# 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 southern mountain yellow legged-frog (*Rana muscosa*). Both species are currently proposed for federal listing as endangered (FWS 2013), while *R. sierrae* is state listed as threatened and *R. muscosa* is state listed as endangered (CFGC 2012).

The two genetically distinct species can be distinguished visually (Vredenburg et al. 2007). The Sierra Nevada yellow-legged frog tends to be dark with light spots dorsally (Fig. 1), and the legs are shorter than the southern mountain yellow-legged frog. The southern mountain yellow-legged frog has dark spots on a light background (Figure 16), and the legs are longer than the Sierra Nevada yellow-legged frog.

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. Some red may be present. Larvae (tadpoles) are very dark (nearly black) during their first year. Older larvae are slightly lighter and resemble large ripe olives with tails (Figure 16; Stebbins and McGinnis 2012).



#### 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 7,513 ft (2,290 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 occupying montane meadows such as Round Meadow at Giant Forest and meadows at Grant Grove (Vredenburg et al. 2007, Vredenburg unpublished data).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 17.

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 life history 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 2006). Adult MYLFs eat beetles, flies, ants, bees, wasps, bugs (Jennings and Hayes 1994), and treefrogs (Vredenburg V., pers. comm., 2007). 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



Figure 2. 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.

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).

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. 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 (Vredenburg V., pers. comm., 2007). 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 Sequoia and Kings Canyon National Parks during 1989-1990 failed to find MYLFs in forty-eight percent of the sites where they were found between 1955 through 1979. Within the Tablelands portion of the Kaweah drainage, MYLFs declined ninety-six percent 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 these parks, 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 frog has been extirpated from 92.5% of its



Figure 3. Historic range of mountain yellowlegged 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).

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 (Fig. 3; Vredenburg 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 3 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 Policy 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" (NPS 2007).* 

Of the two species and six minor clades in the MYLF complex, Sequoia and Kings Canyon National Parks include 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 southern 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 will be limited to the nearest genetic matches to restore the ecological function the frogs provided. Again, this is consistent with NPS Policy 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"* (NPS 2007).

# Known Causes of the Decline

Introduced trout are a major factor contributing to the declines of MYLFs (Knapp and Matthews 2000). Introduced predators are not a problem unique to MYLFs. Declines in a variety of western of 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 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. Later, brook trout (*Salvelinus fontinalis*) were added to the species planted in the mid-elevations, and cutthroat trout (*Oncorhynchus clarki* subsp.) to the high elevations (NPS, U. S. Department of the Interior 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 Sequoia and Kings Canyon National Parks.

Most lakes within the parks have relatively 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 became 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). Other species generally sustain themselves at a level that maintains healthy-looking fish. A survey of 137 lakes in Sequoia and Kings Canyon National Parks showed that 61% were self-sustaining, 10% were probably self-sustaining, 12% showed little evidence of fish reproduction, 4% were not producing any new fish, and 13% were barren of fish (Zardus et al. 1977). Knapp and Matthews (2000) found fish in 20% of the 1,059 lakes and ponds, representing more than 50% of the total waterbody surface area, surveyed in northeastern Kings Canyon National Parks. The results from these two studies are not that different since Zardus et al. (1977) excluded ponds.

One consequence of the general low productivity of Sierran waterbodies was 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 the *Hyalella* are quite abundant in these waters.

If fish planting has been going on since the mid to late 1800s, why didn't the dramatic declines begin to occur until the last two decades? The answer probably lies in the cumulative effects of fragmentation. Within their habitat, not all water carries equal value: some ponds are better homes or serve certain functions better than others. 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, some sites may produce many frogs; others maintain their populations by immigration from more productive sites. Before fish were introduced, there was connectivity, and thus potential for effective movement, between the ponds. Frogs could freely move between ponds to achieve their seasonal needs, and ponds that lost frogs could be recolonized from nearby locations.

Because trout and MYLFs are largely mutually exclusive (Bradford et al. 1994, Knapp 1996), the introduction of trout fragmented the historic connectivity. Gauntlets of predatory fish impeded seasonal movements. Fish populated most deep water over-wintering sites. Frog populations became fragmented and isolated. Then, if the population of a pond was wiped out by a catastrophic event like drought, disease, or winter-kill, often there were no adjacent source sites for recolonization. The addition of environmental stressors, such as chytrid 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). Without connectivity, the means to recover from an inherent drop in numbers would be more limited. What might have been a downward fluctuation two-hundred years ago could today, in the absence of connectivity, easily turn into another local extirpation. As isolated populations disappeared, the distance between potential sources increased. As isolation and environmental stressors increased, it is likely that the rate of frog declines increased.

The recently discovered amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Bd), has compounded the effects of nonnative fish on MYLFs, causing serious losses in many populations throughout their ranges. Bd infection can result in chytridiomycosis (chytrid), which 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 chytrid 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).

Virtually all remaining MYLF populations in SEKI are infected with chytrid. Studies indicate it recently spread through the Sierra Nevada (Morgan et al. 2007) and has infected nearly all MYLF populations including in SEKI. Most MYLF populations crashed within a few years after becoming infected, and many populations were extirpated. Chytrid fungus has thus been a major factor in accelerating the decline of MYLFs caused by non-native trout in the Sierra Nevada (Knapp et al. 2011).

Virtually all of SEKI's infected MYLF populations have experienced severe die-offs, and the remaining remnant populations 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 the second 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 show promise for future management application. A larger-scale study and is expected to be initiated in 2013.

In addition, climate change has recently begun to affect MYLFs by drying and freezing small, shallow ponds to which MYLF populations are restricted in many basins because nonnative trout occupy all adjacent lakes (Lacan et al. 2008). The trout severely limit frog distribution and abundance by excluding them from large and deep lake habitat, while at the same time restricting them to pond habitat that is highly vulnerable to climate change. These ponds can completely dry up in even relatively short droughts as 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 as 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 this area. Eradicating non-native trout as quickly as possible in such areas will allow MYLF populations to expand (Knapp et al. 2007) and recolonize large lake habitat that is much more protected from climate effects.

#### Potential Contributing Factors

Pesticides may be also 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 a statistical association between pesticides and amphibian decline in the Sierra (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 within 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 PCBs and 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 polychlorinated biphenyl (PCBs) 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 a high of 15.6 ng/g to a low of 1.5 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 boylii*). 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.

These concentrations are well above those reported for the southern Sierra Nevada (Fellers et al. 2004, McConnell et al. 1998, LeNoir et al. 1999, Zabik and Seiber 1993) which are normally reported in nanograms, values that are one millionth of a milligram and sometimes in picograms, numbers that are 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 4 species of frogs, including the MYLF. This relationship was strongest for the cholinesterase inhibiting pesticides. The low concentrations of pesticides found in the southern Sierra suggest that pesticide effects are caused by sublethal effects. 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 (*Rana 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 pesticides and predation by introduced fish. They found that both stressors caused significant loss of MYLFs, but the effect from pesticide application was stronger than the effect from introduced fish. 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 than upwind along the coast or north of the Valley. They found higher levels of cholinesterase suppression in populations that were doing poorly compared to healthy populations. Over half of the specimens from Yosemite National park contained residues of organophosphorus pesticides compared to only 9% along the California coast.

For over two decades, scientists have been noting the dramatic world-wide decline of amphibians (Blaunstein and Wake 1990, Stuart et al. 2004, Wake and Vredenburg 2008). Many different theories have been developed to explain the losses, and in many cases there was local data to support one or more of the hypotheses, but scientists often have few clues. The list of possible causes omits few options: increased UV-B radiation as a consequence of the thinning stratospheric ozone layer, pollution, acid deposition, pesticides, introduced diseases, introduced predators, global climate change, habitat destruction, and various combinations of the above postulates (Collins and Storfer 2003). Occasionally one sees articles in the popular media that compare amphibians to the coal miners' canary and warn that amphibian losses are an early warning for humans (Kerby et al. 2009).

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. This has been attributed to some of the same processes believed to be effecting declines (e.g., UV-B radiation; Ankley 1996-1997), but natural causes like trematodes have also been identified (Sessions 1996-1997). 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 some amphibian larvae sensitive to elevated nitrite and nitrate concentrations. The effects increased with both concentration and time.

Climate change is exacerbated partly by anthropogenic generation of greenhouse gases like methane and carbon dioxide. Pounds et al. (1999) demonstrated the loss golden toads and associated species in Costa Rica due to global warming. As the climate warmed, these mountaintop species were stranded as their climatic requirements moved above the mountaintop. Pounds et al. (2006) demonstrated that the changing climate created conditions that were optimal for amphibians to contract chytridiomycosis. Changes in the climate of the Sierra Nevada could affect MYLFs in a variety of ways (Lacan et al. 2008). Losses in the snow pack could affect the availability of shallow ponds or the connectivity of deep lakes. On a positive side, lakes at higher elevations might become available habitat. The broad historic elevation range of the MYLFs suggests that climate projections may be tolerable for the species if adequate aquatic habitat persists, but we do not know 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).

Other diseases have been blamed for the destruction of many amphibian populations. Red leg disease 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 it to cause losses of MYLFs. They reported it as localized cases and not likely to cause widespread declines. Red leg disease is often a consequence of immune systems being weakened by stress (Corn 1994). The fungus that causes Red leg disease, *Saprolegnia*, is frequently found in hatcheries, and its spread may be another consequence of planting trout (Knapp 1996). 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 (Family includes *Ranavirus*) could be transmitted from introduced trout to amphibians.

The waters of Sequoia and Kings Canyon National Parks 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 fourteen 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 (Blaustein and Wake 1990; Carey 1993; Harte and Hoffman 1989; Wyman 1990), 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 9 lakes and ponds in the Middle Fork Kaweah drainage were surveyed in 1993, 8 of 9 field pH measurements were below 6 (unpublished data). The one

high pH of 8.1 was from a pond in marble, a rock that neutralizes acidity. MYLFs are believed extirpated from the Kaweah drainage.

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. (1994, 1995, and 1997) attributed the decline of several amphibians to UV-B radiation. However, other investigators have not been able to replicate his experimental results (Grant and Licht 1995, Ovaska et al. 1997, Corn 1998, Vredenburg 2002). Exposing embryonic stages to ultraviolet radiation does reduce survival for some species (Worrest and Kimeldorf 1975, 1976; Blaustein et al. 1994, 1995; Hayes et al. 1996) and can cause developmental malformations (e.g., extra or missing limbs) in a laboratory situation (Ankley et al. 1998). 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, but that does not mean that they are not potentially sensitive to changes in ambient UV-B levels.

Direct habitat destruction is one of the most visible causes of amphibian attrition. 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". This would appear to be an unlikely cause that is unlikely to be a factor in the loss of MYLFs because they occupy high-elevations sites, most of which are far removed from human engineering projects. Many of the historic populations occurred in remote basins that infrequently see human use. Most populations are within federal wilderness or in national parks where they, and their habitat, are fully protected.

When thinking about amphibian declines, one must also consider that not all losses are necessarily the result of human intervention. Fluctuations have been reported in amphibian populations (Pechmann et al. 1991, Pechmann and Wilbur 1994). It is important to be able to distinguish between natural events and loses attributable to human impacts. In many cases, a combination of causes may be contributing to amphibian losses (Collins and Storfer 2003, Blaustein et al. 2011). As stated above, we know that chytridiomycosis and predation by introduced fish are primary causes of decline of MYLF species. However, other causes likely contributed to amphibian declines in the Sierra, and some factors not previously considered may also be contributing. We are especially concerned about atmospheric transport of toxic compounds.

#### Loss of Aquatic and Terrestrial Biodiversity

Introduced trout not only contributed to the decline of MYLFs (Bradford et al. 1993, Knapp 1996, Vredenburg V., pers. comm., 2007), they contributed to a general loss of biodiversity in aquatic biota and associated terrestrial fauna. In the northern Sierra Nevada, the long-toed salamander appears to be found primarily in fishless lakes (Bradford and Gordon 1992). Epanchin (2010) found rosy-crowned 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 finch feeds during mayfly emergence. The mountain garter snake feeds on MYLFs. Matthews et al. (2002) found that mountain garter snake abundance is directly related to frog abundance.

While quantitative data is lacking, the abundance of other alpine/subalpine species are likely to be affected by losses of frog populations. Both Brewer's blackbirds and Clark's nutcrackers feed on MYLFs (Jennings and Hayes 1994, NPS unpublished data). While the high elevations of the southern Sierra Nevada seem to provide little natural food for black bears (*Ursus americanus*), they have been observed foraging for MYLFs (Knapp R., pers. comm., 2010). Before frog populations crashed, they may have

been an important high elevation food for bears. Additionally, MYLFs are not only prey for a variety of alpine/subalpine vertebrates, they are also a predator. Much of their food is insects, but they feed also on small vertebrates, such as Pacific treefrogs (Pope 1999, Vredenburg V., pers. comm., 2007).

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 species occurrence, with small-bodied species being found in association with fish and large-bodied species occurring only where fish are absent. Likewise, Bradford et al. (1994, 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 6 taxa. The strongest effects appeared to be on taxa endemic to the Sierra Nevada, which had no coevolutionary history that would have facilitated their 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 a threat to native trout, as well. 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. To this day there is 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. Many of the fish in those streams show evidence of hybridization with golden trout.

The impacts of trout can be broader than the direct loss of the organisms they eat or displace. Those organisms are important components of the ecosystem. Once removed, their loss will affect the other native organisms on which they fed, as well as the creatures that depended on them for food. 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 anyone's control, such as chytrid, the removal of introduced 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.