

## APPENDIX J: LIFE HISTORY OF MOUNTAIN YELLOW-LEGGED FROGS

### Description

Mountain yellow-legged frogs (MYLFs) are a native amphibian species complex that includes two species (Vredenburg et al. 2007): the Sierra Nevada yellow-legged frog (*Rana sierrae*) and the mountain yellow-legged frog (*Rana muscosa*). Both species are federally listed as endangered (FWS 2014), while *R. sierrae* is state listed as threatened and *R. muscosa* is state listed as endangered (CFGF 2012). The two species are difficult to distinguish visually within SEKI, although some phenotypic differences have been established between the two species (Vredenburg et al. 2007, pgs. 370-371). *R. sierrae* (Figure J-1, A and B) have relatively shorter legs when compared with *R. muscosa* (Figure J-1, C and D), and the two species have different calls. However, visual differences are slight, both species only call when underwater, and there is considerable color and pattern variation within both species (NPS aquatics staff, pers. obs., Stebbins and McGinnis 2012). The typical way to differentiate between the two species involves a priori knowledge of which species is present within the geographic area being surveyed based on genetic sampling.

Adults are a moderate-sized frog that varies from about 1.5 to 3 inches (40 to 80 mm) snout to vent length (SVL). In general, dorsal color has varying mixes of olive-green, brown, and black. Color varies from mottled to spotted, and they may appear anywhere from drab to colorful. The ventral color is white to yellowish-beige. The underside of legs often contains a yellowish wash. The venter and underside of the upper thighs may also have a bright reddish-orange coloration. Larvae (tadpoles) are very dark (nearly black) during their first year. Older larvae are slightly lighter and resemble large ripe olives with tails (Figure J-1, E; Stebbins and McGinnis 2012).

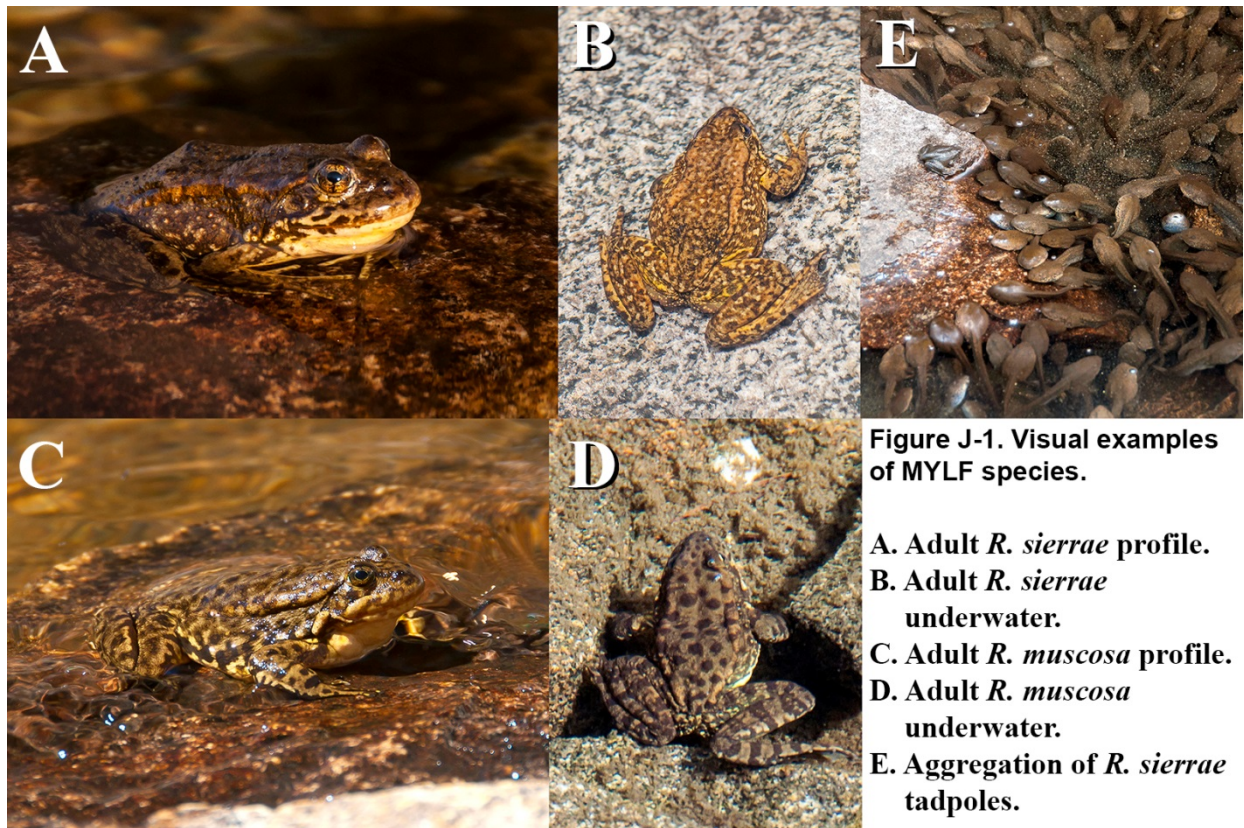


Figure J-1. Visual examples of MYLF species.

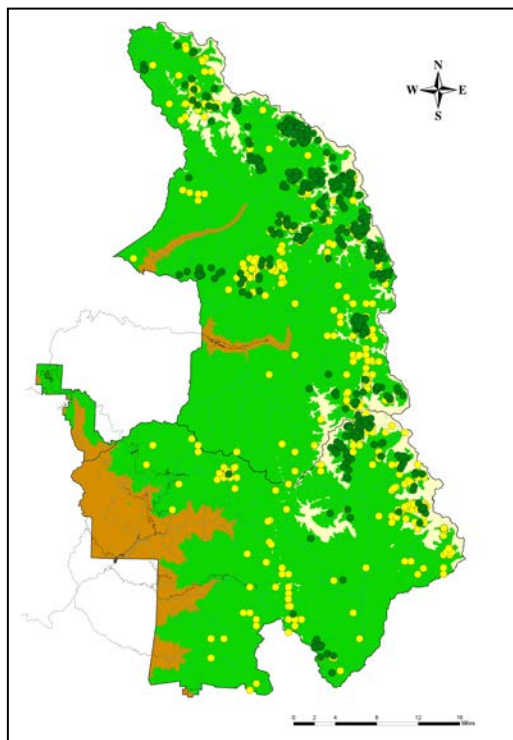
- A. Adult *R. sierrae* profile.
- B. Adult *R. sierrae* underwater.
- C. Adult *R. muscosa* profile.
- D. Adult *R. muscosa* underwater.
- E. Aggregation of *R. sierrae* tadpoles.

## Biology

MYLFs live high in the Sierra Nevada, occupying lakes, ponds, tarns, wet meadows, and streams (Mullally and Cunningham 1956). Though they have been reported between 4,495 ft (1,370 m; Zweifel 1955) to over 11,975 ft (3,650 m; Mullally and Cunningham 1956), MYLFs are normally encountered at the higher end of their elevational range. Historic, and some extant, populations exist in the mountains of southern California (San Gabriel, San Bernardino, San Jacinto, and Mt. Palomar), living at lower elevations from 1,214 ft (370 m) to over 7,500 ft (2,286 m; Zweifel 1955; Jennings and Hayes 1994). The historic records show that they ranged down to 6,400 ft (1,950 m) in Sequoia and Kings Canyon National Parks (SEKI), occupying montane meadows such as Round Meadow in Giant Forest and meadows at Grant Grove (Vredenburg et al. 2007, Vredenburg unpublished data). It is presumed that MYLFs ranged downstream to the upper limits of the natural distribution of trout prior to fish planting. In some drainages, this could have been below 5,000 ft (1,500 m). Known records and the likely historic distribution are shown in Figure J-2.

Within SEKI, the range of *R. sierrae* is generally bordered by ridges that divide the Middle and South Forks of the Kings River and ranges from Mather Pass, west to the Monarch Divide, and north to the northern boundary of the parks; while the range of *R. muscosa* is generally bordered by the crest of Sierra Nevada with the ridges that divide the Middle and South Forks of the Kings River, and ranges from Mather Pass, west to the Monarch Divide, and south to the southern boundary of the parks (Vredenburg et al. 2007).

MYLFs were once the most common frog and large tadpole seen within their range. Given their diurnal nature and historic large numbers, MYLFs were very conspicuous to early travelers. Seeing tens to hundreds of MYLFs leap into the water is an impressive sight for wilderness travelers. Though once common, they were rarely heard, since MYLFs call primarily under water (Stebbins and McGinnis 2012). Most frog calling in the high Sierra is produced by a more diminutive frog found throughout their range, the Pacific treefrog (*Pseudacris regilla*; Recuero et al. 2006). Adult MYLFs eat beetles, flies, ants, bees, wasps, bugs (Jennings and Hayes 1994), and treefrogs (Pope and Matthews 2002). Their predators include mountain garter snakes (*Thamnophis elegans elegans*), Brewer's blackbirds (*Euphagus cyanocephalus*), coyotes (*Canis latrans*), and introduced fish (Jennings and Hayes 1994). Additionally, there are observations of MYLFs being eaten by Sierra garter snakes (*Thamnophis couchii*), Clark's nutcrackers (*Nucifraga columbiana*), and American robins (*Turdus migratorius*) (NPS unpublished data), and also black bears (*Ursus americanus*) (Knapp R., pers. comm., 2010).



**Figure J-1. Estimated historic distribution of MYLFs.**

The estimated historic distribution is in green. Yellow dots mark historic records, and dark green dots mark observations since 1997. Brown areas are lower elevations than they were known to occur though the frogs range may have extended farther downstream prior to the planting of fish above their pristine distribution. The beige areas were generally too high to be inhabited.

Breeding and egg-laying is coincident with late snowmelt and usually occurs in late May or June, but can occur later in summer, depending on conditions and elevation. Egg masses are normally laid in shallow water, especially in tiny springs and streams adjacent to lakes and ponds (Vredenburg V., pers. comm., 2007). Because the growth period is limited to summer and early fall, it can take up to three summers (possibly four) for larvae to metamorphose into adults. During the summer, larvae congregate in shallow waters, where the warmer temperatures facilitate their development (Figure J-1). Sometimes hundreds of larvae can be seen within a few square feet of warm, shallow water (Bradford 1984).

Both adults and larvae must be able to survive the winter. Larvae can survive the loss of oxygen when shallow lakes freeze to the bottom, but adults are much more susceptible to winter kill (Bradford 1983). Adults must have deep lakes or other refugia from anoxic conditions caused by winter ice.

### Significance

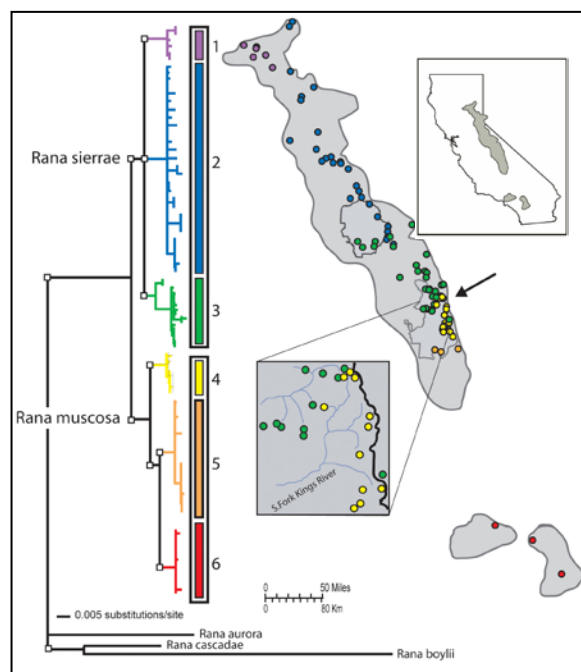
MYLFs are subalpine/alpine predators of both aquatic and terrestrial invertebrates, as well as some vertebrates like the Pacific treefrog (Pope and Matthews 2002). In turn, they are a major source of food to larger alpine predators like the mountain garter snake. The loss of MYLFs is likely to have a measurable impact on the natural functioning of the lakes and streams within their historic range. Their loss could change the abundance of some other species with which they interact. This has been demonstrated for the mountain garter snake (Matthews et al. 2002).

### Ecological Issues

#### *Decline of mountain yellow-legged frogs*

Populations of MYLFs have declined precipitously from their historic abundance, and they are in danger of becoming extinct. Drost and Fellers (1996) found that MYLFs have disappeared from eighty-six percent of the sites where Grinnell and Storer (1924) found them in 1915. Surveys by Bradford et al. (1994B) in SEKI during 1989-1990 failed to find MYLFs in 48% of the sites where they were found between 1955 through 1979. Within the Tablelands portion of the Kaweah drainage, MYLFs declined 96% between the late 1970s and 1989 (Bradford et al. 1994B). During the summers of 1997, 2000, 2001, and 2002, Roland Knapp and his field crews surveyed virtually all lentic sites in SEKI, and found MYLFs at 547 of the 3,639 sites surveyed. Repeat surveys in 2004 and 2005 showed that the frogs had gone extinct at 42% of the frog populations surveyed 3 to 8 years earlier (Knapp 2005B). Many of the sites that remain consist of small numbers of individuals that are vulnerable to extirpation (Knapp R., pers. comm., 2010).

Resurveys of historic localities throughout the ranges of MYLFs show dramatic declines (Vredenburg et al. 2007). Both species are imperiled: the Sierra Nevada yellow-legged



**Figure J-2. Historic range of mountain yellow-legged frogs and collection locations used for genetic analyses.**

From Vredenburg et al. (2007). Historic range (grey) and collection locations (dots) used for genetic analyses. Bayes phylogram shows two major clades (*Rana muscosa*, *Rana sierrae*) and six minor clades (1–6) identified from the mitochondrial DNA analysis. The contact zone (arrow) between the species is located between the Middle and South Forks of the Kings River (inset).



frog has been extirpated from 92.5% of its historic range, and the southern mountain yellow-legged frog has been extirpated from 96.2% of its historic range (Vredenburg et al. 2007).

#### *Need to Preserve Yellow-legged Frog Genetic Diversity*

In addition to the two species within the MYLF complex, each of the two species has three distinct genotypes or minor clades (Figure J-3; Vredenburg et al. 2007), and there is evidence that genetic diversity exists even within individual basins (Vredenburg V., pers. comm., 2007). Additionally, the critically endangered populations of southern mountain yellow-legged frogs in the San Gabriel, San Bernardino, and San Jacinto mountains, each show distinct population structure, which suggests a high level of genetic isolation (Schoville et al. 2011). There has likely been isolation in these populations since the Pleistocene, and the authors recommended that each of the three respective populations be managed as separate conservation units (Schoville et al. 2011). Preserving this diversity is one of the goals of this conservation effort and is consistent with NPS *Management Policies 2006*, which states: “*The Service will strive to protect the full range of genetic types (genotypes) of native plant and animal populations in the parks by perpetuating natural evolutionary processes and minimizing human interference with evolving genetic diversity*” (section 4.4.1.2).

Of the two species and six minor clades in the MYLF complex, SEKI includes the range of the southern minor clade (Number 3 in Fig. 3) of the Sierra Nevada yellow-legged frog and the two most northern minor clades (Numbers 4 and 5 in Fig. 3) of the mountain yellow-legged frog. Much of this genetic diversity is being lost due to the rapid decline of these species. Where genotypes are completely lost, the restoration effort would be limited to the nearest genetic matches to restore the ecological function the frogs provided. Again, this is consistent with NPS *Management Policies 2006*, which states: “*The restoration of native plants and animals will be accomplished using organisms taken from populations as closely related genetically and ecologically as possible to park populations, preferably from similar habitats in adjacent or local areas*” (section 4.4.1.2).

#### *Known Causes of the Decline*

Introduced trout are a major factor contributing to the declines of MYLFs (Bradford et al. 1993, Knapp 1996, Knapp and Matthews 2000, Vredenburg 2004, Knapp et al. 2007). Introduced predators are not a problem unique to MYLFs. Declines in a variety of western frogs have been attributed, in part, to the introduction of predators like bullfrogs, bass and sunfish, catfish, mosquito fish, and red swamp crawfish (Cowles and Bogert 1936, Dumas 1966, Moyle 1973, Hammerson 1982, Hayes and Jennings 1986, Corn 1994, Jennings and Hayes 1994).

Fish are not native to most of the upper elevations of the Sierra Nevada. Pleistocene glaciation and steep topography created barriers to fish moving upstream (Christenson 1977, Moyle et al. 1996). West of the Sierra crest, most native fish (primarily coastal rainbow trout) occurred in streams below 4,900 ft (1,500 m), but may have reached 7,200 ft (2,200 m) in streams in the Kings watershed (Moyle et al. 1996). South of the glaciation, golden trout ranged in streams up to 9,800 ft (3,000 m; Moyle et al. 1996). In the eastern watersheds of the Sierra Nevada, Lahontan cutthroat trout also ranged in streams up to 9,800 ft (3,000 m; Moyle 2002). When settlers of European ancestry first came to the high country, virtually all of the waters in the lake basins, which are perched above the high gradient streams, were naturally fishless.

Planting fish in natural areas of the Sierra Nevada began in 1861 and became widespread in the 1870s (Christenson 1977). The first *recorded* stocking of fish in SEKI was in 1970 (Christenson 1977). Initially, planting was done by stock users, anglers, and anyone else that wanted to move fish into fishless waterbodies. By 1912, the California Department of Fish and Game had become involved in planting fish. In general, brown trout (*Salmo trutta*) were planted at the lower elevations, rainbow trout (*Oncorhynchus mykiss*) at the mid elevations, and golden trout (*Oncorhynchus mykiss aguabonita*) in the high Sierra. Brook trout (*Salvelinus fontinalis*) were later added to the species planted in the mid-elevations, and

cutthroat trout (*Oncorhynchus clarki* subsp.) to the high elevations (NPS 1989). In the early days, fish planting was completed primarily with pack stock, but aircraft began to be used in 1940 (NPS 1989). This increased the efficiency of fish planting efforts and increased access to new planting locations. Since 1988, fish planting was been banned within SEKI.

Most lakes within the parks are relatively oligotrophic (having low biological productivity) because they are primarily in granitic basins at high elevations. In many lakes, nutrients and prey are initially abundant enough to sustain fish populations, but productivity declines over time as fish consume resources (Purkett 1951, Reimers 1958). In some situations, fish become stunted, or their populations decline to where they are in equilibrium with the lake (Pister 1977). Populations of brook trout planted in oligotrophic lakes are particularly prone to becoming stunted (Reimers 1979). The general low productivity of Sierran waterbodies led to an effort to enhance trout food. In 1919, the Department of Fish Culture introduced an amphipod (*Hyalella azteca*) and an alga (*Nitella* sp.) to Rae Lakes to enhance the fish food supply (NPS 1989). Today, these introduced organisms persist, and *Hyalella* is quite abundant in these waters.

Some trout species generally sustain themselves at a level that maintains healthy looking fish. A survey of 137 lakes in SEKI showed that 84 lakes (61%) were definitely self-sustaining, 13 (10%) were probably self-sustaining, 16 (12%) showed little evidence of fish reproduction, six (4%) were not producing any new fish, and 18 (13%) were fishless (Zardus et al. 1977). Armstrong and Knapp (2004) resurveyed all lakes in which there had been direct evidence of trout reproduction from the Zardus et al. (1977) study. Armstrong and Knapp (2004) found reproducing nonnative fish populations in 57 of the 84 resurveyed lakes (68%). Given that at least 20 years had passed since any stocking had occurred in the resurveyed lakes, Armstrong and Knapp (2004) concluded that sufficient natural reproduction was occurring to maintain numerous nonnative trout populations in the long term.

Nearly a century ago, Grinnell and Storer (1924) reported the loss of MYLF following the introduction of nonnative trout. Fish stocking intensity reached its peak following the adoption of airplane stocking in the late 1940s. Dramatic MYLF declines were sometimes not reported until years following initial stocking (Bradford 1989, Drost and Fellers 1996). However, lack of immediate reporting is not surprising, given that many MYLF populations experienced relatively slow declines over time from reduced recruitment in the presence of nonnative fish predators (Knapp and Matthews 2000). Additionally, the remoteness of many sites containing MYLFs (and corresponding difficulty of access) can result in declines not being documented for many years. Therefore, MYLF have been declining primary due to fish stocking between the late 19<sup>th</sup> and late 20<sup>th</sup> centuries. More recently, amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, often abbreviated *Bd*; discussed below) has spread through most remaining frog populations in SEKI and intensified long-term declines (Vredenburg et al. 2010A).

Within MYLF habitat, not all waterbodies provide ideal habitat. Ponds that may be good for feeding may not be the best sites for breeding or over-wintering. Matthews and Pope (1999) documented seasonal movement between ponds. In addition to seasonal movements, recruitment may be high at some locations, while other waterbodies maintain their populations through immigration from more productive sites. Before fish were introduced, there was connectivity, and thus potential for successful movement, between the ponds. Frogs could freely migrate between ponds to achieve their seasonal needs, and ponds that lost frogs could be recolonized from nearby locations. The isolation of frog populations has been exacerbated by the effects of both introduced fish and amphibian chytrid fungus.

Because trout and MYLFs are largely mutually exclusive (Bradford 1989, Bradford et al. 1993, Knapp 1996), the introduction of trout fragments the historic connectivity of MYLF habitat. Dense populations of predatory fish impede seasonal movements and occupy many deep water over-wintering sites formerly used by MYLFs. Frog populations have subsequently become fragmented and isolated. Then, if the MYLF population of a smaller waterbody is wiped out by a catastrophic event like drought, disease, or

winter-kill, often there are no adjacent source sites for recolonization. The addition of environmental stressors, such as amphibian chytrid fungus and climate change, may have increased the frequency of local catastrophic events (Blaustein et al. 2011). Not only do populations become more isolated with the loss of connectivity, but with isolation, there is likely to be increased inbreeding and possible loss of genetic viability. Amphibian populations have been shown to have natural fluctuations (Pechmann et al. 1991), but without connectivity, the means to recover from an inherent drop in numbers would be more limited. What might have been a downward fluctuation 200 years ago could today, in the absence of connectivity, result in extirpation. As isolated populations disappear, the distance between potential sources has increased. Thus, as isolation and environmental stressors continued to increase in magnitude, it is likely that the rate of frog declines will also increase.

Amphibian chytrid fungus has compounded the effects of nonnative fish on MYLFs, causing serious losses in many populations throughout their ranges. Amphibian chytrid fungal infection can result in chytridiomycosis, a disease that causes catastrophic disruption to the skin of infected adult frogs (Voyles et al. 2009, Rosenblum et al. 2012). Both species of MYLFs share the same pathological response to amphibian chytrid fungal infection, including electrolyte imbalance, compromised ability to osmoregulate, and cascading effects on other organ systems. Many of these perturbations appear to be directly linked to physical disruption of the epidermis (Rosenblum et al. 2012).

Studies demonstrate that amphibian chytrid fungus recently spread through the Sierra Nevada (Morgan et al. 2007, Vredenburg et al. 2010A, Voyles et al. 2012) and has infected nearly all MYLF populations, including those in SEKI. Most MYLF populations crash within a few years after becoming infected, and many populations have been extirpated. Amphibian chytrid fungus has thus been a major factor in accelerating the decline of MYLFs already well underway following the introduction of nonnative trout (Knapp et al. 2011).

MYLF populations that persist following chytridiomycosis outbreaks have very low survival and recruitment from year to year, making them extremely vulnerable to extirpation. In addition to trout removal, these MYLF populations would likely benefit from an emerging disease treatment technique using antifungal agents, designed to significantly increase short-term survival, and hopefully long-term recruitment. The first phase involves treating frogs with itraconazole (an antifungal drug) to reduce their infection levels; and a second possible phase involves augmenting the concentration of a common bacterium (*Janthinobacterium lividum*) on the skin of frogs that has protective anti-fungal properties. Combined, the regimen appears to allow development of an immune response on treated frogs, thus changing the outcome for many frogs from mortality to persistence. Preliminary results of several field trials conducted in SEKI from 2009 to 2012 have shown promise for future management application. Immediately following the discovery of a large scale chytridiomycosis die-off in northern Kings Canyon National Park, a larger-scale study was initiated in August 2015 to determine if anti-fungal treatments are a viable option for reducing amphibian chytrid fungal infection intensities, facilitating MYLF adult survival following chytridiomycosis outbreak, and help frogs develop an immune response to the disease (Knapp et al. unpublished data). Follow-up surveys are planned for the summer of 2016 to determine if there was survival among the frogs treated with anti-fungal drugs.

#### *Potential Contributing Factors*

For over two decades, scientists have been noting the dramatic world-wide decline of amphibians (Blaustein and Wake 1990, Stuart et al. 2004, Wake and Vredenburg 2008). Many different hypotheses have been developed to explain the losses, and in many cases there are local data to support one or more potential causal agents, but often there is not enough data to suggest that any given hypothesis fully explains the worldwide decline. The list of commonly cited possible explanations for amphibian declines includes: disease, habitat loss, pesticides, climate change, introduced predators/competitors, acid deposition, increased UV-B radiation (as a consequence of the thinning stratospheric ozone layer), and

various combinations of all these factors (Collins and Storfer 2003). Scientific articles and popular media often refer to amphibians as indicator species or “coal miners' canaries” and warn that amphibian losses are an early warning for humans (Kerby et al. 2010).

Climate change has recently begun to affect MYLFs by drying and/or freezing small, shallow ponds to which MYLF populations are restricted in many basins because nonnative trout occupy all adjacent lakes (Lacan et al. 2008). These ponds can completely dry up in even relatively short droughts, an event that has already occurred in Dusy Basin (Lacan et al. 2008). When this happens, multiple year-classes of MYLF tadpoles are lost, and populations already suppressed by trout can be quickly extirpated. In addition, shallow ponds can freeze solid during atypical climate patterns, which also occurred in Dusy Basin during the winter of 2011 to 2012. This event appears to have killed most of the adult MYLFs that remained in that area of Dusy Basin. Quickly eradicating nonnative trout would allow MYLF populations to expand (Knapp et al. 2007) and recolonize large lake habitat that is much more resistant to the effects of climate change.

Changes in the climate of the Sierra Nevada could affect MYLFs in a variety of ways (Lacan et al. 2008). Snow pack depletion could reduce the availability of shallow ponds or the connectivity of deep lakes. The broad historic elevation range of the MYLFs suggests that climate projections may be tolerable for the species if adequate aquatic habitat persists, but it is not known what indirect effects climate change may cause to the species. How climate change may affect amphibians worldwide is still mostly unknown (Davidson et al. 2002, Carey and Alexander 2003, Lips et al. 2008, Rohr et al. 2008, Blaustein et al. 2010).

Pesticides may also be contributing to the loss of MYLFs (Sparling et al. 2001, Davidson et al. 2002, Davidson 2004, Fellers et al. 2004, Davidson and Knapp 2007) and other California amphibians (Sparling et al. 2001, Davidson et al. 2002, Davidson 2004). However, not all recent studies have found an association between pesticides and amphibian decline in the high elevation regions of the Sierra Nevada (Bradford et al. 2011). Still, the southern and central Sierra Nevada are downwind of one of the most intensely cultivated areas on earth (Cory et al. 1970). Fresno and Tulare Counties used over 43 million pounds of pesticide active ingredients in 2010 (CDPR 2011). Combined with Kings and Kern Counties, nearly 76 million pounds (CDPR 2011) of pesticide active ingredient were used in agricultural areas upwind of the southern Sierra Nevada in 2010 alone.

A variety of pesticides have been found in the parks' water and in the tissues of amphibians and fish. Measurable quantities of organophosphate pesticides were measured in the Sierra Nevada at 6,300 ft with increasing concentrations at lower elevations (Zabik and Seiber 1993). Datta et al. (1998) found polychlorinated biphenyl (PCBs) and dichlorodiphenyldichloroethylene (DDE) in trout in the Kaweah drainage. Pacific treefrog tadpoles contained PCBs, chlorpyrifos, chlorthalonil, and a chloronitrile fungicide. In the late 1960s, Cory et al. (1970) found DDE residues in MYLFs, with the heaviest concentrations being in the southern and central Sierra Nevada. Angerman et al. (2002) found PCB concentrations in Pacific treefrogs to range from 244 ng/g (wet weight) at low elevations to 1.6 ng/g on the eastern slopes of the Sierra Nevada. Likewise, toxaphene in Pacific treefrogs varied from 1.5 ng/g to 15.6 ng/g. The data suggested that rain-shadows may reduce tissue concentrations.

The pesticide drift into these parks includes organophosphorous pesticides. They are highly toxic to amphibians because they deactivate acetylcholinesterase, the enzyme that breaks down acetylcholine. This results in a repeated and uncontrolled firing of neural signals across synapses, causing the animals to die, usually from asphyxiation. Metabolism of organophosphorus pesticides in the liver, plus breakdown by certain bacteria and other environmental conditions, creates oxons that increase the toxicity of the pesticide. Sparling and Fellers (2007) calculated the LC50 (the concentration that kills half of the test animals) for the three most commonly used organophosphorus pesticides in California's Central Valley,

chlorpyrifos, malathion, and diazinon, using larval foothill yellow-legged frogs (*Rana boylei*). Median lethal concentrations were 3.005 mg/l for chlorpyrifos with a 24 hr exposure, 2.14 mg/l for malathion with a 96 hr exposure, and 7.488 mg/l for diazinon with a 96 hr exposure. The oxons of these chemicals were 10 to 100 times more lethal with maloxon showing an LC50 of 0.023 mg/l and diazoxon being 0.760 mg/l. Cloroxon could not be calculated because all the test animals died at the lowest concentration, so cloroxon exceeds being 100 times more toxic than its parental compound.

The LC50 concentrations for *R. boylei* are well above reported southern Sierra Nevada high elevation pesticide concentrations (Zabik and Seiber 1993, McConnell et al. 1998, LeNoir et al. 1999, Fellers et al. 2004), which are normally reported in nanograms (values that are one millionth of a milligram) and sometimes in pictograms (one billionth of a milligram). However, when Davidson (2004) compared extant frog populations with historic pesticide use, he found a significant inverse relationship between pesticide application and amphibian populations for four species of frogs, including MYLFs. This relationship was strongest for the cholinesterase inhibiting pesticides. The low concentrations of pesticides found in the southern Sierra suggest that pesticide effects are sublethal. While those sublethal effects have not been identified, one possibility is reduced immunity.

Pesticide depression of immunity has been demonstrated in some frogs. Gilbertson et al. (2003) demonstrated immunosuppression on the northern leopard frog (*Lithobates pipiens*) using malathion, and Davidson et al. (2007) on the foothill yellow-legged frog using carbaryl. A landscape-scale study by Davidson and Knapp (2007) looked simultaneously at correlations between pesticide use, prevailing wind patterns, and predation by introduced fish. The study found that both stressors caused significant loss of MYLFs, but the landscape-scale correlation with pesticide application was stronger than the effect from introduced fish. However, this association was not based on actual measurement of pesticide concentrations in the field, or within animal tissues (Davidson and Knapp 2007).

A more recent study investigated potential effects of pesticide exposure in MYLFs at 14 high elevation sites in SEKI (Bradford et al. 2011). In this study, the authors measured concentrations of nine different pesticide compounds (only those compounds—of an original 46 pesticides and metabolites targeted for measurement—that were detected >30% of the time in sediment and tadpoles) that were detected in air and sediment samples, plus cholinesterase (ChE) levels (which are used as an indicator of organophosphorus and carbamate exposure) measured in whole Pacific treefrog tadpoles (Bradford et al. 2011). The study concluded that the results “do not support the hypothesis that pesticides have contributed to the population declines of *R. muscosa* and *R. sierrae* in the alpine zone of the southern Sierra Nevada. In particular, no association was found between any pesticide-related metric and population declines. By contrast, the amphibian disease chytridiomycosis has been demonstrated in other studies as the cause for dramatic population declines of many populations of these species in recent years. Moreover, linear distance from the Valley was strongly related to frog population status in the present study, a finding that is consistent with the apparent pattern of spread of chytridiomycosis.” (Bradford et al. 2011, pg. 690).

Bradford et al. (2011) concluded that there is evidence for lethal and sublethal effects of pesticides for other species of amphibian residing in lower elevations and downwind locations closer to where pesticides are being applied (Davidson 2004, Sparling and Fellers 2009). For example, using the Pacific treefrog as a sentinel species, Sparling et al. (2001) demonstrated that cholinesterase activity was significantly more suppressed on the downwind side of the Central Valley in the Sierra Nevada foothills (~1500 m elevation) than upwind along the coast or north of the Valley.

In addition to declines, there is another phenomenon that concerns scientists. In some areas, frogs are developing deformities that range from missing to extra limbs (Lannoo 2008). This has been attributed to some of the same processes believed to be effecting declines (e.g., UV-B radiation; Ankley 1997), but natural causes like trematodes have also been identified (Sessions 1997). Deformities were not believed to



be an issue in the Sierra Nevada until Pacific treefrogs with unnaturally shortened femurs (brachymelia) were found in Sequoia and Yosemite National Parks (Cowman et al. 2002).

Air pollutants may also be cause for concern. Jennings (1996) reported a conversation with T. Cahill stating that studies by the Crocker Nuclear Laboratory have noticed that the pattern of recent frog extinctions in the southern Sierra Nevada corresponds to the patterns of highest concentrations of exhaust pollutants from automobiles. Nitrates and nitrites are associated with automobile pollution. Marco et al. (1999) found that *Rana pretiosa* and *Ambystoma gracile* larvae were sensitive to nitrite and nitrate levels allowable in Environmental Protection Agency (EPA) established drinking water concentrations for these chemicals. The effects increased with both concentration and time.

Another indirect effect of air pollution is the thinning of stratospheric ozone as a consequence of chlorofluorocarbons, resulting in increased ultraviolet radiation, especially the UV-B region of the spectrum. Blaustein et al. (1994B, 1995, and 1997) attributed the decline of several amphibians to UV-B radiation. However, other investigators have failed to find negative effects of UV-B on amphibians (Grant and Licht 1995, Ovaska et al. 1997, Corn 1998, Vredenburg 2002, Vredenburg et al. 2010B). Exposing embryonic stages to ultraviolet radiation does reduce survival for some species (Worrest and Kimeldorf 1975, 1976; Blaustein et al. 1994B, 1995, 1997; Grant and Licht 1995; Hayes et al. 1996) and can cause developmental malformations (e.g., extra or missing limbs) in a laboratory situation (Ankley et al. 1998). However, what can be done in a laboratory does not necessarily resemble what happens in nature. In a natural environment, larvae have the ability to avoid UV-B radiation by shielding themselves under vegetation, in rock crevices, and under mud and detritus. There is no published evidence, to date, that UV-B radiation has caused declines of MYLFs. However, the limited information currently available does not mean that MYLFs are insensitive to changes in ambient UV-B levels.

Diseases other than amphibian chytrid fungus have also been implicated in some amphibian population declines, including ranaviruses and red leg syndrome (Densmore and Green 2007). Red leg syndrome (bacterial dermatosepticemia) may have contributed to losses of several species of toads and larval tiger salamanders in the western United States (Collins et al. 1988, Worthylake and Hovingh 1989, Carey 1993, Kagarise Sherman and Morton 1993). Both Bradford (1991) and Knapp (pers. comm., 2010) reported red leg syndrome to cause losses of MYLFs. They reported red-leg syndrome as localized cases and not likely to cause widespread declines. Red leg syndrome is often a consequence of immune systems being weakened by stress (Corn 1994). In 2001, a major die-off from a ranavirus was reported in Upper Basin in Kings Canyon National Parks (Knapp R., pers. comm., 2010). Mao et al. (1999) demonstrated that Iridoviridae (the virus family that includes *Ranavirus*) could be transmitted from introduced trout to amphibians.

The waters of SEKI are subject to acidic deposition (Melack et al. 1989). Acid deposition is a potential source of stress on frog populations. Tome and Pough (1982) looked at 14 species of amphibians, and found that when pH drops to 4 or less, mortality during embryonic development is over 50%. This increased to 85% with pH between 3.7 to 3.9. Acidic deposition has been suspect as a contributing cause to amphibian declines in a variety of places (Harte and Hoffman 1989, Wyman 1990, Blaustein and Wake 1990, Carey 1993), and episodic acidification does occur in these parks in basins with low acid neutralizing capacity (Melack et al. 1989, Stoddard 1995). Bradford et al. (1992) found that *Rana muscosa* embryos and hatchlings were not sensitive to pH values recorded in high-elevation Sierra Nevada lakes. From an analysis of pH at 235 potential breeding sites, Bradford et al. (1994A) concluded that acidic deposition is not a likely cause of amphibian declines in the Sierra Nevada. However, Bradford et al. (1998) reported that MYLF tadpoles were absent in acidic lakes (pH <6) in the vicinity of Mt. Pinchot (Bradford et al. 1998). When nine lakes and ponds in the Middle Fork Kaweah drainage were surveyed in 1993, eight of nine field pH measurements were below six (NPS unpublished data). The one

high pH of 8.1 was from a pond in marble, a rock that neutralizes acidity. Given a lack of any observations for many years, MYLFs are assumed to be extirpated from the Kaweah drainage.

Direct habitat destruction is one of the leading factors responsible for amphibian declines (Lehtinen et al. 1999, Davidson et al. 2002, Cushman et al. 2006, Gallant et al. 2007, Sodhi et al. 2008). Some amphibian losses can be attributed to the conversion of wetlands to urban or agricultural use (Corn 1994). Other alterations are subtler. Jennings (1996) noted that: “*aquatic habitats of the Sierra Nevada have been greatly altered through dams, diversions, channelizations, siltation, livestock grazing, timber harvest, placer mining, and many other factors.*” Although certainly a factor in MYLF declines outside of protected areas, habitat loss and alterations appear to be an unlikely factor in the loss of MYLFs in SEKI because they occupy high-elevations sites far removed from human engineering projects. Many of the historic populations occurred in remote basins that infrequently see human use. Most populations are within designated wilderness where they, and their habitat, are fully protected.

Not all amphibian declines are the result of direct or indirect human activities. Fluctuations have been reported in amphibian populations (Pechmann et al. 1991, Pechmann and Wilbur 1994). It is important to distinguish between natural events and losses attributable to anthropogenic effects. In many cases, a combination of causes may be contributing to amphibian losses (Collins and Storfer 2003, Blaustein et al. 2011). As stated above, chytridiomycosis and predation by introduced fish are the primary causes of decline of MYLF species. However, other causes may have contributed to amphibian declines in the Sierra, and some factors not previously considered may also be contributing.

#### *Loss of Aquatic and Terrestrial Biodiversity*

Introduced trout not only contribute to the decline of MYLFs (Bradford et al. 1993, Knapp and Matthews 2000), they contribute to a general loss of biodiversity in aquatic biota and associated terrestrial fauna (Knapp et al. 2001, Knapp et al. 2005, Knapp and Sarnelle 2008, Epanchin et al. 2010). For example, long-toed salamander (*Ambystoma macrodactylum*) populations are greatly reduced when fish are present, and fish can occasionally completely exclude long-toed salamanders from breeding sites (Lannoo 2005, pg. 620). Epanchin et al. (2010) found gray-crowned rosy finch to be more common at lakes without fish than at lakes with fish. This is because introduced fish populations limit mayfly populations on which the finches feed during mayfly emergence.

While quantitative data is lacking, the abundance of other alpine/subalpine species are likely to be affected by losses of MYLF populations. Both Brewer’s blackbirds and Clark’s nutcrackers feed on MYLFs (Jennings and Hayes 1994, NPS unpublished data). Black bears (*Ursus americanus*) have also been observed foraging for MYLFs (Knapp R., pers. comm., 2010). MYLFs may have been an important high elevation food for bears prior to nonnative trout and disease caused declines. Mountain garter snakes feeds on MYLFs (Jennings et al. 1992), and Matthews et al. (2002) found that mountain garter snake abundance is directly related to frog abundance. Additionally, MYLFs have historically been important predatory species in the high elevations of the Sierra Nevada. Most of their prey consists of insects (Finlay and Vredenburg 2007), but they feed also on small vertebrates, such as Pacific treefrogs (Pope 1999, Pope and Matthews 2002).

Trout virtually eliminate large-bodied invertebrates from lakes. When Stoddard (1987) surveyed zooplankton in 75 Sierra Nevada lakes, he found fish to be important predictors of zooplankton species occurrence, with small-bodied species being found in association with fish and large-bodied species occurring only where fish were absent. Likewise, Bradford et al. (1998) found large-bodied planktonic microcrustaceans (e.g., *Hesperodiaptomus shoshone* and *Daphnia middendorffiana*) and epibenthic and limnetic macroinvertebrates (e.g., back swimmers, water boatmen, predaceous diving beetles, and larvae of some families of caddis flies and mayflies) to be relatively common in lakes without trout, but rare or absent in lakes with trout.

Herbst et al. (2009) found that the presence of introduced trout in streams resulted in decreased density for 20 invertebrate taxa and increased abundance for six taxa. The strongest effects appeared to be on taxa endemic to the Sierra Nevada, which have no coevolutionary history that would have facilitated the development of mechanisms to deal with fish predation. The study found that streams containing introduced trout had significantly more algae density and cover, increased abundance of midges, and reduced density of the most common large invertebrate predator, the stonefly *Doroneuria baumanni*.

Introduced trout are also a threat to native trout. On the Kern Plateau, introduced brown trout threatened the golden trout native to the South Fork Kern River. Programs to remove brown trout were necessary to manage the native fishery. In the Little Kern River drainage, the Little Kern golden trout became federally listed as threatened because of genetic introgression from planted rainbow trout (FWS 1978). Today, there remains an interagency effort to restore the Little Kern golden trout. Likewise, the original genotypes of rainbow trout native to the Parks' western drainages are unlikely to have persisted following a century of planting non-indigenous rainbow and golden trout (Christenson 1977). Many of the fish in those streams show evidence of hybridization with golden trout (Gall et al. 1976)

The impacts of trout can be broader than the direct loss of the organisms they consume or displace. The native organisms preyed on by nonnative trout are important, interconnected components of the ecosystem. Once removed, their loss affects the other native organisms on which they feed, and the native predators that depend on the trout-consumed native species. Knapp (1996) cites several published examples of these cascading effects.

Alone or in combination, both the introduction of trout and the decline of MYLFs threaten the natural biodiversity of the southern Sierra Nevada. Even if the frog were to go extinct for reasons beyond our control, the removal of nonnative trout would benefit restoration of more natural assemblages of invertebrates and go a long way toward restoring alpine/subalpine community structure and biodiversity to a more natural condition.

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