical localities that are no longer occupied.

# C. Clade 3 (Rana sierrae)

Clade 3 extends from the Merced River watershed in Yosemite National Park through the Sierra National Forest and northern Kings Canyon National Park to Monarch Divide east to Mather Pass in the Middle Fork of the Kings River. This clade includes portions of Yosemite and Kings Canyon National Parks, and the Sierra, Inyo, and Sequoia National Forests. In general, Clade 3 habitats primarily include high elevation, alpine regions comprised mainly of lakes, although there are several *R. sierrae* populations that inhabit streams and streams within meadows on the west side of the Sierra National Forest. As with other parts of the species' range, Bd, introduced fish and the prevalence of small isolated populations are the dominant threats in Clade 3. In some regions such as the west-side Sierra National Forest, more overlap occurs with common land use

activities such as livestock grazing and vegetation and fuels management.

Clade 3 FCAs encompass most of the known occupied habitats and include additional areas identified for potential translocations (Figures 8-9). Some FCAs were identified in the region of overlap between Clades 2 and 3 (unknown lineage based on the mtDNA marker) (Figure 2). Most populations in this clade are centered around lakes but there are some stream-based populations. Multiple successful restoration projects have been implemented in Clade 3 in Yosemite National Park, Inyo National Forest, and Sequoia and Kings Canyon National Parks, and include fish removal and translocations. Further assessment and/or research to develop effective conservation actions are identified in some cases.

# Yosemite National Park

The northern boundary of Clade 3 is the divide between the Tuolumne and Merced River drainages in Yosemite National Park. Historically, R. *sierrae* occurrences were scattered across much of the Clade 3 portion of the park, but only a few occupied areas currently remain, mostly in the of the headwaters of the Merced River and its tributaries. R. *sierrae* are completely extirpated from the South Fork Merced River. Only a few large populations remain across the Yosemite National Park portion of Clade 3. FCAs encompass most of the currently occupied habitats. The majority of the current populations are lake-based, though there are a few stream and meadow populations near the Glacier Point Road (Summit, McGurk Meadow FCAs) and in West Horse Ridge FCA to the south. Similarly, FCAs identified for restoration focus primarily on lakes. As with Clade 2, fish removal and translocation options were evaluated for the various FCAs. About half of the FCA's identified are currently fishless. Compared with the Clade 2 portion of the park, few restoration actions have been implemented. Translocations of frogs from the Hutchings Creek FCA into lakes in the South Lyell FCA have been completed. Reestablishing *R. sierrae* populations in additional previously occupied stream/meadow habitats is a priority but cannot be accomplished without the use of piscicides to remove fish from streams.

### Sierra National Forest

R. sierrae historical localities were known from the central portion of the Sierra National Forest around Shaver Lake, Huntington Lake, and the Kaiser Pass area, and were scattered throughout the John Muir Wilderness. Currently in these areas, only small isolated populations remain. In addition, small populations have recently been found in several locations in the northwestern portion of the forest (e.g., Hoggem, Soquel, Willow FCAs). FCAs on the Sierra National Forest encompass most known occupied areas as well as unoccupied areas with high quality habitat suitable for translocations. In contrast with the high elevation alpine John Muir Wilderness, the western portion of the Sierra National Forest outside the Wilderness has not been extensively surveyed and evaluated for frogs, fish, and restoration options. Compared with other R. sierrae populations in the surrounding region, the Sierra National Forest is somewhat unusual in that a relatively large proportion of known populations inhabit stream/meadow complexes, particularly on the west side of the forest. Several of these populations have only recently been discovered, and because this area contains privately owned land inholdings, large-scale frog management and monitoring would require written permission from landowners. These areas are subject to a variety of common land management activities such as livestock grazing, timber management, OHV use, developed campgrounds, and roads. Most of the historical records and known large populations were in the John Muir Wilderness, where frogs primarily inhabit lakes and there is a large amount of high quality lake habitat. There are only a few large populations remaining on the Sierra National Forest, and frogs are either greatly reduced in number or are extinct in locations that used to have hundreds of frogs (e.g., Humphreys Basin).

Because information is lacking for substantial portions of the Sierra National Forest (e.g., lower elevation areas), several of the initial conservation actions proposed are to gather more information. These include identifying and surveying areas of the forest that have not been comprehensively surveyed by the USFS or by CDFW to assess frog and fish presence and restoration options. Specific known areas requiring additional

information include the Nelson, Hopkins, and Lakecamp FCAs. Conserving R. *sierrae* in the greater San Joaquin watershed is an important spatial goal, and several conservation actions have been identified including fish removal in the Golden Lake FCA, and further assessment of options in the Hopkins FCA specifically and the Mono Creek watershed more generally. Watershed improvement projects may be warranted, particularly in stream/meadow complexes inhabited by frogs. Much of the pioneering restoration research occurred in Humphreys Basin including extensive surveys, fish removal, and translocations. This area historically contained large populations (Brown *et al.* 2014) and fish removal experiments in the late 1990's resulted in the expansion of populations to include many lakes with large frog abundances. Many of these populations were extirpated by Bd outbreaks in 2010-2011. As a result, there is now a large amount of high quality fishless habitat suitable for translocation. Translocations would ideally be conducted using frogs from nearby populations that are persisting with Bd, but no such populations exist in the vicinity.

### **Inyo National Forest**

On the Inyo National Forest, historical R. *sierrae* localities occurred throughout the east side of the Sierra Nevada south to Lone Pine Creek. Records are scarce between Lone Pine Creek and Big Pine Creek, possibly because of the steeper topography with fewer lakes, meadows, and marshes. R. *sierrae* is now found in only a few remaining locations. Because of fish removal and translocation activities, several large populations occur on the northern portion of the Inyo National Forest. Bd has recently infected east side populations near Bishop and has wiped out at least two formerly large populations (Coyote Flat FCA, Big Pine FCA). Populations on this forest are predominantly lake based, although one of the largest known stream-based populations was in the Coyote Flat FCA. FCAs on the Inyo National Forest include most currently occupied areas, several formerly occupied areas suitable for translocations, and potential high quality habitats identified for fish removal and translocations.

Multiple successful fish removal and/or translocation projects have resulted in large population responses including in the Gable Lakes, Big Pine, Treasure Lakes, and Independence Creek FCAs. Other FCAs where fish removal and/or translocations have been implemented or are ongoing include Thousand Island Lake, Horton Lakes, Shadow Creek, Witcher Hole, Rock Creek, and Wonder Lakes. This Strategy proposes to complete ongoing projects on the Inyo National Forest and fish removal and/or translocation actions identified for three additional FCAs (Treasure Lakes, Clark Lake). Finally, given the recent invasion of Bd in the southern portion of the forest, continued monitoring of large populations and restoration areas is warranted for potentially implementing intervention measures.

# Sequoia and Kings Canyon National Parks

The southern extent of Clade 3 within Kings Canyon National Park encompasses the headwaters of the South Fork of the San Joaquin River and the Middle Fork of the Kings River. The boundary between Clade 3 (*R. sierrae*) and Clade 4 (*R. muscosa*) is roughly from the crest of the Monarch Divide northeast to Mather Pass. Historically, *R. sierrae* occurrences were scattered in much of the Clade 3 portion of the park. Several occupied areas currently remain in fish-free areas. Most of the extant populations are infected by Bd and have relatively low abundance.

FCAs encompass nearly all of the currently occupied habitats, several formerly occupied areas suitable for translocations, and potential high quality habitats identified for fish removal. All of the populations are lakebased, therefore, FCAs identified for restoration focus primarily on lakes. About half of the FCA's identified are currently fishless. Several restoration actions have been implemented. Fish were removed for upper LeConte Canyon and Amphitheater. The LeConte population expanded substantially and, prior to a Bd outbreak in 2015, was thought to be the largest population in the range of *R. sierrae*. Second, a translocation suspected as a contributing cause. Third, disease treatments were conducted in the Barrett, Dusy and LeConte Canyon FCAs, which may have contributed to persistence of the Barrett and LeConte Canyon populations.



Figure 8. Sierra Nevada Yellow-legged Frog Conservation Areas: Northern Clade 3.



Figure 9. Sierra Nevada Yellow-legged Frog Conservation Areas: Southern Clade 3.

The priority actions for Clade 3 in Kings Canyon National Park are: 1) undertake fish removals in the Upper Evolution, McGee, Dusy, Barrett, Horseshoe, Amphitheater, Rambaud, Swamp, and Slide FCAs; 2) translocations into existing or newly created fishless habitat if viable source populations exist; 3) antifungal treatment and/or bioaugmentation of frogs in Bd-negative areas if interventions can be timed with the onset of Bd arrival and frog die-offs; and 4) antifungal treatment and/or bioaugmentation of frogs methods shown to increase survival.

# D. Clade 4 (Rana muscosa)

Clade 4 encompasses portions of Sequoia and Kings Canyon National Parks and Sequoia National Forest (Figure 10). Within Sequoia and Kings Canyon National Parks, Clade 4 extends from the Monarch Divide northeast to Mather Pass and south through Kings Canyon National Park to Sequoia National Park, with a rough terminus at the southern ridges of the Kern-Kaweah River and Whitney Creek, which are tributaries of the upper Kern River. Clade 4 overlaps with Clade 5 in the south between the Kings-Kern and Great Western Divides, and the southern ridges of the Kern-Kaweah River and Whitney Creek. Within Sequoia National Forest, Clade 4 extends south from the Middle Fork Kings River canyon to the North Fork of the Kaweah River canyon. Within Sequoia and Kings Canyon National Parks, Clade 4 habitats primarily include high elevation, alpine regions comprised mainly of lakes, although there are a few R. *muscosa* populations that inhabit meadows.

Historically, R. *muscosa* occurrences in Clade 4 were scattered throughout much of the habitat in the parks; at present, several occupied areas remain fish-free. Most of the extant populations are infected by Bd and have relatively low abundance. A few populations are currently uninfected and have abundance ranging from relatively high (Upper Bubbs FCA) to moderate (Kern Point FCA). Within Sequoia National Forest, Clade 4 habitats primarily include meadows and streams. Historically, *R. muscosa* occurrences were scattered in some Clade 4 habitats in Sequoia National Forest; at present, the species is thought to be extirpated from this area. As with *R. sierrae*, in addition to Bd, introduced fish and the prevalence of small isolated populations are the dominant threats in Clade 4. On National Forest lands in Clade 4, overlap occurs with common land use activities such as livestock grazing and vegetation and fuels management.

Clade 4 FCAs encompass nearly all of the known occupied habitats, several formerly occupied areas suitable for translocations, and potential high quality habitats identified for fish removal (Figure 10). Of these, several FCAs were identified in the region of overlap between Clades 4 and 5 (unknown lineage based on the mtDNA marker) (Figure 3). For example, the Tablelands and Crescent FCAs are located in the Kaweah River watershed, for which no frog genetic samples were available.

Most populations in this clade are centered around lakes but there are some stream- and/or meadow-based populations. Several restoration actions have been implemented. First, fish removal was conducted in the Upper Basin, Pinchot, Sixty Lake, Upper Bubbs, and Kern Point FCAs. The Sixty Lake, Upper Bubbs, and Kern Point populations expanded substantially, and the population in Upper Bubbs is currently thought to be the largest population in the range of *R. muscosa*. Second, several translocations were conducted, including to lakes in the Upper Basin, Pinchot, Sixty Lake, Vidette, Upper Bubbs, Milestone, and Tyndall Creek FCAs, and to lakes in Gardiner Basin and near Mount Ruskin. The frogs translocated in 2004 to Vidette and Gardiner are barely extant or may have died out. The frogs translocated to Upper Basin in 2013 appear to have died out. However, frogs in Pinchot and near Mount Ruskin have thus far survived. Frogs in the more recent translocations in Upper Bubbs, Milestone, and Tyndall Creek FCAs appear to be persisting, but more time will be needed to determine the status of these populations. A 2016 translocation in Sixty Lake FCA may have been unsuccessful but more surveys are needed. Third, disease treatments were conducted in the Sixty Lake FCA that may have contributed to current population persistence.



Figure 10. Northern DPS of Mountain Yellow-legged Frog Conservation Areas: Clade 4.

The priority actions for Clade 4 are: 1) undertake fish removals in the Sixty Lake, Vidette, Brewer, Upper Bubbs, Upper Kern, Milestone, East Wright, Tablelands, and Crescent FCAs; 2) translocations into existing or newly created fishless habitat if viable source populations exist; 3) captive-rearing of early *R. muscosa* lifestages in Bd-positive areas to facilitate population recruitment; 3) antifungal treatment and/or bioaugmentation of frogs in Bd-negative areas if intervention can be timed with the onset of Bd arrival and frog die-offs; and 4) antifungal treatment and/or bioaugmentation of frogs in Bd-positive areas if current research develops methods shown to increase survival. Further assessment and/or research to develop effective conservation actions are identified in some cases.

# E. Clade 5 (Rana muscosa)

Clade 5 includes the Kern River watershed from its headwaters south of Clade 4 to Taylor and Dunlap Meadows (Figure 11). Clade 5 overlaps with Clade 4 in the north between the Kings-Kern and Great Western Divides and the southern ridges of the Kern-Kaweah River and Whitney Creek. R. *muscosa* populations in the overlap zone and are described in the Clade 4 summary, above. Clade 5 includes the southern part of Sequoia National Park, and the southern portions of the Inyo and Sequoia National Forests. Habitats in the northern regions of Clade 5 in the park are typically higher elevation alpine lakes and some meadows. Fewer lakes occur outside the park and in these areas; historical populations were generally associated with stream/meadow complexes. In general, moving south into Sequoia National Forest, habitats become lower elevation, drier than other parts of the species' Sierran range, and with steep terrain and steep canyon streams.

Historically, R. *muscosa* occurrences were scattered throughout Clade 5; at present, only four occupied areas are thought to remain, all in fish-free areas, including two in Sequoia National Park, one in Inyo National Forest, and one in Sequoia National Forest. All of the extant populations are Bd-positive and have low abundance. Threats in Clade 5 include introduced fish, Bd, and the prevalence of small isolated populations. On the National Forest lands, the existing populations are inside the Golden Trout Wilderness where there is overlap with common land use activities such as livestock grazing. Historical populations outside the wilderness also would have been potentially exposed to other common management activities such as vegetation management.

Clade 5 FCAs include the known occupied habitats, a few formerly occupied areas suitable for translocations, and a few potential high quality habitats identified for fish removal. Several FCAs were identified in the region of overlap between Clades 4 and 5 (unknown lineage based on the mtDNA marker) (Figure 3).

Known populations in this clade are lake and stream/meadow-based. The only frog restoration action currently implemented in Clade 5 is a captive-rearing and reintroduction project in which *R. muscosa* tadpoles were collected from Mulkey Meadow FCA. These individuals are being reared in captivity, with the intent to translocate them to Rocky Basin Lakes FCA. Restoration options for all of Clade 5 are limited due to presence of so few frog populations, and additional captive breeding and/or rearing may be warranted. The priority actions for Clade 5 include several restoration actions in the park and further inventory needed to develop effective conservation actions in the National Forests.

# Sequoia and Kings Canyon National Parks

The northern extent of Clade 5 is within Sequoia National Park from the headwaters of the Kern River watershed south to the park boundary. Historically, R. *muscosa* occurrences were scattered within the Clade 5 habitat in the park. The two known occupied areas are Funston Creek and west of Crytes Basin. These extant populations are in fish-free areas, are infected by Bd, and have low abundance. Threats to Clade 5 habitat in the park include introduced fish, Bd, and the prevalence of small isolated populations. FCAs encompass the two currently occupied habitats (Funston Creek and west of Crytes Basin), one formerly occupied fishless

area suitable for translocations (Hockett FCA), and three potential high quality habitat areas identified for fish removal (Laurel, Crytes, and Blossom FCAs). Note that the Hockett and Blossom FCAs are located in the Kaweah River watershed, for which no frog genetic samples were available. It is currently unknown whether these historical populations genetically grouped with Clade 4, Clade 5, or a unique clade that went extinct. For this Strategy, the Hockett and Blossom FCAs were placed in Clade 5. FCAs identified for restoration focus primarily on lakes (and connecting streams). Half of the FCA's identified are currently fishless. No frog restoration actions have yet been implemented. The priority actions for Clade 5 in Sequoia National Park are: 1) undertake fish removal in Laurel, Crytes, and Blossom FCAs; 2) translocations into existing or newly created fishless habitat if viable source populations exist; and 3) antifungal treatment and/or bioaugmentation of frogs if current research develops methods shown to increase survival.

# **Inyo National Forest**

On the Inyo National Forest, *R. muscosa* historical localities occurred in multiple locations within the Golden Trout Wilderness. Many of these populations occupied meadow/stream habitats, but large populations also were known from the few lakes that occur in this area (e.g., Rocky Basin Lakes). The species is now only found in small numbers in Mulkey Meadows, a large meadow complex. Because there are few lakes in this region, the species primarily has been associated with stream/meadow complexes. FCAs identified for the Inyo National Forest include Mulkey Meadow to encompass the currently occupied habitat, and Rocky Basin Lakes, which includes fishless lakes into which *R. muscosa* will be translocated. Additional surveys for frogs and restoration options are needed for the Clade 5 portion of the Inyo National Forest.

# Sequoia National Forest

There are a few known historical *R. muscosa* localities scattered in the Sequoia National Forest. Similar to Clade 1, there are few lakes in this region, and many of the known localities are from stream/meadow complexes. Also similar to Clade 1, this area has not been extensively surveyed when compared with other parts of the species (and *R. sierrae*'s) range and frogs are currently known to occur in only one location (Bullfrog Lakes). No frog restoration actions have yet been implemented in Clade 5. However, a self-sustaining population of introduced brook trout was eradicated using piscicides (antimycin A) from Bullfrog Lakes in 1975 to create habitat for threatened Little Kern golden trout. Although subsequently introduced, Little Kern golden trout have remained undetected or died out, and thus Bullfrog Lakes currently provide habitat for a small population of *R. muscosa*. Bullfrog Lakes has a Bd-positive population with low abundance. This population has potential to serve as a source population for future translocations. Given the paucity of available information on *R. muscosa* from this region, the priority conservation actions focus on collecting additional information options). First priority areas include historically occupied sites, fish-free lakes, and then other fish-free areas.



*Figure 11.* Northern DPS of Mountain Yellow-legged Frog Conservation Areas: Southern Clade 5.

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