Ranaviruses Infect Mountain Yellow-legged Frogs (Rana muscosa and Rana sierrae) Threatened by Batrachochytrium dendrobatidis

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Abstract.—Amphibians are declining worldwide, and pathogens are one of the most important causes. Disease-driven declines are attributed to ranaviruses in the family Iridoviridae and to chytridiomycosis caused by the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, *Bd*). Epizootics associated with *Bd* are the main driver of current declines of two species of endangered mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) in California. However, during 15 y of amphibian population surveys in Kings Canyon National Park (KCNP), California, USA, we observed occasional tadpole mortality not associated with *Bd* epizootics. In 2001 and 2005 we collected tadpoles from five lakes in a single, large basin (containing 83 ponds and lakes within a 1,000 ha catchment) during tadpole mortality events, and we detected ranavirus in these animals. To better understand the distribution and occurrence of ranaviruses in other mountain yellow-legged frog populations in KCNP, in 2006 we sampled for ranaviruses in 17 populations located within 25 km of the lake basin in which we originally detected ranaviruses. In this survey, we detected ranaviruses in just six (of 174) tadpoles, all from the same basin where ranavirus was originally detected; we detected ranavirus-*Bd* coinfections in five of those tadpoles. To compare the population level effects of ranavirus and *Bd* epizootics, we examined frog population and *Bd* occurrence data in 1997–2009 for the same basin where we observed ranavirus in 2001, 2005, and 2006. Despite ranavirus epizootics and tadpole mortality, these populations did not decline until *Bd* epizootics caused dramatic adult frog mortality. Although a ranavirus is present and has the potential to reduce recruitment of mountain yellow-legged frogs in KCNP, compared with the severe impact of Bd, ranavirus has likely not contributed to mountain yellow-legged frog population declines.

Key Words.—amphibian declines; coinfection; Iridoviridae; pathogens; Sierra Nevada; tadpoles

Introduction

Approximately one-third of all known amphibian species are experiencing declines in abundance and distribution (Stuart et al. 2004). One of the primary causes of these declines is the emergence of infectious diseases that can be lethal to amphibians, including ranaviruses (family Iridoviridae) and the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, *Bd*; Collins and Storfer 2003; Stuart et al. 2004). Although both ranaviruses and *Bd* can kill individuals, the two pathogens can affect populations differently because each targets a different life stage. In anurans, ranavirus-caused mortality generally affects larvae but not adults, so epizootics can decimate tadpole abundance and may reduce subsequent recruitment (Brunner et al. 2015).

However, populations generally do not decline to local extinction because adults survive and continue to reproduce (Brunner et al. 2011; Brunner et al. 2015). In contrast, mortality from infection with *Bd* is generally associated with post-metamorphic frogs but not with tadpoles (Berger et al. 1998; Blaustein et al. 2005; Rachowicz et al. 2006). Thus, a *Bd* epizootic may kill the adults in a population but leave the tadpoles alive. Post-epizootic, such a population may decline to local extinction because the loss of adults ends reproduction, and as remaining tadpoles metamorphose they generally die from chytridiomycosis, the disease caused by *Bd* (Briggs et al. 2005; Rachowicz et al. 2006; Rosenblum et al. 2010).

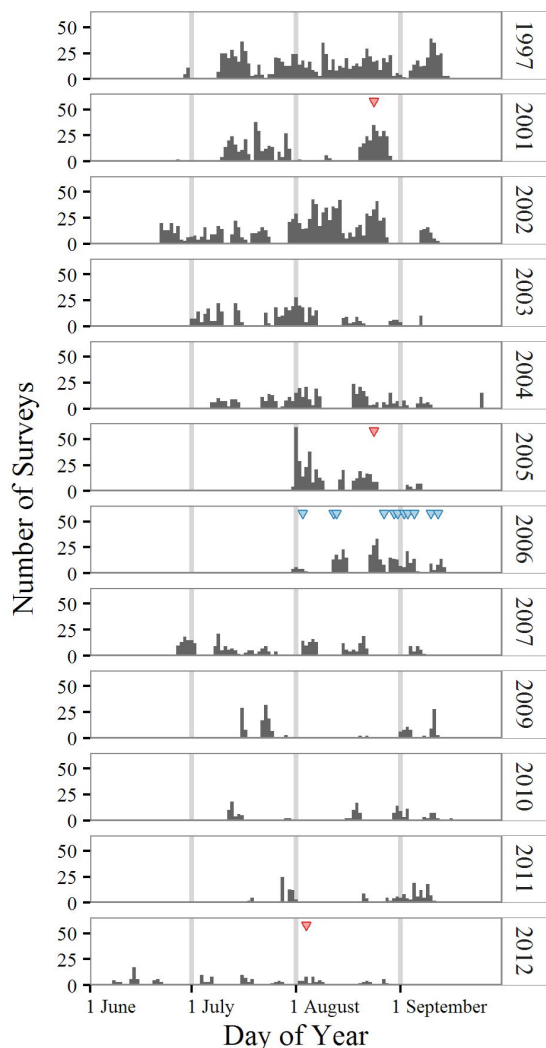Given the different population-level effects of ranaviruses and *Bd*, the fate of a frog population

Figure 1. Occurrence of amphibian visual encounter surveys, with respect to year and day of year. Triangles indicate dates of ranavirus sampling: red triangles indicate opportunistic sampling during mortality events in 2001, 2005, and 2012, and blue triangles indicate 2006 ranavirus survey dates.

and emerging infectious disease. Historically, mountain yellow-legged frogs were among the most abundant amphibians in the Sierra Nevada (Grinnell and Storer 1924), but they were extirpated from many localities by the introduction of non-native trout (Bradford 1989; Knapp and Matthews 2000; Vredenburg et al. 2007). The emergence of *Bd* in the Sierra Nevada in the 1970s (Ouellet et al. 2005; Vredenburg et al. 2010) caused extensive population extirpations that continue to the present day (Vredenburg et al. 2010). During *Bd* outbreaks in mountain yellow-legged frog populations, adult mortality is high, rapid, and can lead to extirpation within 1−5 y (Rachowicz et al. 2006; Vredenburg et al. 2010). Both mountain yellow-legged frog species are now listed as endangered under the U.S. Endangered Species Act (United States Fish and Wildlife Service 2002, 2014).

In contrast to the well understood population-level effects of *Bd* in mountain yellow-legged frogs, the distribution and potential effects of ranaviruses are unknown. Mass mortality of mountain yellow-legged frog tadpoles is rare, even during *Bd* epizootics (Rachowicz and Vredenburg 2004; Briggs et al. 2005). However, during our studies of these species, we occasionally observed tadpole mortality and tadpoles with abnormalities consistent with ranavirus infection (small lesions, bleeding, swelling, erythema, edema, and anorexia; Chinchar 2002; Gray et al. 2009; Miller et al. 2011; Lesbarrères et al. 2012). Our objective in this study was to describe the occurrence of ranaviruses in extant *R. muscosa* and *R. sierrae* populations in Kings Canyon National Park (KCNP). We also used a time series of frog population and disease occurrence data to describe how both tadpole mortality caused by ranavirus epizootics and adult mortality caused by *Bd* epizootics affected the long-term persistence of mountain yellow-legged frog populations.

Materials and Methods

Amphibian population surveys.—As part of ongoing, long-term monitoring of Sierra Nevada amphibian populations, we conducted > 5,800 visual encounter surveys to describe the abundance of amphibians in > 2,200 high-elevation water bodies throughout KCNP in 1997, 2001−2007, and 2009−2012 (Knapp et al. 2003; Davidson and Knapp 2007; Roland Knapp, unpubl. data). On average, a lake was surveyed 1.8 times (± 0.04 SE, range: 1–16 surveys) in this period. We surveyed lakes between ice-melt, which varied yearly but typically occurred in late June, and mid-September. Tadpoles and frogs were active from ice-melt through mid-October, and our surveys spanned most of that period of activity (Fig. 1). The earliest date on which we began surveys in KCNP was 6 June (in 2012) and the
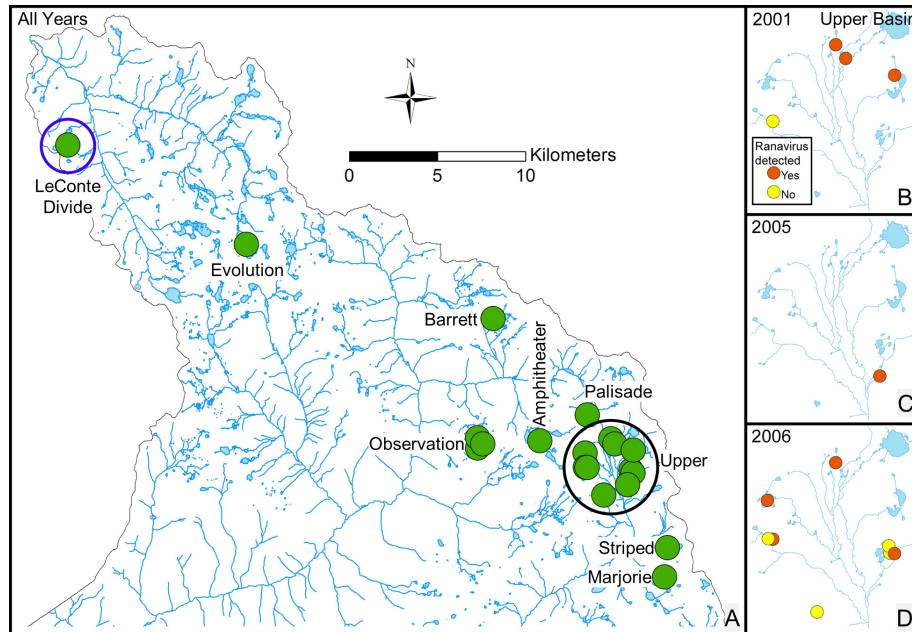
may depend on the pathogen(s) present. Despite the association of ranaviruses with amphibian population declines in North America and worldwide, ranavirus outbreaks are rarely linked to local frog population extinctions (Gray et al. 2009; Miller et al. 2011; Brunner et al. 2015). However, *Bd* is implicated as a cause of population declines, local extinctions, and species extinctions worldwide (Skerratt et al. 2007; Wake and Vredenburg 2008; Smith et al. 2009).

In the Sierra Nevada of California, USA, population declines and local extinctions of mountain yellow-legged frogs (the Southern Mountain Yellow-legged Frog, *Rana muscosa*, and the Sierra Nevada Yellow-legged Frog, *R. sierrae*) have been caused by introduced predatory fish

FIGURE 2. Map of study area in Kings Canyon National Park, California, USA. A) Green dots indicate all lakes surveyed for ranavirus in all study years (2001, 2005, 2006, 2012); large circles indicate basins where ranavirus was detected. The large black circle indicates Upper Basin, where ranavirus was detected in 2001, 2005, and 2006. The large blue circle indicates LeConte Divide basin, where ranavirus was detected in 2012. B-D) Red and yellow dots indicate lakes in Upper Basin where tadpoles were collected and whether ranavirus was detected, in 2001, 2005, and 2006. Blue polygons indicate lakes; blue lines represent streams.

latest date on which we ended surveys in KCNP was 24 September (in 2004); the mean and median survey date was 7 August.

Surveyors walked the entire shoreline of each lake and counted all individuals of each species and life stage observed, alive or dead. In these lakes, clear water, unvegetated shorelines, and basking behavior of frogs and tadpoles allowed a single visual survey to provide a repeatable estimate of the relative abundance of frogs and tadpoles in each lake (Bradford 1989; Knapp and Matthews 2000; Knapp et al. 2003). We calculated frog and tadpole densities by dividing observed abundances by the lake perimeter (derived from an ArcGIS 10 geographic information system; Esri, Redlands, California, USA). To calculate a rough metric of how common tadpole mortality is among mountain yellow-legged frogs, we examined our visual encounter survey abundance data for tadpoles, alive and dead. For each survey, we then calculated the proportion of tadpoles that were dead, and examined corresponding field observations for possible causes of tadpole mortality.

Concurrent with our amphibian visual encounter surveys in 2001−2012, we sampled tadpoles or adults to describe *Bd* occurrence in a population. To evaluate the presence of *Bd* on an individual, we used either tadpole mouthpart inspections (Knapp and Morgan 2006) in 2001−2005, or swabbing of adults (Hyatt et al. 2007) in 2006–2012.

Sampling for ranavirus during tadpole mortality events.— In our surveys of amphibian populations, we encountered 20 tadpole mortality events that we did not associate with lake freezing or drying or with predation. Predation was suggested by both the presence of non-intact carcasses and observations of predation (generally by Brewer's Blackbirds, *Euphagus cyanocephalus*). We opportunistically sampled tadpoles from six of those 20 mortality events to test for ranavirus infection (Fig. 1). In late August 2001, we observed tadpole mass mortality in several *R. muscosa* populations in Upper Basin, a large and remote lake basin in KCNP (Table 1; Fig. 2 and Fig. 2B) that contains 83 ponds and lakes and covers approximately 1,000 ha. We returned to Upper Basin 18 d later (early September) to collect live tadpoles, and found the die-off still in progress. We collected 37 live tadpoles from four lakes; in three of these lakes we observed numerous dead or dying tadpoles, and in the fourth we observed no dead or dying tadpoles. We held tadpoles in coolers, which were flown out of the backcountry on a helicopter. We euthanized, froze, and shipped these tadpoles to the US Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA. Necropsies were performed on 20 tadpoles, and included both histological examinations of liver, mesonephroi, spleen, and gills, and culture of virus from a pool of those tissues.

TABLE 1. *Rana muscosa* and *R. sierrae* tadpole mortality events in lakes in Kings Canyon National Park, California, USA, 2000–2012, in which 1% or more of observed tadpoles were dead and mortality could not be attributed to either predation or habitat freezing or drying. Lake basins are listed from north to south.

Basin	Species	Year	Lake ID	Dead	Alive	% Dead
LeConte Divide	*Rana sierrae*	2007	11988	4	9	31
		2012	10011	20	197	9
			11987	17	280	6
Barrett Basin	*R. sierrae*	2009	11470	3	14	18
Observation Basin	*R. sierrae*	2004	11444	6	59	9
Upper Basin	*R. muscosa*	2001	10272	50	1,950	3
			10276	5	134	4
			10279	6	5	55
			11332	10	980	1
			11347	30	990	3
			12357	100	90	53
			12624	22	112	16
		2003	11332	2	120	2
		2005	12624	2	7	22
		2006	11332	4	169	2
		2007	10272	2	12	14
			11342	2	12	14
			12624	4	4	50
Sixty Lake Basin	*R. muscosa*	2003	10418	3	286	1
Forester Basin	*R. muscosa*	2000	13025	23	52	31

In August 2005, we observed abnormal and dead tadpoles in a different *R. muscosa* population in Upper Basin (Figs. 1, 2B). We collected five tadpoles from this population, held them in a 1-L water bottle, and carried them from the backcountry on foot. We euthanized and dissected these tadpoles, froze the liver, kidneys, and tadpole body, and sent them to Arizona State University, Tempe, Arizona, USA, where we tested them for the presence of ranavirus using PCR (described below). We visited Upper Basin only once in 2005. In 2012 we observed a tadpole mass mortality event in an *R. sierrae* population in the LeConte Divide area of northern KCNP (Figs. 1, 2A). We collected and preserved in 95% ethanol a single recently deceased tadpole for use in PCR testing.

***Survey for ranavirus and* Bd**.—To assess the distribution of ranavirus throughout KCNP, in 2006, we sampled for ranaviruses at 17 of > 300 extant mountain yellow-legged frog localities in KCNP (about 5%; Fig. 2). Our survey included 1) populations in which we had previously observed tadpole mortality or tadpoles with abnormalities (e.g., skin lesions and edema) that suggested ranavirus infection, 2) populations in basins adjacent to those in which ranavirus had been observed or was suspected, and 3) populations in non-adjacent basins and in which no signs of ranavirus had been observed. Generally, we sampled tadpoles from the largest population in each selected basin, except in two cases. In Observation Basin, we sampled tadpoles in the three largest populations in the basin because we had observed tadpole mortality in these populations in previous surveys and at that time we saw a high proportion (about 50%) of abnormal individuals (ragged and bleeding tails, blood in the body cavity or eyes). In Upper Basin, where ranavirus was previously documented, we sampled tadpoles in each population where they were observed (Fig. 2D). We visited each survey lake once in August or early September (Table 2, Fig. 1); in 2006 most lakes were ice-free by mid-July, after which frogs and tadpoles would have been active.

To sample tadpoles for ranavirus, we captured up to 20 tadpoles in each study lake; the actual number was limited by the difficulty of capture or by very low abundance (Table 2). These within-population sample sizes were not large enough to detect a pathogen at (or below) a 5% prevalence with 95% confidence; the sample size required to make that conclusion is about 60 individuals in a large population (Cannon and Roe 1982). We caught tadpoles in a hand held net, and we determined the Gosner stage (Gosner 1960) of each tadpole after examination under a 10× hand lens.

TABLE 2. Results from the 2006 survey for ranaviruses and *Batrachochytrium dendrobatidis* (*Bd*) in mountain yellow-legged frog tadpoles (*Rana muscosa* and *R. sierrae*). Tadpoles in Evolution, Barrett, Palisade, Observation, and Amphitheater basins were all *R. sierrae*, and tadpoles in Upper, Striped, and Marjorie basins were all *R. muscosa*. We conducted tadpole visual encounter surveys on the same day as the ranavirus and Bd sampling. Prevalence confidence intervals (CI) are Clopper-Pearson binomial confidence intervals. Abbreviations are NoObs = Number Observed, NoSamp = Number Sampled, NoInft = Number Infected, %Prev = 95% CI for Prevalence (%).

Basin Name	Lake ID	Date	Tadpoles		Ranavirus		*Bd*	
			NoObs	NoSamp	NoInft	%Prev	NoInft	%Prev
Evolution	10090	3 August	2,519	19	0	0–18	0	0–18
Barrett	10223	12 August	5,316	19	0	0–18	0	0–18
Palisade	10263	13 August	499	10	0	0–31	1	0.3–45
Observation	10247	12 September	100	5	0	0–52	5	48–100
	10249	10 September	1,010	20	0	0–17	1	62–97
	10514	10 September	565	5	0	0–52	5	48–100
Amphitheater	11392	31 August	347	10	0	0–31	10	70–100
Upper	10272	2 September	193	2	1	13–99	1	13–99
	10284	5 September	232	15	0	0–22	15	78–100
	11329	5 September	204	14	0	0–23	7	23–77
	11330	5 September	1	1	0	0–98	0	0–98
	11342	3 September	15	11	2	2–52	11	72–100
	11347	2 September	219	14	2	2–43	14	77–100
	12519	3 September	1	1	0	0–98	0	0–98
	12538	5 September	7	2	1	1–99	2	16–100
Striped	11228	30 August	144	4	0	0–60	4	40–100
Marjorie	11221	27 August	684	20	0	0–17	8	19–64

Using tadpoles between stages 26–42 (mean 36.3 ± 0.30 SE, n = 174), we collected the distal centimeter of tail tissue from each tadpole (mountain yellow-legged frog tadpoles are large: 5–9 cm), and stored it in 95% ethanol. We also visually inspected tadpoles and noted abnormalities.

For all ranavirus-sampled tadpoles, we also sampled for *Bd* by swabbing mouthparts of individuals 30 times with a synthetic swab (Retallick et al. 2006). Swabs were air-dried in the field and stored in 1.5 mL microcentrifuge tubes until analysis. Our 2006 *Bd* occurrence data was complemented by our previously and subsequently collected *Bd* occurrence data that we collected throughout KCNP between 2001 and 2012.

Pathogen diagnostics and identification.—We used PCR to detect ranavirus in tissues collected from tadpoles in 2006. We extracted DNA from tail tips using a salt-extraction protocol (Sambrook and Russell 2001), and screened samples for ranavirus using standard PCR of the major capsid protein (MCP) for ranaviruses (Mao et al. 1997). The PCR products were visualized on a 1% agarose gel, where no band indicated the absence of ranaviruses and an approximately 500bp band revealed the presence of ranaviruses in the sample. To confirm the identity of amplified virus, we sequenced the MCP of each sample in which virus was detected, and compared sequences to known ranavirus MCP sequences from wild populations. For the single tadpole collected in 2012, we used a Taqman real-time quantitative PCR (qPCR) assay to test for the presence of ranavirus. The DNA was extracted from the liver, interrenal glands, and the upper intestine using the Qiagen DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, California, USA). The qPCR assay used primers that amplify a 70-bp region within the major capsid protein of all known ranaviruses (Brunner and Collins 2009). To quantify *Bd* occurrence in sampled tadpoles, we extracted DNA from swabs using a PrepMan DNA extraction kit (Applied Biosystems, Foster City, California, USA), and quantified *Bd* infection intensity using a qPCR assay (Boyle et al. 2004; Hyatt et al. 2007).

Estimating pathogen occurrence.—To estimate confidence in our ability to detect pathogens when they were present, we calculated the observed infection prevalence and confidence intervals. We calculated prevalence as the proportion of sampled individuals that were infected with *Bd* or ranavirus. For each prevalence estimate, we calculated a Clopper-Pearson binomial confidence interval (Clopper and Pearson 1934), which indicated our ability to detect the presence of ranavirus or *Bd* in each survey lake given our sampling effort.
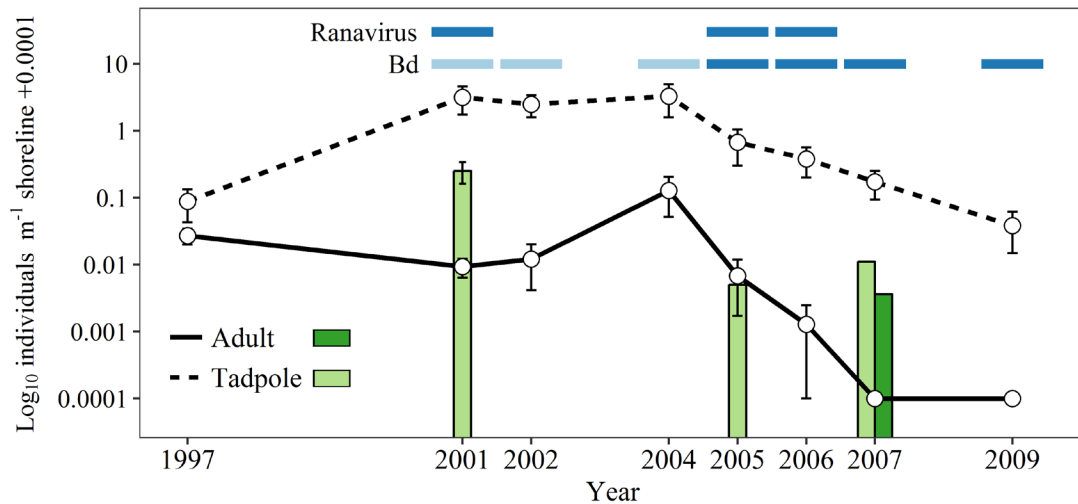
FIGURE 3. Mean densities (± 1 SE, points connected by lines) of live and dead tadpole and adult mountain yellow-legged frogs in six lakes in Upper Basin, Kings Canyon National Park, California, USA, based on shoreline visual encounter surveys conducted during 1997–2009. Density values of 0.0001 are equivalent to population density of zero individuals. Horizontal blue bars at top of figure indicate years in which we tested for ranavirus or *Batrachochytrium dendrobatidis*; dark blue bars indicate years in which we detected the pathogen, and light blue bars indicate years in which we tested for but did not detect the pathogen. Green bars indicate observations of dead individuals that exceeded 0.003 individuals m-1 shoreline; for 2001, the column shows the mean (± 1 SE) density of dead tadpoles observed in five lakes, and for 2005 and 2007, the columns show density of dead tadpoles or adults observed in only one lake.

RESULTS

Based on our long-term amphibian population surveys, we found that mountain yellow-legged frog tadpole mortality events were rare in KCNP. Of 1,872 amphibian surveys in which we observed tadpoles (conducted in 1997 and from 2001 to 2012), we found dead tadpoles in only 73 surveys (3.9%). Of the surveys in which we observed that ≥ 1% of observed tadpoles were dead, we could not attribute mortality to predation or to habitat freezing or drying in 20 cases (Table 1).

In Upper Basin in 2001, tadpole mortality was as high as 55% (Table 1). Eighty-one percent (13 of 16) of the tadpoles we collected from the three high-mortality populations displayed signs of ranavirus infection, and none of the four tadpoles we collected from the mortality-free population displayed signs of infection (Fig. 2A; Converse and Green 2005). No signs of *Bd* were found in any of those tadpoles collected in 2001 (Converse and Green 2005). In the 2005 Upper Basin die-off event, mortality was approximately 22% (Table 1), and two of the three tadpoles we collected were infected with a frog virus 3 (FV3)-like ranavirus (Fig. 2B), based on PCR and sequencing.

In our 2006 large-scale sampling and PCR analysis, we found ranavirus in few tadpoles and in few localities (Table 2). Based on samples from 174 tadpoles that we collected from 17 lakes located in seven lake basins, we detected ranavirus in only six *R. muscosa* individuals (overall prevalence 3%, 95% binomial confidence interval: 1–7%). All ranaviruses detected were FV3-like. We found these six infected individuals in four Upper

Basin lakes (Table 2; Fig. 1C). In 2006, we observed tadpole mortality in only one lake, in Upper Basin (Table 1). In marked contrast to the limited distribution of ranavirus found in animals sampled during the 2006 survey, *Bd* was widely distributed. We detected *Bd* in 13 of the 17 (76%) populations sampled, and in six of the seven basins sampled. The overall *Bd* prevalence in the 174 sampled tadpoles was 58% (95% binomial confidence interval: 50–66%), and *Bd* epizootics were occurring in five of the sampled populations (in Upper, Amphitheater, Observation, Striped, and Marjorie basins; Table 2). Of the six sampled tadpoles that we found to be infected with ranavirus, five were also infected with *Bd*.

In Upper Basin, the decline of *R. muscosa* populations was more closely associated with the arrival of *Bd* than with the presence of ranavirus (Fig. 3). As described above, in 2001, we saw large numbers of dead tadpoles in several Upper Basin frog populations (Table 1; Fig. 3), and this die-off was associated with ranavirus infection (and not with *Bd*). Despite the observed tadpole mass mortality, tadpole and adult abundances did not decline in the following years (Fig. 3). In 2005 and 2006 we observed ranavirus-associated tadpole mortality in a small number of populations. Samples collected during 2001 to 2004 failed to detect *Bd*, but in 2005, we detected *Bd* in Upper Basin for the first time. We simultaneously detected *Bd* and observed large numbers of dead adult frogs, and adult abundances declined markedly in subsequent years (Fig. 3). We continued to detect *Bd* throughout the basin from 2006 until 2009, the last year we sampled Upper Basin. Adult frogs

were last observed in 2006, and throughout this period (2006−2009) tadpole abundances continued to decline (Fig. 3). The tadpole mass mortality event observed in 2012 in a previously unsampled part of KCNP (LeConte Divide basin; Fig. 2) was also associated with ranavirus, and resembled the 2001 die-offs in scale. Although only a single sample was available for testing (using qPCR), this sample was positive for ranavirus.

DISCUSSION

Our results indicate that an FV3-like ranavirus is present in endangered mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) in the Sierra Nevada. We found ranavirus-infected tadpoles in two widely separated lake basins in KCNP, and tadpoles at these sites were simultaneously infected with *Bd*. These two pathogens co-occur within individuals in several other frog species, habitats, and regions (Miller et al. 2008; Schock et al. 2010; Souza et al. 2012; Reshetnikov et al. 2014; Warne et al. 2016). When both do occur in a region, there can be substantial variation in their co-occurrence within individuals, populations, species, and years (Souza et al. 2012; Hoverman et al. 2012; Reshetnikov et al. 2014; Warne et al. 2016). Of the tadpoles we found that were infected with ranavirus, 83% were also infected with *Bd*, but because ranavirus infection was relatively rare, only a few of those infected with *Bd* were also infected with ranavirus. The mean within-population prevalence of *Bd* in mountain yellow-legged frog tadpoles during epizootics is generally high (50−100%; Rachowicz and Vredenburg 2004; Knapp and Morgan 2006) so it is not surprising that, in populations where both pathogens occurred, most virus-infected tadpoles were also infected with *Bd*.

Although each pathogen was associated with amphibian mortality, their population-level impacts differed. The ranavirus-associated mortality we saw in *R. muscosa* tadpoles did not lead to local extinction. When *Bd* later emerged in those same populations, mortality from chytridiomycosis in adult frogs led to local extinctions as adults died, tadpoles metamorphosed, metamorphs succumbed to chytridiomycosis, and individuals were not replaced by reproduction. Ranaviruses could contribute to amphibian declines in the Sierra Nevada, but when compared to *Bd*, ranaviruses probably have a relatively small long-term impact on mountain yellow-legged frog populations.

Our results also indicate that ranavirus occurs at low prevalence within populations and sparsely across the landscape: in our 2006 survey, we detected ranavirus in relatively few of the mountain yellow-legged frog individuals and populations we sampled. However, we probably underestimated the occurrence

and distribution of ranavirus in mountain yellow-legged frogs for several reasons. First, a PCR test of tadpole tail tissue to diagnose ranavirus infection can produce false negatives if tadpoles are not viremic (Greer and Collins 2007); of the tadpoles we sampled, few displayed abnormalities indicative of a viremic infection (Chinchar 2002; Gray et al. 2009; Miller et al. 2011; Lesbarrères et al. 2012). We tested small numbers of tadpoles (< 20) from each surveyed tadpole population. Thus, we had limited power to show that ranavirus was absent from a population when we failed to detect it, and when we detected it, our confidence intervals surrounding observed prevalences were wide (Cannon and Roe 1982). Third, we surveyed tadpole populations on only one day per year, so we could have missed ranavirus outbreaks or tadpole mortality events due to a mismatch of surveys to disease phenology (Green et al. 2002; Collins et al. 2004; Brunner et al. 2007; Greer et al. 2009; Brunner et al. 2015) or the short duration of an epizootic (Gray et al. 2009) and rapid decay of tadpole carcasses. Lastly, because we prioritized sampling in and near lakes in which we had previously observed signs of potential ranavirus infection, rather than sampling across the entire range of the frogs, we limited our ability to describe the range of ranaviruses across the range of mountain yellow-legged frogs. We know the distribution of ranavirus in mountain yellow-legged frogs is wider than shown by our 2006 sampling, as revealed by our 2012 observation of ranavirus in the LeConte Divide basin, which lies beyond the northern-most lake that we sampled in 2006. Therefore, although we sampled some of the largest, then-extant populations of mountain yellow-legged frogs in KCNP, the distribution of ranavirus across the rest of the range of populations in the Sierra Nevada remains unknown. Collectively, because of our limited and somewhat targeted sampling, we likely underestimated the distribution of ranavirus in mountain yellow-legged frogs. Whatever is the actual prevalence and distribution, ranavirus is present and thus merits consideration in planning future disease sampling in Sierra Nevada amphibian populations.

In the Sierra Nevada and other landscapes, amphibian populations infected with ranavirus, *Bd*, or both, occur near populations free from either or both pathogens (Schock et al. 2010; Vredenburg et al. 2010; Hoverman et al. 2012). This has implications for disinfection of equipment when researchers travel between amphibian populations, and argues for the use of a disinfection protocol that kills both ranavirus and *Bd*. There are several choices, but no one option is clearly superior. Both quaternary ammonia and chlorhexidine (Nolvasan) are effective against *Bd* and enveloped ranavirus particles (but a higher concentration of quaternary ammonia is needed to kill enveloped viruses than is needed to kill

Bd; Johnson et al. 2003; Bryan et al. 2009; Phillott et al. 2010; Bowker et al. 2014). However, both disinfectants may be ineffective against non-enveloped ranavirus particles (Shirai et al. 2000; Chinchar 2002; Bryan et al. 2009), introducing uncertainty about their ability to completely prevent ranavirus dispersal. Bleach is completely effective against both pathogens, but the high concentration (3%) needed to kill both pathogens (Phillott et al. 2010; Bryan et al. 2009) can destroy frequently cleaned field equipment. Virkon Aquatic (1% solution) is effective against enveloped and non-enveloped forms of ranavirus and against *Bd*, and it is reportedly equipment-friendly (Gold et al. 2013; Bryan et al. 2009). But the volume of Virkon Aquatic powder needed to mix several batches is large, and inhalation hazard of the powder warrants use of a respirator, qualities that could burden researchers doing extended trips in remote field settings. Of the commonly used disinfectants effective against ranavirus and *Bd*, each has pros and cons that will determine under what conditions they are best used. All should be paired with careful removal of organic material from field equipment to enhance the performance of chemical disinfectants (Pessier 2008).

In summary, the presence of a ranavirus in tadpoles of endangered mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) may seem to be a significant concern, given the potential for ranaviruses to cause amphibian mortality and population declines (Gray et al. 2009; Miller et al. 2011; Brunner et al. 2015). Although both ranavirus and *Bd* threaten these frogs, we suggest that, compared to the negative effects of *Bd*-epizootics and chytridiomycosis (Rachowicz et al. 2006; Briggs et al. 2010; Vredenburg et al. 2010), the negative effect of ranaviruses on long-term abundance of mountain yellow-legged frogs will be less severe.

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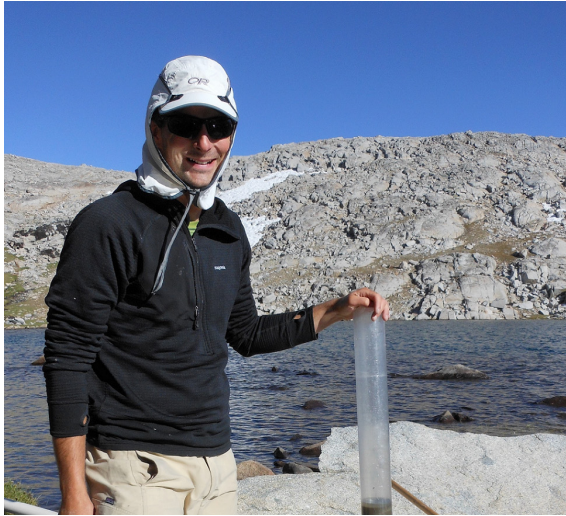
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Angela M. Picco is the Regional Recovery Coordinator and Deputy Division Chief of Listing and Recovery in the Pacific Southwest Regional Office of the US Fish and Wildlife Service. She received her B.S. in Evolution and Ecology at the University of California, Davis, USA, and she received her Ph.D. in Biology from Arizona State University, Tempe, USA. Angela's dissertation research focused on amphibian diseases, trade, and conservation. Her current work focuses on Listing and Recovery under the Endangered Species Act. (Photographed by Steve Henry).

Roland A. Knapp is a Research Biologist at the Sierra Nevada Aquatic Research Laboratory of the University of California, USA. He received his B.A. and Ph.D. from the University of California, Santa Barbara, USA. His research interests include the population and conservation biology of endangered mountain yellow-legged frogs in the Sierra Nevada of California, and the community ecology of montane lake ecosystems. The landscape-scale surveys of aquatic habitats in the southern Sierra Nevada (7,000+ lakes and ponds) that he led form the basis for ongoing amphibian and lake recovery efforts in Sequoia, Kings Canyon, and Yosemite National Parks, and beyond. His current research focuses on the recovery of mountain yellow-legged frogs in the presence of emerging pathogens, in particular the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). (Photographed by Anand Varma).

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