

Population dynamics of *Rana sierrae* at Dusy Basin: influence of non-native predators, drought, and restoration potential

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Abstract. The Sierra Nevada yellow-legged frog (*Rana sierrae*) was once an abundant and widely distributed amphibian in California's alpine ranges. *Rana sierrae* is adapted to high-elevation, fishless habitats. Its adaptations are reflected in a unique life cycle that involves a flexible, extended juvenile phase due to the short growing season typical of its alpine habitat. However, today this species is critically endangered, and most populations have been extirpated from their native range. Here, we present an 18-yr-long demographic study of a *R. sierrae* population in 15 lakes at Dusy Basin in Kings Canyon National Park. We focused on the period leading up to the arrival of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), and the subsequent local extinction of *R. sierrae*. We used N-mixture abundance modeling, which accounts for detection probabilities, to quantify factors affecting frog abundance at different life stages. The abundance of subadult and adult frogs was negatively associated with the presence of introduced trout (*Oncorhynchus mykiss* and *O. aquabonita*). Frog abundance in all life stages was positively associated with lake surface area. The propensity of lakes drying correlated negatively with abundance of eggs, subadults, and adults in the following year. Moreover, drought years, characterized by longer summers and less winter snowpack, led to higher rates of lakes drying. Finally, our results suggest that the frequency of such droughts in the region has increased since 1937. Increased frequency or severity of droughts is expected to decrease the value of shallow lakes for Sierra Nevada yellow-legged frogs because these habitats are prone to drying. We discuss our results in terms of future restoration strategies, including reintroduction and probiotic treatment, in this changed and changing ecosystem.

Key words: Chytrid fungus; demography; drought; extirpation; introduced trout; N-mixture modeling; *Rana sierrae*; season length; Sierra Nevada yellow-legged frog; snowpack.

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INTRODUCTION

Human-induced global changes are threatening the persistence of amphibians around the globe (Lips 2018). These changes include habitat

loss and fragmentation, animal trade, the spread of diseases and pesticides, the movement and spread of non-native species, and climate change (Wake and Vredenburg 2008, Ceballos et al. 2015, O'Hanlon et al. 2018). The local extirpation

of the Sierra Nevada yellow-legged frog (*Rana sierrae*) at more than 90% of historic sites throughout its range exemplifies the consequences of multiple anthropogenic stressors (Blaustein and Wake 1995, Drost and Fellers 1996, Fellers et al. 2013, Schmeller et al. 2018). *Rana sierrae* was once highly abundant in the Sierra Nevada mountains (Grinnell and Storer 1924). However, over the last 100 yr, frog numbers have declined throughout their range (Drost and Fellers 1996), initially due to non-native predators (Drost and Fellers 1996, Knapp and Matthews 2000) and later due to the arrival of chytrid fungus (Vredenburg et al. 2010). Climate change is an added stressor. By increasing the frequencies of droughts and accelerating the spread of novel diseases, climate change limits the amount of rearing habitat for this slow-developing amphibian.

The High Sierra ecosystem where *R. sierrae* is found harbors around 10,000 lakes and ponds at >2500 m above sea level (Pope and Matthews 2001). The lakes are remote and not easily accessible for people. However, today non-native trout are present in many lakes due to stocking. Settlers introduced trout into fishless lakes as early as 1850 to create a reliable food supply (Halverson 2010). Stocking of non-native fish peaked in the 1960s when aerial planting by planes became possible (Knapp et al. 2001). Many fish populations established self-sustaining populations that persisted even after stocking ceased (Vredenburg 2004). Today, the majority of permanent lakes in the High Sierra harbor rainbow trout and golden trout (*Oncorhynchus mykiss* and *O. aquabonita*), as well as hybrid lineages. These non-native fish are now the top predators in the aquatic food web, and they prey upon several taxa including aquatic invertebrates, amphibian eggs, tadpoles, and sometimes even adult frogs (Drost and Fellers 1996, Knapp and Matthews 2000). Due to concerns about the top-down effects of non-native trout on native amphibians, stocking was halted within the National Parks in California in the late 1970s (Knapp and Matthews 2000). In some parts of the Sierra Nevada, fish were actively removed as part of amphibian conservation efforts (Knapp and Matthews 1998, Knapp et al. 2007). However, shortly after these management interventions to restore *R. sierrae*, the chytrid fungus

Batrachochytrium dendrobatidis (*Bd*) arrived in the Sierra Nevada.

Batrachochytrium dendrobatidis causes the amphibian skin disease chytridiomycosis and is a contributing factor to the global decline in amphibian populations (Stuart et al. 2004, Scheele et al. 2019). Amphibian population declines, and die-offs due to chytridiomycosis can occur very rapidly, sometimes within just a few weeks after initial infection (Lips et al. 2006). The chytrid fungus was first detected in California as early as in the 1960s (Padgett-Flohr and Hopkins 2009) and in the Sierra Nevada mountains in 1990; however, there is no evidence for it causing population extinctions during that time (Scheele et al. 2019). The arrival of a fatal *Bd* strain in central California has been linked to tree frogs, for example, *Pseudacris regilla* (a reservoir host of *Bd*; Padgett-Flohr and Hopkins 2009). Eventually, this detrimental *Bd* strain made its way into one of the most remote environments of North America, the Dusy Basin within Kings Canyon National Park (Olson et al. 2013). Following the arrival of this strain in the National Park, populations were monitored for *Bd* and frog die-offs were detected (Vredenburg et al. 2010), including local (basin-wide) extirpations. One such local extirpation occurred within Dusy Basin where we had been monitoring *R. sierrae* populations since 1997. In Dusy Basin, *Bd* was first detected in 2008 (Jani 2014). The population decline due to *Bd* spanned two full years before all adult individuals disappeared with relatively high infection intensity and load (Jani 2014, Jani and Briggs 2018).

Here, we present a demographic survey of a *R. sierrae* population from Dusy Basin within Kings Canyon National Park, California, USA. In particular, we monitored the abundance of unmarked individuals from 1997 to 2015, which encompasses the period leading up to and including the arrival of *Bd* and subsequent local extirpation of the frogs. The objective of this study was to explore the influence of multiple factors on frog abundance at different age classes. The age classes were categorized into three groups that have been shown to differ in survival depending on life stage-specific factors (Schoch 2009): (1) egg masses, which represent reproductive effort in a given year. Eggs can only survive submerged in water. (2) Tadpoles, which are fully aquatic and depend on their respective water bodies not drying out for several

consecutive years. (3) Subadults and adults, which represent semi-aquatic life stages that can move among lakes. Subadults and adults were grouped together to avoid misclassifications due to variation in individual growth rates. Accordingly, we examined how biotic and abiotic factors would affect these life stages independently. For biotic factors, we included the observed presence or absence of non-native predatory fish and the arrival of *Bd* at Dusy Basin. Abiotic factors included mean lake surface area and the proportion of lakes in the basin that dried out completely in the previous season. We hypothesized that the effects of abiotic factors would be more pronounced at the early, aquatic life stages and impair reproduction because individuals at these life stages cannot migrate to other lakes in the basin. We expected a similar outcome for biotic factors, that is, more predation on egg masses and tadpoles as they represent easy targets for fish. Dusy Basin consists of a mosaic of permanent and ephemeral lakes, the balance of which depends on snowpack and summer season length, so we also explored how different types of lakes respond to droughts in order to inform future conservation efforts.

METHODS

Study sites

We surveyed lakes within the Dusy Basin (37°5'40" N, 118°33'45" W) between 1997 and 2015. The study area ranges from 3383 to 3585 m above sea level, and air temperatures varied from −30 to +27°C (mean highs and lows) throughout the year (Lacan et al. 2008). Snowpack and lake levels in Dusy Basin fluctuate widely from year to year (20–200% of average compared with the previous year). The surveyed area comprises ~0.8 km². We surveyed 12 lakes in upper Dusy Basin (lakes 1–12 in Fig. 1; the numbers correspond to Matthews and Preisler 2010) and three lakes in lower Dusy Basin (lakes 20–22 in Fig. 1). Eight out of the 15 lakes are ephemeral; that is, they dry out during summers following winters with low snowpack. Sites in Fig. 1 with identification numbers between 12 and 20 designate streams and were not surveyed for this study. Of the ephemeral lakes, lake 2 had the largest mean surface area throughout the study period (1737 m²). The other lakes in the same size range

are all permanent lakes (lake 1 49,615 m², lake 2 1635 m², lake 3 1462 m², and lake 4 1425 m² at their maximum size during our study period). Lake size information is based on estimates presented in Lacan et al. (2008).

The food web in these mountain lakes is characterized by primary producers including algae, aquatic plants, and phytoplankton. Primary consumers include zooplankton and aquatic invertebrates, and higher-level consumers include native amphibians and garter snakes (*Thamnophis elegans elegans*; Matthews et al. 2002). These lakes were historically fishless. However, fish were stocked into the lakes to create fishing opportunities (Halverson 2010) until the 1970s when stocking was halted in national parks within California to prioritize native species and eliminate exotic species (Knapp and Matthews 1998, 2000, Knapp et al. 2001). During the course of this study, lakes 1 and 3 (Fig. 1) represented the main source of introduced fish (rainbow trout–golden trout hybrids, *Oncorhynchus mykiss* × *O. aquabonita*). The other lakes were typically fishless. However, when water levels are high, fish can temporarily invade adjacent waterbodies (Knapp and Matthews 2000).

Rana sierrae

Rana sierrae is endemic to the Sierra Nevada and transverse ranges of California and Nevada found up to 3600 m above sea level (Zweifel 1955). *Rana sierrae*'s adaptations to the alpine environment are reflected in its unique life cycle with slow development times of the early life stages (Bradford 1983, Pope and Matthews 2001). As soon as ponds and lakes become ice-free at the end of the winter, females lay 40–300 eggs (Pope and Matthews 2001). This usually occurs after snowmelt at high elevation from late June through July. Two to three weeks later, larvae metamorphose into large tadpoles (more than 76 mm in diameter; Pope and Matthews 2001). Tadpoles overwinter for 2–3 yr depending on ambient temperature and food availability (Zweifel 1955). Once they metamorphose into the next life stage, they can remain as juveniles (subadults) for up to four years (Pope and Matthews 2001). All life-history stages are found within or adjacent to water, including the adults, which are considered semi-aquatic (Knapp et al. 2003). When temperatures approach freezing in the fall, frogs retreat into lakeside

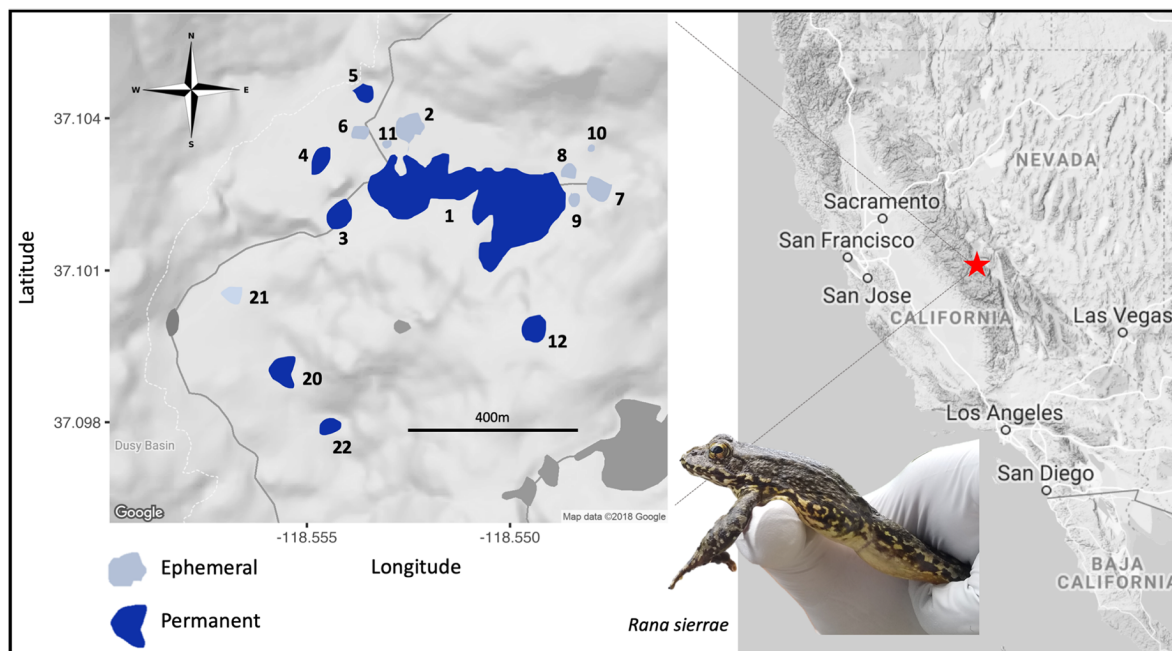


Fig. 1. Study site of *Rana sierrae* population monitoring. Dusy Basin is an elevated plateau of lakes and rivers in Kings Canyon National Park, California, USA. Elevation ranges from 3383 to 3585 m above sea level. Fifteen lakes were monitored for this study (marked in blue): lakes 1–12 and 20–22. Lakes 2, 6–11, and 21 dry out regularly (ephemeral). Bishop Pass is marked with a red star.

crevices where they accumulate at high densities and overwinter (Matthews and Pope 1999). During summer, they move among lakes and across terrestrial terrain for breeding, feeding, and to find overwintering habitats (Matthews 2003, Matthews and Preisler 2010).

Population monitoring at Dusy Basin

We measured frog abundance separately for the following three life stages: (1) egg masses, (2) tadpoles, and (3) subadults and adults. These categories were chosen in order to capture differences in the life-history traits of age classes and to minimize recounting the same individuals within a season without marking them (Knapp et al. 2003). Counts were conducted at least three times during the summer season, that is, in July (breeding season), August (feeding season), and September (start of overwintering), except for 2004 when no surveys were conducted. In some years, there were more than three visits to Dusy Basin. The counting of frogs had been standardized for the Sierra Nevada and is described in detail in Knapp and Matthews (2000). Briefly,

surveys were conducted by one person walking the complete perimeter of a lake and counting all life stages of *Rana sierrae*. At large lakes (i.e., lake 1 and lake 20), the perimeter was subdivided into two sections and two observers performed the surveys. Individuals were counted from 10 am until 4 pm when frogs are generally active (*R. sierrae* is a diurnal species). Surveys were not performed if it was stormy or cold (i.e., conditions when frogs take cover). During surveys, adults can be found either on the shore, rocks, or floating in the water; that is, they are easily visible. Tadpoles were identified to the species level and counted after capturing with a hand net. All of the lakes had been gill-netted at the beginning of the project in 1997 to determine whether fish were initially present, and subsequent surveys were done visually to determine whether fish were present. Shallow lakes never contained fish as these lakes usually dry out by the end of the summer and freeze in the winter. Counting effort was recorded in minutes, and fieldworker identity was noted. As there were different crews each summer, all surveyors were trained by one

of us (KRM) or a long-term crew leader at the beginning of a season to standardize counts. No frogs of any life stage were found after 2012; hence in 2013, 2014, and 2015, surveys were conducted by KRM alone without a crew.

Abundance modeling

The number of individuals counted in a survey depends on the partially observed true abundance of a population and the detection rate of individuals (Dénes et al. 2015). To incorporate the detection error in our analysis, we analyzed the number of egg masses, tadpoles, and frogs (subadults and adults combined) using the N-mixture model following Royle (2004). Subadults and adults were grouped into one category to avoid issues of misclassification (Matthews and Miaud 2007, Matthews and Preisler 2010). The `pcount` function in the R package `unmarked` v.0.12-2 (Fiske and Chandler 2011) was used to fit the N-mixture models. These models are hierarchical and are composed of two submodels, one estimating abundance and the other estimating rates of detection. Multiple visits per site and year (mean visits per year = 4.4, minimum of zero and maximum of 14) were used to adjust for detection error. As lake 12 had not been surveyed consistently, we removed it from the abundance modeling. We employed the stacking method where each lake season is considered a site sensu Fogg et al. 2014 and Ahlring and Merkord 2016. This approach assumes independence of abundance within a lake between years. This assumption is best satisfied for the egg life stage where there is complete turnover each year. For the larval and frog life stages, this modeling approach may lead to low variance estimates of model parameters since individuals may be recounted in subsequent years.

For the abundance submodel of each life stage, we used a negative binomial mixture to account for overdispersion. The detection submodel also suffers from overdispersion, but this is not addressed directly as the detection submodel is restricted to the binomial distribution. By accounting for detection probability, bias in the abundance parameters, which are of primary interest here, is minimized (Dénes et al. 2015). These estimates and their relation to ecological and environmental covariates allow us to uncover what factors influencing population

dynamics and hinder persistence of local populations. Before including any factors into our models, we tested for the correlations between factors. As lake surface area and lake depth were highly correlated (>0.8), we ran two independent models including only one of the two correlated factors to investigate which types of lakes supported frogs at different life stages. In addition to either lake surface area or lake depth, we included the proportion of lakes in the basin that had dried out in the previous year and observed fish presence/absence during the survey year as predictors of abundance. Linear and quadratic terms for Julian day, survey length (log minutes), and number of observers were included as predictors of detection rates. Model selection was based on AIC values.

Effects of environmental variation on lake characteristics

Lake characteristics emerged as important in predicting frog abundance. Consequently, we investigated how associated environmental variables (precipitation and season length) have changed over time. For precipitation, we used the maximum water content and snow water equivalent in a given year (Appendix S1: Fig. S1). Summer season lengths were inferred from snow cover data collected at Bishop Pass (Appendix S1: Fig. S2). We defined the summer season at Dusy Basin for *R. sierrae* as extending from the time when all the snow is melted in spring (Lacan et al. 2008) until the next snowfall. The summer season includes both the frog breeding and feeding seasons. Precipitation data were collated from public sources. The first source provided temperature and snowpack data since 1937; however, it was limited to a few data points per year (Appendix S1: Fig. S1). These data were downloaded from the National Operational Hydrologic Remote Sensing Center for the measuring station at Bishop Pass, which is located at the southwestern edge of Dusy Basin (Fig. 1; www.noahrs.noaa.gov on 7 April 2017). The second source contributed daily snowpack and temperature data since 1988 with a few missing values. It was downloaded from the Department of Water Resources at the Division of Flood Management, California Data Exchange Center (<http://cdec.water.ca.gov/index.html> on 12 October 2017, including sensor

numbers 3, 30, 31, and 32 for daily snow data, daily average air temperature, daily maximum air temperature, and daily minimum air temperature). These two datasets were merged with a third source from the California Nevada River Forecast Center (<https://www.cnrfc.noaa.gov/> on 7 April 2017) to fill gaps in daily temperature and precipitation data. It resulted in continuous precipitation measures from 1937 through the end of our study (2015).

To test for the effects of precipitation and season length on lake characteristics at Dusy Basin, we used general linear mixed models in the R package lme4 (Bates et al. 2015). We represented whether a lake dried out or not in a given year as a binary response variable. The explanatory covariates were either season length (for current summer season) or the maximal water equivalent of the previous season (snowpack) as fixed effects in two separate models. Lake ID was added to the models as a random effect. Additionally, we included lake surface area or lake depth (as selected by AIC) as covariate. Season length of a given year and snowpack in the previous year, as well as lake surface area and lake depth, could not be included in the same models due to high collinearity. Consequently, we ran four different models (model A.1, lake depth, lake identity, and summer season length; model A.2, lake depth, lake identity, and maximum snowpack in the previous year; model B.1, lake surface area, lake identity, and summer season length; and model B.2, lake surface area, lake identity, and maximum snowpack in the previous year; Table 1). Model fits were estimated using likelihood ratio tests, log likelihoods, and AIC values by comparing models with and without the factor of interest.

Availability of data and scripts

All statistical analyses were done in R v.3.4 (R Development Core Team 2013). Scripts and all data are openly available on the GitHub repository: <https://github.com/megaptera-helvetiae/DusyBasin>.

RESULTS

Population monitoring

Surveys of Dusy Basin between 1997 and 2012 resulted in raw (i.e., before correcting for

detection rates) counts of four life stages. No individuals of any life stage were found following 2012. Mean (90% inter-quantile range) counts per visit were 1.06 (0–4) egg masses, 126 (0–626) tadpoles, 6.52 (0–33) subadults, and 9.76 (0–47) adults (Appendix S1: Fig. S3). Means (90% inter-quantile ranges) of annual maximums (i.e., the most fruitful visit each year) were 4 (0–26) eggs, 233 (0–1013) larvae, 14 (0–67) subadults, and 15 (0–67) adults. Variation in counts was consistently high between years and could vary by 12 egg masses, 2000 tadpoles, 120 subadults, and 100 adults counted at the same site between two years (Appendix S1: Figs. S4, S5). Counts among sites were also variable (Appendix S1: Fig. S6, Fig. S7). We did not find any egg masses after 2009 and no adults after 2010 (Fig. 2). Tadpoles and subadults disappeared in 2012 (Fig. 2 and Appendix S1: Fig. S3). During 1997, 2005, and 2007, there were no egg masses detected (Fig. 2, Appendix S1: Fig. S5, S7). Lakes 2 and 20 harbored the highest numbers of egg masses and tadpoles consistently across the study period. Lake 1 harbored most of the subadults and adults despite its depth and the presence of fish in this lake. The proportion of occupied lakes was highest in 1998, 2003, 2005, 2006, and 2007 for adult frogs; 1998 and 2002 for subadults; 1998, 2002, and 2003 for tadpoles; and 1998 and 2006 for egg masses (Fig. 2). The proportion of occupied lakes ranged from zero to 70% for egg masses, 30–80% for tadpoles and subadults, and zero to 100% for adult frogs across sampling years.

Abundance modeling

Before modeling abundance, correlations among covariates were inspected (Appendix S1: Fig. S8). Accordingly, we ran separate models for correlated factors >0.8; that is, lake surface area and lake depth were not combined in the same model. Lake surface area was chosen over lake depth for the abundance modeling because it resulted in a better model fit (difference in AIC values of surface models vs. depth models: eggs = 4.46, larvae = 1.63, frogs = 42.52). The final model included the effects of fish presence, lake surface area, and proportion of lakes drying out in the previous year on abundance in all age classes (Fig. 3). The effect of fish presence was estimated to be significantly negative for adult

Table 1. Likelihood ratio tests on generalized linear mixed model regression modeling whether lakes at Dusy Basin dried out in a given year.

Model	Effect tested	Log(likelihood)	AIC	χ^2	df	P-value
(A) Lake depth models						
(A.1) Summer length						
Depth + lake + sum length		–46.88	101.77			
Depth + lake	Sum length	–62.28	130.56	30.79	1	<0.01**
Lake + sum length	Depth	–53.12	112.25	12.48	1	<0.01**
(A.2) Snowpack						
Depth + lake + snow max		–50.12	108.25			
Depth + lake	Snow max	–62.28	130.56	24.31	1	<0.01**
Lake + snow max	Depth	–55.74	117.49	11.42	1	<0.01**
(B) Lake surface area models						
(B.1) Summer length						
Surf + lake + sum length		–49.37	106.74			
Surf + lake	Sum length	–64.75	135.50	30.75	1	<0.01**
Lake + sum length	Surf	–51.12	110.25	4.51	1	0.02*
(B.2) Snowpack						
Surf + lake + snow max		–52.28	112.56			
Surf + lake	Snow max	–64.75	135.5	24.94	1	<0.01**
Lake + snow max	Surf	–55.74	15.89	4.1	1	0.06

Notes: Current summer length and snowpack of the previous winter at Bishop Pass, maximum lake surface area in a given year, and lake depth were entered as fixed effects, while lake identity was entered as a random effect. We had to run four independent models as lake surface area and lake depth were highly correlated (see Results section). Within the lake depth and the lake surface area models, we ran two submodels because summer season length and snowpack were correlated as well. The response variable was binary for a lake drying out or not. To test for the significance of an effect, a reduced model was compared to the reference model (in bold). Akaike's information criterion (AIC) and log likelihoods, which provide a measure of model fit and complexity (lower values indicate a better fit to the data) and likelihood ratio tests (LRT), were used to compare model fits (χ^2). Abbreviations are depth, lake depth; lake, lake ID according to Fig. 1; sum length, summer season length in a given year; snow max, maximum snowpack at Bishop's pass the previous year; and surf, maximum lake surface area in a given year. Significance levels: * < 0.05, ** < 0.01.

frogs (–0.916, P -value < 0.001). Estimated effects for eggs (–0.801, P -value = 0.11) and larvae (–0.877, P -value = 0.11) were also negative but with greater model uncertainty. Abundance was positively related to lake area for eggs (1.23, P -value < 0.001), tadpoles (1.36, P -value < 0.001), and frogs (1.08, P -value < 0.001; Fig. 4). The proportion of basin lakes drying in the previous year was negatively related to egg (–0.435, P -value < 0.05) and frog abundance (–0.179, P -value < 0.05) but its relation to larval abundance was highly uncertain (0.236, P -value = 0.35). Estimates for all model parameters including effects of Julian day and sampling effort on detection are tabulated in Appendix S1: Table S1. Mean sampling effort is shown in Appendix S1: Table S2.

Environmental variation

On average, there was 220 cm of snow each year during the winter season at Bishop Pass

across the period 1937–2012. Maximum snow depth was 480 cm in 1969 (Fig. 5). The lowest snowpack was measured in the drought years of 1977 (76 cm), 1976 (79 cm), and 2015 (80 cm; Fig. 5). During our study period (1997–2015), maximum snow height was measured in 1998 (339 cm) and minimum snowpack was recorded in 2012 (115 cm). Snow-free summer seasons ranged from 23 (1998, Appendix S1: Fig. S2) to 44 d (1999).

Current summer length and snowpack in the previous year both had a significant effect on whether lakes within Dusy Basin dried or not (summer length P -value < 0.01 and snowpack P -value < 0.01; Table 1). Lake depth was a significant covariate in these models; the deeper the lake, the less they dried out (P -value < 0.01). However, not all models provided evidence for lakes with a larger surface area to dry out less than lakes with a smaller surface area (P -value = 0.02 and 0.06; Table 1).

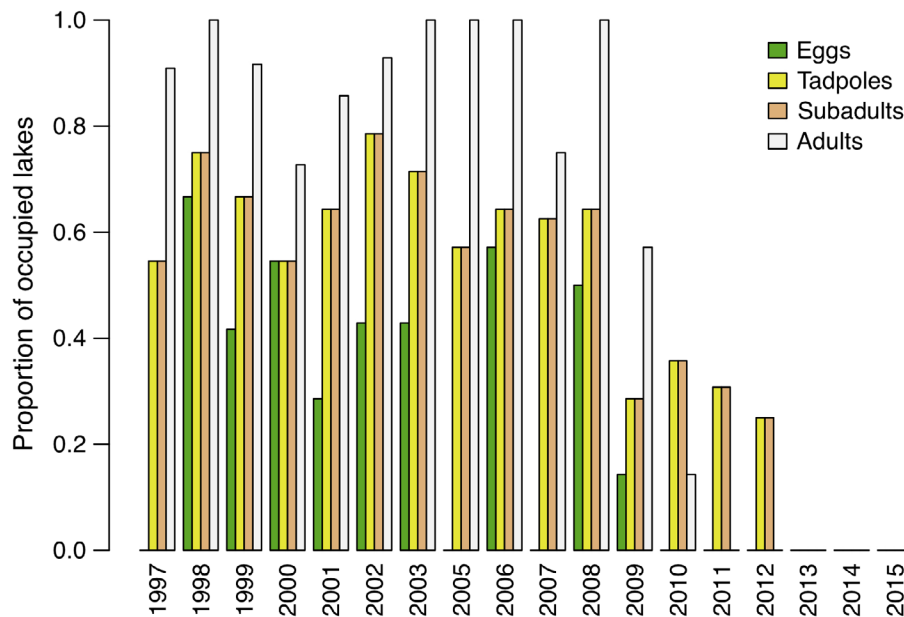


Fig. 2. Proportion of occupied lakes at Dusy Basin. Shown is the proportion of occupied lakes for each life stage in a given summer, averaged over three counting events in July (breeding), August (feeding), and September (overwintering). For egg masses, we only included counts during the breeding season (July) in this figure. Fifteen lakes were surveyed for 18 years (1997–2015; Fig. 1). No frogs survived after 2012. Reproduction was low in 1997, 2005, 2007, 2010, 2011, and 2012.

DISCUSSION

We explored the factors influencing the population dynamics of *R. sierrae* at Dusy Basin before its extirpation due to the arrival of *Batrachochytrium dendrobatidis* (*Bd*). We found significant effects of drought-related environmental variables on frog abundance during the 14 yr prior to extirpation, including lake surface area and the propensity of lakes to dry out. Lake surface area was positively related to frog abundance for all life stages. However, large shallow lakes often dried during the summer season, which resulted in lower numbers of subadult and adult frogs, due to either mortality or migration to other lakes. In contrast, deep lakes never dried, but they tended to contain predatory, non-native fish. Fish presence in lakes significantly reduced subadult and adult frog numbers, and likely reduced abundance of the egg and larval life stages as well. Ultimately, it was the arrival of *Bd* that caused the local extinction of this frog population. With evidence of persistence with *Bd* emerging in some nearby populations (Joseph

and Knapp 2018) as well as active treatment methods to counteract the effects of *Bd* (Woodhams et al. 2011, Knapp et al. 2016), there is growing interest in re-introducing frogs to areas where they were extirpated. Our results suggest that deeper and bigger lakes are those most likely to persist with climate change, and that actively removing fish from these lakes (Knapp et al. 2007) is critical for frog reintroduction success in the Dusy Basin.

Climate-related effects on *Rana sierrae* demography

Because of their extended juvenile life history, *R. sierrae* population dynamics are strongly influenced by availability of persistent aquatic habitat. For this reason, we also explored the factors influencing lake persistence. We found that lower snowpack in the previous winter and longer summer seasons led to more lakes drying out in the Dusy Basin. The number and surface area of lakes was the main driver of frog abundance, and lakes with a larger surface area supported higher numbers of Sierra Nevada yellow-legged

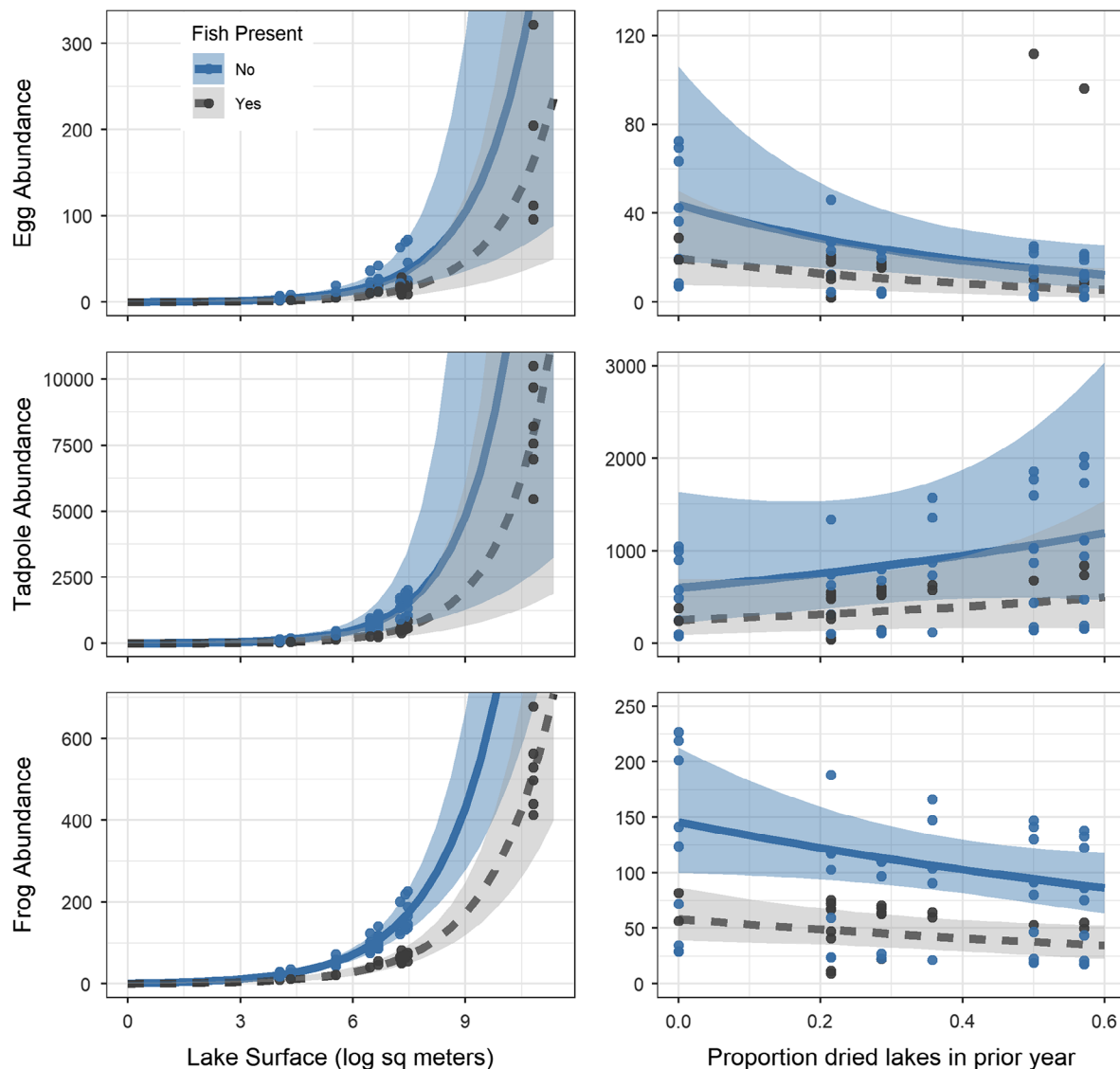


Fig. 3. Abundance modeling of frogs at Dusky Basin. Marginal effects of lake surface area, depth, and fish presence for the three life stages modeled. Mean predicted abundance for each lake number and fish presence condition is shown as points, shaded area depicts 95% confidence intervals.

frogs at the egg, tadpole, subadult, and adult stages. We expected that the effects of droughts would be more pronounced at the early aquatic life stages because these individuals cannot migrate to other lakes. However, we found that lake surface area significantly positively correlated with frog abundance in all life stages. This observation is likely attributed to three main features of these habitats: (1) Large, shallow lakes show increased water temperatures which

allows for faster developmental times in frogs (Wheeler et al. 2015). (2) Large water bodies boost algal growth in spring, which is a primary food source for early life stages most amphibians, including *R. sierrae* (Porej and Hetherington 2005). (3) Shallow lakes at Dusky Basin dry out regularly and are therefore generally predator-free, even in a basin where introduced fish have become self-sustaining in many of the large, permanent lakes (Fig. 4). Lake 2 was a major driver

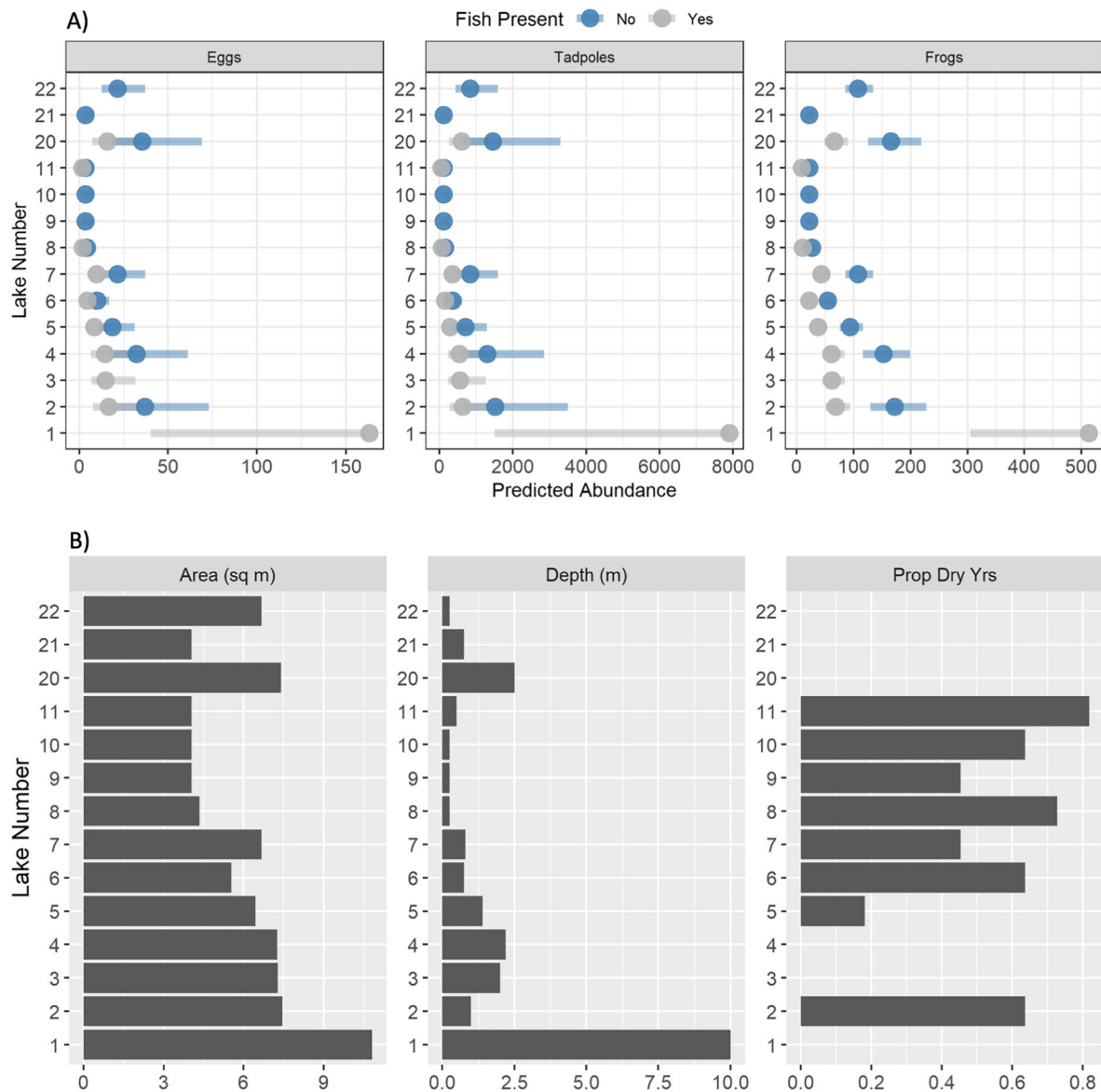


Fig. 4. Predicted abundance of eggs, tadpoles, and frogs for each sampled lake at Dusky Basin. (A) Points and error bars represent mean predictions and 90% confidence intervals, respectively. (B) Bar plots show lake surface area, lake depth, and the proportion of sample years each lake dried out.

of population persistence at Dusky Basin and exemplifies these three points in practice.

The natural habitat of *R. sierrae* is characterized by large fluctuations in lake habitat due to inter-annual variation in snowpack and rainfall. After wet winters, reproduction was highest in ephemeral lakes especially lake 2. However, after dry winters, these lakes dried out and any eggs or tadpoles died. In lake 2, larger size measured in

surface area does not prevent a lake from drying out due to its shallow depth. Hence, any increase in drought frequency and severity will severely decrease frog reproduction especially given the observation that deeper, permanent lakes are now of lower quality to the frogs due to the presence of predatory, non-native fish (see *Biotic effects on R. sierrae population dynamics*). Similar patterns were described by Lacan et al. (2008) for the years of

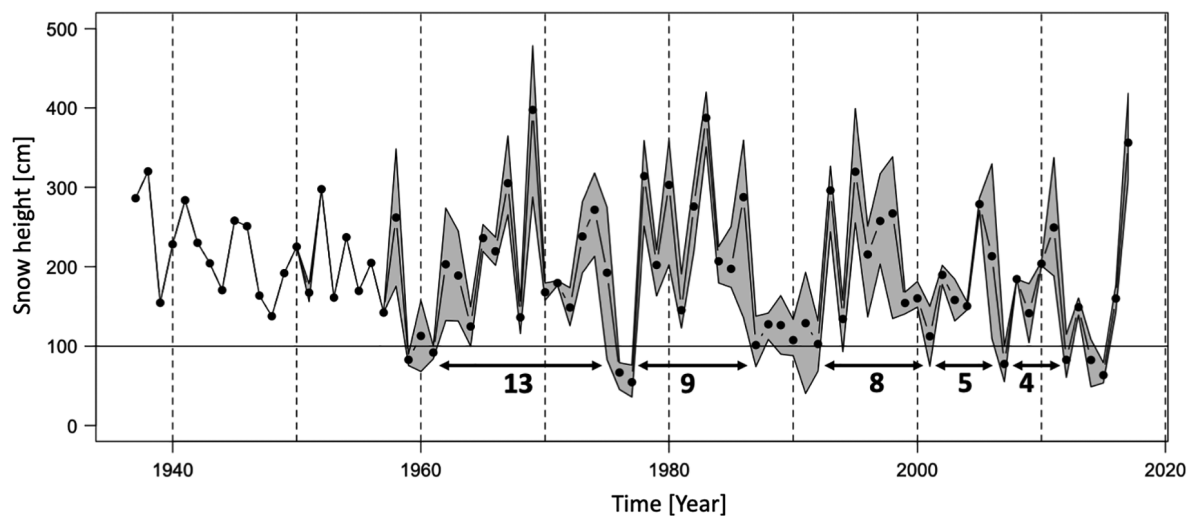


Fig. 5. Snow height at Bishop Pass between 1937 and today. Using publicly available snowpack data (see Methods), we show the average snow height for each winter since 1937 at Bishop Pass. The shaded areas delimit minimum and maximum snow levels in a given year. Years with <1 m of snow are marked with arrows. The time in between drought years is indicated with the arrow widths and associated years in text, which reveals that the time in between droughts has been decreasing since 1960.

2002–2003 at Dusy Basin, and our analysis strengthens these results when considering the longer time period including up until the local extirpation of the frogs due to the arrival of *Bd*. In summary, a good year for *R. sierrae* is defined by a sufficient amount of water so that shallow lakes will not dry, but also high enough temperatures to allow the metamorphosis of larvae to tadpoles, which can survive their first winter. The longer the summer season, the stronger possibility that the lakes will dry and impair recruitment.

Global climate change is expected to increase variation in precipitation in California with fewer but more intense rainfall or snowfall (Diffenbaugh et al. 2015). Moreover, drought events are expected to become more severe because it is getting hotter, snow will melt earlier, and water will evaporate faster. Together, these changes may lead to longer and drier summers and shorter winters at high-elevation habitats in the Sierra Nevada (Maurer 2007). In fact, using publicly available snowpack data, we visualized the average snow depth for each winter since 1937 at Bishop Pass, which revealed that the time intervals in between drought events have been decreasing since 1960 (Fig. 5). Overall, our findings suggest droughts will decrease the value of

shallow lakes for Sierra Nevada yellow-legged frogs because these habitats are prone to drying. Moreover, warmer summers in the future may increase water temperatures such that larger lakes may become more suitable for frog reproduction (in the absence of fish) and shallow lakes too warm for optimal frog development. In summary, climate change and reduced persistence of shallow lakes in the coming years may render large shallow lakes as sink habitat for yellow-legged frogs under most conditions, which points to the importance of managing the larger, persistent lakes for frogs.

Biotic effects on Rana sierrae population dynamics

We hypothesized that biotic factors that is, fish predation, would show more pronounced, negative effects on the early life stages of frogs as these individuals are easy targets for predation. Our results show that fish presence resulted in significantly lower numbers of subadult and adult frogs, most probably due to predation and avoidance as individuals in these life stages might actively select fishless lakes. Abundance of earlier life stages was negatively affected by fish presence but with greater model uncertainty (i.e., 89%

estimated likelihood of a negative effect). This more uncertainty result likely reflects measurement error with the detection of fish in the field, but we were not able to account for it in our models. Also, presence/absence data have less power, and for future studies, we suggest that quantitative measures of fish abundance would be ideal. Combined, our results add modest support to a robust literature showing the negative effects of non-native fish on *Rana sierra* populations (Knapp and Matthews 1998, Knapp and Matthews 2000, Knapp et al. 2007), with the strongest effects detected at the subadult/adult stage.

We assume that before the introduction of fish to the lakes within Dusy Basin, *R. sierrae* relied on the mosaic of habitats and was characterized by source-sink dynamics that allowed frogs to hedge their investment into reproduction (Martínez-Solano and González 2008, Griffiths et al. 2010). During wet and warm years, the population would grow due to the high productivity of many shallow lakes that persisted, while during dry years, these habitats dried, and the population was maintained by less productive but persistence deep lakes. Moreover, deeper lakes often do not completely freeze during winter and thus serve as refugia for adult frogs (Pope and Matthews 2001). Since the introduction of predatory fish, however, these safe source lakes have become less available (Vredenburg 2004). Moreover, with more drought events in the future, shallow lakes will likely dry more often, which will constrain frog recruitment to the deepest lakes that currently contain predatory, non-native fish (e.g., lake 1). We recorded such dramatic events in 1997, 2005, and 2007, when all ephemeral lakes dried out and no egg masses were encountered in the deep lakes in July and August, presumably because they were consumed by fish predators. Lake 1 showed up as an outlier in our dataset given its extraordinarily large depth and high numbers of frogs (at all life stages) that were consistently found throughout the study period. Historically, lake 1 most probably served as a reliable, constant breeding pool and numbers must have been substantially higher. Today, recruitment at lake 1 is restricted to shallow areas along the lake edges where fish cannot feed on eggs and tadpoles (Matthews and Pope 1999).

In 2009, the chytrid fungus *Bd* arrived at Dusy Basin (Joseph and Knapp 2018), and by 2013, *R.*

sierrae was locally extirpated. *Bd* has been linked to dramatic population declines and extinctions of amphibian species globally (Stuart et al. 2004). Disease outbreaks in the High Sierras could all be linked to the same genetic lineage, GPL-1 (global pandemic lineage 1; Knapp et al. 2011). *Bd* lineage GPL-1 had already developed an extraordinary virulence by the time it arrived at Dusy Basin (Schloegel et al. 2012, James et al. 2015, Miller et al. 2018). GPL-1 infection loads can reach lethal levels quickly in susceptible species and hence crash frog populations before the chytrid becomes limited by density-dependent factors (Lips et al. 2006, Vredenburg et al. 2010). In 2010, one year after *Bd* was first observed at Dusy Basin (Jani 2014, Jani and Briggs 2018), there was a recruitment failure; that is, no egg masses could be found during our surveys. Tadpoles seemed not to be affected at first; however two years later, the tadpoles were gone too. *Bd* typically does not result in mortality in tadpoles due to the localization of infection of keratinized tissues of the mouthparts (Berger et al. 1998). During metamorphosis, there is evidence of reduced immune functioning. Hence, when tadpoles metamorphose, they become vulnerable, typically leading to mortality as juveniles (Ellison et al. 2019). As a consequence, it is possible to encounter new juveniles after arrival of *Bd* because tadpoles metamorphosed, but those juveniles typically succumb to *Bd* before becoming adults. Amphibian population declines due to chytridiomycosis can occur very rapidly, sometimes within just a few weeks (Stuart et al. 2004, Lips et al. 2006, Olson et al. 2013) and, in fact, this is what happened at Dusy Basin. The rapid decline of frogs observed at Dusy Basin exemplifies how *Bd* can rapidly wipe out populations that are rare, specialized, and endemic. The *R. sierrae* population at Dusy Basin, which already faced reduced habitat availability due to the increasing frequency of drought events and predation by introduced non-native fish, could not cope with the *Bd* invasion. Interestingly, nearby populations in Desolation Wilderness did not go extinct and seem to persist even in the presence of *Bd* (Jani 2014). While no relationship could be demonstrated between elevation and temperature on *Bd* prevalence in the High Sierras (Knapp et al. 2011), host-associated skin microbes seem to play a significant role in

population survival (Jani and Briggs 2018, Ellison et al. 2019).

Implications for management

To date, no large-scale translocations of frogs into Kings Canyon National Park have been attempted, but there is interest in doing so. Translocation experiments in Yosemite National Park are underway in an attempt to re-populate suitable, historic habitat (Joseph and Knapp 2018). Initial results of these attempts provide evidence that introductions can succeed even in habitats where *Bd* persists. Important to reintroduction efforts is the observation that different frog species show varying degrees of susceptibility to chytridiomycosis (Fisher et al. 2009). Even within species, some populations show different responses. This variation has recently also been shown to exist across populations of *R. sierrae* (Joseph and Knapp 2018). For example, while the Dusy Basin population of *R. sierrae* was entirely wiped out, small populations of *R. sierrae* have persisted in other parts of the Sierra Nevada (e.g., Desolation Wilderness or Yosemite; Lam et al. 2010, Jani 2014). Hence, attempts are being made to keep infection intensities of *Bd* in wild *R. sierrae* populations low with the idea that previously exposed individuals that survived will show lower infection intensities in the future (Vredenburg et al. 2010). Understanding the mechanisms by which some populations resist *Bd* could inform management strategies for threatened amphibians. Sierra Nevada yellow-legged frogs are one of the most well-studied systems to give insight into chytridiomycosis progression and the role of the host microbiome on disease outcome in the wild (Jani and Briggs 2018). Whether individual frogs survive or succumb to disease may be in part a result of the rate of their microbiome response to *Bd* proliferation (Jani and Briggs 2014). The rate of increase in *Bd* load, the maximum load reached, and the clinical outcome of a given pathogen load is not only driven by host genes and phenotype, but also driven by pre-existing properties of its associated skin microbiome (Jani and Briggs 2014, Jani et al. 2017). Recent studies have examined correlations between skin-associated bacterial communities and *Bd* infection status, intensity, prevalence, and susceptibility. It could be shown that *Bd* disturbs the frog skin microbiome during natural

epidemics and experimental infection in the laboratory with the same members of the microbiome reacting to infection (Jani and Briggs 2014). Yellow-legged frogs that were given a probiotic bath in local bacterial strains isolated from resistant individuals were better able to clear infection by *Bd* and less likely to suffer mortality (Bletz et al. 2013, Kueneman et al. 2016, Rebollar et al. 2016). Strains common to amphibian skin such as *Janthinobacterium lividum*, *Serratia* spp., and *Pseudomonas* spp. can produce antifungal compounds that reduce *Bd* load (Brucker et al. 2008, Woodhams et al. 2018). Transplantation of *J. lividum* from survivors to susceptible individuals has been shown to be most effective in protecting against new *Bd* outbreaks when applied to the tadpole stage (Jin Song et al. 2019). It was also shown in an *in vitro* inhibition experiment that there are nine natural members of the *R. sierrae* skin microbiome that are capable of inhibiting *Bd* growth (Ellison et al. 2019). This is promising for the development of probiotic treatments *in vivo*. Manipulation of the natural microbiome before disease outbreak (preventive prebiotics) or after (restorative probiotics) represents a powerful, emerging conservation strategy to make reintroductions possible with the continued presence of *Bd* in the environment and in reservoir hosts like the Sierran treefrog (Song et al. 2019). However, California's alpine range is getting warmer and non-native fish are still present in many lakes. While a few populations of *R. sierrae* are growing in some parts of the Sierra Nevada (Yosemite National Park; Knapp et al. 2016), Sierra Nevada yellow-legged frogs have been extirpated from more than 90% of their historic range. In light of ongoing efforts to manage frog populations in the face of *Bd*, it is important to also consider the other factors that are threatening yellow-legged frogs. Changing climate and loss of predator-free shallow lake habitat will limit the success of ongoing restoration efforts, which points to the need to continue efforts to remove non-native predatory fish from deep, persistent lakes to sustain *R. sierrae* into the future.

CONCLUSIONS

The aim of our study was to document the factors influencing the dynamics of an imperiled population of the Sierra Nevada yellow-legged

frog prior to its local extirpation ultimately due to chytrid fungus. We presented modeled abundance estimates at three key frog life stages in lakes with and without fish at a high-elevation habitat. Our analyses revealed lake surface area is crucial for frog survival and reproduction, and that this habitat will decrease with climate change. This work highlights the importance of managing deep lakes that persist even during extreme drought for yellow-legged frogs—as long as there are fish in the deeper lakes, they will not serve as source habitats for *R. sierrae*. In short, the habitat to support *Rana sierrae* is there, but is effectively unavailable to the frogs due to the presence of predatory, non-native fishes. With the emergence of *Bd*-resistant *Rana sierrae* populations and the potential for future reintroductions, creating fish-free habitats in the short-term and reducing greenhouse gas emissions in the long-term are feasible goals to promote the persistence of the Sierra Nevada yellow-legged frog in the future.

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