mountain yellow-legged frog
(*Rana muscosa*)

Southern California Distinct Population Segment

5-year Review:
Summary and Evaluation

Mountain yellow-legged frog (*Rana muscosa*) and habitat. Photocredit: Adam Backlin (USGS).

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U.S. Fish and Wildlife Service
Carlsbad Fish and Wildlife Office
Carlsbad, California

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5-YEAR REVIEW
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I. GENERAL INFORMATION

Purpose of 5-year Reviews:

The U.S. Fish and Wildlife Service (Service) is required by section 4(c)(2) of the Endangered Species Act (Act) to conduct a status review of each listed species at least once every 5 years. The purpose of a 5-year review is to evaluate whether or not the species' status has changed since it was listed (or since the most recent 5-year review). Based on the 5-year review, we recommend whether the species should be removed from the list of endangered and threatened species, be changed in status from endangered to threatened, or be changed in status from threatened to endangered. Our original listing of a species as endangered or threatened is based on the existence of threats attributable to one or more of the five threat factors described in section 4(a)(1) of the Act, and we must consider these same five factors in any subsequent consideration of reclassification or delisting of a species. In the 5-year review, we consider the best available scientific and commercial data on the species, and focus on new information available since the species was listed or last reviewed. If we recommend a change in listing status based on the results of the 5-year review, we must propose to do so through a separate rule-making process defined in the Act that includes public review and comment.

Species Overview:

As summarized in the final listing rule, mountain yellow-legged frog (*Rana muscosa*), is a medium-sized amphibian in the family Ranidae (true frogs). The listed entity is a Distinct Population Segment (DPS) of the *R. muscosa* species and is currently limited to nine populations in the San Gabriel, San Bernardino, and San Jacinto Mountains in southern California. The southern California DPS was listed as Endangered due to substantial population declines thought to be caused primarily by nonnative trout predation and habitat impacts associated with recreation. *Rana muscosa* also exists in the southern Sierra Nevada in isolation from the listed entity. This species is a member of the mountain yellow-legged frog complex, which is comprised of two species: *R. muscosa* and *R. sierrae*. Populations of mountain yellow-legged in southern California occupied a wide elevational range historically (370 meters (m) to 2,290 m (1,200 feet (ft) to 7,500 ft)) with use of rocky, shaded streams and cool waters originating from springs and snowmelt. Hibernation occurs under water or in streambank crevices during the coldest winter months. Individuals emerge from hibernation in the spring and begin breeding shortly after. All known extant populations of the listed entity occur within the San Bernardino and Angeles National Forests.

The southern California DPS of *Rana muscosa* was listed as Endangered under the Act in 2002, and *R. muscosa* is not listed by the State of California.

Methodology Used to Complete This Review:

This review was prepared by Susan North, Fish and Wildlife Biologist at the Carlsbad Fish and Wildlife Office, following the Region 8 guidance issued in March 2008. To update the status and threats to mountain yellow-legged frog, information was gathered from the following sources: the final listing rule; the final critical habitat rule; peer-reviewed and published scientific studies; survey information and knowledge from species experts who monitor the DPS and captive populations (i.e., Adam Backlin, Elizabeth Gallegos, and Robert Fisher, United States Geological Survey (USGS); Leslie Welch, Nathan Sill, Anne Poopatanapong, and Kathie Meyer, United States Forest Service (USFS); Mike Giusti, Jeff Brandt, Tim Hovey, John O'Brien, Curtis Milliron, and Mitch Lockhart, California Department of Fish and Game (CDFG); Frank Santana and Jeff Lemm, San Diego Zoo Institute for Conservation Research (SD Zoo ICR); Ian Recchio, Los Angeles Zoo (LA Zoo); Andy Snider, Fresno-Chaffee Zoo (Fresno Zoo); and Becca Fenwick (University of California, James San Jacinto Mountains Reserve)); and other information in our files. We received no information from the public in response to our Federal Notice initiating this 5-year review. This 5-year review contains updated information on the species' biology and threats, and an assessment of that information compared to that known at the time of listing. We focus on current threats to the species identified under the Act's five listing factors. The review synthesizes all this information to evaluate the listing status of the species and provide an indication of its progress towards recovery. Finally, based on this synthesis and the threats identified in the five-factor analysis, we recommend a prioritized list of conservation actions to be completed or initiated within the next 5 years.

Contact Information:

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Federal Register (FR) Notice Citation Announcing Initiation of This Review:

A notice announcing initiation of the 5-year review of this taxon and the opening of a 60-day period to receive information from the public was published in the **Federal Register** on May 21, 2010 (USFWS 2010, p. 28636). No information relevant to the taxon reviewed here was received in response to the **Federal Register** notice.

Listing History:

Federal Listing
FR Notice: 67 FR 44382
Date of Final Listing Rule: July 2, 2002

Entity Listed: Frog, mountain yellow-legged (southern California DPS) (*Rana muscosa*), an amphibian DPS
Classification: Endangered

State Listing
The California Fish and Game Commission recently concluded that a petition to list *Rana muscosa* as an endangered species is warranted under CESA (CFCG 2012, p. 1).

Associated Rulemakings:

Proposed Critical Habitat
FR Notice: 70 FR 54106
Date of Proposed Critical Habitat Rule: September 13, 2005

Final Critical Habitat
FR Notice: 71 FR 54344
Date of Final Critical Habitat Rule: September 14, 2006

Review History:

No previous 5-year reviews have been completed for the southern California DPS of mountain yellow-legged frog.

Species' Recovery Priority Number at Start of 5-year Review:

The recovery priority number for the listed entity is 3 according to the Service's 2011 Recovery Data Call for the Carlsbad Fish and Wildlife Office, based on a 1–18 ranking system where 1 is the highest-ranked recovery priority and 18 is the lowest (USFWS 1983a, pp. 43098–43105; USFWS 1983b, p. 51985). This number indicates that the taxon is a DPS that faces a high degree of threat and has a high potential for recovery.

Recovery Plan or Outline:

There is no approved Recovery Plan for the southern California DPS of mountain yellow-legged frog.

II. REVIEW ANALYSIS

Application of the 1996 Distinct Population Segment (DPS) Policy:

The Act defines "species" as including any subspecies of fish or wildlife or plants, and any DPS of any species of vertebrate wildlife. This definition of species under the Act limits listing as a DPS to species of vertebrate fish or wildlife. The 1996 Policy Regarding the Recognition of Distinct Vertebrate Population Segments under the Endangered Species Act (USFWS 1996, p. 4722) clarifies the interpretation of the phrase "distinct population segment" for the purposes of listing, delisting, and reclassifying species under the Act.

The Service listed mountain yellow-legged frog (*Rana muscosa*) in southern California as a DPS in 2002 (USFWS 2002, p. 44382). This determination was based on the geographic isolation of this population in relation to the remainder of the species to which it belongs, the significance of the population segment to the species to which it belongs, and the population's conservation status in relation to the Act's standards for listing (USFWS 2002, p. 44384). For additional information regarding this determination please refer to the discussion in the final listing rule (USFWS 2002, p. 44384).

Information on the Species and its Status:

Much of what was known about the biology of mountain yellow-legged frog (*Rana muscosa*) at the time of listing was based on research focused in the Sierra Nevada. As described below, mountain yellow-legged frogs are now represented by two species (*Rana muscosa* and *Rana sierrae*) comprising the mountain yellow-legged frog complex, of which both species are the only members. Therefore, a significant amount of research that took place prior to the taxonomic change in 2007 (Vredenburg *et al.* 2007, p. 361) now applies to a different species. However, because both species remain in the mountain yellow-legged frog complex, we continue to consider much of this research applicable to the following discussion, although species-specific details are noted where possible. For the purposes of this review, the listed entity will hereafter be referred to as southern *R. muscosa*. Any reference to the populations of *R. muscosa* that persist in isolation from the listed entity in the Sierra Nevada will be of northern *R. muscosa*. Any reference to the newly described species, which occupies the northern and central Sierra Nevada, will be of *R. sierrae*. Details related to the biology of all mountain yellow-legged frogs will be discussed as such.

Changes in Taxonomic Classification

Prior to listing, Macey *et al.* (2001, pp. 131–143) performed a phylogenetic analysis of mitochondrial DNA sequences on mountain yellow-legged frogs through the range and found statistical support for four evolutionarily distinct lineages from the northern Sierra Nevada, central Sierra Nevada, southern Sierra Nevada, and southern California mountains (the latter including the San Gabriel, San Bernardino, and San Jacinto Mountains). The southern Sierra Nevada and southern California subgroups were estimated to have diverged about 1.4 million years ago (Macey *et al.* 2001, p. 131) and are currently separated by the Tehachapi Mountains, a distance of about 225 kilometers (km) (140 miles (mi)). Therefore, at the time of listing the DPS represented the southernmost subgroup in what were thought to be four evolutionarily distinct subgroups of *R. muscosa*.

Since listing, Vredenburg *et al.* (2007, p. 361) further clarified the taxonomy of mountain yellow-legged frogs. After analyzing mitochondrial DNA, acoustic data, and morphological characteristics of museum specimens, the study recognized two distinct species of mountain yellow-legged frog: *Rana sierrae* in the northern and central Sierra Nevada, and *R. muscosa* in the southern Sierra Nevada and southern California. The analysis indicated that there is no range overlap between the two species (Vredenburg *et al.* 2007, p. 361). Within *R. muscosa*, three clades were identified (two in the southern Sierra Nevada, and one in southern California). The southern California clade (the listed entity) is described as occurring in the Transverse Ranges of Los Angeles, San Bernardino, and Riverside Counties, and as disjunct from the Sierra Nevada.

The new taxonomic distinction determined by Vredenburg *et al.* (2007, p. 361) was recently adopted by the American Society of Ichthyologists and Herpetologists, the Herpetologists' League, and the Society for the Study of Amphibians and Reptiles (Crother *et al.* 2008, p. 11). The Service recently accepted this new taxonomic distinction in a status assessment of the Sierra Nevada populations of *Rana muscosa* (USFWS 2011b, p. 5). This taxonomic change will be proposed concurrently with a proposed listing rule for the Sierra Nevada populations (USFWS 2011b, p. 5). Neither the taxonomy of southern *R. muscosa*, nor its status as a DPS will be affected should the Service finalize its recognition of two distinct mountain yellow-legged frog species.

Species Description

Adult mountain yellow-legged frogs are moderately sized, about 40 to 80 millimeters (mm) (1.5 to 3 inches (in)) from snout to urostyle (the pointed bone at the base of the backbone) (Zweifel 1955, p. 230; Jennings and Hayes 1994b, p. 74). Females are slightly larger (up to 95 mm (3.75 in)) than males (up to 85 mm (3.35 in)) on average (Wright and Wright 1949, pp. 424–430). *Rana muscosa* have slightly longer legs than *R. sierrae* (Vrendendburg *et al.* 2007, p. 371). Male mountain yellow-legged frogs have swollen and darkened nuptial pads (thumb bases) for gripping the female during copulation. Although lacking vocal sacs, individuals can make both terrestrial and underwater vocalizations, which have been described as a flat clicking sound (Zweifel 1955, p. 234; Ziesmer 1997, pp. 46–47; Stebbins 2003, p. 233). *Rana muscosa* produces a significantly different call from that of *R. sierrae*, in that it has discrete pulsed and noted sounds with no transitions (Vrendendburg *et al.* 2007, p. 371). Mountain yellow-legged frog males can be heard from a short distance (less than 2 m) above water, and may produce a distinctive garlic-like odor when disturbed (Wright and Wright 1949, p. 432; Stebbins 2003, p. 233).

The skin pattern of mountain yellow-legged frog is variable, ranging from discrete dark spots that can be few and large, to smaller and more numerous with a mixture of sizes and shapes, to irregular patches or a poorly defined network (Zweifel 1955, p. 230). Body color is also variable, usually a mix of brown and yellow, but often with gray, red, or green-brown. Some individuals may be dark brown with little pattern (Jennings and Hayes 1994b, p. 74). Dorsolateral (on back and side) folds are present, but usually are not prominent (Stebbins 2003, p. 233). The throat is white or yellow, sometimes mottled with dark pigment (Zweifel 1955, p. 230). The belly and ventral surface (underside) of the hind limbs range in hue from pale lemon yellow to an intense sun yellow. Eye coloration consists of a gold-colored iris with a horizontal, black counter shading stripe (Jennings and Hayes 1994b, p. 74).

The tadpoles (larvae) of mountain yellow-legged frogs generally are mottled brown in dorsal (back) coloration with a golden tint and a faintly-yellow venter (underside) (Zweifel 1955, p. 231; Stebbins 2003, p. 460). Total tadpole length reaches 72 mm (2.8 in); the body is flattened, and the tail musculature is wide, about 25 mm (1 in) or more, before tapering into a rounded tip (Wright and Wright 1949, p. 431). The mouth has a maximum of 8 labial (lip) tooth rows (2 to 4 upper and 4 lower) (Stebbins 2003, p. 460).

Eggs of the mountain yellow-legged frog are laid in globular clumps, which are often somewhat flattened, roughly 25 to 50 mm (1 to 2 in) across (Stebbins 2003, p. 444). When eggs are close to hatching, egg mass volume may average 198 cubic cm (12 cubic in) (Pope 1999a, p. 30). Eggs have three firm jelly-like transparent envelopes surrounding a grey-tan or black vitelline (egg yolk) capsule (Wright and Wright 1949, pp. 431–433).

Habitat and Life History

Southern *Rana muscosa* historically inhabited rocky and shaded streams on desert and coastal slopes from 370 to 2,290 m (1,200 to 7,500 ft) in elevation, with cool waters originating from springs and snowmelt (Zweifel 1955, p. 237; Jennings and Hayes 1994a, p. 194; Jennings and Hayes 1994b, p. 74). Mountain yellow-legged frogs are diurnal and are rarely found more than 1 m (3 ft) away from water (Mullally and Cunningham 1956, p. 191; Bradford *et al.* 1993, p. 886; Stebbins 2003, p. 233). Individuals are most often found in creeks with permanent (perennial) water in at least some portion of the reach (A. Backlin, USGS, 2012, pers. comm.). Perennial flows are needed for reproduction, larval growth and survival, and hydration of juveniles and adults (Vredenburg *et al.* 2005, p. 564). Water depth, persistence, and configuration (i.e., gently sloping shorelines and margins) are important factors for overwintering (hibernation), thermoregulation (regulation of body temperature through behavior), reproduction and development, foraging, and protection from predation (Jennings and Hayes 1994b, p. 77). Mountain yellow-legged frogs seem to be absent from the smallest creeks, probably because these have insufficient depth for adequate refuge and overwintering habitat (Jennings and Hayes 1994b, p. 77).

Streams utilized by adults vary from those having steep gradients with numerous pools, rapids, and small waterfalls, to those with low gradients with slow flows, marshy edges, and sod banks (Zweifel 1955, p. 237; Mullally 1959, p. 78). Aquatic substrates vary from bedrock to fine sand, rubble, rocks, and boulders (Zweifel 1955, p. 237), any of which may serve as basking areas for thermoregulation (Zweifel 1955, p. 237). Zweifel (1955, p. 237) noted that the high stream gradient and large boulders testify to the heavy rains of winter and early spring that are sent down the canyons in southern California. Although mountain yellow-legged frogs may use a variety of shoreline habitats, both tadpoles and adults are less common at shorelines which drop abruptly to a depth of 60 cm (2 ft) than at open shorelines that gently slope up to shallow waters of only 5 to 8 cm (2 to 3 in) deep (Mullally and Cunningham 1956, p. 191; Jennings and Hayes 1994b, p. 77). USGS (2004, p. 21) reported creeks occupied with southern *Rana muscosa* were generally narrow, with an average width of 1 to 3 m (3 to 10 ft). Stream reach lengths containing mountain yellow-legged frog populations reportedly varied from approximately 250 m (Dark Canyon) to over 5,000 m (East Fork City Creek) (820 ft to over 16,400 ft respectively) (USGS 2004, p. 21). Pools were typically 1 to 10 m (3 to 32 ft) long, 0.5 to 7 m (2 to 23 ft) wide, and 1 to 180 cm (0.4 to 71 in) deep. Pools usually had some type of structure that could function as refugia (cover from predators) such as bank overhangs, rocks, and downfall logs or branches, although aquatic vegetation was minimal (USGS 2004, p. 21).

At lower elevations, the non-aquatic habitat is characterized by common species such as *Baccharis viminea* (seep willow), *Alnus rhombifolia* (white alder), *Pseudotsuga macrocarpa* (big-cone spruce), and *Populus* spp. (cottonwood) (Zweifel 1955, p. 237; Jennings and Hayes 1994a, p. 195). At higher elevations, the streamside habitat is dominated by species such as *Pinus contorta* (lodgepole pine), *Pinus jeffreyi* (Jeffery pine), *Pinus lambertiana* (sugar pine), *Pinus ponderosa* (yellow pine), *Abies concolor* (white fir), and *Calocedrus decurrens* (incense cedar) (Zweifel 1955, p. 237). USGS (2004, p. 21) reported that in mountain yellow-legged frog occupied habitat, riparian zone widths ranged from 8 to 25 m (26 to 82 ft), with canyon walls typically rising steeply on either side. The riparian zone, with the associated vegetation canopy, is necessary to maintain the prey base needed for the nutritional requirements of the mountain yellow-legged frog. An open or semi-open canopy (not exceeding 85 percent of riparian vegetation) is needed to ensure that adequate sunlight reaches the stream to allow for basking behavior and for photosynthesis of benthic algae (USFWS 2006a, p. 54351).

Reproduction and Development

Mountain yellow-legged frog breeding activity typically occurs from April (at lower elevations), to June or July (at higher elevations) and continues for approximately a month (Zweifel 1955, p. 243). Mountain yellow-legged frogs deposit their eggs in clusters (masses) in shallow waters of inlet streams where they may attach to rocks, gravel, vegetation, under banks, or similar substrates (Wright and Wright 1949, p. 431; Zweifel 1955, p. 243; Pope 1999a, p. 30; Vredenburg *et al.* 2005, p. 565). Egg masses vary in size from as few as 15 eggs to 350 eggs per mass (Livezey and Wright 1945, p. 703; Vredenburg *et al.* 2005, p. 564). This is considered low, relative to a range of several hundred to several thousand for other true frogs such as the California red-legged frog (*Rana draytonii*). Egg hatching time ex situ ranged from 18 to 20 days at 5 to 13.5°C (41 to 56°F) for mountain yellow-legged frogs (Zweifel 1955, p. 265). Field observations show similar results (Pope 1999a, p. 31).

Time to develop from fertilization to metamorphosis (transformation from tadpole to frog) is variable and dependent upon temperature. Metamorphosis may occur in a single season at low elevations because of the longer summer (the active season) (Storer 1925, p. 265). However, in the higher elevation areas of the Sierra Nevada, metamorphosis may take up to 3 years (Zweifel 1955, p. 245; Cory 1962b, p. 515; Bradford 1983, pp. 1171, 1182; Bradford *et al.* 1993, p. 883; Knapp and Matthews 2000, p. 435). Southern *Rana muscosa* tadpoles have been observed in two size classes (first year tadpoles and second year tadpoles), hence metamorphosis likely occurs at the end of the second summer when second year tadpoles are 1.5 years old (Backlin 2012, pers. comm.). Individuals are referred to as metamorphs during the short period of time when a second-year tadpole is morphing into a juvenile frog. After this individual has survived one overwintering period it is referred to as a juvenile (subadult). Reproductive maturity may be reached after 2 years as a juvenile, when frogs are approximately 4 years of age (Zweifel 1955, p. 245). Little is known about the lifespan of mountain yellow-legged frog, but it is presumed to be long-lived due to high adult survivorship from year to year (Pope 1999a, p. 619). One southern *R. muscosa* individual was PIT (Passive Integrated Transponder) tagged as an adult and has been observed annually for the past 11 years; thus, it was about 14 years old (Backlin 2012, pers. comm.).

Foraging

Juveniles and adults of southern *Rana muscosa* appear to be principally insectivorous, feeding on a wide variety of invertebrates, including beetles (*Coleoptera*), ants (*Formididae*), bees (*Apoidea*), wasps (*Hymenoptera*), flies (*Diptera*), true bugs (*Hemiptera*), and dragonflies (*Odonata*) (Long 1970, p. 7). Terrestrial insects and adult stages of aquatic insects may be the preferred food for adult mountain yellow-legged frogs (Bradford 1983, p. 1171); larger frogs consume more aquatic true bugs likely because of their more aquatic behavior (Jennings and Hays 1994b, p. 77). Adult mountain yellow-legged frogs have been found to eat Pacific treefrog (*Pseudocris regilla*) tadpoles (Pope 1999b, p. 163) and can be cannibalistic (Heller 1960, p. 127; Vredenburg *et al.* 2005, p. 565). Predation of conspecific (of the same species) eggs by *R. muscosa* tadpoles may occur (Vredenburg 2000, p. 170). Tadpoles graze on benthic detritus and algae along rocky bottoms in streams (Bradford 1983, p. 1171; Zeiner *et al.* 1988, p. 88).

Movement

Pope (1999a, p. 45) suggests that mountain yellow-legged frogs may have strong site fidelity. In Sierra Nevada aquatic habitats, mountain yellow-legged frog adults typically move only a few hundred meters (about 900 feet) (Matthews and Pope 1999, p. 623; Pope 1999a, p. 45), but distances of up to 1 km (0.62 mi) have been recorded (Vredenburg 2002, p. 4). Though adults are usually found within 1 m (3 ft) of water, overland movements of over 65 m (215 ft) have been recorded in the Sierra Nevada (Pope 1999a, p. 45); the furthest reported distance of a mountain yellow-legged frog from water is 400 m (1,300 ft) (Vredenburg 2002, p. 4). In southern California, USGS (2004, p. 7) examined the movement patterns of PIT tagged southern *Rana mucosa* between the 2000 and 2003 field seasons. Of 42 individuals that were recaptured, 17 individuals showed no measurable movement over 4 years, while the 23 individuals moved an average of only 68 m, and the 2 remaining individuals moved longer distances (1,494 m and 512 m) (USGS 2004, p. 20). These results confirm that adult southern *R. muscosa* are also highly philopatric, but may travel longer distances, perhaps in search of new territories and mates (USGS 2004, p. 26). Movement patterns suggest that the longer dispersal events occur just after emergence from hibernation in the spring and just before returning to hibernacula in the winter, with high site fidelity occurring during in the middle of the active season (Matthews and Pope 1999, p. 615). In a study of displaced mountain yellow-legged frogs, Matthews (2003, p. 621) concluded that stress due to a homing response in adults may preclude translocation as an effective conservation tool. However, other research in the Sierra Nevada has found that if translocations occur an adequate distance from the source population, the homing mechanism will not function (R. Knapp, UCSB, 2012, pers. comm.). Almost no data exist on the dispersal of juvenile mountain yellow-legged frogs; however, in the Sierra Nevada juveniles from small intermittent streams are thought to disperse to permanent water (Bradford 1991, p. 176). Ecologists from USGS continue to PIT tag and track the movement patterns of southern *R. muscosa* (Backlin 2012, pers. comm.).

Egg masses and tadpoles are difficult to detect due to their cryptic nature, although USGS (2004, p. 27) noted that when southern *Rana muscosa* tadpoles are detected, they tend to be found further and further downstream as the season progresses. This indicates that downstream currents may contribute to tadpole dispersal, especially after summer rains (USGS 2004, p. 27).

In streams of the Sierra Nevada, tadpoles have been observed more than 1 km (0.6 mi) downstream from the initial point of observation (Knapp 2012, pers. comm.). Tadpoles may disperse continually downstream over time, unless they are limited by the presence of predators, such as nonnative trout (Knapp 2012, pers. comm.).

Hibernation

The coldest winter months are spent in hibernation, probably underwater or in crevices in the streambanks (Zweifel 1955, p. 242; Bradford 1983, p. 1171; Matthews and Pope 1999, p. 615). In lakes and ponds of the Sierra Nevada, which do not freeze to the bottom in winter, mountain yellow-legged frogs may overwinter in the shelter of bedrock crevices as a behavioral response to the presence of introduced fishes (Vredenburg *et al.* 2005, p. 565). Individuals emerge from overwintering sites immediately following snowmelt in early spring and breeding begins soon after. Tadpoles may survive overwintering better than juveniles and adults (Bradford 1983, p. 1171). Ex situ experiments demonstrate that mountain yellow-legged frog tadpoles in the Sierra Nevada have a higher tolerance to hypoxia and reduced energy use and oxygen intake during hibernation when compared to juveniles and adults (Bradford 1983, p. 1171). A recent ex situ study of the dormancy requirements of adult southern *R. muscosa* found that reproductive output was significantly higher when adults are hibernated prior to the breeding season (F. Santana, SD Zoo ICR, 2012a, pers. comm.). Individuals may also aestivate (become dormant) during especially dry periods of late summer (Mullally 1959, p. 79).

Batrachochytrium dendrobatidis (Bd) in the Environment

The amphibian fungal pathogen *(Batrachochytrium dendrobatidis (Bd)),* which causes the disease chytridiomycosis, is strongly associated with amphibian declines in seemingly pristine environments on all continents where amphibians occur (Fisher *et al.* 2009, p. 291). While other diseases are known to cause amphibian declines (Daszak *et al.* 2000, p. 444), *Bd* is the first emerging disease to cause the decline or extinction of hundreds of species. This includes over 200 anurans over the past 30 years, many of which were not otherwise threatened and some declines occurred within a single year (Skerratt *et al.* 2007, p. 125). Though unknown in the habitat of southern *Rana muscosa* at listing, *Bd* may have been present but undetected in some portion of the range. Since listing, *Bd* has been identified on individuals from all extant localities (Backlin 2012, pers. comm.) and is expected to become a permanent and ubiquitous habitat feature in the waterways of the San Gabriel, San Bernardino, and San Jacinto Mountains.

This pathogen has the ability to become pervasive across landscapes due to the mode of infection and dissemination, persistence on alternate substrates and in vector species, and a wide tolerance of environmental conditions. The life cycle of *Bd* includes the flagellated zoospore, which encysts on the skin surface, and the zoosporangium, which forms under the skin and eventually opens to the surface, releasing zoospores (Berger *et al.* 2005, p. 56). Zoospores may reinfect the same host (autoinfection) or disperse into the environment. In addition to growing on amphibian skin, *Bd* may survive as a saprobe in moist soil, or on bird feathers or other substrates (Longcore *et al.* 1999, p. 227; Johnson and Speare 2005, p. 181). Water flow is likely the main method of dissemination although spread of the disease through movement of vector organisms has been suggested (humans, birds, fish, and crustaceans) (Laurance *et al*. 1997, p. 1030; Johnson and

Speare 2005, p. 181). In the Sierra Nevada, the pattern of spread has been upstream, suggesting there has been an overland transmission (Vredenburg *et al.* 2010, p. 9690). Researchers are currently investigating the possibility that dissemination is occurring through a vector (carrier) species, the Pacific treefrog (*Pseudacris regilla*) (Padgett-Flohr and Hopkins 2009, p. 1; V. Vredenburg, SFSU, 2012, pers. comm.). Another ranid, the American bullfrog (*Rana catesbeiana*) may be a global vector for the disease as it is among one of the earliest infected specimens known from the United States (1961, from central California) and today carries multiple genotypes of *Bd* (Rosenblum *et al.* 2010, p. 1). Both the Pacific treefrog and the American bullfrog occur in waterways occupied by southern *R. muscosa*. Additionally, individual mountain yellow-legged frogs maintaining low infection intensities can also serve as vectors. The timing and method of introduction of *Bd* into mountain yellow-legged frog habitat is unclear. No analyses of museum specimens have occurred to identify when *Bd* was introduced in southern California. The effects of *Bd* on southern *R. muscosa* will be discussed further under the section titled Disease in **Factor C**.

Spatial Distribution

Southern *Rana muscosa* was known from an estimated 166 historical localities from creeks and drainages in the San Gabriel, San Bernardino, San Jacinto, and Palomar Mountains of Los Angeles, San Bernardino, Riverside, and San Diego counties. In the 1994 assessment Amphibian and Reptile Species of Special Concern in California, Jennings and Hayes (1994b, p. 77) estimated that southern *R. muscosa* had been extirpated from more than 99 percent of its previously documented range. Between 1970 and 1993, southern *R. muscosa* was thought to be extirpated from the San Bernardino Mountains (Jennings and Hayes, 1994b, p. 77) until a single small population was rediscovered at East Fork City Creek (a tributary of the Santa Ana River) in 1998 (USGS 1999, p. 6).

At the time southern *Rana muscosa* was listed as endangered in 2002, it was known from only 7 of the 166 historical localities in southern California including 5 small streams in the San Gabriel Mountains (Bear Gulch, Vincent Gulch, South Fork Big Rock Creek, Little Rock Creek, and Devil's Canyon), 1 stream in the San Bernardino Mountains (East Fork City Creek), and 1 stream in the upper reaches of the San Jacinto River system in the San Jacinto Mountains (Fuller Mill Creek) (USGS 2002a, p. 1). Other populations observed in the 1990s could not be detected in surveys performed by USGS in 2000 and 2001 (including Prairie Fork, East Fork San Gabriel River, Fish Fork, Alder Gulch, Dark Canyon (the uppermost reach of the North Fork San Jacinto River), North Fork San Jacinto River) (Jennings and Hayes 1994b, p. 78; USGS 1995, p. 2; USGS 1999, p. 3; USGS 2001, p. 5; USGS 2002a, p. 6). Additionally, the only two populations on Palomar Mountain were thought to be extirpated (USFWS 2002, p. 44382), shifting the latitudinal distribution of extant populations approximately 45 km (30 mi) north of the historical southern extent. At listing, all of the known locations of southern *R. muscosa* occurred on lands administered by the USFS; however, the headwaters of Fuller Mill Creek flowed through private inholdings in San Bernardino National Forest.

Since listing, USGS has identified two additional waterways occupied by southern *Rana muscosa*, both in the San Jacinto Mountains. Dark Canyon, which was known to be occupied in 1998 and 1999 (USGS 2001, p. 5), was not found again until 2003 even though annual surveys occurred at this site (USGS 2004, p. 6). Then in 2009, one adult was found at Tahquitz Creek (USGS 2009b, p. 2). Extensive surveys have been performed by USGS at over 200 additional locations in all four mountain ranges within the historical distribution (Figure 1) of the species. No other occupied areas have been identified (USGS 1998–2012; USGS GIS data). However, because survey intensity has varied between sites and many areas have not been surveyed, there may be additional unoccupied areas that are currently unaccounted for. Therefore, southern *R. muscosa* is currently known to be extant at only nine locations within the San Gabriel, San Bernardino, and San Jacinto Mountains (Table 1, Figure 1).

In 2010, an experimental re-establishment effort began at the recently occupied Indian Creek in Hall Canyon. Tadpoles collected during a salvage operation at Dark Canyon were raised at SD Zoo ICR and bred in captivity. Progeny of these animals, including 300 eggs and 36 tadpoles were released at this location in 2010; however, these individuals were not detected during surveys in 2011. In 2011, 313 tadpoles and 270 eggs were placed at the Indian Creek location; tadpoles were detected during subsequent monitoring in 2011 (Santana 2012a, pers. comm.). While southern *Rana muscosa* may become established here in the future, this site currently remains an experimental re-establishment area (Table 1, Figure 1).

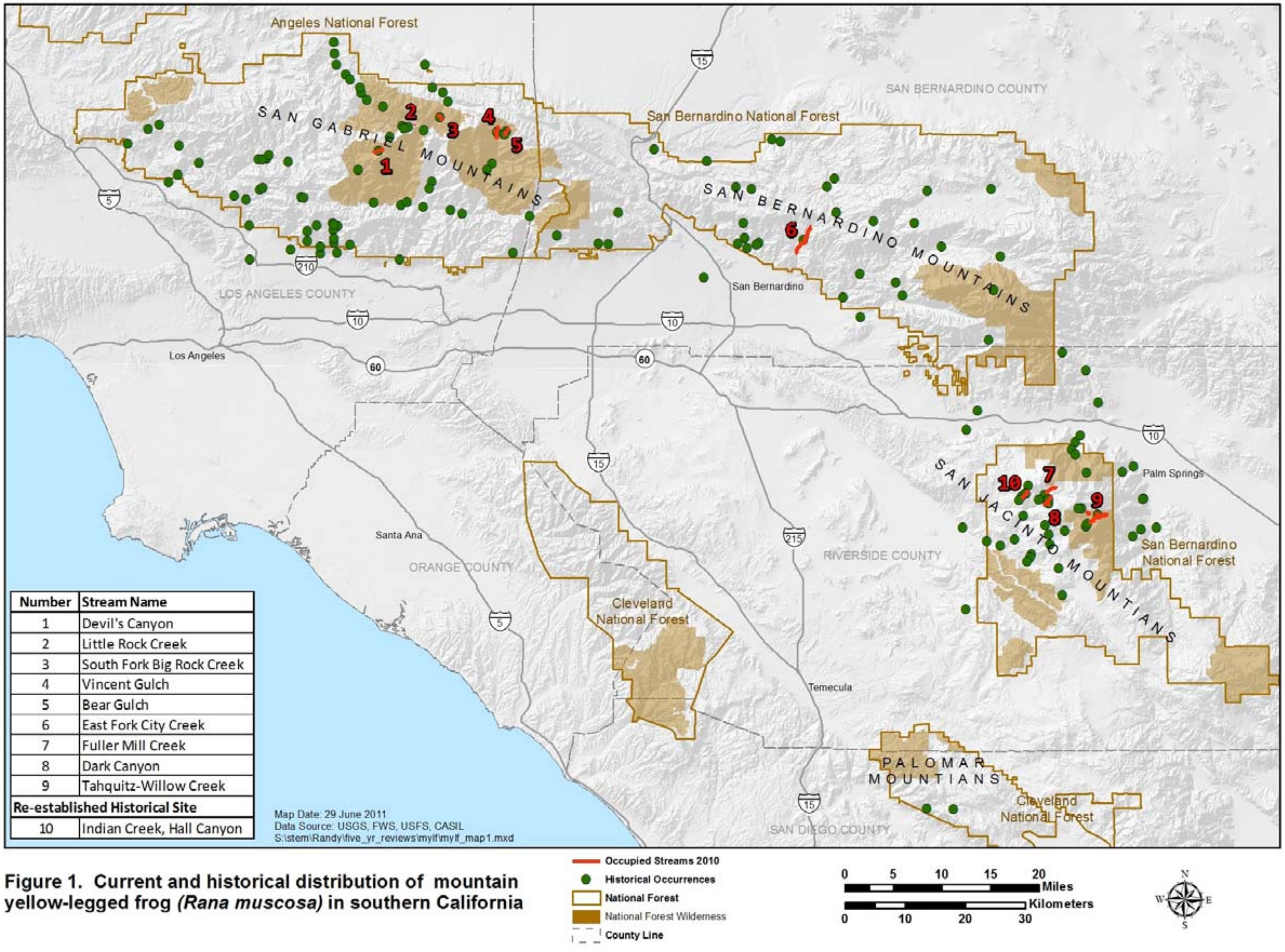
Table 1. Current distribution of mountain yellow-legged frog (*Rana muscosa*) populations in southern California. For historical distribution see Figure 1 (USGS 2004, pp. 84–85).

	National Forest	Mountain Range	Population	Known to be Occupied at Listing
1	Angeles	San Gabriel Mountains	Devil's Canyon	Yes
2	Angeles	San Gabriel Mountains	Little Rock Creek	Yes
3	Angeles	San Gabriel Mountains	South Fork Big Rock Creek	Yes
4	Angeles	San Gabriel Mountains	Vincent Gulch	Yes
5	Angeles	San Gabriel Mountains	Bear Gulch	Yes
6	San Bernardino	San Bernardino Mountains	East Fork City Creek	Yes
7	San Bernardino	San Jacinto Mountains	Fuller Mill Creek	Yes
8	San Bernardino	San Jacinto Mountains	Dark Canyon	No
9	San Bernardino	San Jacinto Mountains	Tahquitz/Willow Creeks	No
10	San Bernardino	San Jacinto Mountains	Indian Creek, Hall Canyon	No*

*****Individuals reintroduced in 2010 and 2011 from captive-bred mountain yellow-legged frogs. Re-establishment success is unknown as yet.

Metapopulation Structure

Regionally, mountain yellow-legged frogs are thought to exhibit a metapopulation structure (Bradford *et al.* 1993, p. 886; Drost and Fellers 1996, p. 424). In describing the metapopulation concept, Hanski and Simberloff (1997, p. 6) stated: “...the two key premises in this approach to population biology are that populations are spatially structured in assemblages of local breeding populations and that migration among the local populations has some effect on local dynamics, including the possibility of population re-establishment following extinction.” Both genetic and



Number	Stream Name
1	Devil's Canyon
2	Little Rock Creek
3	South Fork Big Rock Creek
4	Vincent Gulch
5	Bear Gulch
6	East Fork City Creek
7	Fuller Mill Creek
8	Dark Canyon
9	Tahquitz-Willow Creek
Re-established Historical Site	
10	Indian Creek, Hall Canyon

Map Date: 29 June 2011
Data Source: USGS, FWS, USFS, CASIL
S:\stem\Randy\five_yr_reviews\mylf\mylf_map1.mxd

Figure 1. Current and historical distribution of mountain yellow-legged frog *(Rana muscosa)* in southern California

demographic factors are important to ensure the long-term viability of individual populations and the metapopulation as a whole. The potential for increased inbreeding (mating between close relatives) and genetic drift (random changes in genetic frequencies) accompanies decreasing population sizes, and can lead to decreasing levels of heterozygosity (a measure of genetic diversity) that may have negative demographic effects through inbreeding depression (reduction in fitness due to mating among relatives) (Soulé 1987, p. 96) and loss of adaptability. There is also growing evidence that the level of heterozygosity affects the disease resistance of a population (Allentoft and O'Brien 2010, p. 47).

A small amount of genetic exchange among populations via movements by adults, juveniles, or more commonly, dispersal of tadpoles downstream, can counteract inbreeding and associated decreases in genetic diversity that might otherwise develop within small isolated populations. If geographic distance between populations is not great, gene migration via dispersing individuals occurs readily. However, any interference of movement between groups can quickly eliminate genetic diversity through genetic drift (Epps *et al.* 2005, p. 1035). In the absence of an operable metapopulation structure, isolated subpopulations may benefit from genetic enrichment via induced migration of individuals translocated between subpopulations (Epps *et al.* 2006, p. 4300). Another important long-term process in metapopulation dynamics is the balance between rates of natural extinction and colonization among constituent subpopulations. Colonization rates must exceed extinction rates for a metapopulation to persist (Hanski and Gilpin 1991, p. 5). Disruption of metapopulation dynamics diminishes natural recovery options and increases the extinction risk of species that exhibit this population structure (Noss and Cooperrider 1994, pp. 61–62).

The metapopulation structure of southern *Rana muscosa* is currently not functional in natural circumstances. Populations of southern *R. muscosa* are highly isolated in the headwaters of tributaries above barriers that prevent the upstream movement of predatory nonnative trout. Trout dominate the downstream habitat below barriers at the majority of occupied localities. Surveys performed by USGS have shown that with very little exception, nonnative trout and southern *R. muscosa* do not currently coexist in the same reach of a stream or creek. Previous co-occurrence between the two has resulted in the displacement of frogs by nonnative trout through predation. Currently, nonnative trout act as barriers to dispersal and recolonization by tadpoles. The extensive landscape scale occupancy of nonnative trout in southern *R. muscosa* habitat has severely interrupted the metapopulation dynamics that would allow for natural colonization of new or previously occupied areas and genetic exchange between populations. This issue will be discussed further under the section titled Predation in **Factor C**.

Abundance

A review of museum specimens by USGS found that collections of southern *Rana muscosa* decreased substantially in the late 1960s, indicating this period was the start of their overall decline (Backlin 2012, pers. comm.). However, a quantitative evaluation of the decline did not occur until the 1990s when the total representative abundance was thought to be less than 100 individuals across all populations (Jennings and Hayes 1994b, p. 78; USGS 1995, p. 2; USGS 1999, p. 3).

At listing, statistical analyses of observational data provided an estimate of approximately 79 adult frogs total in 5 of the 7 occupied localities (Little Rock Creek, South Fork Big Rock Creek, Vincent Gulch, Bear Gulch, and East Fork City Creek) (USFWS 2002, p. 44384) in addition to direct observations of 4 adults in Devil's Canyon and 1 adult in Fuller Mill Creek (USGS 2002a, p. 5; USFWS 2002, p. 44384). Three of the seven localities were estimated to have less than 10 adults each, although upper 95 percent confidence intervals estimated a maximum population size of 7 (South Fork Big Rock Creek and Vincent Gulch) and 20 (Little Rock Creek) (USGS 2002, p. 5).

Determining accurate population estimates has been a challenge due to exceedingly low numbers at almost all nine currently extant localities (Backlin 2012, pers. comm.). Regardless, it is clear that every population remains precariously small today (Table 2 and Table 3). Two of the larger populations at listing now may have less than five adults remaining (Bear Gulch and East Fork City Creek). Tahquitz-Willow Creek also appears to have less than five adults remaining. Three additional populations may have 15 or fewer adults (Vincent Gulch, Fuller Mill Creek, and Dark Canyon). However, threat abatement including increased restrictions on recreation and trout removal at Dark Canyon may have reversed the decline of this population as evidenced by a recent increase in abundance (Backlin 2012, pers. comm.). South Fork Big Rock Creek appears to be stable at a low abundance of less than 30 adults and may be on an upward trajectory. Only Little Rock Creek has experienced a substantial increase since 2001; this increase is a result of trout removal efforts performed by CDFG and the creek closure enforced by the USFS at this location (USGS 2012, p. 18). The status of the Devil's Canyon is unclear although it also persists at a very low abundance. Therefore, although population trends are difficult to discern, all populations are considered very small and are at risk from a number of factors discussed below.

Captive propagation is being investigated for southern *Rana muscosa* and a total of 54 animals are currently being held at two facilities (SD Zoo ICR and LA Zoo). Individuals were obtained through emergency salvages, conducted in response to stochastic events and environmental conditions. This is discussed below under the section titled *Captive Breeding, Reintroduction, Augmentation, and Translocation Program*.

Genetics

Schoville *et al.* (2011, p. 2031) used mitochondrial and microsatellite data to examine patterns of genetic variation in multiple populations of northern and southern *Rana muscosa*. The study found low levels of genetic variation within each population compared to other montane ranid populations (Zhan *et al.* 2009, p. 2; Zhao *et al.* 2009, p. 270; Schoville *et al.* 2011, p. 71). Their work concluded that inbreeding in southern *R. muscosa* is not strong, but the highest degree of inbreeding was found in the East Fork City Creek, Little Rock Creek, and Dark Canyon. However, genetic bottlenecks occur in all populations. Populations were found to have diversified within the Pleistocene, with little gene flow during population divergence, indicating that unique evolutionary lineages of *R. muscosa* exist in each mountain range in southern California. The study analyzed the biogeography of southern *R. muscosa* and estimated that the

Table 2. Population estimates for adult mountain yellow-legged frogs (*Rana muscosa*) in southern California from 2001 to 2009 (Backlin 2011a, p. 1).

	National Forest	Mountain Range	Occurrence	2001	2002	2003	2004	2005	2006	2007	2008	2009
1	Angeles	San Gabriel	Devil's Canyon	9	15	12	0	12	11	5	11	20
2	Angeles	San Gabriel	Little Rock Creek	7	9	5	3	4	12	27	28	42
3	Angeles	San Gabriel	South Fork Big Rock Creek	4	26	24	16	17	28	21	21	20
4	Angeles	San Gabriel	Vincent Gulch	6	3	1	3	2	3	2	1	3
5	Angeles	San Gabriel	Bear Gulch	27	18	24	0	0	1	2	3	1
6	San Bernardino	San Bernardino	East Fork City Creek	15	22	2	0	0	11	11	3	0
7	San Bernardino	San Jacinto	Fuller Mill Creek		8	8	0	8	7	10	14	10
8	San Bernardino	San Jacinto	Dark Canyon			33	20	21	8	8	11	39
9	San Bernardino	San Jacinto	Tahquitz-Willow Creek									1*
10	San Bernardino	San Jacinto	Indian Creek, Hall Canyon									

*Observed, not estimated statistically. No data is available for the areas blocked out (brown) because *Rana muscosa* was not known to occur at these localities during these years. Status of individuals released at Indian Creek, Hall Canyon in 2010 and 2011 (blue) is unknown.

Table 3. Number of unique adult mountain yellow-legged frogs (*Rana muscosa*) observed from 2001 to 2011 (not population estimates) (adapted from USGS 2011, p. 16). Number of days surveyed (effort) varies.

	Mountain Range	Location	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
1	San Gabriel	Devils Canyon	3	5	4	0	4	8	3	8	11	12	4
2	San Gabriel	Little Rock Creek	5	8	7	3	5	10	15	15	24	48	51
3	San Gabriel	South Fork Big Rock Creek	4	22	25	17	14	20	15	12	11	12	26
4	San Gabriel	Vincent Gulch	4	2	1	1	2	3	2	1	2	7	3
5	San Gabriel	Bear Gulch	21	14	17	0	1	1	1	1	1	0	1
6	San Bernardino	East Fork City Creek	11	18	4	0	1	8	12	2	1	1	7
7	San Jacinto	Fuller Mill Creek		3	6	0	8	5	9	14	9	6	1
8	San Jacinto	Dark Canyon			11	23	20	14	17	16	14	9	14
9	San Jacinto	Tahquitz/Willow Creeks									1	4	0
		Total	48	72	75	44	55	69	74	69	74	99	107

No data is available for the areas blocked out (brown) because southern *Rana muscosa* was not known to occur at these localities during these years. Five of seven adults at East Fork City Creek (blue) were collected from the wild in 2011.

San Bernardino and San Jacinto Mountains split 47,000 years before present, the San Gabriel and combined San Bernardino-San Jacinto lineage split 289,423 years before present, and the southern California lineage split from the Sierra Nevada lineage 1.42 million years before present. Due to very small populations and the high probability of catastrophic events to eliminate or reduce remaining populations, the study stated that loss of genetic diversity is likely to be rapid in the future. As a result, Schoville *et al.* 2011 (p. 2038) recommend that translocations between populations be considered to avoid inbreeding depression; however, because populations represent unique evolutionary lineages, care must be taken to avoid outbreeding depression that might result from mixing between populations. If significant genetic differences are tied to ecological adaptations to different habitat types, then mixing between lineages of northern and southern *R. muscosa* could cause outbreeding depression (Backlin 2011b, p. 1). Other recent genetic research related to *R. muscosa* is discussed above under the section titled Changes in Taxonomic Classification.

Species-specific Research and/or Grant-supported Activities

Various funding sources and collaboration with numerous Federal, State, and private agencies have supported recovery related activities for southern *Rana muscosa*. Such activities include: 1) monitoring extant populations; 2) surveying suitable habitat for additional populations; 3) research of ecological requirements and biological characteristics; 4) salvage operations for at-risk populations; 5) captive breeding programs at the San Diego, Los Angeles, and Fresno zoos; 6) habitat assessments for reintroduction and potential trout removal; 7) trout barrier construction; 8) trout removal operations; 9) monitoring of released individuals; 10) genetics research; 11) testing for infectious disease (*Bd* and viruses); 12) closures to public access and fencing to reduce recreational pressures at extant populations; and 13) other recovery related activities. Partners supporting various recovery related activities include USGS, USFS (Angeles and San Bernardino National Forests), CDFG (Regions 5 and 6), University of California James Reserve, SD Zoo ICR, LA Zoo, Fresno Zoo, Caltrans, and the Service.

The activities listed above are ongoing and contribute to our knowledge of the southern *Rana muscosa* population to help conserve this imperiled species. Each of the nine extant southern *R. muscosa* populations is isolated and supports an alarmingly small population. Additionally, the small populations appear increasingly less capable to cope with environmental events that would generally have a minimal impact on a healthy metapopulation, an aspect that may necessitate additional emergency salvages. As a result of these concerns, partners (listed above) for the conservation of this DPS determined that an essential component for recovery is the maintenance of captive populations that might one day facilitate the re-establishment of historically occupied areas, augmentation of existing populations, and the restoration of connectivity between populations. The central means to accomplish these goals is through a captive breeding program and possibly future translocation between existing populations.

Captive Breeding and Reintroduction/Population Augmentation Program

In accordance with the Service's Policy Regarding Controlled Propagation of Species Listed under the ESA (USFWS and NOAA 2000, p. 56921), we requested and obtained Regional Director [Manager] approval for use of captive propagation for the conservation and recovery of *Rana muscosa* (USFWS 2007, pp. 1–4). The approved program includes translocation (USGS 2007a, pp.1-32). We had previously issued a 10(a)(1)(A) permit to USGS to facilitate a captive breeding, reintroduction, and population augmentation program for southern *Rana muscosa* (USFWS 2006b pp. 1–12), and amended this permit in 2011 to include additional recovery actions for the DPS (USFWS 2011a, pp. 1–7). Thus far, the program has helped to maintain captive populations collected in emergency salvages, allowed for the breeding of individuals in captivity and concurrent research of biological requirements, and helped to introduce the first re-establishment effort at Indian Creek in Hall Canyon. The initiation of this program originally occurred in 2003 after the Old Fire burned the habitat at East Fork City Creek and subsequent flooding scoured the area. Ten adults were salvaged, although all were infected with both mycobacteriosis and the pathogenic chytrid fungus, *Bd* (A. Pessier, SD Zoo ICR, 2006, pers. comm.; J. Lemm, SD Zoo ICR, 2006, pers. comm.). The health of these frogs deteriorated in captivity. Individuals were treated for chytridiomycosis in anti-fungal baths; however, the treatment did not clear all infections. All animals eventually perished as a result of mycobacteriosis by 2006.

Eighty first-year tadpoles collected in the Dark Canyon emergency salvage of 2006 were raised in captivity at the SD Zoo ICR and are the source captive population for the first experimental re-establishment at Indian Creek in Hall Canyon. In captivity, 73 salvaged tadpoles metamorphosed into frogs in 2008. By December 2010, 56 adults remained alive in captivity, 10 of which were transferred to the LA Zoo in order to protect this genetic line at two facilities (Santana 2012a, pers. comm.). The first successful breeding occurred in 2008, with a single female producing a clutch of 100 eggs, only 3 of which developed into tadpoles because the eggs were overcome with *Saprolegnia* (water mold), which is common in aquatic environments. One adult from this cohort remains alive in captivity today.

In 2010, an experiment at the SD Zoo ICR demonstrated significantly higher reproductive output after captive individuals were hibernated at 40°F (4.5°C), compared to non-hibernated individuals (Santana 2012a, pers. comm.). In 2010, six females (all hibernated) produced approximately 870 eggs. In May and August of 2010, 300 eggs and 36 tadpoles were released at Indian Creek, and monitored post-release until December 2010 (Santana 2012a, pers. comm.). A subset of tadpoles were caged and fed in pools in Indian Creek for 3 months prior to the December release; 100 percent of caged animals survived until December. Survivorship of uncaged tadpoles was less clear, although three of these animals were observed before the 2010 monitoring period was complete. Released animals have not been detected in subsequent surveys in 2011, though this not surprising, due to the low detection rate of tadpoles (Santana 2012a, pers. comm.).

After the hibernation experiment of 2010 revealed that reproductive output is higher if hibernation occurs, all mature individuals at SD Zoo ICR were hibernated prior to the 2011 breeding season (Santana 2012a, pers. comm.). Forty individuals were bred (22 females) produced 4,846 eggs, an approximately six-fold increase in reproductive output. In 2011, 600 eggs and 310 surviving tadpoles were released at Indian Creek in Hall Canyon (Santana 2012a, pers. comm.). At the LA Zoo, 10 individuals (6 females) bred to produce approximately 1,000 eggs, 200 of which produced tadpoles (I. Recchio, LA Zoo 2012, pers. comm.). Progeny (160 tadpoles) from the LA Zoo were also released at Indian Creek in Hall Canyon in 2011 (Recchio 2012, pers. comm.). During monitoring, these individuals can be distinguished from those released in 2010 as they belong to different age classes.

After the Station Fire in 2009, the population in Devil's Canyon was potentially at risk to catastrophic flooding and habitat alteration (Cannon *et al.* 2010, p. 1). Thus, a third emergency salvage took place and 106 first-year tadpoles were collected from Devil's Canyon. These individuals were housed at the Fresno Zoo. Many of these individuals lived until the juvenile stage; however, the remaining juveniles died in captivity. This may have resulted from an accidental exposure to high levels of phosphate added to the municipal water source. However, no other amphibian species cared for in captivity by the Fresno Zoo exhibited any negative effects similar to that of southern *Rana muscosa* despite being exposed to the same water. The cause of death of these animals remains unclear.

A fourth emergency salvage was approved in 2011 to collect any remaining individuals from East Fork City Creek because reproduction had not been detected at this locality in over 7 years, the number of adults had been low for many years (Table 2), and this is the last known population in the San Bernardino Mountains. After intensive surveys in 2011, five adults and six metamorphs were found dispersed throughout the 5,000 m (16,250 ft) creek and were taken into captivity. This captive population suffered mortalities due to unknown causes. Metamorphs may have perished due to either water quality issues or exposure to a skin irritant (Santana 2012b, pers. comm.). Four adult frogs (one female) are now a part of the captive breeding program at the SD Zoo ICR.

Therefore, the current captive breeding program at two facilities represents approximately 50 individuals from one population (Dark Canyon) from the San Jacinto Mountains, and 4 individuals from the last known population in the San Bernardino Mountains (East Fork City Creek). Future efforts will also include translocations within and between populations to increase the size, distribution, and connectivity of populations, as well as to ameliorate genetic effects associated with small population size.

Vulnerability Factors

Species may be vulnerable to threats for a variety of reasons. Primack (2006, p. 159) outlined five categories of species considered most vulnerable to extinction as:

1) Species with very narrow geographical ranges;
2) species with only one or a few populations;
3) species in which population size is small (identified as one of the best predictors of species extinction rate);
4) species in which population size is declining; and
5) species that are hunted or harvested by people.

Consideration of these categories in conjunction with life history traits can provide a vulnerability profile for southern *Rana muscosa.* Nonnative trout, few and very small populations, disease, and catastrophic events are among the factors affecting the rarity and leading to the decline of the DPS. Several factors make southern *R. muscosa* vulnerable to extinction, including:

1) *Rana muscosa* is known from four mountain ranges in southern California, and persists in only three;
2) each mountain range supports very few populations, and all populations are highly isolated in the headwaters of tributaries;
3) each population is very small (one population supports approximately 50 adults; two support approximately 25 adults; three may support less than 15 adults; three may support less than 5 adults);

4) metapopulation dynamics have been severely interrupted by the presence of predatory nonnative trout in intervening waterways (trout eliminated and replaced populations rangewide, fragmented the remaining habitat, isolated extant populations in marginal habitat, and currently inhibit natural dispersal, recolonization, and recruitment in the historical range);
5) physical isolation of populations has caused genetic isolation (inbreeding has been detected in three populations, and genetic bottlenecks have been detected in all populations);
6) a virulent fungal pathogen has been detected in all populations and appears to be inhibiting recruitment of the juvenile lifestage; and
7) catastrophic natural events such as wildfires and flooding greatly increase the likelihood that the small, isolated populations will become extirpated.

The most significant vulnerabilities of southern *Rana muscosa* are comprised by the threats imposed on few, small, and highly isolated populations. The principle threats include predatory nonnative trout, which inhibit metapopulation dynamics, genetic effects, disease, wildfires, and flooding. The threats described below in the **Five-factor Analysis** section likely have the greatest impacts on southern *R. muscosa*. Those threats in the listing rule are described below and addressed for all populations (Appendix 1).

Five-factor Analysis

The listing rule asserted that unless threats to the species were moderated or reversed, a high probability existed that southern *Rana muscosa* would go extinct in the near future, and that consequently, additional research on the effects of the factors at work on amphibian populations was necessary (USFWS 2002, p. 44383). Current population and genetics data indicate that southern *R. muscosa* continues to be critically endangered and that the manageable threats must be ameliorated in order to prevent extinction.

Prior to listing, the USFS finalized the Mountain Yellow-Legged Frog Conservation Assessment and Strategy (CAS) for the Angeles and San Bernardino National Forests, which satisfied a term and condition outlined by the Service in the Biological and Conference Opinions for the Four Southern California Land and Resource Management Plans (USFWS 2001, p. 326). Threats at listing were identified in the listing rule and were also described in detail in the CAS; therefore, both documents are used here to clearly describe the threats as they were understood at that time. The following five-factor analysis is provided to elucidate the threats attributable to one or more of the five listing factors outlined in section 4(a)(1) of the Act and to evaluate the current status and conservation needs of southern *R. muscosa*.

FACTOR A: Present or Threatened Destruction, Modification, or Curtailment of Habitat or Range

At listing, the remaining populations of southern *Rana muscosa* were found primarily on public land within the Angeles and San Bernardino National Forests (USFWS 2002, p. 44383). Therefore, the majority of habitat was protected or managed through management plans established for the national forests and sensitive species and habitat contained therein.

Habitat destruction related to activities such as logging and commercial development does not appear to have been a significant factor in the precipitous decline of southern *Rana muscosa* because these activities are not prominent within the habitat. Most alteration or degradation of habitat occurring at listing was associated with recreational activities in the forests, such as hiking, mountain climbing, camping, swimming, suction dredge mining for gold, stocking of nonnative trout for fishing, or other human-related impacts such as the dumping of trash and toxic materials (i.e., soap, motor oil, and mercury) into waterways (Jennings 1995, p. 6; USGS 2002a, p. 10; USFWS 2002, p. 44387). Nonnative trout stocking for recreational purposes was identified as a threat to the habitat in the listing rule. This was likely because nonnative trout minimize the area available for occupancy due to competition with and predation of southern *R. muscosa*, and because anglers can degrade sensitive streambank habitat utilized by southern *R. muscosa*. Ultimately predation is the most direct effect of nonnative trout presence in mountain yellow-legged frog habitat; thus, this threat is discussed further in the section titled Predation in **Factor C**. Wildfire was considered a threat to the habitat at listing, although fire management (abatement) activities, which can also impact habitat were not discussed in the listing rule. A new threat to the habitat since listing is the proliferation of illegal marijuana plantations on the national forests. All threats to southern *R. muscosa* habitat are described in detail below.

Recreational Activity

Human use in and along streams (hiking, mountain climbing, camping, swimming, and suction dredge mining for gold) can disrupt the development, survivorship, and recruitment of eggs, larvae, and adult frogs (Jennings 1995, p. 5; Rodriguez-Prieto and Fernandez-Juricic 2005, p. 1), and can change the character of a stream and its banks and associated vegetation in ways that make whole sections of a stream less suitable habitat for southern *Rana muscosa*. At the time of listing, six of the seven known occupied areas were experiencing impacts caused by recreational activities, including: Devil’s Canyon (minimal), Little Rock Creek, South Fork Big Rock Creek, Bear Gulch, East Fork City Creek (minimal), and Fuller Mill Creek. Dark Canyon, which was not thought to be occupied at listing, was also impacted by recreational activities.

Since listing, Vincent Gulch has been identified as an additional site experiencing recreational pressure as there is a trail crossing through the stream, increasing use at that location (N. Sill, USFS 2011, pers. comm.). The USFS implements management and close monitoring of recreational pressure at many locations, minimizing the level of this impact substantially since listing. For example, threats associated with human presence (including fouling of the water with human waste, disturbance, and trampling of progeny and adults) led the USFS to close Williamson’s Rock climbing area at Little Rock Creek in December of 2005, substantially reducing or eliminating this impact to the habitat. Since approximately 2008, following the closure of this area to recreation and downstream trout removal efforts, the southern *Rana muscosa* population has increased at Little Rock Creek.

East Fork City Creek was one of the largest populations of southern *Rana muscosa* at listing, and the only known extant population in the San Bernardino Mountains, elevating its conservation value. Therefore, though recreational impacts were considered minimal at this location, the USFS took steps to address this concern by closing this area to the public from February to

October annually (USFS 2011, p. 1). East Fork City Creek has received little recreation during the summer months since this closure went into effect in 2006 (K. Meyer, USFS 2011a, pers. comm.).

At Fuller Mill Creek the USFS removed picnic tables and barbeque pits near the creek and prohibits recreational use in the water. At Dark Canyon, the USFS removed camp sites adjacent to the creek and also prohibits recreational use in the water at this location. The USFS monitors creek closures and surveys the habitat condition during the breeding season at both Fuller Mill Creek and Dark Canyon. The installation of interpretive educational signage and increased communication with recreationalists has also been beneficial at these locations (A. Poopatanapong 2011, pers. comm.). These areas receive visits from many of the same members of the public annually, increasing the effectiveness of the education outreach (Poopatanapong 2011, pers. comm.). To create additional connectivity of protected habitat in the headwaters of Fuller Mill Creek, the USFS purchased approximately 24 hectares (ha) (60 acres (ac)) of land that was within private inholdings within the San Bernardino National Forest boundary. Camp sites remain open near Prairie Fork, an area occupied by southern *Rana muscosa* as recently as 1997 (USGS 1998, p. 3), although road access is limited to this area.

Suction dredge mining is a method of extracting minerals, commonly gold, from water bodies. Harvey (1986, p. 407) found that suction dredging may affect habitat suitability conditions for fish and invertebrates by altering substrates and drafting water away from a source. Potential impacts to the habitat include alteration of stream channel morphology, turbidity, sedimentation, and impacts to the benthic community. Direct impacts may also occur to southern *Rana muscosa*, including behavioral disturbances, physical entrainment or excavation, and exposure to contamination (toxicological effects). At listing, recreational suction dredge mining for gold occurred in at least one area occupied by southern *R. muscosa* (Bear Gulch), as well as a portion of the nearby East Fork San Gabriel River, which was occupied as recently as 1998 (USGS 1999, p. 3). Both waterways are within the Sheep Mountain Wilderness Area.

The CDFG currently enforces a moratorium on suction dredge mining (CDFG 2011a, p.1), though illegal mining continues to be a problem in the more accessible parts of East Fork San Gabriel River (Sill 2011, pers. comm.). Miners may be causing significant habitat destruction in this area and other accessible reaches of the Sheep Mountain Wilderness because portions of the stream are excavated in search for gold (Sill 2011, pers. comm.).

The USFS is implementing some protective measures at Little Rock Creek, East Fork City Creek, Fuller Mill Creek, Dark Canyon, and Prairie Fork. Although protective measures do not occur at Devil's Canyon, South Fork Big Rock Creek, and Bear Gulch, recreation is no longer considered a predominant threat at these locations (Sill 2011, pers. comm.). Currently, recreational impacts are a concern at three of the nine localities known to support southern *Rana muscosa*, including: Vincent Gulch, Dark Canyon, and Fuller Mill Creek.

Illegal Marijuana Plantations

Although not identified as a threat to the habitat at listing, illegal marijuana plantations have many potentially negative impacts to southern *Rana muscosa* habitat. Cultivation sites often have terracing which involves ground disturbance, water diversions, native vegetation removal, all of which may result in riparian habitat degradation, weed infestations, increased sedimentation, and reduced water quality and quantity (Sill 2011, pers. comm.). There is also the potential for contamination associated with pesticide and fertilizer use as these chemicals are routinely found at cultivation sites (Sill 2011, pers. comm.); impacts associated with contaminants will be discussed below under **Factor E**. Though secondary to loss of habitat, direct injury or mortality to southern *R. muscosa* can also occur through the displacement of egg masses when growers walk through waterways, suction of individuals into water diversion pipes, physically stepping on individuals, or exposing animals to lethal levels of contamination.

The presence of illegal marijuana plantations in the forest has impeded the ability to monitor southern *Rana muscosa* habitat to search for additional populations in remote areas on USFS lands. In 2009 and 2011, the Devil's Canyon population had a grow site adjacent to the stream. Piping for water diversion ran through the creek and drew water to plants planted in large holes filled with fertilizer on the slopes of the creek (Backlin 2012, pers. comm.). Thus, this site may have been subject to some of the impacts described. The cultivation site at Devil's Canyon appears to have been abandoned and impacts from pesticides were not observed here; however, no tests for pesticide contamination have been conducted (Sill 2011, pers. comm.). Marijuana cultivation also occurred at Bear Gulch in 2009 and a tributary of Vincent Gulch in recent years (Backlin 2012, pers. comm.). City Creek (East Fork and West Fork) has had multiple marijuana plantations between 2001 and 2011 (Backlin 2012, pers. comm.). Therefore, since listing illegal marijuana cultivation has impacted the habitat at four localities supporting southern *R. muscosa*: Devil's Canyon, Bear Gulch, Vincent Gulch, and City Creek.

Impacts from Road Construction and Maintenance

The CAS (USFS 2002, pp. 25–30; Appendix 1–9) outlined required actions to work with Caltrans to promote awareness and understanding of the impacts from road construction and maintenance, including toxic materials spills into southern *Rana muscosa* habitat, and to develop an action plan for prevention, notification, and containment of spills before they enter creeks or tributaries, particularly at the following locations: 1) State Route 2 above Little Rock Creek; 2) Highway 330 above East Fork City Creek; and 3) Highway 243 above Dark Canyon and Fuller Mill Creek. Though an action plan has not been developed, the USFS has made efforts to inform Caltrans workers regarding this issue.

The dumping of trash and toxic materials (soap, motor oil, and mercury), which can degrade water quality and cause adverse effects to eggs and developing tadpoles, was known to occur in the East Fork San Gabriel River (occupied in the 1990s) (Jennings 1995, p. 5) and was identified as a threat to the habitat at listing (USFWS 2002, p. 44387). Though disposal of toxic materials has not occurred in southern *Rana muscosa* habitat since listing, other spills associated with roadwork (sedimentation from construction sites) have impacted occupied habitat on at least three separate occasions during road repair work on State Route 2 in the San Gabriel Mountains, and Highway 330 in the San Bernardino Mountains. In 2009, a spill involving spoil piles (excess soil from an excavation) stored along Highway 330 resulted in soil entering into Schenk Creek, which enters East Fork City Creek, though the extent of the effects to southern *R. muscosa* and its habitat could not be determined (Meyer 2011b, pers. comm.). In 2010, a large culvert failure above Schenk Creek required the initiation of a slope repair project. Established Best Management Practices (BMPs) were not implemented, allowing a large amount of silt from the construction site to become transported from a tributary into Schenk Creek, and to enter the preferred pool-riffle habitat of southern *R. muscosa* more than 500 m (1,640 ft) downstream (Meyer 2011b, pers. comm.). In June 2011, in response to a landslide, emergency road repair work occurred along a segment of State Route 2 above Little Rock Creek without the use of silt fencing. An occupied portion of Little Rock Creek was negatively affected by sediment and southern *R. muscosa* individuals were found in impacted waters.

Repeated impacts to southern *Rana muscosa* habitat as a result of road work activity demonstrate that continued management is needed to prevent impacts in the future. As outlined in the 2002 CAS, this includes measures to improve communication between the USFS and road workers, and continue to implement defined BMPs. As a result of the recent impacts to habitat at Little Rock Creek, Caltrans has agreed to post materials provided by the USFS regarding sensitive species and habitats in maintenance yards. In addition, the USFS will consider whether roadside markers might be beneficial to identify sensitive habitat to help prevent future impacts on the Angeles National Forest (S. Brown, USFWS 2011, pers. obs.). Similar measures are needed to address impacts at occurrences in the San Bernardino National Forest.

Wildfire

Wildfire is a natural occurrence across California, although historically significant fires (greater than 40,000 ha (100,000 ac)) have increased in frequency during the last century. Of the 20 largest wildfires recorded in California since 1932, 12 occurred between 1999 and 2009 (CalFire 2009, p. 1). In southern *Rana muscosa* habitat, wildfire can reduce or eliminate riparian vegetation; increase water temperature through shade reduction; increase sedimentation, flooding, and debris in waterways; eliminate refugia; and alter stream channel morphology. In some systems, fire is thought to be important in maintaining open aquatic and riparian habitats for amphibians (Russel *et al.* 1999, p. 378). Amphibians display adaptive behavior that may minimize mortality from fire, by taking cover in wet habitats or taking shelter in subterranean burrows, though the moist and permeable skin of amphibians increases their susceptibility to heat and desiccation (Russell *et al.* 1999, p. 374). Severe and intense wildfires may reduce the ability of amphibians to survive the extensive habitat impacts of such a fire. Southern *R. muscosa* is likely to be extra sensitive to stream alterations from wildfire due to its highly aquatic nature.

Prior to listing, high fuel loads were identified as high wildfire risks in the watersheds of Hall Canyon (unoccupied at listing), East Fork City Creek, Dark Canyon, Fuller Mill Creek (the last two occupy the same watershed, and all four are within the San Bernardino National Forest) (USFS 2002, pp. 25–30). The listing rule identified East Fork City Creek and Fuller Mill Creek to be at risk of wildfire (USFWS 2002, p. 44387). The USFS initiated development of a fuel reductions plan for East Fork City Creek; however, prior to the completion of this plan two fires occurred in this watershed in 2003. This population declined substantially as a result of severe habitat alterations associated with the larger of the two fires (the Old Fire), which burned approximately 37,000 ha (91,000 ac). The habitat in East Fork City Creek has been recovering naturally over the past several years and the area now appears visually to be suitable for southern *R. muscosa* (Meyer 2011a, pers. comm.; R. Taylor, USFS 2011, pers. comm.). However, because no other southern *R. muscosa* populations exist nearby, this area will not be recolonized naturally and will require either reintroductions or translocations to fully recover.

No other occupied habitat identified as having high fuel loads in the San Bernardino National Forest has burned since listing (i.e., Dark Canyon, Fuller Mill Creek, and Hall Canyon). The USFS initiated the North Fork Fuel Reductions Project to address the high fuel levels and high wildfire concern within the watershed (affecting Dark Canyon and Fuller Mill Creek). Full implementation of this plan has not occurred and budget constraints may prevent this in the near term (Poopatanapong 2011, pers. comm.). Thus, a high wildfire risk remains at Fuller Mill Creek and Dark Canyon. Hall Canyon occurs within the James Reserve, part of the University of California Reserve System within the San Bernardino National Forest; the James Reserve does not manage the fuel load or fire risk in the Reserve area.

Wildfire was not identified as a risk on the Angeles National Forest at listing. In 2009, the largest fire in Los Angeles County history (the Station Fire) burned the entire watershed at Devil's Canyon, prompting the third emergency salvage of tadpoles. All extant populations in the San Gabriel Mountains, with the possible exception of Devil's Canyon, are at risk of wildfire because these populations are within wilderness areas, where fuel loads are not actively managed (Sill 2011, pers. comm.). The USFS is currently preparing a fuels management plan; however, they do not implement fuel reduction projects in wilderness areas unless there is a community within or adjacent to the Wilderness Area (Sill 2011, pers. comm.).

The California Department of Forestry and Fire Protection (CalFire) developed a rating of wildfire threat based on the combination of potential fire behavior (fuel rank) and expected fire frequency (fire rotation) to create a four-class risk index (extreme, very high, high, and moderate) (CalFire 2005, p. 1). The majority of southern *Rana muscosa* occupied and unoccupied habitat falls within the "extreme risk" category, emphasizing the significance of this threat rangewide.

Fire Management Activities

Although not described as a threat at listing, fire management activities (particularly former fire suppression policies) changed the forest structure and conditions, resulting in increased fuel loads and the risk of high intensity wildfire (McKelvey *et al.* 1996, pp. 1034–1035). Fire management activities also have the potential to impact southern *Rana muscosa* habitat during fire-fighting events, including: water drafting from occupied streams, resulting in direct mortality or rendering the habitat unsuitable for reproduction and survivorship; construction of fuel breaks, potentially resulting in erosion and siltation of habitat; fire suppression with water applications or fire retardants; and increased human activity in the area, potentially altering streamside habitat or disrupting southern *R. muscosa* behavior. Long-term impacts may result from the use of fire retardant chemicals, the effects of which will be discussed in the section titled Contaminants under **Factor E**. Due to the significant rangewide wildfire risk to occupied and unoccupied southern *R. muscosa* habitat, fire management activities will likely continue to be utilized in the future. However, activities occurring in response to a wildfire may cause minimal or short-term impacts compared to the effects of a large wildfire.

Nonnative Plants

Nonnative plants are present in the habitat of southern *Rana muscosa* at City Creek. These include *Tamarix aphylla* (tamarisk), *Spartium junceum* (Spanish broom), and *Ricinus communis* (castor bean). Tamarisk significantly reduces or eliminates the standing water and it grows to thick, often impenetrable stands (Sanchez 1975, p. 12; Lovich *et al.* 1994, p. 168). The rapid reproductive and dispersal rates of tamarisk allow it to outcompete native plant species. The USFS and Caltrans have cut and removed tamarisk from this site but herbicide application is likely needed for successful removal (Meyer 2011b, pers. comm.). Spanish broom and castor bean also alter the habitat by outcompeting native plants for resources. Spanish broom occurs along Highway 330, 18, and 38. Herbicide application was approved to remove Spanish broom within 30 m (100 ft) of the highways and a buffer area was established to protect aquatic species in the creeks from exposure to herbicides (Meyer 2011b, pers. comm.). This precludes the removal of Spanish broom from City Creek using herbicide application. Castor bean is also hand pulled from this location when possible (Meyer 2011b, pers. comm.). Nonnative plants are not substantially altering the habitat at any other occupied sites. The potential impacts associated with herbicides will be discussed further under the section titled Contaminants under **Factor E**.

Summary of Factor A

The USFS has protected and managed the majority of southern *Rana muscosa* habitat since before listing. Recreational activities continue to impact habitat at three of nine southern *R. muscosa* populations (Vincent Gulch, Dark Canyon, and Fuller Mill Creek). Illegal marijuana cultivation has impacted four occupied sites since listing (Devil’s Canyon, Bear Gulch, Vincent Gulch, and City Creek). Repeated impacts from roadwork activities (sedimentation, contamination, and introduction of invasive plant species) have continued in two occupied southern *R. muscosa* occurrences. Long-term fire suppression caused the increase of fuel loads and wildfire risk rangewide. Fire thoroughly burned two occupied sites (Devil's Canyon and East Fork City Creek) resulting in a decline of *R. muscosa* at City Creek. The remaining seven *R. muscosa* populations remains at an extreme risk of fire and could be exposed to impacts from fire management activities in the future. This is the most significant risk to the habitat of southern *R. muscosa*.

Impacts to the habitat (negative and positive) have resulted from management decisions, such as opening or closing areas to recreation, including suction dredge mining; implementing BMPs during road work; and suppressing fire over time, and implementing fire management activities. Though all southern *Rana muscosa* populations are at risk from habitat threats, most of these threats are largely controllable.

FACTOR B: Overutilization for Commercial, Recreational, Scientific, or Educational Purposes

As noted in the listing rule, numerous museum specimens from many localities document that southern *Rana muscosa* were collected for scientific purposes for decades (Jennings and Hayes 1994b, pp. 74–78). Overutilization for any purpose was identified as a potential threat to the listed entity, although it was thought that scientific collecting was not likely to be authorized due to the precipitous decline of the DPS (USFWS 2002, p. 44388).

Overutilization is not a threat at this time. Since listing, authorized collecting of southern *Rana muscosa* took place during four emergency salvages. Due to the uncertainties related to the very small sizes of most southern *R. muscosa* populations, future collection of individuals may be necessary to assist the captive breeding and augmentation program or to prevent loss of individuals that might otherwise perish in the wild (i.e., in drying pools). Long-term recovery of this DPS may require breeding between populations in captivity to increase genetic robustness of bottlenecked or inbred populations. Therefore, additional collection of individuals may be necessary.

Scientific research may cause stress to mountain yellow-legged frogs through disturbance, including disruption of the species' behavior, handling individuals, and injuries associated with marking and tracking individuals. Of greater concern is the possibility that researchers may be contributing to the spread of pathogens via clothing and sampling equipment as they move between water bodies and populations (Bradford *et al.* 1994a, p. 326; Fellers *et al.* 2001, p. 952). Given the uncertainty surrounding the potential for researchers to contribute to the spread of pathogens, equipment sterilization procedures are implemented between survey sites (Backlin 2011c, pp. 1–2). For further discussion concerning the threat of disease, see **Factor C** below.

FACTOR C: Disease or Predation

Predation

Native predators of mountain yellow-legged frogs include the two-striped garter snake (*Thamnophis hammondii*), Brewer's blackbird (*Euphagus cyanocephalus*), Clark's nutcrackers (*Nucifraga columbiana*), raccoons (*Procyon lotor*), and coyotes (*Canis latrans*) (Mullally and Cunningham 1956, p. 197; Jennings *et al.* 1992, p. 503; Matthews *et al.* 2002, p. 16). These species, which may be heavily dependent on mountain yellow-legged frog as a food source, are not likely to have contributed to the decline of the DPS. Rather, the decline of mountain yellow-legged frogs has been shown to adversely impact native predators dependent upon the frogs as a food source in the Sierra Nevada (Matthews *et al.* 2002, p. 16). Garter snakes feed extensively on mountain yellow-legged frogs and are commonly found near large numbers of tadpoles (Jennings *et al.* 1992, p. 503). A study performed by Matthew *et al.* (2002, p. 16) supported the hypothesis that the presence of amphibians is a prerequisite for garter snake persistence in high elevation portions of the Sierra Nevada, and that the introduction of trout can have serious effects on snake prey and other predators in the ecosystem. Similar research has not been performed in southern California; however, given the former widespread abundance of mountain yellow-legged frogs throughout its historical range, an impact on predator-prey interactions has almost certainly occurred.

At the time of listing, predation by nonnative fish (rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*)) was thought to be the principal cause of the decline of southern *Rana muscosa* from most of its previous historical range. Most research supporting this hypothesis was focused on the interactions between trout and mountain yellow-legged frogs in the Sierra

Nevada, although occurrence data (distribution and abundance) suggested the same interactions were taking place in southern California. Since listing, research has shown that the interaction between nonnative trout and southern *R. muscosa* is extremely detrimental to southern *R. muscosa* and the two taxa cannot co-occur indefinitely. Nonnative bullfrogs (*R. catesbeiana*), which were considered a threat to most western frogs (Kiesecker and Blaustein 1998, p. 776), were also a predatory concern at listing. Bullfrogs do not co-occur with southern *R. muscosa* at any localities and are no longer considered an imminent threat.

Nonnative Trout

Careful study of the interactions between nonnative trout and mountain yellow-legged frogs has shown conclusively that trout have negative impacts on the frogs (Bradford 1989, p. 777; Bradford *et al.* 1993, pp. 882–888; Knapp 1994, p. 3; Knapp 1996, pp. 13–15; Knapp and Matthews 2000, p. 428). Bradford (1989, p. 775) and Bradford *et al.* (1993, p. 886) concluded that introduced trout eliminate many populations of mountain yellow-legged frogs through the consumption of eggs and tadpoles, the latter likely being the principal dispersing lifestage of southern *R. muscosa*. In both the Sierra Nevada and southern California, the distribution of trout and mountain yellow-legged frog illustrates that the two rarely co-occur in the same stream reach. Once introduced, trout will reduce or eliminate mountain yellow-legged frog populations and then occupy the intervening habitat between populations that persist without trout (Grinnell and Storer 1924, p. 664; Mullally and Cunningham 1956, p. 190; Cory 1962a, p. 401, 1963, p. 172; Bradford 1989, pp. 775–777; Bradford and Gordon 1992, p. 65; Bradford *et al.* 1993, pp. 882–888, 1994a, p. 326; Drost and Fellers 1996, p. 422; Jennings 1996, p. 940; Knapp 1996, pp. 13–15; Knapp and Matthews 2000, p. 428; Knapp *et al.* 2001, p. 401; Vredenburg 2004, p. 7649). Bradford *et al. (*1993, p. 886) concluded that the presence of trout in intervening waterways sufficiently isolates other frog populations so that recolonization after local extirpations is likely impossible. This pattern of occupancy has relegated mountain yellow-legged frogs to the less preferable, marginal habitat (Knapp and Matthews 2000, p. 436).

Knapp and Matthews (2000, p. 436) suggested that mountain yellow-legged frog populations co-occurring with trout generally represent ‘‘sink’’ populations (a population in which the growth rate is negative in the absence of immigration). Such a population will eventually decline to the point of extirpation. Persistence of these frog populations is likely dependent on immigration from “source” populations (Bradford *et al.* 1998, p. 2489; Knapp and Matthews 2000, p. 436). USGS (2004, p. 20) found that adults have the longest migratory and dispersal movements immediately after emerging from hibernation in the spring, likely travelling to search for a mate. This indicates that movement from “source populations” into new areas is intended to be followed by reproductive events, which should they occur in trout-occupied waters, would probably be unsuccessful as a result of predation. Additionally, the long larval stage of southern *Rana muscosa* increases its susceptibility to predation. Thus, southern *R. muscosa* has a limited ability to successfully recruit outside the isolated habitat it currently occupies. Such isolation and fragmentation of the habitat has severed the metapopulation structure and increased the vulnerability of each population to extirpation from random events (such as fire or flood) compared to large, unfragmented metapopulations (Wilcox 1980, pp. 114–115; Bradford *et al.* 1993, p. 887; Hanski and Simberloff 1997, p. 21; Knapp and Matthews 2000, p. 436). Such an effect is thought to have occurred after the Old Fire at East Fork City Creek in 2003.

Furthermore, the physical isolation of these very small populations has also increased the potential for inbreeding. Therefore, nonnative trout have substantial impacts on the metapopulation structure of southern *R. muscosa*.

Trout stocking

Nonnative trout were introduced into the habitat of southern *Rana muscosa* from 1940s to the late 1990s, during which time CDFG routinely stocked at least 115 waterways for recreational fishing. These waterways included Little Rock Creek, City Creek, Dark Canyon, and Fuller Mill Creek (USFWS 2002, p. 44388; USGS 2004, pp. 87–89). These actions likely contributed to the widespread occupancy of trout in the San Gabriel, San Bernardino, and San Jacinto Mountains.

Surveys performed by USGS in 2001 and 2002 identified nonnative trout immediately downstream from southern *Rana muscosa* at five of the seven localities extant at that time (Little Rock Creek, South Fork Big Rock Creek, Vincent Gulch, and Bear Gulch in the San Gabriel Mountains; Fuller Mill Creek in the San Jacinto Mountains). In 2002, all remaining southern *R. muscosa* populations were known to be in the small headwater sections of streams where barriers restricted upstream movement of trout (USGS 2002a, p. 5; USFWS 2002, p. 44388); however, trout were not found downstream at East Fork City Creek or at Devil’s Canyon.

Since listing, surveys performed by USGS (from 2002 to 2010) identified nonnative trout downstream of southern *Rana muscosa* in eight of nine extant localities (Devil’s Canyon, Little Rock Creek, South Fork Big Rock Creek, Vincent Gulch, Bear Gulch, City Creek, Fuller Mill Creek, and Tahquitz-Willow Creek) (USGS 2003–2010). However, USGS biologists have not observed trout immediately downstream of frogs at Little Rock Creek since 2009, after an extensive trout removal project; or at City Creek since 2003, when the entire watershed burned thoroughly (USGS 2004, p. 96). Trout were not observed during recent surveys at Devil’s Canyon in 2011 (USGS 2012, p. 3); they may have been extirpated from this site after the 2009 Station Fire. Trout are thought to occur in almost all waterways with perennial water in the San Jacinto Mountains (M. Giusti, CDFG, 2012, pers. comm.). Some are potentially native steelhead trout, although a genetic analysis has not occurred (Giusti 2012, pers. comm.). Trout currently occupy habitat downstream of southern *R. muscosa* at five of nine occupied sites (South Fork Big Rock Creek, Bear Gulch, Vincent Gulch, Fuller Mill Creek, and Dark Canyon), and upstream at one site (Tahquitz-Willow Creek) (Backlin 2012 pers. comm.). However, at Vincent Gulch there is an approximate half-mile extent of stream available above a trout barrier and below the southern *R. muscosa* population where frogs do not occur (USGS 2011a, p. 6). The reasoning for this is unknown. All nine extant localities remain isolated in fishless headwaters of tributaries (Backlin 2012, pers. comm.).

CDFG has ceased trout stocking in all localities currently occupied by mountain yellow-legged frog rangewide. No new areas have been stocked in the San Gabriel Mountains since 1998, although other locations continue to be stocked in the San Gabriel Mountains outside the historical range of southern *Rana muscosa* (J. O’Brien, CDFG, 2012, pers. comm.). Trout stocking continues in the San Jacinto Mountains, including at Lake Fulmor, a high use recreational fishing area downstream of the Hall Canyon re-establishment site (Giusti 2012, pers. comm.). Stocking at Lake Fulmor resumed with concurrence from the Service in 2010 (USFWS

2010, p. 1). CDFG plans to convert all fish stock to triploid (sterile) rainbow trout by 2013 unless there is an unforeseen need to use diploid (reproductively sound) stock (Giusti 2012, pers. comm.; O'Brien 2012, pers. comm.). This may be a beneficial action, as it will prevent the massive reproductive capabilities of trout from coming to fruition after future stocking. However, a major concern remains that trout are adept at upstream dispersal where no barrier impedes their movement; therefore, any reach they can disperse into has the potential to remain occupied for the lifespan of the individual. Movement of trout between water bodies by anglers is also thought to have curtailed substantially in the San Jacinto Mountains (Giusti 2012, pers. comm.); however, education and outreach to the angler community should occur to prevent reintroductions in areas restored to fishless conditions (Meyer 2011b, pers. comm.).

Trout removal

Trout-induced declines of the mountain yellow-legged frog may be reversed in some locations with an intensive and focused effort to restore fishless conditions (Knapp and Matthews 1998, p. 207; 2000, p. 437; Knapp *et al.* 2001, p. 418; Knapp *et al.* 2007, p. 17). At Little Rock Creek, CDFG led a trout removal effort between two trout barriers immediately downstream of the southern *Rana muscosa* population. Movement of adults out of the isolated headwaters into the fish removal area and recruitment of young in the trout removal reach has slowly occurred. A rapid response was not expected due to the small population available for recolonization (possibly less than 10 adults), the steep and complex topography of the area which could cause a challenge for migration (USGS 2004, p. 24), and *Bd* presence in the area, which likely diminishes recruitment rates. Trout removal continued almost annually at Little Rock Creek until 2010, at which time trout appeared to be successfully eradicated between the two barriers. From 2005 until 2011, the number of southern *R. muscosa* found within the trout removal reach continually increased. Tadpoles were first detected in the area in 2008 (USGS 2008a, p. 10), and approximately eight adults occupied the trout removal area in 2010 (USGS 2011b, p. 8). Little Rock Creek is now the largest southern *R. muscosa* population and the length of the occupied area supports all lifestages.

Since 2009, CDFG has also taken the lead on trout removal in the San Jacinto Mountains. Trout removal has occurred between the Fuller Mill Creek and Dark Canyon populations with the help of volunteers and partners from the USFS, Western Riverside County Multiple Species Habitat Conservation Plan (MSHCP), and the Service. Southern *Rana muscosa* has yet to recolonize the intervening area; however, the population at Dark Canyon does appear to be slowly increasing (Backlin 2012, pers. comm.). Additionally, the population at Fuller Mill Creek is very small, which will probably slow recolonization, and portions of the creek become dry in the summer, potentially inhibiting survival of tadpoles after dispersing downstream (Giusti 2012, pers. comm.). In 2012, Tahquitz Creek will be evaluated for future trout removal (Giusti 2012, pers. comm.).

In southern California, trout removal efforts in the immediate future should focus on areas adjacent to existing populations in order to aid survivorship of individuals naturally dispersed downstream and thus facilitate the expansion of these populations. Trout removal near existing populations can also provide opportunities for augmentation of very small populations, such as Bear and Vincent Gulch, using either individuals bred in captivity or individuals translocated from other populations. In the long term, trout removal between existing populations will allow these populations to re-establish connectivity and increase genetic interchange. Expanding the number of existing southern *Rana muscosa* populations is vital to the long-term recovery of this DPS; therefore, it is essential that trout removal occur at many additional locations rangewide.

Summary of Predation

In summary, the widespread introduction of nonnative trout has undoubtedly contributed to the decline of southern *Rana muscosa*. This is a well-documented cause of decline of mountain yellow-legged frogs in the Sierra Nevada (Bradford 1989, pp. 775–778; Bradford *et al.* 1993, pp. 882–888; Knapp and Matthews 2000, p. 435). In summarizing the effects of nonnative fish on the mountain yellow-legged frog, it is important to recognize that:

1) The vast majority of mountain yellow-legged frog populations did not evolve with trout;
2) water bodies throughout the range of the mountain yellow-legged frog have been intensively stocked with nonnative trout by both CDFG and private citizens, and where stocking has been terminated, self-sustaining trout populations continue to persist;
3) the long larval stage of the mountain yellow-legged frog increases their susceptibility to predation by trout where they co-occur;
4) the introduction of nonnative trout has fragmented mountain yellow-legged frog habitat, isolated populations from each other, and generally restricted remaining frog populations to marginal habitats, thereby increasing the likelihood of localized extinctions without the possibility of recolonization.

Nonnative trout currently occupy downstream areas at five of nine southern *Rana muscosa* localities (South Fork Big Rock Creek, Bear Gulch, Vincent Gulch, Fuller Mill Creek, and Dark Canyon), and a meadow upstream of one locality (Tahquitz-Willow Creek). Nonnative trout removal has occurred at Little Rock Creek, Dark Canyon, and Fuller Mill Creek. These efforts appear to be critical in assisting the rebound of these populations. Trout removal is also being considered as a management tool at other localities occupied by southern *R. muscosa*. The decline of southern *R. muscosa* is no longer attributed to predation by bullfrogs or native predators. To the contrary, the widespread reduction of southern *R. muscosa* has almost certainly interrupted trophic interactions in its former habitat.

Disease

Global amphibian population declines and extinctions have been increasingly attributed to disease (Bradford 1991, pp. 174–177; Blaustein *et al.* 1994, pp. 251–254; Muths, *et al.* 2003, p. 357; Weldon *et al.* 2004, p. 2100; Rachowicz *et al.* 2005, p. 1446). Various pathogens including fungi, ranavirus, iridovirus, and bacteria have been isolated from infected individuals. The most notable is the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). Although mentioned as a potential concern in the listing rule, *Bd*, which was found to infect mountain yellow-legged frogs in the Sierra Nevada (Fellers *et al.* 2001, p. 945), had not yet been detected in southern California. At listing, nothing indicated that disease was responsible for the precipitous decline of the listed entity. Since listing, all populations have tested positive for *Bd*. No retrospective analysis of museum specimens has occurred to identify when *Bd* was introduced into southern *Rana muscosa* habitat and to help determine the degree to which it has contributed to the historical rangewide decline. No other pathogens of serious concern (ranavirus, iridovirus, bacteria, or fungi) have been identified on southern *R. muscosa* in the wild.

Chytridiomycosis

Effects of *Bd* on host populations of the mountain yellow-legged frog are variable, ranging from extinction to persistence with a high level of infection to persistence with low levels of infection (Briggs *et al.* 2010, p. 9696; Vredenburg *et al*. 2010, pp. 9689–9694). In southern California, all southern *Rana muscosa* populations appear to be persisting with low levels of *Bd* infection. Although positive identification of *Bd* on southern *Rana muscosa* did not occur until 2007 (USGS 2007b, p. 1; USGS 2007c, p. 1), all populations have now tested positive with results dating back to 2001 (Backlin 2012, pers. comm.). However, only 6 percent of southern *R. muscosa* adults are infected rangewide (Backlin 2012, pers. comm.). This low infection rate is true for all other anurans in southern California (1 to 12 percent) with *Pseudacris cadaverina* having a 1 percent infection rate (Backlin 2012, pers. comm.).

In the Sierra Nevada, *Bd* has been observed to result in overwinter mortality and mortality during metamorphosis of mountain yellow-legged frogs (Rachowicz *et al*. 2006, p. 1671). Past research has shown that this pathogenic fungus is widely distributed throughout the Sierra Nevada, and that infected mountain yellow-legged frogs die soon after metamorphosis in that region (Rachowicz *et al.* 2006, p. 1671). The USGS, which has been testing southern *Rana muscosa* for *Bd* since 2000, has found that post-metamorphic individuals (juveniles) represent a missing age class in most populations. The most probable cause for this is infection from *Bd*, which is known to have the greatest impact at this lifestage.

The infection rate of southern *Rana muscosa* may be higher than currently understood because not all individuals detected in the field are tested for *Bd*. Although it appears that southern *R. muscosa* populations (adults) are infected at low rates, and that the juvenile age class may be succumbing to disease, additional information regarding infection rates and intensities for other age classes is needed. This may be the most significant stressor to southern *R. muscosa* because it affects all extant populations, is likely hindering recruitment, and will have a significant impact on animals released from captive biosecure conditions.

Batrachochytrium dendrobatidis infects the keratinized (outermost epidermal) tissue on amphibians. This tissue first develops in the mouthparts of larvae, potentially causing depigmentation (Fellers *et al*. 2001, p. 945; Rachowicz and Vrendenburg 2004, p. 78) and defects that can affect feeding capabilities. Tadpoles of ranids are not typically susceptible to disease from *Bd* because they have a minimal amount of keratin on which to focus infection. Therefore, *Bd* infection in larval ranids usually has no effect on activity or mortality (Andre *et al.* 2008, p. 716); however, this type of infection may provide a method for autoinfection (whereby zoospores released from the skin reinfect the host) once metamorphosis occurs (Marantelli *et al.* 2004, p. 178). As tadpoles metamorphose the remainder of the body becomes keratinized and infection across the body becomes possible; this is usually concentrated on the ventral side (abdomen, digits, and pelvic “drink patch”) (Berger *et al.* 1998, p. 9034). The skin on infected individuals becomes thicker (hyperkeratosis) and will slough off in stages (Berger *et al.* 1998,

p. 9034). Sloughing may hinder sampling detection depending on what stage of infection samples are taken, thereby potentially producing false negatives or artificially low infection intensities. In heavy infections (10,000 zoospores) osmotic regulation becomes increasingly compromised and electrolyte blood levels drop, causing death from cardiac arrest (Voyles *et al.* 2009, p. 582; Vredenburg *et al.* 2010, p. 9691).

For some species and populations *Bd* has been a highly virulent pathogen, yet in others it has caused only light infections (Lips *et al.* 2006, pp. 3167–3168; Rachowicz *et al.* 2006, p. 1671). The variation in pathogenicity may be a reflection of both environmental factors (air and water temperature, water pH, and climate change) and host factors (behavioral adaptations and natural defenses). In culture, *Bd* achieves maximum growth in a wide pH range (4 to 8) and in the temperature range of 17 to 25°C (63 to 77°F), but can grow and reproduce at temperatures ranging from 4 to 25°C (39 to 77°F) (Johnson and Speare 2003, p. 185; Piotrowski *et al.* 2004, p. 9). Occupied streams in southern California are not known to freeze over in the winter, which may allow *Bd* to survive in overwintering hosts such as southern *Rana muscosa*. In the summer, the range of water temperatures recorded in occupied streams has been recorded from 9 to 30°C (48 to 86°F) (USGS 2004, p. 22), providing a wide temperature range for optimal growth of *Bd*.

Infection prevalence is highest during cool temperature periods (Berger *et al.* 2004, pp. 434–439; Woodhams and Alford 2005, p. 1449; Kriger and Hero 2007, p. 352). Pathogenicity decreases at temperatures greater than 29°C (84°F) (Longcore *et al.* 1999, p. 223). Exposure to high temperatures (27 to 37°C (81 to 99°F)) has been shown to clear other species of infection (Woodhams *et al.* 2003, p. 65; Berger *et al.* 2004, p. 434). Experimental infection of mountain yellow-legged frog tadpoles kept at 17°C (63°F) and 22°C (72°F) demonstrated that individuals kept at 22°C exhibited significantly lower mortality (50 percent) than those housed at 17°C (95 percent) (Andre *et al.* 2008, p. 716). The authors of this study suggested that because both temperatures are within the optimal range for growth of *Bd*, the difference in outcome reflects the effect of temperature on the host's resistance to *Bd*, rather than an effect on *Bd* alone (Andre *et al.* 2008, p. 716).

Although much remains unknown regarding the interaction between *Bd* and southern *Rana muscosa*, recent research on northern *R. muscosa* indicates that a strategy termed "bioaugmentation" may be an effective management tool to control chytridiomycosis in captive and wild populations (Harris *et al*. 2009, p. 1). This replicated experiment showed that adding an antifungal bacterial species, *Janthinobacterium lividum*, which occurs naturally on the skin of many species of amphibians including northern *R. muscosa*, to the skin of northern *R. muscosa* (at higher densities than it naturally occurs) prevented morbidity and mortality associated with *Bd*. In high densities, this bacterium produces an anti-*Bd* metabolite, violacein, which was strongly associated with the survival of frogs that were infected with *Bd* (Harris *et al*. 2009, p. 4). This research demonstrated that cutaneous microbes a part of the innate immune system of amphibians, that this microbial community on the frog skin is a determinant of disease outcome, and that altering the microbial interactions on frog skin can prevent a lethal disease outcome (Harris *et al*. 2009, p. 1). Field research in the Sierra Nevada supports these results (Vredenburg 2012, pers. comm.). Thus far, bioaugmentation has been focused on prevention of *Bd* infections, rather than treatment of animals infected with *Bd*. Given that the research was performed on *R. muscosa* in the Sierra Nevada, it may prove to be a useful tool in southern California for preventing infection on captive animals released into the wild, or potentially as a treatment to increase survivorship in wild populations.

Summary of Disease

In summary, the chytrid fungus, *Bd*, has been identified as having potentially catastrophic effects (localized extinction) on mountain yellow-legged frog populations. Populations in southern California have low infection rates, indicating that some adults are persisting and are likely capable of reproducing. The offspring of these individuals will likely be vulnerable to mortality caused by chytridiomycosis until they reach adulthood but are particularly susceptible immediately following metamorphosis. Therefore, while *Bd* poses a significant risk to the small and isolated populations, persistent individuals may be able to replenish these populations with time if enough survive to reproductive maturity. Additional information is needed regarding the effects of *Bd* on southern *Rana muscosa*, particularly with consideration of reintroduction, augmentation, and translocation efforts occurring. Other pathogens could have negative effects on southern *R. muscosa*, although they currently appear to have little to no impact on the wild populations.

Summary of Factor C

The widespread introduction of nonnative trout contributed to the decline of southern *Rana muscosa* through predation. All populations of southern *R. muscosa* are now isolated in marginal habitat in the headwaters of tributaries. Nonnative trout occupy the downstream waters (dispersal and migratory routes) at five of nine occupied localities (South Fork Big Rock Creek, Bear Gulch, Vincent Gulch, Fuller Mill Creek, and Dark Canyon) and upstream at one (Tahquitz-Willow Creek). Nonnative trout have already fragmented and reduced available habitat. They currently prevent recolonization of historically occupied areas, disrupting metapopulation dynamics, and as such, increase the vulnerability of southern *R. muscosa* to wildfire and flooding, and the likelihood of inbreeding. Trout removal efforts have seen beneficial results at two locations thus far (Little Rock Creek and Dark Canyon). At listing, the amphibian fungal pathogen, *Bd*, was not detected on any populations of southern *R. muscosa*. Currently, all populations are known to have *Bd*, although infection rates are low. This pathogen is likely to be exerting the greatest impact on the juvenile lifestage, and may ultimately be preventing the replacement of an aging adult population. Therefore, all populations are impacted by Factor C threats. Trout eradication must continue downstream of occupied areas as this effort has been the greatest tool available to increase the abundance of existing populations. Additional research is needed on the effects of *Bd* on southern *R. muscosa* and methods to ameliorate the effects should be considered.

FACTOR D: Inadequacy of Existing Regulatory Mechanisms

At the time of listing, regulatory mechanisms thought to have some potential to protect southern *Rana muscosa* included: 1) the California Environmental Quality Act (CEQA); 2) section 1603 of the California Department of Fish and Game Code (California Lake and Streambed Alteration Program); 3) the National Environmental Policy Act (NEPA); 4) section 404 of the Federal Clean Water Act; 5) local land use processes and ordinances; and 6) the Endangered Species Act in those cases where southern *R. muscosa* occurs in habitat occupied by a listed wildlife species. The listing rule (USFWS 2002, p. 44388) provides an analysis of the level of protection that was anticipated from those regulatory mechanisms. There are several State and Federal laws and regulations that were not described in the listing rule, but are pertinent to the conservation of southern *R. muscosa* in varying degrees. All such regulatory mechanisms are described below.

State Protections in California

The State's authority to conserve rare wildlife comprises three major pieces of legislation: California Endangered Species Act (CESA), CEQA, and the Natural Community Conservation Planning (NCCP) Act.

California Endangered Species Act (CESA)

The State of California was petitioned under CESA to list *Rana muscosa* in June 2010, and determined that the petitioned action may be warranted (CDFG 2010). In February 2012, the California Fish and Game Commission (CFGC) found that the petition to list *Rana muscosa* as an endangered species is warranted under CESA (CFGC 2012, p. 1). Although State regulations are yet to be formally amended, *Rana muscosa* is now afforded all of the same protections as a State-listed species (M. Lockhart, CDFG, 2012, pers. comm.). An amendment to Section 670.5, Title 14, California Code of Regulations, is expected in the summer of 2012 (Lockhart 2012, pers. comm.).

Under CESA, there are take restrictions for State-listed species, where take is defined as "to hunt, pursue, catch, capture, or kill, or attempt to hunt, pursue, catch, capture, or kill," and includes killing that is incidental to an otherwise lawful activity. It is unlawful to import or export, take, possess, purchase, or sell any species or part or product of any species listed as endangered or threatened under CESA. CESA requires State agencies to consult with the CDFG on activities that may affect a State-listed species and mitigate for any adverse impacts to the species or its habitat. The State may authorize permits for scientific, educational, or management purposes, and to allow take that is incidental to otherwise lawful activities. CESA (California Fish and Game Code, section 2080 *et seq*.) prohibits the unauthorized take of State-listed threatened or endangered species. However, sections 2081(b) and (c) of CESA allow the CDFG to issue incidental take permits for State-listed threatened and endangered species if:

1) Authorized take is incidental to an otherwise lawful activity;
2) impacts of the authorized take are minimized and fully mitigated;
3) measures required to minimize and fully mitigate the impacts of the authorized take are roughly proportional in extent to the impact of the taking on the species, maintain the applicant's objectives to the greatest extent possible, and are capable of successful implementation;
4) adequate funding is provided to implement the required minimization and mitigation measures, and to monitor compliance with and the effectiveness of the measures; and
5) issuance of the permit will not jeopardize the continued existence of a State-listed species.

Species of Special Concern

The State of California does consider *Rana muscosa* (rangewide) to be a "Species of Special Concern", an administrative designation assigned to focus attention, research, and conservation on at risk species prior to their meeting criteria for listing under CESA (Comrack *et al.* 2008, p. 1; CDFG 2011b, p. 35). This designation is given to a species, subspecies, or distinct population of an animal native to California that satisfies one or more of the following (not necessarily mutually exclusive criteria):

1) is extirpated from the State;
2) is federally, but not State-listed as threatened or endangered; meets the State definition of threatened or endangered but has not formally been listed;
3) is experiencing, or formally experienced, serious (noncyclical) population declines or range retractions (not reversed) that, if continued or resumed, could qualify it for State threatened or endangered status;
4) has naturally small populations exhibiting high susceptibility to risk from any factor(s), that if realized, could lead to declines that would qualify it for State threatened or endangered status.

The Species of Special Concern designation carries no formal legal status. The intent of designating this title is to focus attention on animals at conservation risk by CDFG, other State, local and Federal government entities, regulators, land managers, planners, consulting biologists, and others; stimulate research on poorly known species; and achieve conservation and recovery of these animals before they meet CESA criteria for listing as threatened or endangered. However, Species of Special Concern must be considered during the CEQA environmental review process.

California Sport Fishing Regulations do not include *Rana muscosa* as a species that may be taken or possessed (CDFG 2011c, p. 1). However, the protection afforded by this regulation does not address the threats to the DPS presented by factors related to sport fishing such as habitat alteration by anglers.

California Environmental Quality Act (CEQA)

CEQA is the principal statute mandating environmental assessment of projects in California. The purpose of CEQA is to evaluate whether a proposed project may have an adverse effect on the environment and, if so, to determine whether that effect can be reduced or eliminated by pursuing an alternative course of action or through mitigation. CEQA applies to projects proposed to be undertaken by, or requiring the approval of, State and local public agencies (*http://www.ceres.ca.gov/topic/env_law/ceqa/summary.html*). CEQA requires disclosure of potential environmental impacts and a determination of "significant" if a project has the potential to reduce the number or restrict the range of a rare or endangered plant or animal. However, projects may move forward if there is a statement of overriding consideration. If significant effects are identified, the lead agency has the option to require mitigation through changes in the project or decide that overriding considerations make mitigation infeasible (CEQA section 21002). In the latter case, projects may be approved that cause significant environmental damage, such as elimination of endangered species or their habitats. Protection of listed species through CEQA is, therefore, dependent upon the discretion of the lead agency involved. CEQA provides that, when overriding social and economic considerations can be demonstrated, project proposals may go forward, even in cases where the continued existence of the species may be threatened, or where adverse impacts are not mitigated to the point of insignificance.

Natural Community Conservation Planning (NCCP) Act

In 1991, the State of California passed the NCCP Act to address the conservation needs of natural ecosystems throughout the State (CFG 28002835). The NCCP program is a cooperative effort involving the State of California and numerous private and public partners to protect regional habitats and species. The primary objective of NCCPs is to conserve natural communities at the ecosystem scale, while accommodating compatible land uses. NCCPs help identify, and provide for, the regional or area-wide protection of plants, animals, and their habitats, while allowing compatible and appropriate economic activity. Many NCCPs are developed in conjunction with Habitat Conservation Plans (HCPs) prepared pursuant to the Act. The specific measures under each plan that afford protection to *R. muscosa* are discussed in the **Federal Protections** section below.

California Lake and Streambed Alteration Program (CDFG Code sections 1600–1616)

The Lake and Streambed Alteration Program (CDFG Code sections 1600–1616) may promote the recovery of listed species in some cases. This program provides a permitting process to reduce impacts to fish and wildlife from projects affecting important water resources of the State, including lakes, streams, and rivers. This program also recognizes the importance of riparian habitats to sustaining California's fish and wildlife resources, including listed species, and helps prevent the loss and degradation of riparian habitats. Therefore, potential projects that may substantially modify a river, stream, or lake would be evaluated and must comply with CEQA.

The California Porter-Cologne Act of 1969

The primary law regulating water quality in California is the California Porter-Cologne Act (CPCA) of 1969 (Section 13000 *et seq.,* California Water Code). The CPCA authorizes the State Water Resources Control Board to establish water quality standards and guidelines for resource planning, management, and enforcement for surface water, ground water, and wetlands. The CPCA establishes the nine Regional Water Quality Control Boards (Regional Boards) as the principal state agencies with the responsibility for controlling water quality at the local level in California. Occupied southern *Rana muscosa* habitat falls within the jurisdiction of four Regional Boards (Lahontan, Los Angeles, Santa Ana, and Colorado River). Regional Boards are responsible for preparing and updating Basin Plans (water quality control plans), each of which establishes: 1) beneficial uses of water designated for each protected water body; 2) water quality standards for both surface and groundwater; and, 3) actions necessary to maintain these standards to control non-point and point sources of pollution to waters. One of many identified beneficial uses of protected waters is the designation as "RARE", defined as "uses of water that support habitats necessary, at least in part, for the survival and successful maintenance of plant or animal species established under State or Federal law as rare, threatened, or endangered."

Regional Boards are required to protect the designated beneficial uses of waterbodies in their decisionmaking, including issuance of National Pollutant Discharge Elimination System permits. Therefore, those waterbodies known to harbor federally or state-listed threatened or endangered species should be maintained such that the waterbodies are capable of supporting the survival and recovery of those species. We note that none of the waterways supporting southern *R. muscosa* have been designated as RARE water bodies. We will work with the Lahontan, Los Angeles, Santa Ana, and Colorado River Regional Water Quality Control Boards to have them recognize the occurrences of *R. muscosa* and, hence, designate the beneficial use of "RARE" for those waterbodies known to harbor *R. muscosa*. Therefore, CPCA provides an existing regulatory mechanism whereby water quality can be maintained to support the long-term survival and recovery of aquatic-dependent endangered species, including *R. muscosa.*

Federal Protections

National Environmental Policy Act (NEPA)

All Federal agencies are required to adhere to the NEPA of 1970 (42 U.S.C. 4321 *et seq.*) for projects they fund, authorize, or carry out. The Council on Environmental Quality's regulations for implementing NEPA state that agencies shall include a discussion on the environmental impacts of the various project alternatives (including the proposed action), any adverse environmental effects that cannot be avoided, and any irreversible or irretrievable commitments of resources involved (40 CFR part 1502). NEPA is a disclosure law, and does not require subsequent minimization or mitigation measures by the Federal agency involved. Although Federal agencies may include conservation measures for southern *Rana muscosa* as a result of the NEPA process, any such measures are typically voluntary in nature and are not required by the statute. NEPA does not itself regulate activities that might affect southern *R. muscosa,* but it does require full evaluation and disclosure of information regarding the effects of contemplated Federal actions on sensitive species and their habitats.

Endangered Species Act of 1973, as amended (Act)

At the time of listing, southern *Rana muscosa* was known to co-occur with the federally listed arroyo toad (*Anaxyrus californicus* (formerly *Bufo californicus*)) in the San Gabriel Mountains. However, this benefit has always been limited due to the difference in habitat types utilized and areas occupied (USFWS 2002, p. 44389). Furthermore, the two species were not known to co-occur in the other mountain ranges occupied by southern *R. muscosa*.

The Act is the primary Federal law that provides protection for southern *Rana muscosa*. The Service is responsible for administering the Act, including sections 7, 9, and 10. Section 7(a)(1) of the Act requires all Federal agencies to utilize their authorities in furtherance of the purposes of the Act by carrying out programs for the conservation of endangered and threatened species. Section 7(a)(2) requires Federal agencies to consult with the Service to ensure any project they fund, authorize, or carry out does not jeopardize a listed species. A jeopardy determination is made for a project that is reasonably expected, either directly or indirectly, to appreciably reduce the likelihood of both the survival and recovery of a listed species in the wild by reducing its reproduction, numbers, or distribution (50 CFR 402.02). A non-jeopardy opinion may include reasonable and prudent measures that minimize the amount or extent of incidental take of listed species associated with a project.

In 2006, critical habitat (3,352 ha (8,283 ac)) was designated in the San Gabriel, San Bernardino, and San Jacinto Mountains of southern California (USFWS 2006a, p. 54356). Since the designation of critical habitat, the Service has analyzed the potential effects of Federal projects under section 7(a)(2) of the Act, which requires Federal agencies to consult with the Service prior to authorizing, funding, or carrying out activities that may destroy or adversely modify areas designated as critical habitat.

Section 9 of the Act and Federal regulations pursuant to section 4(d) of the Act prohibit the "take" of federally listed wildlife. Section 9 of the Act prohibits the taking of any federally listed endangered or threatened species. Section 3(18) defines "take" to mean "to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct." Service regulations (50 CFR 17.3) define "harm" to include significant habitat modification or degradation which actually kills or injures wildlife by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering. Harassment is defined by the Service as an intentional or negligent action that creates the likelihood of injury to wildlife by annoying it to such an extent as to significantly disrupt normal behavioral patterns which include, but are not limited to, breeding, feeding, or sheltering. The Act provides for civil and criminal penalties for the unlawful taking of listed species. Under the terms of section 7(b)(4) and section 7(o)(2) of the Act, taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking under the Act provided that such taking is in compliance with the terms and conditions of an incidental take statement.

For projects without a Federal nexus that would likely result in incidental take of listed species, the Service may issue incidental take permits to non-Federal applicants pursuant to section 10(a)(1)(B). To qualify for an incidental take permit, applicants must develop, fund, and implement a Service-approved HCP that details measures to minimize and mitigate the project's adverse impacts to the listed species. Therefore, HCPs provide an additional layer of regulatory protection to plants as well as animals. The MSHCP is a large-scale, multi-jurisdictional HCP permitted under section 10(a)(1)(B) of the Act and is discussed below.

Western Riverside County Multiple Species Habitat Conservation Plan (MSHCP):

The MSHCP was permitted on June 22, 2004, and is a regional, multi-jurisdictional HCP encompassing about 510,000 ha (1.26 million ac) in western Riverside County. This Plan addresses 146 listed and unlisted ''covered species,'' including *Rana muscosa* (southern *R. muscosa*), and was designed to establish a multi-species conservation program that minimizes and mitigates the effects of expected habitat loss and associated incidental take of covered species. The MSHCP will establish approximately 61,917 ha (153,000 ac) of new conservation lands ("Additional Reserve Lands") to complement the approximately 140,426 ha (347,000 ac) of pre-existing natural and open space areas ("Public/Quasi-Public" (PQP) lands) to form the overall MSHCP Conservation Area over the 75-year permit period (USFWS 2004, p. 2).

Within the MSHCP, southern *Rana muscosa* have been observed only in the upper reaches and tributaries of the North Fork of the San Jacinto River, including Dark Canyon, Hall Canyon, Fuller Mill Creek, and the North Fork above Highway 74. Since 2004, the only locations in MSHCP known to support southern *R. muscosa* are Dark Canyon and Fuller Mill Creek. Dark Canyon is entirely on USFS lands, while Fuller Mill Creek is on land owned by the USFS, Riverside County, and private owners. Modeled southern *R. muscosa* habitat includes all portions of streams and habitat within 100 m (984 ft) of the streams, between 370 to 2,290 m (1,214 to 7,513 ft) in elevation, and within riparian scrub/woodland/forest, montane coniferous forests, woodlands and forest, and chaparral habitats. Modeled southern *R. muscosa* habitat within the MSHCP totals approximately 12,516 ha (30,927 ac), of which approximately 8,498 ha (21,001 ac) (68 percent) occur on PQP lands. The population at Dark Canyon is entirely on PQP lands, whereas the population at Fuller Mill Creek is on both private and PQP lands.

Outside of the MSHCP Conservation Area, an estimated 3,275 ha (8,094 ac (26 percent)) of southern *Rana muscosa* modeled habitat could be impacted or lost (USFWS 2004, p. 182). Development and modification of these acres may result in the loss of suitable and/or historically occupied habitat. Habitat fragmentation and degradation would result from urban development, water diversion/flood control projects, fill of aquatic habitat, construction projects, sand and gravel mining practices, recreation, and other urban and agricultural activities. However, the loss of additional southern *R. muscosa* populations outside the MSHCP Conservation Area is not anticipated (USFWS 2004, p. 182).

To offset the loss of southern *Rana muscosa* modeled habitat within the MSHCP, implementation of the MSHCP will conserve and manage areas containing modeled habitat for southern *R. muscosa* (USFWS 2004, p. 182). Core areas have been identified to be set aside and preserved within the MSHCP. The MSHCP proposes the San Jacinto Mountains Bioregion Core Area and the San Bernardino Mountains Bioregion to support southern *R. muscosa* within the MSHCP Conservation Area (Dudek and Asssociates 2003, p. A-51). In total, the MSHCP Conservation Area will include (9,241 ha) 22,834 ac (74 percent) of the total modeled habitat for southern *R. muscosa*. The MSHCP Conservation Area includes a total of 1,832 acres (6 percent) of Additional Reserve Lands and 21,001 acres (68 percent) of PQP Lands. Of the Additional Reserve Lands, 1,714 acres (94 percent) occurs within the San Jacinto Mountains Bioregion and 118 acres (6 percent) occurs within the San Bernardino Mountains Bioregion.

The MSHCP identifies six conservation objectives that will be implemented to provide long-term conservation for southern *Rana muscosa* (Dudek and Associates 2003, p. A-48), which are outlined as follows:

1) Conserve 136 ha (335 ac) of primary breeding habitat above 370 m (1,214 ft) (riparian scrub woodland and forest) within the San Jacinto Mountains for southern *Rana muscosa*. Of the 136 ha (335 ac), 108 ha (268 ac) are PQP lands, and 27 ha (67 ac) are Additional Reserve Lands to be assembled from within the Criteria Area;
2) Conserve the Cores Areas above 370 m (1,214 ft) at the North Fork San Jacinto River (including Dark Canyon), Hall Canyon, Fuller Miller Creek, and other perennial water streams in the San Jacinto Mountains;

3) Conserve at least (13,111 ha) 32,399 ac of secondary wooded habitat above 370 m (1,214 ft) within the North Fork of the San Jacinto River (including Dark Canyon), Hall Canyon, Fuller Mill Creek, and other perennial water streams in the San Jacinto Mountains. Of the 13,111 ha (32,399 ac), 13,026 ha (32,189 ac) are on PQP lands, and 85 ha (210 ac) are Additional Reserve Lands to be assembled from within the Criteria Area;
4) Conduct surveys as part of the project review process for public and private projects within the amphibian species survey area where suitable habitat is present and conserve southern *Rana muscosa* localities identified as a result of the survey efforts;
5) Maintain, or if feasible, restore ecological processes (with a particular emphasis on removing nonnative predatory fish and bullfrogs) within occupied habitat and suitable new areas within the Criteria Area. At a minimum, these areas will include areas above 370 m in the North Fork of the San Jacinto River (including Dark Canyon), Fuller Mill Creek, and Hall Canyon above Lake Fulmor; and
6) Maintain successful reproduction as measured by the presence/absence of tadpoles, egg masses, or juvenile frogs once a year for the first 5 years after permit issuance (but not less frequently than every 8 years) within the MSHCP Conservation Area.

The MSHCP considers southern *Rana muscosa* an Additional Survey Needs and Procedures species (Dudek and Associates 2003, Section 6, p. A-47). Surveys for southern *R. muscosa* will be conducted, as appropriate until the Additional Reserve Lands are assembled and conservation objectives for this species are met (USFWS 2004, p. 181). For those locations found to contain large numbers of individuals or otherwise determined to be important to the overall conservation of the species, the MSHCP allows flexibility to acquire these locations for inclusion into the Additional Reserve Lands (Dudek and Associates 2003, Section 6, pp. 6–70).

The MSHCP permittees will implement management and monitoring practices within the Additional Reserve Lands including surveys for southern *Rana muscosa*. Cooperative management and monitoring are anticipated on PQP Lands. Surveys will be conducted (as described above) to verify occupancy at a minimum of 75 percent of the known locations. If a decline in the distribution of southern *R. muscosa* is documented below this threshold, management measures will be triggered, as appropriate, to meet the species-specific objectives identified in Section 9, Table 9.2 of the MSHCP. Other management activities (USFWS 2004, pp. 182–183) will be conducted to benefit southern *R. muscosa* within the MSHCP Conservation Area.

Clean Water Act (CWA)

Under section 404 of the CWA, the U.S. Army Corps of Engineers (Corps) regulates the discharge of fill material into waters of the United States, which include navigable and isolated waters, headwaters, and adjacent wetlands (33 U.S.C. 1344). In general, the term "wetland" refers to areas meeting the Corps' criteria of hydric soils, hydrology (either sufficient annual flooding or water on the soil surface), and hydrophytic vegetation (plants specifically adapted to growing in wetlands). Any action with the potential to impact waters of the United States must be reviewed under the CWA, NEPA, and the Act. These reviews require consideration of impacts to listed species and their habitats, and recommendations for mitigation of significant impacts.

The Corps interprets "the waters of the United States" expansively to include not only traditional navigable waters and wetlands, but also other defined waters adjacent or hydrologically connected to traditional navigable waters. At the time of listing, section 404 of the CWA, in concordance with the Fish and Wildlife Coordination Act, provided some protection to southern *Rana muscosa* where they occurred in waters that require a permit from the Corps. Through the Fish and Wildlife Coordination Act, the Service may recommend discretionary conservation measures to avoid, minimize, and offset impacts to fish and wildlife resources resulting from a water development project authorized by the Corps. This protection continues to provide some benefit to southern *R. muscosa* because the majority of this species' habitat occurs on Federal land.

Rivers and Harbors Act

The Rivers and Harbors Act of 1899 is the oldest environmental law in the United States. Section 9 regulates the construction of bridge, dam, dike, or causeway over or in navigable waterways of the United States without Congressional approval. Section 10 regulates the obstruction or alteration of any navigable water of the United States, such as building of any wharf, pier, jetty, or other structure without Congressional approval. The U.S. Coast Guard and the Corps authorize such actions, respectively. This Federal regulation prohibits the use of Federal funds for activities, which may have an adverse effect on those characters which cause a river to be classified as wild, scenic, or recreational. Southern *Rana muscosa* may benefit indirectly from this regulation because portions of its occupied range are considered wild and scenic, such as Little Rock Creek, North Fork San Jacinto River, and Fuller Mill Creek (USFS 2005).

Multiple-use Sustained Yield (MUSY) Act

The MUSY Act of 1960, as amended, provided direction that the national forests be managed using principles of multiple use, and to produce a sustained yield of products and services. Specifically, the MUSY Act gives policy that the national forests are established and shall be administered for outdoor recreation, range, timber, watershed, wildlife, and fish purposes. Land management for multiple uses has inherent conflicts. However, the MUSY Act directs resource management not to impair the productivity of the land; while giving consideration to the relative values of the various resources, and not necessarily in terms of the greatest financial return or unit output. The MUSY Act provides direction to the USFS that wildlife is a value that must be managed. However, discretion is given to each National Forest when considering the value of the southern *R. muscosa* relative to the other uses for which they must manage. The MUSY Act does not have any provisions specific to the protection of southern *Rana muscosa* or its habitat.

Wilderness Act

The Wilderness Act of 1964 established a National Wilderness Preservation System made up of Federal-owned areas designated by Congress as "wilderness" for the purpose of preserving and protecting designated areas in their natural condition. Commercial enterprise, road construction, use of motorized vehicles or other equipment, and structural developments are generally prohibited within designated wilderness. Livestock grazing is permitted within designated wilderness, subject to other applicable laws, if it was established prior to the passage of this act. The Wilderness Act does not specifically mention fish stocking although it does state that it shall not affect the jurisdiction or responsibilities of States with wildlife and fish responsibilities in the national forests. Whether fish stocking is permitted under the Wilderness Act is an issue that has been debated (Bahls 1992, p. 188; Landres *et al.* 2001, p. 287). Nevertheless, fish stocking does not occur in wilderness areas in the three mountain ranges supporting southern *Rana muscosa* habitat, including: Pleasant View Ridge Wilderness, Sheep Mountain Wilderness, San Gabriel Wilderness, and Cucamonga Wilderness in the San Gabriel Mountains; San Gorgonio Wilderness in the San Bernardino Mountains; and San Jacinto Wilderness, and South Fork San Jacinto Wilderness in the San Jacinto Mountains. The Wilderness Act has likely helped to protect southern *R. muscosa* habitat from development or other types of habitat conversions and disturbances.

Federal Land Policy and Management Act (FLPMA)

The FLPMA of 1976, as amended, gives management direction to the Bureau of Land Management; however, its application is to all Federal lands, including those managed by the USFS. FLPMA includes a provision requiring that 50 percent or $10,000,000 per year, whichever is greater, of all moneys received through grazing fees collected on Federal lands (including the USFS-administered lands within the range of southern *Rana muscosa*) be spent for the purpose of on-the-ground range rehabilitation, protection, and improvement. This includes all forms of rangeland betterment such as fence construction, water development, and fish and wildlife enhancement. Half of the appropriated amount must be spent within the National Forest where such moneys were derived. FLPMA provides for some rangeland improvements intended for the long-term betterment of forage conditions and resulting benefits to wildlife, watershed protection, and livestock production. Land improvements initiated pursuant to FLPMA may have benefited southern *R. muscosa* and its habitat; however, some historical habitat was likely impacted due to livestock grazing on lands subject to FLPMA. We are unaware of any USFS-initiated projects developed under FLPMA for the specific benefit of southern *R. muscosa*.

National Forest Management Act (NFMA)

The National Forest Management Act (36 C.F.R. 219.20(b)(i)) (NFMA) requires the USFS to incorporate standards and guidelines into Land and Resource Management Plans, including provisions to support and manage plant and animal communities for diversity and for the long-term, rangewide viability of native species. On January 5, 2005, USFS revised National Forest land management planning under NFMA. Because the planning rule continues to be litigated, uncertainty regarding the future of regulations under the NFMA remains. Therefore, the impact of any revisions of this rule to this species is unknown at this time.

Since listing, non-jeopardy biological and conference opinions were issued that addressed the Revised Land Management Plans for the four southern California national forests (USFWS 2005b, p. 1). The Revised Land Management Plans included strategic direction in the form of land use zoning and standards (USFWS 2005b, p. 8). The land use zoning and standards indicated that for projects on USFS lands under the Land Management Plans, potential impacts should be minimized due to dispersed recreation activities, and expansion of existing facilities or new facilities will focus recreational use away from southern *Rana muscosa*. No new permanent loss of occupied or designated critical habitat is expected. Future projects will be implemented to promote the recovery of southern *R. muscosa* with the potential exception of fire abatement activities (fuel treatments) in wildland-urban interface areas (USFWS 2005b, p. 79). All southern *R. muscosa* habitat that overlaps with existing facilities occurs within Critical Biological Zones and all activities within such zones will be managed to be neutral or beneficial to southern *R. muscosa*. The primary impacts are expected to be those associated with recovery actions that result in long-term benefits to southern *R. muscosa*. Impacts due to ground disturbance activities (roads, trails, and recreation sites) in critical habitat areas will be minimized by conservation measures to specific sites and activities as determined through site-specific section 7 consultations with the Service. Many potential impacts are expected to be minimal due to the lack of direct instream impacts, the low impact nature of the activities involved, and implementation of appropriate minimization measures. The USFS will undertake measures to prevent, control, and eradicate noxious weeds associated with activities in these areas, including tamarisk. Although actions could still occur outside the parameters of the revised Land Management Plans, we anticipate implementation of the management outlined in these documents will reduce threats to southern *R. muscosa*.

Mountain Yellow-legged Frog Conservation Assessment and Strategy—Angeles and San Bernardino National Forests

The Mountain Yellow-legged Frog Conservation Assessment and Strategy for the Angeles and San Bernardino National Forests outlines tasks and actions to improve southern *Rana muscosa* habitat and promote recovery through cooperation with partners such as CDFG, USGS, Caltrans, and the Service. These specific actions address habitat suitability and occupancy assessments, land acquisition, nonnative trout impacts, recreational impacts, fire risk, mining, and toxic spills (USFS 2002, pp. 23–30).

Summary of Factor D

In summary, the Act is the primary Federal law that provides protection for *Rana muscosa* since its listing as endangered in 2002. Other Federal and State regulatory mechanisms provide discretionary protections for the species based on current management direction, but do not assure protection for the species absent its status under the Act. The State of California is currently proposing that *R. muscosa* be listed as Endangered. All populations in southern California are on USFS land with the exception of one population that also occurs partially on private land (Fuller Mill Creek). Two of the nine extant southern *R. muscosa* populations (Fuller Mill Creek and Dark Canyon) are within the Western Riverside MSHCP Plan Area. In absence of the Act, other laws and regulations have limited ability to protect the species.

FACTOR E: Other Natural or Manmade Factors Affecting Its Continued Existence

Other natural or manmade factors thought to be affecting southern *Rana muscosa* at listing were encompassed in threats to few and small populations (fire, flooding, and drought; demographic events; genetic risks; and increased disruption of metapopulation dynamics). Most of these effects will be discussed below under the section titled Small Population Size, which is currently the most predominant and imminent Factor E threat. Fire and flooding were discussed above under the section titled Fire under **Factor A**. Drought is addressed below under the section titled Climate Change, which was not discussed in the listing rule. Ultraviolet-B (UV-B) radiation and pesticides, which were mentioned as possible threats at listing though not analyzed under the five factors, are discussed below. Additionally, contamination, which was considered only as it related to spills or dumping, was discussed under **Factor A** in the listing rule as well as this review. However, additional possible sources of contamination impacting southern *R. muscosa* are discussed below.

Small Population Size

At listing, southern *Rana muscosa* was thought to have a high extinction risk because of the extremely limited number, small size, and isolation of the remaining populations (USFWS 2002, p. 44389). Although two additional populations have been discovered since listing, the risk of extinction to the DPS remains high. All nine extant populations are very small and highly isolated, and the additional populations do not appreciably increase the representative abundance of the listed entity. Southern *R. muscosa* populations are small and vulnerable to extirpation (local extinction) from environmental, demographic, and genetic stochasticity (random, natural occurrences), and unforeseen (natural or unnatural) catastrophes (Shaffer 1981, p. 131). Additionally, because there are so few populations of southern *R. muscosa*, the loss of individual populations increases the risk of extinction to the DPS as a whole. The potential effects associated with environmental, demographic, and genetic stochasticity; natural catastrophes; and the lack of interconnectedness of small populations are discussed in detail below.

Environmental, Demographic, and Genetic Stochasticity

Environmental stochasticity refers to annual variation in birth and death rates in response to weather, disease, competition, predation, or other factors external to the population (Shaffer 1981, p. 131). Small populations may be less able to respond to natural environmental changes (Kéry *et al.* 2000, p. 28), such as a prolonged drought or even a significant natural predation event. Periods of prolonged drought are more likely to have a significant effect on southern *R. muscosa* because drought conditions occur on a landscape scale and all life stages are dependent on habitat supporting a perennial water source.

Demographic stochasticity is random variability in survival or reproduction among individuals within a population (Shaffer 1981, p. 131) and could increase the risk of extirpation of the remaining populations. For example, the introduction of a novel fungal pathogen (*Bd*) into the habitat of southern *Rana muscosa* may have resulted in disproportionate reduction in the survivorship of juveniles. Population monitoring indicates that juveniles of southern *R. muscosa*, which are more susceptible to *Bd*, are rarely observed (Backlin 2012, pers. comm.). Therefore, the small number of adults currently present in each population will be further reduced if younger generations are not available to succeed aging adults. If a population does not recruit individuals from every lifestage, demographic stochasticity can lead to the extirpation of that population.

Genetic stochasticity results from changes in gene frequencies due to founder effect (loss of genetic variation that occurs when a new population is established by a small number of individuals) (Reiger *et al.* 1968, p. 163); random fixation (the complete loss of one of two alleles in a population, the other allele reaching a frequency of 100 percent) (Reiger *et al.* 1968, p. 371); or inbreeding depression (loss of fitness or vigor due to mating among relatives) (Soulé 1987, p. 96). Additionally, small populations generally have an increased chance of genetic drift (random changes in gene frequencies from generation to generation that can lead to a loss of variation) and inbreeding (Ellstrand and Elam 1993, p. 225). Evidence of inbreeding within southern *Rana muscosa* populations is not strong; the highest inbreeding has occurred at Little Rock Creek, East Fork City Creek, and Dark Canyon (Schoville *et al.* 2011, p. 7). However, every southern *R. muscosa* population has low levels of genetic variation (a measure of the genetic differences within populations or species) (Schoville *et al.* 2011, p. 1). This could impair the ability to adapt to changes in the environment, such as the introduction of a novel disease, or contribute to more pronounced inbreeding depression over time (Shaffer 1981, p. 133; Noss and Cooperrider 1994, p. 6; Primack 1998, p. 305). In every population there is some evidence of recent genetic bottlenecking (an event in which a population's size is radically reduced causing gene frequencies to change by random chance and ultimately reducing genetic variation) (Schoville *et al.* 2011, p. 5). It is currently unknown whether the effects of reduced genetic variability in each population will affect fitness (Schoville *et al.* 2011, p. 7).

Natural Catastrophes

In southern *Rana muscosa* habitat, natural catastrophes such as regional fires tend to be followed by large flooding events, which could result in extirpation of small populations (Shaffer 1981, p. 131). Habitat alterations caused by natural catastrophes have direct effects (exposure to fire, increase in water temperature, flooding individuals from the habitat, and sediment covering tadpoles or egg masses) and indirect effects (debris and sediment filling in pools and reduction of refugia) all of which can result in mortality of individuals. The streams inhabited by the southern *R. muscosa* flow through narrow canyons that provide little opportunity for off-channel refuge for the species during fire and flood events (USFS 2002, p. 22). Two large fires decimated the habitat in two occupied areas since listing, and at least one fire initiated the downward trajectory of a population. Ultimately, even a small fire or flood event occurring directly in southern *R. muscosa* habitat can have significant effects to this taxon due to the few remaining individuals available to support recovery in most populations.

Connectivity

The extinction risk of a species represented by few small populations is magnified when those populations are also isolated from one another. This is especially true for species whose populations function in a metapopulation structure, whereby dispersal or migration of individuals to new or formerly occupied areas is necessary. Connectivity between these populations is essential to increase the number of reproductively active individuals in a population; mitigate the genetic, demographic, and environmental effects of small population size; and recolonize extirpated areas. Genetic data indicate that there is no migration occurring between the small, highly isolated southern *R. muscosa* populations (Schoville *et al.* 2011, p. 6) and functional self-sustaining metapopulations no longer exist. Every population, with the exception of Bear and

Vincent Gulch, appears to be genetically isolated with very little inter-population gene flow (Schoville *et al.* 2011, p. 8).

Summary

Southern *Rana muscosa* remain at a high extinction risk due to the vulnerabilities associated with few, small, isolated populations. The listed entity is at risk from natural environmental fluctuations that *R. muscosa* would likely recover from under normal circumstances whereby many more and larger populations exist in closer proximity to one another. A significant gap in the juvenile lifestage indicates an important demographic weakness. Genetic variability is low in all populations and each appears to be bottlenecked. Inbreeding thus far has been minimal but is evident in three of the nine populations. Finally, metapopulation dynamics are severely inhibited, possibly preventing the natural recovery of populations through recolonization. Therefore, southern *R. muscosa* is likely to be significantly affected by small population size.

Climate Change

Our analyses under the Act include consideration of ongoing and projected changes in climate. The terms “climate” and “climate change” are defined by the Intergovernmental Panel on Climate Change (IPCC). The term “climate” refers to the mean and variability of different types of weather conditions over time, with 30 years being a typical period for such measurements, although shorter or longer periods also may be used (IPCC 2007, p. 78). The term “climate change” thus refers to a change in the mean or variability of one or more measures of climate (e.g., temperature or precipitation) that persists for an extended period, typically decades or longer, whether the change is due to natural variability, human activity, or both (IPCC 2007, p. 78).

Scientific measurements spanning several decades demonstrate that changes in climate are occurring, and that the rate of change has been faster since the 1950s. Global mean surface temperatures have increased 0.3 to 0.7°C (0.6 to 1.2°F)) since the late 19th century (USEPA 1997, p. 1). Results of scientific analyses presented by the IPCC show that most of the observed increase in global average temperature since the mid-20th century cannot be explained by natural variability in climate, and is “very likely” (defined by the IPCC as 90 percent or higher probability) due to the observed increase in greenhouse gas (GHG) concentrations in the atmosphere as a result of human activities, particularly carbon dioxide emissions from use of fossil fuels (IPCC 2007, pp. 5–6 and figures SPM.3 and SPM.4; Solomon *et al*. 2007, pp. 21–35). Further confirmation of the role of GHGs comes from analyses by Huber and Knutti (2011, p. 4), who concluded it is extremely likely that approximately 75 percent of global warming since 1950 has been caused by human activities.

Scientists use a variety of climate models, which include consideration of natural processes and variability, as well as various scenarios of potential levels and timing of GHG emissions, to evaluate the causes of changes already observed and to project future changes in temperature and other climate conditions (e.g., Meehl *et al*. 2007, entire; Ganguly *et al*. 2009, pp. 11555, 15558; Prinn *et al*. 2011, pp. 527, 529). All combinations of models and emissions scenarios yield very similar projections of increases in the most common measure of climate change, average global surface temperature (commonly known as global warming), until about 2030. Although projections of the magnitude and rate of warming differ after about 2030, the overall trajectory of all the projections is one of increased global warming through the end of this century, even for the projections based on scenarios that assume that GHG emissions will stabilize or decline. Thus, there is strong scientific support for projections that warming will continue through the 21st century, and that the magnitude and rate of change will be influenced substantially by the extent of GHG emissions (IPCC 2007, pp. 44–45; Meehl *et al*. 2007, pp. 760–764 and 797–811; Ganguly *et al*. 2009, pp. 15555–15558; Prinn *et al*. 2011, pp. 527, 529). Most models generally predict that the southwest United States will become drier, and that extreme events such as heavier storms, heat waves, and regional droughts will become more common (Glick *et al.* 2011, p. 7). Moreover, it is generally expected that the duration, frequency, and intensity of droughts will increase in the future (Glick *et al.* 2011, p. 45; PRBO 2011, p. 21).

Global climate projections are informative, and, in some cases, the only or the best scientific information available for us to use. However, projected changes in climate and related impacts can vary substantially across and within different regions of the world (e.g., IPCC 2007, pp. 8–12). Therefore, we use “downscaled” projections when they are available and have been developed through appropriate scientific procedures, because such projections provide higher resolution information that is more relevant to spatial scales used for analyses of a given species (see Glick *et al*. 2011, pp. 58–61, for a discussion of downscaling). With regard to our analysis for southern *Rana muscosa* downscaled projections are available for southern California.

In the southwestern California ecoregion, climate models predict that mean annual temperatures will increase from 1.7 to 2.2°C (3.1 to 4.0°F) by 2070 (PRBO 2011, p. 41). High temperature events are expected to become more common in southern California and species with narrow temperature tolerance levels may experience thermal stress (PRBO 2011, p. 42). Increases in extremely high temperature events may cause direct mortality or halt or diminish reproduction (PRBO 2011, p. 42). There is a general lack of consensus of the effects of future climate change on precipitation patterns in southern California. Regional models suggest a decrease in mean annual rainfall of 51 to 184 mm (2 to 7.2 in) (a reduction by 10 to 37 percent) by 2070 (PRBO 2011, p. 41). Snyder *et al.* (2004, p. 594) has projected that snowpack will decrease by 90 percent in the South Coast hydrologic region of California. There is currently no published literature on the predicted effects of climate change on stream flow in southwestern California; however, snow-fed rivers and streams are expected to have less water. There is currently no consensus regarding how climate change will influence wildfire events or Santa Ana events (high winds combined with low humidity, typically following a wet rainy season) in southern California. However, historically significant wildfires in California have increased in frequency in the last century, as discussed under **Factor A** above, in the section titled Fire.

Various changes in climate may have direct or indirect effects on species. These effects may be positive, neutral, or negative, and they may change over time, depending on the species and other relevant considerations, such as interactions of climate with other variables (e.g., habitat fragmentation) (IPCC 2007, pp. 8–14, 18–19). Identifying likely effects often involves aspects of climate change vulnerability analysis. Vulnerability refers to the degree to which a species (or system) is susceptible to, and unable to cope with, adverse effects of climate change, including climate variability and extremes. Vulnerability is a function of the type, magnitude, and rate of climate change and variation to which a species is exposed, its sensitivity, and its adaptive capacity (IPCC 2007, p. 89; see also Glick *et al.* 2011, pp. 19–22). There is no single method for conducting such analyses that applies to all situations (Glick *et al.* 2011, p. 3). We use our expert judgment and appropriate analytical approaches to weigh relevant information, including uncertainty, in our consideration of various aspects of climate change.

Changes in climate that occur faster than the ability of endangered species to adapt could cause local extinctions (USEPA 1989, p. 145). Amphibians are extra-sensitive to certain environmental changes, such as slight shifts in temperature and moisture due to their permeable skin, biphasic lifecycles (aquatic and terrestrial), and unshelled eggs (Carey and Alexander 2003, pp. 113–114). Emergence from hibernation and breeding cues are initiated by changes in the environment. As a species that inhabits relative climate extremes, some conditions may directly push southern *Rana muscosa* past physiological or ecological tolerance thresholds, and therefore risk from climate change is theoretically enhanced. In the summer, reduced snowpack and enhanced evapo-transpiration following high temperature events may dry out pools which otherwise would have sustained rearing tadpoles (Lacan *et al.* 2008, p. 220) and may also reduce fecundity (egg production) (Lacan *et al.* 2008, p. 222). Predicted increases in mean annual temperatures, high temperature events, and potentially decreased precipitation could also diminish the volume and timing of water availability to support all lifestages. Portions of some occupied streams currently become dry in the summer, potentially inhibiting survival of tadpoles dispersing downstream. Recolonization of such sites will be further inhibited if drought conditions become more common. This is because increased exposure to high temperature events may appreciably reduce the availability of suitable habitat and may cause direct mortality from desiccation. Furthermore, an increase in the frequency, intensity, and duration of droughts would magnify stresses associated with such conditions. Earlier snowmelt would cue breeding earlier in the year, on average, and advancing this primary signal for breeding phenology in montane and boreal habitats (Corn 2005, p. 61) may have both positive and negative effects. Additional time for growth and development may render larger individuals more fit to overwinter; however, earlier breeding may also expose young tadpoles to killing frosts in more variable conditions of early spring (Corn 2005, p. 60). Severe winters would force longer hibernation times and could stress mountain yellow-legged frogs by reducing the time available for them to feed and breed.

It is unclear if there are dependencies on interacting species that may be affected either positively or negatively by climate change. Climate change may alter invertebrate communities (Porinchu *et al.* 2010 in PRBO 2011 p. 24). In one study, an experimental increase in stream water temperature was shown to decrease density and biomass in invertebrates (Hogg and Williams 1996, p. 401). Thus, global climate change might have a negative impact on the prey base of southern *Rana muscosa*.

Changes in temperature may also affect virulence of pathogens (Carey 1993, p. 359), which could make amphibians such as the southern *Rana muscosa* more susceptible to disease. Climate change could affect the distribution of pathogens and their vectors, exposing mountain yellow-legged frogs (potentially with weakened immune systems as a result of other environmental stressors) to new pathogens (Blaustein *et al.* 2001, p. 1808). Climate change may result in a range shift of *Bd* (Pounds *et al.* 2006, p.161; Bosch *et al.* 2007, p. 253), and could also lead to increased virulence of *Bd* (Fisher *et al.* (2009, p. 299).

The key risk factor for climate change impacts to southern *Rana muscosa* is likely the interaction between reduced water levels, and the relative inability of individuals to disperse and colonize across longer distances in order to occupy more favorable habitat conditions (e.g., move higher in latitude and/or elevation). Although this range shift has been observed in some plant and animal species, the changes observed amongst amphibians to date have been more associated with changes in timing of breeding (phenology) (Corn 2005, p. 60). This reduced adaptive capacity for mountain yellow-legged frogs is a function of high site fidelity, and the extensive habitat fragmentation facilitated by introduction of nonnative fishes throughout much of the frog's range, as discussed under **Factor C** above.

Thus, an increase in the frequency, magnitude, and duration of droughts caused by global warming may have compounding effects with respect to populations of southern *Rana muscosa* already in decline. In situations where other factors have resulted in the isolation populations to marginal habitats, localized population crashes, or extirpations due to droughts may exacerbate their isolation and preclude recolonization or immigration from other populations (Bradford *et al.* 1993, p. 887; Drost and Fellers 1996, p. 424; Lacan *et al.* 2008, p. 222).

Ultraviolet-b (UV-B) Radiation

Ambient ultraviolet-b (UV-B) radiation (280–320 nanometers (11.0–12.6 microinches)) has increased at north temperate latitudes in the past two decades (Adams *et al.* 2001, p. 521). Melanic pigment on the upper surfaces of amphibian eggs and larvae protects these sensitive life stages against UV-B damage, an important protection for normal development of amphibians exposed to sunlight, especially at high elevations in clear and shallow waters (Perotti and Diéguez 2006, p. 2064). If UV-B radiation is contributing to amphibian population declines, the declines would likely be greater at higher elevations and more southerly latitudes because UV-B exposure is greatest where the thinner atmosphere allows greater penetration of UV-B (Davidson *et al.* 2001, p. 474; Davidson *et al.* 2002, p. 1589). In California, where there is a north-to-south gradient of increasing UV-B exposure, amphibian declines would also likely be more prevalent at southerly latitudes (Davidson *et al.* 2001, p. 474; Davidson *et al.* 2002, p. 1589). Given the expected pattern of exposure it is possible that UV-B radiation may have negatively impacted southern *Rana muscosa*; however, additional research on the effect of this threat is needed.

Acid Precipitation

Acidic precipitation (acid deposition) has been suggested as a contributor to amphibian declines in the western United States (Blaustein and Wake 1990, p. 204; Carey 1993, p. 357; Alford and Richards 1999, pp. 139), including the Sierra Nevada (Bradford *et al.* 1994b, p.156). Bradford *et al.* (1998, p. 2482) found mountain yellow-legged frog tadpoles to be sensitive to naturally acidic conditions, and they were not found in lakes with a pH less than 6 in the Sierra Nevada. Laboratory studies have documented sublethal effects (reduced growth) on mountain yellow-legged frog embryos at pH 5.25 (Bradford *et al* 1992, p. 369). Survivorship of embryos and tadpoles was negatively affected as acidity increased (at approximately pH 4.5 or lower), with embryos being more sensitive to increased acidity than tadpoles (Bradford and Gordon 1992, p. 3; Bradford *et al.* 1992, pp. 374–375).

An evaluation of water quality at known extant southern *Rana muscosa* sites in 2003 found that water chemistry parameters were within the expected range for the species (USGS 2004, p. 21). The most consistent water quality parameter between all sites was pH, generally measuring from 7 to 8. Thus, the occupied waters indicate a hospitable aquatic environment for southern *R. muscosa*. Excluding two streams with water quality differences based on other parameters, there were no discernible differences in water quality between sites that supported southern *R. muscosa* and those that did not (USGS 2004, p. 22). Therefore, it is unlikely that acid precipitation is a current threat to southern *R. muscosa*.

Contaminants

Environmental contaminants (e.g., pesticides, heavy metals, and nitrogen based fertilizers) have been suggested, and in some cases documented, to negatively affect amphibians by causing: direct mortality (Hall and Henry 1992, pp. 66–67; Berrill *et al.* 1994, p. 663, 1995, pp.1016–1018; Carey and Bryant 1995, p. 16; Relyea and Mills 2001, p. 2493); immune system suppression—making amphibians more vulnerable to parasites, disease, and UV radiation (Carey 1993, pp. 358–360; Carey and Bryant 1995, p. 15; Carey *et al.* 1999, p. 9; Daszak *et al.* 1999, p. 741; Taylor *et al.* 1999, p. 540; Blaustein *et al.* 2003, pp. 123–140; Christin *et al.* 2003, p. 1127; Gendron *et al.* 2003, p. 469); disruption of breeding behavior and physiology (Berrill *et al.* 1994, p. 663; Carey and Bryant 1995, p. 16; Hayes *et al.* 2003a, p. 5479); disruption of growth or development (Hall and Henry 1992, p. 66; Berrill *et al.* 1993, p. 537; 1994, p. 663; 1995, pp. 1016–1018; Berrill *et al.* 1998, pp. 1741–1744; Sparling *et al.* 2001, p. 1595; Brunelli *et al.* 2009, p. 135); disruption of predator avoidance behavior (Hall and Henry 1992, p. 66; Berrill *et al.* 1993, p. 537; 1994, p. 663; 1995, p. 1017; Berrill *et al.* 1998, p. 1744; Relyea and Mills 2001, p. 2493; Sparling *et al.* 2001, p. 1595); disruption of the endocrine system resulting in sexual malformations, such as hermaphroditism (Hayes *et al.* 2003a, p. 5476; Hayes *et al.* 2003b, p. 568); and alteration of food web dynamics (Boone and Bridges 2003, p. 2700). In addition to interfering with nerve function, contaminants such as industrial and agricultural chemicals may act as estrogen mimics (Jobling *et al.* 1996, p. 194), causing abnormalities in reproduction and disrupting endocrine functions (Carey and Bryant 1995, p. 16; Jobling *et al.* 1996, pp. 198–200; Hayes *et al.* 2003a, p. 5479).

Wind-borne pesticides from agricultural areas in the Imperial Valley may be deposited in the mountains of southern California. Evidence of the effects of wind-borne pesticides deposited from upwind agricultural sources are suggested as a cause of measured sublethal effects to amphibians in the Sierra Nevada (Davidson *et al.* 2001, pp. 474–475; Sparling *et al.* 2001, p. 1591; Davidson 2004, p. 1892; Fellers *et al.* 2004, p. 2176).

In southern California, nitrate (NO_3^-) and other oxidized compounds of nitrogen (N) are the primary nitrogenous pollutants, although ammonium (NH_4^+) also occurs in significant concentrations in some places (Padgett *et al.* 1999, p. 770). Smog generated in the Los Angeles region causes an estimated 35 to 45 kg N ha^{-1} yr^{-1} in dry deposition is added to the forests surrounding the Los Angeles Basin (Padgett *et al.* 1999, p. 770). Air pollution has caused damage to ponderosa pine in the San Bernardino National Forest since the 1960s (Fenn and Bytnerowicz 1993, p. 277). High levels of nitrogenous compounds are also known to be deposited in the San Gabriel Mountains (Fenn and Bytnerowicz 1993, p. 277). Nitrate concentrations in streamwater in southern California are the highest for wildland watersheds in North America (Fenn *et al.* 2005, p. 269). In the western San Bernardino Mountains, average concentrations of nitrate in streams fall within the low side of the range that could cause developmental, physical, or behavioral abnormalities in sensitive amphibians (Fenn *et al.* 2005, p. 270). Peak concentrations of nitrate in these streams are double the average values that may have acute effects on amphibians (Fenn *et al.* 2005, p. 270). Studies on the effects of elevated streamwater nitrate on southern *Rana muscosa* have not occurred; however, extensive research elsewhere demonstrates that compounds of nitrogen and other contaminants can have extremely harmful effects on amphibians.

It appears that exposure of southern *Rana muscosa* to nitrogenous pollutants is likely to have occurred in the San Gabriel and San Bernardino Mountains, although the magnitude of the impacts on southern *R. muscosa* have not been measured. It is hypothesized that such pollutants contributed to the decline of southern *R. muscosa*, and may continue to limit dispersal potential. Water quality testing at extant localities has not identified contaminants; however, only basic variables are tested (pH, conductivity, and dissolved oxygen). Pesticides, herbicides, and nitrogen-based fertilizers are used directly adjacent to streams where illegal marijuana cultivation sites are planted (Devil's Canyon, Bear Gulch, Vincent Gulch, and City Creek). Any waterways where these contaminants are used in the future should be tested to evaluate the effects on southern *R. muscosa*. Future impacts may result from the increased air pollution and the use of fire retardant chemicals, which contain nitrogen compounds and surfactants.

Summary of Factor E

While all nine populations are at risk to Factor E threats, the small populations of southern *Rana muscosa* are particularly at risk of effects associated with environmental, demographic, and genetic stochasticity; natural catastrophes; and the lack of interconnectedness that would allow for recolonization in natural circumstances. Impacts from climate change are also likely through an increase in the frequency, magnitude, and duration of droughts that may have compounding effects with southern *R. muscosa* already in decline. In the southwestern California ecoregion, climate models predict an increase in annual temperatures (1.7 to 2.2°C (3.1 to 4.0°F) by 2070), more frequent high temperature events, potentially decreased precipitation, and decreased snowpack leading to decreased stream flows in snow-fed waters. All of these changes in climate would be especially relevant to southern *R. muscosa*, which is already isolated in the headwaters of waterways, thus making a shift in distribution to accommodate climatic changes less possible. Furthermore, an increase in high temperature events (potentially affecting drought and fire frequency) could easily cause the extirpation of any of the remaining populations. Classically recognized threats to amphibians are largely unstudied in southern *R. muscosa*, including exposure to UV-B radiation, acid precipitation, and contamination. Though these threats may potentially impact southern *R. muscosa*, there is insufficient data to determine how they have contributed to past declines throughout the range of the DPS.

Cumulative Effects

Combinations of stressors working in concert with one another have the ability to negatively impact species to a greater degree than individual stressors operating alone. Much research has been focused on how these cumulative or synergistic effects are connected to global amphibian declines. Such cumulative effects, alluded to throughout this review, are applicable to southern *Rana muscosa*. For example, extirpation of populations can be caused by disease (particularly *Bd*), predation, or natural events (e.g., fire, flooding, and drought). Extirpated localities can no longer be recolonized under natural circumstances because nonnative trout occupy most connected areas. This further increases the risk of extirpation to the remaining populations. Southern *R. muscosa* populations are challenged in their ability to replace aging adults, because all remaining occupied areas are infected with *Bd*, which appears to be causing a low recruitment rate of juveniles. Thus, isolated populations may continue to decrease in size over time and may begin to experience genetic defects associated with small population size including increased inbreeding and loss of genetic variation if they diminish to below threshold levels. An increased loss in genetic variation may increase the susceptibility of this species to additional diseases, more intense *Bd* infections, and potentially greater sensitivity to UV-B light or contamination. Furthermore, as climate change becomes more severe (greater frequency and intensity of droughts, decreased snowpack causing decreased flow in snow-fed streams), successful reproduction may be further inhibited if breeding phenology is altered. Additionally, decreased water availability has the potential to increase exposure to UV-B where embryos develop in shallow water. Climate change may also increase the spread or virulence of *Bd* and the likelihood of wildfires. Cumulative effects are likely responsible for the historical rangewide decline of southern *R. muscosa* (introduction of nonnative trout, *Bd*, and stochastic processes), and the persistence of these stressors coupled with additional stressors, that are likely to be at play but are less understood (climate change, UV-B exposure, and contaminants), continue to threaten southern *R. muscosa* with extinction throughout its range.

III. RECOVERY CRITERIA

There is no approved recovery plan for the mountain yellow-legged frog.

IV. SYNTHESIS

When the southern *Rana muscosa* DPS was listed in 2002, there were seven known populations (five in the San Gabriel Mountains, one in the San Bernardino Mountains, one in the San Jacinto Mountains, and none remaining in the Palomar Mountains). All populations at listing occurred entirely on USFS lands and remain so, with the exception of Fuller Mill Creek, a portion of which occurs on county land and private property. Since listing, two additional populations were discovered in the San Jacinto Mountains, for a total of nine extant populations. All populations are isolated from one another in the headwaters of streams or tributaries due to the extensive distribution of predatory nonnative trout downstream in historical southern *R. muscosa* habitat. Such isolation and fragmentation coupled with the inability to recolonize areas now occupied by trout likely increases the risk of extinction for this taxon. Bear Gulch and East Fork City Creek were the largest populations at listing but now are hovering near extirpation. Vincent Gulch and Fuller Mill Creek also appear to be declining. The status of the populations at Devil's Canyon and Tahquitz-Willow Creek remains unknown. South Fork Big Rock Creek appears to be stable at low numbers. Little Rock Creek, and seemingly Dark Canyon, have upward population trajectories and appear to be benefitting from both recreational closures and trout removal efforts at these sites. All extant populations remain very small regardless of the population trend.

Each southern *Rana muscosa* population is highly susceptible to stochastic events, especially wildfire, which probably initiated the decline of the East Fork City Creek population. Measures have been taken to reduce the impact of certain threats since listing, including recreation. However, threats to the habitat remain, including marijuana cultivation, suction dredge mining, recreational and fire management activities, and roadwork construction. The most significant stressors to southern *R. muscosa* are related to the constraints on recruitment by predation and disease. Where adults reproduce in trout-occupied waters, or where tadpoles disperse downstream into trout-occupied waters, those tadpoles are likely to be preyed upon by trout. Additionally, all populations are positive for *Bd*, and although infection rates are low, the juvenile lifestage, which experiences the highest mortality from *Bd*, is usually undetected during annual population surveys. Small population sizes and a fragmented metapopulation structure are a great impetus for threat abatement, including trout removal and recreational closures adjacent to extant populations. Trout removal in additional locations should be utilized to facilitate the reintroduction of additional populations in historically occupied areas, with the ultimate goal of restoring metapopulations in each mountain range. Additional research and experimentation should be attempted to increase our understanding of lesser known threats. Southern *R. muscosa* remains in danger of becoming extinct throughout its range and no status change is recommended at this time.

V. RESULTS

Recommended Listing Action:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist (indicate reason for delisting according to 50 CFR 424.11):
    ____ *Extinction*
    ____ *Recovery*
    ____ *Original data for classification in error*
__X__ No Change

New Recovery Priority Number and Brief Rationale: No change.

VI. RECOMMENDATIONS FOR ACTIONS OVER THE NEXT 5 YEARS

The recommended actions listed below are to be completed over the next 5 years. Successful implementation of these actions will reduce threats to southern *Rana muscosa* and provide information to better understand the biological and physical factors limiting the population growth and distribution in southern California. We recognize that conservation of this taxon will require cooperation and coordination with partners (Federal, State, and local agencies) to minimize impacts from current threats, aid future restoration, and maximize effectiveness of limited funding.

1) Nonnative trout removal and barrier construction:
    a) Continue trout removal efforts adjacent to extant populations.
    b) Prioritize future trout removal locations according to areas needed to re-establish connectivity and maintain self-sustaining metapopulations.

2) Continue to survey for and monitor existing populations annually.

3) Survey for unidentified extant populations. Use information on previous survey extent and effort, and the expertise of field biologists to prioritize additional survey areas potentially supporting as yet unidentified extant populations.

4) Increase "assisted rearing" capacity:
    a) Maintain representatives from each distinct population in biosecure captive settings (assurance populations) in order to safeguard against catastrophic impacts (fire, flooding, and drought). Increase space available to breed and support all lifestages of captive individuals and increase care staff. Establish pedigrees for captive breeding.
    b) Experiment with alternate breeding techniques such as creating and utilizing outdoor ponds, utilizing existing pools in or along streams, seeking assistance from previous or current research/hatchery facilities, soliciting the assistance of private breeders, or other novel concepts for breeding southern *Rana muscosa*.

5) Experiment with release strategies including releasing multiple life stages and greater numbers of individuals per release. Consider new techniques for increasing survivorship in the wild (e.g., caging tadpoles or providing other protections from predation or disease). Identify specific populations where translocation from and to will be a more viable option than captive rearing.

6) Use modeling as a tool to guide management actions and determine where the re-establishment of southern *Rana muscosa* populations should occur to establish and maintain self-sustaining connectivity.

7) Analyze the effects of *Bd* on southern *Rana muscosa*:
    a) Historical effects: Analyze museum specimens to address the possibility that *Bd* caused the historical rangewide decline of southern *Rana muscosa*.
    b) Current effects: Test all animals found in the wild strategically to determine the infection intensity of each population and each age class within a population.

8) Develop an approved Recovery Outline for southern *Rana muscosa*.

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Appendix 1: Southern DPS of Mountain Yellow-legged Frog (southern *Rana muscosa*) Occurrence Table; prepared for 5-year review, 2012.

Occurrence	Detected at Listing	Currently Detected	Threats Known at Listing	Significant Changes since Listing	Current Threats	Current Conservation	Population Trend
Devil's Canyon San Gabriel Mountains	Yes	Yes	**Factor A:** Recreation (minimal). **Factor C:** None known, although trout were present downstream.	Large wildfire in 2009 results in emergency salvage of tadpoles. Fire appears to have eradicated trout here.	**Factor A:** Illegal marijuana cultivation. **Factor C:** Chytrid. **Factor E:** Small population size; climate change.	Angeles National Forest San Gabriel Wilderness Area	**Unknown**
Little Rock Creek San Gabriel Mountains	Yes	Yes	**Factor A:** Recreation; potential mudslides from SR-2. **Factor C:** Trout	Climbing area at Mt. Williamson Rock closed. USFS coordinates some with Caltrans to prevent spills from entering creek but roadwork in 2011 caused mass sedimentation to enter and severely impact habitat. Trout removal likely achieved eradication.	**Factor A:** Sedimentation in waterway during roadwork on SR-2; wildfire. **Factor C:** Chytrid. **Factor E:** Small population size; climate change.	Angeles National Forest	**Increasing**
South Fork Big Rock Creek San Gabriel Mountains	Yes	Yes	**Factor A:** Recreation. **Factor C:** Trout stocking; trout predation.	CDFG discontinued trout stocking program at this location.	**Factor A:** Wildfire. **Factor C:** Nonnative trout; chytrid. **Factor E:** Small population size; climate change.	Angeles National Forest Pleasant View Ridge Wilderness Area	**Increasing**
Vincent Gulch San Gabriel Mountains	Yes	Yes	**Factor C:** Trout		**Factor A:** Recreation (minimal); illegal marijuana cultivation; wildfire. **Factor C:** Nonnative trout; chytrid. **Factor E:** Small population size; climate change.	Angeles National Forest Sheep Mountain Wilderness Area	**Declining**

Bear Gulch San Gabriel Mountains	Yes	Yes	**Factor A:** Recreation. **Factor C:** Trout predation.		**Factor A:** Illegal marijuana cultivation; wildfire. **Factor C:** Nonnative trout; chytrid. **Factor E:** Small population size; climate change.	Angeles National Forest Sheep Mountain Wilderness Area	**Declining**
East Fork City Creek San Bernardino Mountains	Yes	Yes	**Factor A:** Recreation; High wildfire concern; potential hazardous material spill from Hwy 330. **Factor C:** Possible introduction of trout, bass, or bullfrogs above existing barrier at East Fork.	Recreational area closed by USFS. Entire watershed burns in 2003.	**Factor A:** Illegal marijuana cultivation; habitat impacts during roadwork. **Factor C:** Chytrid. **Factor E:** Small population size; climate change.	San Bernardino National Forest	**Declining** Four adults currently in captivity.
Fuller Mill Creek San Jacinto Mountains	Yes	Yes	**Factor A:** Recreation; High wildfire concern. Potential hazardous material spill from highway; discontinuous habitat in private inholdings not protected. **Factor C:** Trout stocking, trout predation.	Removal of picnic tables and barbeque pits near waterway. Recreational use within water prohibited. Interpretive educational signage installed and communication with recreationalists increased. USFS purchased approximately 60 ac (24 ha) of land in private inholdings. CDFG discontinued trout stocking. Trout removal in process since 2009.	**Factor A:** Recreation; wildfire. **Factor C:** Nonnative trout; chytrid. **Factor E:** Small population size; climate change.	San Bernardino National Forest	**Declining**
Dark Canyon (Upper North Fork San Jacinto River) San Jacinto Mountains	No	Yes	**Factor A:** Recreation; high wildfire concern; potential hazardous material spill from Hwy 243. **Factor C:** Trout stocking, trout predation.	Camp sites adjacent to creek removed. Recreational use within water prohibited. Interpretive educational signage installed and communication with recreationalists increased. CDFG discontinued trout stocking. Trout removal in process since 2009.	**Factor A:** Recreation; wildfire. **Factor C:** Nonnative trout; chytrid. **Factor E:** Small population size; climate change.	San Bernardino National Forest	**Unknown, possibly increasing** About fifty adults raised from tadpoles currently in captivity.

Tahquitz/ Willow Creeks San Jacinto Mountains	No	Yes	**Factor A:** Recreation; wildfire. **Factor C:** Trout predation.		**Factor C:** Nonnative trout; chytrid. **Factor E:** Small population size; climate change.	San Bernardino National Forest San Jacinto Wilderness Area	**Unknown.**
Indian Creek Hall Canyon San Jacinto Mountains	No	No	**Factor A:** High wildfire concern. **Factor C:** Potential upstream migration of trout or other nonnative species from Lake Fulmor.		**Factor A:** Wildfire. **Factor C:** Nonnative trout downstream; chytrid may be present. **Factor E:** Small population size; climate change.	San Bernardino National Forest	**Unknown.** Experimental reintroduction site for captive-bred animals. Releases occurred in 2010 and 2011, survivorship unknown.

U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW

mountain yellow-legged frog (*Rana muscosa*)
Southern California Distinct Population Segment

Current Classification: Endangered

Recommendation Resulting from the 5-year Review:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist
__X__ No change needed

FIELD OFFICE APPROVAL:

ACTING **Lead Field Supervisor, U.S. Fish and Wildlife Service**

Approve ______ Scott A. Sobiech ______ Date JUL 13 2012