

# Large-scale recovery of an endangered amphibian despite ongoing exposure to multiple stressors

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Amphibians are one of the most threatened animal groups, with 32% of species at risk for extinction. Given this imperiled status, is the disappearance of a large fraction of the Earth's amphibians inevitable, or are some declining species more resilient than is generally assumed? We address this question in a species that is emblematic of many declining amphibians, the endangered Sierra Nevada yellow-legged frog (*Rana sierrae*). Based on >7,000 frog surveys conducted across Yosemite National Park over a 20-y period, we show that, after decades of decline and despite ongoing exposure to multiple stressors, including introduced fish, the recently emerged disease chytridiomycosis, and pesticides, *R. sierrae* abundance increased sevenfold during the study and at a rate of 11% per year. These increases occurred in hundreds of populations throughout Yosemite, providing a rare example of amphibian recovery at an ecologically relevant spatial scale. Results from a laboratory experiment indicate that these increases may be in part because of reduced frog susceptibility to chytridiomycosis. The disappearance of nonnative fish from numerous water bodies after cessation of stocking also contributed to the recovery. The large-scale increases in *R. sierrae* abundance that we document suggest that, when habitats are relatively intact and stressors are reduced in their importance by active management or species' adaptive responses, declines of some amphibians may be partially reversible, at least at a regional scale. Other studies conducted over similarly large temporal and spatial scales are critically needed to provide insight and generality about the reversibility of amphibian declines at a global scale.

amphibians | recovery | disease | *Rana sierrae* | Yosemite

The Earth's biodiversity is imperiled by pervasive and increasing human pressures, with current rates of species loss unprecedented since the last mass extinction event 65 Mya (1). Although all classes of vertebrates contain a significant fraction of threatened species, amphibians stand out as the most at risk. Of 5,743 amphibian species reviewed in the first global assessment of amphibians (2, 3), >30% were categorized as globally threatened with extinction, and several hundred may already be extinct (4). This proportion is far higher than that for birds or mammals, an alarming statistic given that most amphibian lineages persisted through the last four mass extinctions (5). Primary drivers of these ongoing declines include habitat loss, overutilization, introduced species, contaminants, and the infectious disease chytridiomycosis (5). Chytridiomycosis is caused by the amphibian chytrid fungus [*Batrachochytrium dendrobatidis* (Bd)], a recently emerged pathogen that is now present in amphibian populations worldwide. This disease is implicated in the decline or extinction of hundreds of amphibian species, and threatens hundreds more (4, 6). This impact of Bd has been described as "the most spectacular loss of vertebrate biodiversity due to disease in recorded history" (4).

The Sierra Nevada yellow-legged frog (*Rana sierrae*) (7) is emblematic of the global decline of amphibians. A century ago, *R. sierrae* was one of the most abundant amphibians in lakes, ponds, meadows, and streams in California's Sierra Nevada mountains,

including in Yosemite National Park (8, 9), the location of the current study. Despite most of its range being highly protected, *R. sierrae* has disappeared from >93% of its historical distribution (7). This decline is characterized by precipitous decreases in both occupancy and abundance across the species' range, including within the most protected landscapes (10–12). To prevent its extinction and facilitate recovery, *R. sierrae* was recently listed as "endangered" under the US Endangered Species Act (13) and as per the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (3). As with many declining amphibian species, it remains an unanswered question whether *R. sierrae* can recover given the ongoing impacts of multiple stressors.

The decline of *R. sierrae* in the Sierra Nevada, including in the protected habitats of Yosemite National Park, is the result of several factors. Declines began in the early 1900s (8) with the introduction of nonnative fish (predatory trout: primarily *Oncorhynchus* spp. and *Salvelinus* sp.) into naturally fishless habitats (14–16), including the majority of perennial lakes, ponds, and streams that are the main habitat for *R. sierrae* (17). More recently, available information suggests that Bd emerged in the Sierra Nevada in the 1970s (18, 19), and its ongoing spread has driven additional declines and extirpations of hundreds of *R. sierrae* populations (18, 20). In Yosemite, Bd is now essentially ubiquitous

## Significance

Human influences are causing the disappearance of species at a rate unprecedented in millions of years. Amphibians are being particularly affected, and extinctions of many species may be inevitable. The Sierra Nevada yellow-legged frog (*Rana sierrae*) was once common in the mountains of California (United States), but human impacts have driven it near extinction. Repeated surveys of thousands of water bodies in Yosemite National Park show that the decline of *R. sierrae* has recently reversed and that population abundance is now increasing markedly in part because of reduced influence of stressors, including disease and introduced fish. These results suggest that some amphibians may be more resilient than is assumed, and with appropriate management, declines of such species may be reversible.

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Data deposition: The frog resurvey and frog susceptibility datasets reported in this paper are available at <http://dx.doi.org/10.5061/dryad.rm382>.

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(21), and *R. sierrae* populations that survived initial Bd-caused die offs currently display enzootic frog–Bd dynamics (22) with ongoing impacts to populations (23). Airborne contaminants (including pesticides) originating in California’s agricultural Central Valley are transported downwind to the Sierra Nevada (24, 25) and may also have impacts on amphibians (26, 27). However, an explicit link between pesticides and *R. sierrae* declines is lacking.

The specific objectives of our study were to (i) describe the trajectory of *R. sierrae* population sizes in Yosemite National Park over the last 20 y and (ii) relate those changes in population size to changes in stressors and the response of frogs to those stressors. Our results on the long-term dynamics of this particularly well-studied species provide an important case study and may yield more general insights into the reversibility of amphibian declines in other species facing similar stressors. To achieve our objectives, we conducted thousands of *R. sierrae* population surveys throughout Yosemite over a 20-y period. The large spatial and temporal scales that characterize our study are important, because although several studies have shown that amphibians can recover at local scales (28–31), studies of imperiled amphibians at the more relevant landscape scale are lacking. In addition, because landscape-scale population processes are often characterized by transient dynamics, short-term studies may fail to accurately describe longer-term trends. Our long-term dataset allows strong inferences to be made about current and future *R. sierrae* population trajectories.

The dataset used in this study included 7,678 frog population surveys conducted at 2,154 water bodies across Yosemite National Park, a highly protected 3,027-km<sup>2</sup> landscape in California’s Sierra Nevada that constitutes ~13% of the range of *R. sierrae*. Surveys were conducted from 1993 to 2012 and included all 483 *R. sierrae* populations known to occur in Yosemite (16). We estimated trends in *R. sierrae* abundance using a hierarchical spatiotemporal modeling framework and fit models using integrated nested Laplace approximation (32, 33). Given our results showing that these populations increased substantially over the study period, we also provide results from an experiment that may explain, at least in part, how frog populations could increase in the presence of ongoing stressors, specifically disease.

## Results and Discussion

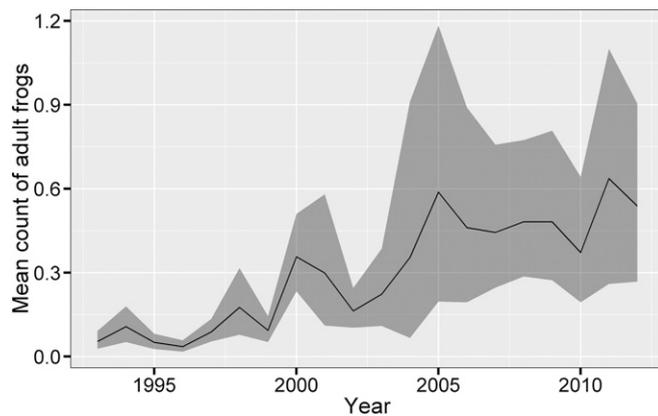
**Trends in Frog Abundance.** Across Yosemite, the total abundance of *R. sierrae* increased markedly over the 20-y study period (Fig. 1). The majority of the observed increase occurred between 1996 and 2005. The estimated intrinsic rate of growth ( $r$ ) for the number of adult *R. sierrae* across all water bodies was 0.105 [95% credible interval (95% CI) = 0.075–0.134]. This rate is equivalent to an 11.0% annual increase in population size or a more than sevenfold increase in abundance over 20 y. We found a similar rate of increase for the number of juveniles ( $r = 0.119$ ; 95% CI = 0.075–0.163; 12.6% annual increase) and an even higher rate of increase for the number of tadpoles ( $r = 0.227$ ; 95% CI = 0.177–0.277; 25.5% annual increase). Counts at three relatively intensively surveyed sites that now contain some of the largest *R. sierrae* populations in Yosemite provide examples of these increases (Fig. 2). Substantial increases in abundance at these sites often occurred over the span of only 2–5 y and were temporally asynchronous between basins.

Positive population growth occurred broadly over most of Yosemite. For 34 of 41 grid cells arrayed across the park (Fig. 3 and details in *SI Text*), average estimated counts of adult *R. sierrae* during the last 3 y of the study (2010–2012) were higher than those during the first 3 y (1993–1995). Grid cell values also indicate large spatial variation across Yosemite in average abundance and changes in abundance during the study period (Fig. 3). Growth rates were relatively uniform and moderate across most of the northern half of the park (primarily the Tuolumne River watershed), but growth rates in the southern half of the park (Merced River watershed) showed substantial variability. In this watershed, growth rates were much faster in the eastern (headwaters) portion than in the western

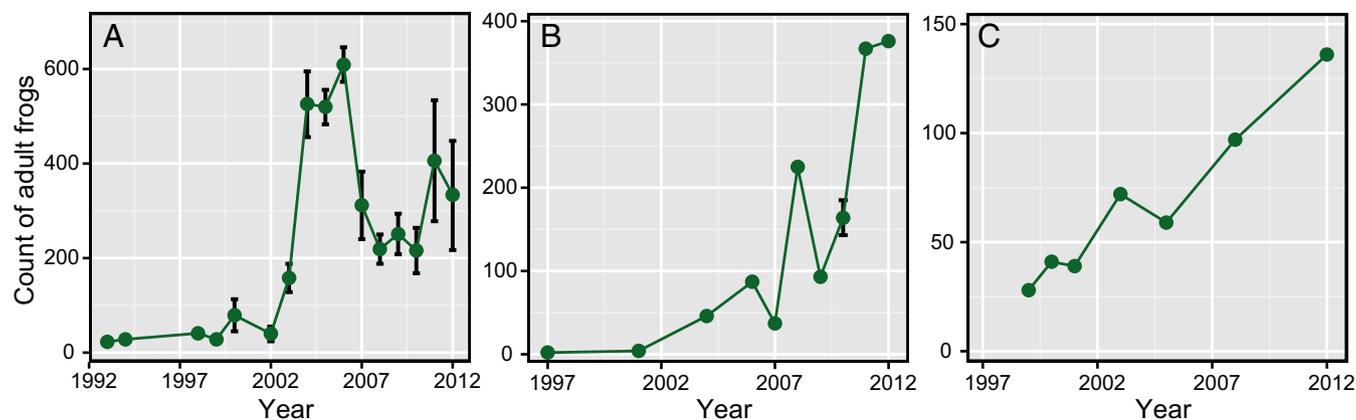
(downstream) portion, with five of the downstream cells exhibiting negative growth.

The addition of five environmental covariates to the base model (fish presence, water depth, elevation, watershed, and precipitation) (additional details are in *SI Text*) indicated that two covariates had important effects on population abundance: abundance was negatively related to fish presence and positively related to water depth (Tables S1 and S2; see also Table S3; additional details are in *SI Text*), consistent with known effects of these factors on *R. sierrae* occupancy and population size (16, 17, 29). More importantly, the inclusion of covariate  $\times$  year interactions allowed us to examine whether trends in abundance differed with respect to each of the covariates. Results indicated different growth rates in water bodies with and without fish, but the other covariates had little influence on growth rate (Table S2).

**Factors Responsible for Frog Recovery.** After a century of decline, what allowed *R. sierrae* populations in Yosemite to begin to recover? A change in the distribution of nonnative fish is one possibility. All lakes and most streams in Yosemite were naturally fishless (16), including all 296 larger perennial water bodies ( $\geq 1$  ha and  $\geq 3$ -m deep) that are essential habitat for the highly aquatic *R. sierrae* (16, 17). Starting in the late 1800s and continuing for a century, several species of nonnative trout (*Oncorhynchus* spp., *Salvelinus* sp., and *Salmo* sp.) were introduced repeatedly into the majority of these habitats. This practice was greatly curtailed by the National Park Service in the late 1970s and stopped entirely in 1991. By the time that fish introductions were halted, only 43% of larger lakes remained in a fishless condition. As a result of this policy change, by 2000 and 2001, the percentage of fishless lakes had increased to 56% (34). Although fish disappeared from a relatively small proportion of lakes (because most introduced fish populations were self-sustaining) (34), this decrease in fish occupancy, nonetheless, created important habitats that, in some cases, were recolonized by *R. sierrae*. Of the 39 lakes that reverted to a fishless condition (“stocked-now-fishless” lakes), *R. sierrae* were detected at 20 (51%) during at least one survey over the study period. In contrast, at 128 lakes that contained fish throughout the study period, *R. sierrae* were detected at only 11 (8%;  $\chi^2$  test,  $\chi^2 = 33.2$ ,  $P < 10^{-8}$ ). In addition, model-estimated frog abundances in the median



**Fig. 1.** Increase in abundance of adult *R. sierrae* across all surveyed water bodies in Yosemite National Park from 1993 to 2012. Values in each year are the estimated numbers of frogs observed per water body during a survey (averaged across all surveys conducted per year), with a bootstrapped 95% CI. Estimates are for all 2,154 water bodies in Yosemite that were surveyed at least twice during the study period, including those that were not visited in a given year and/or where adult *R. sierrae* were never observed in any surveys. The relatively low predicted counts are in part because of the existence of many surveyed water bodies that are only marginally suitable and consequently, in which *R. sierrae* were never detected during the study. Fig. S1 shows the actual distribution of frog counts for water bodies visited in each year and the predicted counts for all water bodies.

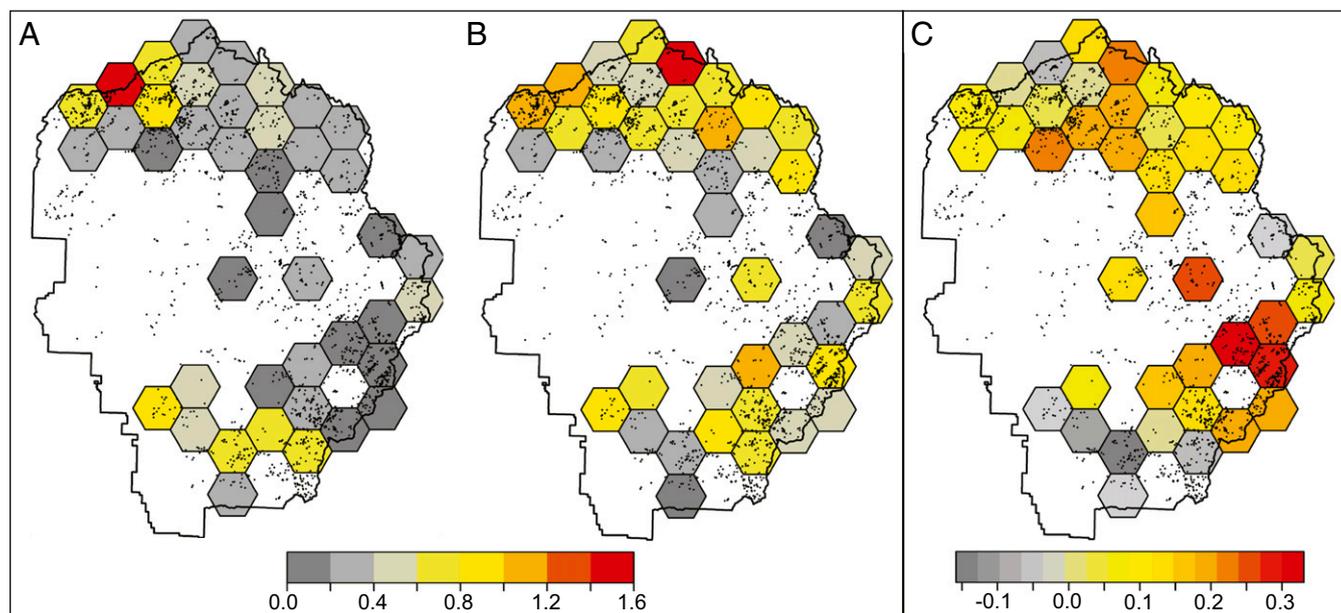


**Fig. 2.** Counts of adult *R. sierrae* at three relatively intensively surveyed sites (A–C) showing marked increases in abundance. These sites are fishless and currently contain some of the largest frog populations in Yosemite National Park. When multiple surveys were conducted in a single year, counts were averaged and are shown as mean  $\pm$  1 SE. In A, the decrease in *R. sierrae* counts after 2006 may be in part because of the removal of  $\sim$ 200 adults between 2006 and 2012 for use in translocations aimed at reestablishing *R. sierrae* populations in nearby habitats.

fish-containing lake was 28.3% (95% CI = 10.1–72.4%) of that in the median fishless lake that had never been subject to fish introductions. Frog abundances in stocked-now-fishless lakes were similar to those in never-stocked lakes (93.4%; 95% CI = 46.9–180.8%), suggestive of full recovery of *R. sierrae* abundance after fish disappearance. Collectively, these results indicate that the cessation of stocking likely contributed to the recovery of *R. sierrae* (28, 34). However, given that 20 *R. sierrae* populations occupying stocked-now-fishless lakes represent only a small fraction of 483 *R. sierrae* populations detected during our surveys, other factors likely had stronger overall influences on the observed trends in population abundance.

Unlike fish, which are relatively limited in their distribution across Yosemite, Bd infects all or nearly all *R. sierrae* populations (21), suggesting the potential for a strong effect of Bd on *R. sierrae*. In particular, a change in frog–Bd dynamics since Bd’s arrival could be

an important driver of frog population increases. For example, evolutionary and immunological changes in frogs in response to Bd may have reduced their susceptibility (35, 36). We conducted a laboratory experiment to test the hypothesis that *R. sierrae* from populations with a long history of exposure to Bd that are persisting, despite ongoing Bd infection (such as those in Yosemite), are less susceptible than those from populations in which frogs have never been exposed to Bd. We refer to these frog types as “persistent” and “naïve,” respectively. In this experiment, we collected *R. sierrae* adults from each of three persistent and three naïve populations (Table S4) and exposed pairs of frogs (one from a persistent population and one from a naïve population) to one of four strains of Bd. Bd was cultured from two persistent frog populations and two populations in which frogs had recently experienced Bd-caused mass die offs (“die-off” populations). After Bd

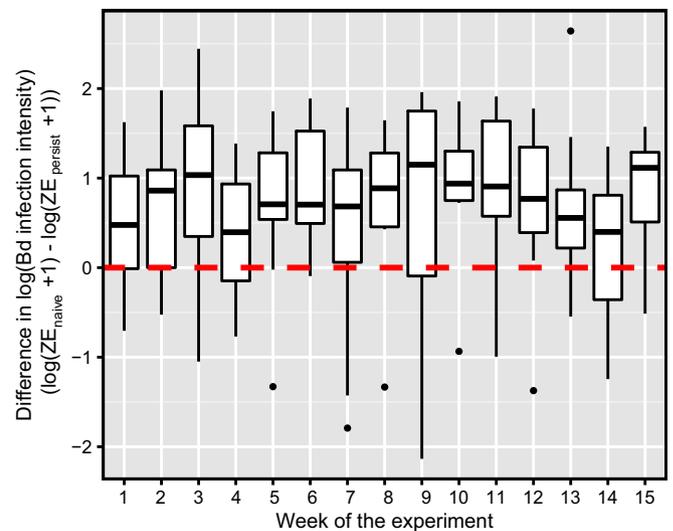


**Fig. 3.** Spatial variation in population abundance and growth rate from 1993 to 2012 in counts of adult *R. sierrae* across Yosemite National Park. Grid cells in A and B depict the estimated counts of *R. sierrae* averaged across all water bodies in the cell for the first 3 y of the study (1993–1995) and the last 3 y of the study (2010–2012), respectively. Average counts are low (zero to two frogs per water body), because only a minority of water bodies in the cell provide high-quality habitat. C depicts the trend in population abundance between those two time points. Values in C are the intrinsic rates of growth ( $r$ ), where values greater than zero indicate positive growth and values less than zero indicate negative growth. Frog abundance was notably higher at the end of the study compared with the beginning as reflected in the positive growth rates for most cells.

exposure, we measured Bd infection intensities (“loads”) on frogs over a 15-wk period.

Frog type strongly influenced Bd load (Table 1), with the average Bd load being five times higher on naïve frogs compared with persistent frogs (Fig. 4). The magnitude of this difference was similar, regardless of the Bd strain to which frog pairs were exposed. The population from which Bd was collected affected loads on frogs (Table 1), but loads did not differ consistently between Bd cultured from persistent vs. die-off populations (see also Tables S5 and S6; details are in *SI Text*). During the first half of the experiment, Bd loads on frogs generally increased exponentially. We expected loads to continue to increase in subsequent weeks, but during the second half of the experiment, loads leveled off or in some cases, declined in all frog pairs (as indicated by a significant negative effect of week<sup>2</sup>) (Table 1). Regardless, loads remained higher on the Bd-naïve frog of each pair compared with the persistent frog (Fig. 4). Frogs in unexposed control tanks remained uninfected for 15 wk of the experiment. These results support the hypothesis that *R. sierrae* adults from persistent populations, such as those in Yosemite, are significantly less susceptible to Bd infection than those from Bd-naïve populations. In addition, since the initial collection of frogs from the three persistent and three naïve populations for use in this experiment (in 2009), Bd-caused epizootics have occurred in all three Bd-naïve populations, and all have been extirpated or nearly extirpated. In contrast, the three persistent populations have maintained stable or increasing frog numbers, and these populations continue to be characterized by enzootic host–pathogen dynamics. Collectively, these results suggest that the reduced susceptibility of *R. sierrae* to Bd infection may have contributed to the recovery of populations in Yosemite.

The reduced susceptibility of frogs from persistent populations could be caused by several nonmutually exclusive factors. First, the reduced susceptibility of frogs from persistent populations could be an outcome of natural selection (36) on *R. sierrae* that occurred after the arrival of Bd in Yosemite’s frog populations, selection to which the naïve frog populations would not have been subject. For example, if the initial invasion of Bd into Yosemite decades ago resulted in high levels of frog mortality, then the remaining populations may be descendants of individuals that possessed more effective innate or adaptive defenses against Bd (35, 37). Second, the lower susceptibility could be the result of an acquired immune response in frogs from persistent populations after their exposure to Bd in the wild, a response that itself could be subject to natural selection. If *R. sierrae* are, in fact, capable of mounting such a response (as is the case in other anurans) (35, 38), frogs from the persistent populations could have had at least partial immunity against Bd before the start of the experiment, and those from naïve populations would not have. A series of experiments is underway to describe adaptive immunity against Bd in *R. sierrae*. Third, the persistent populations from which *R. sierrae* were collected may have inherently lower susceptibility to Bd than those Bd-naïve



**Fig. 4.** Results of the laboratory frog susceptibility experiment showing that, when exposed to Bd, frogs from Bd-naïve populations consistently had higher infection intensities than those from populations persisting with Bd. Each box plot displays the average difference in Bd infection intensity (expressed as ZEs) between the frog from a naïve population and the frog from a persistent population [ $\log_{10}(\text{ZE}_{\text{naïve}} + 1) - \log_{10}(\text{ZE}_{\text{persistent}} + 1)$ ] for all tanks by week. Results from weeks 1–6 include those from all 16 replicate tanks, and results from weeks 7–15 include those from 8 tanks remaining after frogs in the other 8 tanks were killed for a separate gene expression study. Each box plot shows the mean (horizontal line), first and third quartiles (bottom and top of box: “hinge”), lowest and highest values within  $1.5 \times$  interquartile range of the lower and upper hinges (vertical lines), and any outliers (points). The horizontal dashed red line indicates a value of zero, the expected value if Bd loads were the same between paired naïve and persistent frogs.

populations from which *R. sierrae* were also collected, differences that could predate the arrival of Bd in the Sierra Nevada (36, 39).

In addition to our study, two other recent studies have also provided compelling evidence that other anuran species (both from Australia) are recovering after Bd-caused declines (30, 31). Unfortunately, neither allows insights into which (if any) of the above-described mechanisms might be associated with recovery. Elucidating the relative importance of these potential mechanisms would provide important insights into the means by which amphibians being impacted globally by Bd could eventually recover from the effects of chytridiomycosis.

## Conclusions

Amphibian declines have proven difficult to reverse, especially when the causes of decline are poorly understood or challenging to ameliorate (e.g., disease, introduced species, airborne contaminants,

**Table 1.** Parameter estimates for fixed effects in the linear mixed model used to describe the effects of frog type (persistent vs. naïve) on Bd infection intensities in the 15-wk frog susceptibility experiment

Fixed effect	Estimate	SE	df	Likelihood ratio test: $\chi^2$ (df)	P value
Intercept	1.77	0.30	28.4		
Frog type (persistent)	0.87	0.15	41.9	26.1 (1)	<0.0001
Bd source				20.4 (3)	<0.001
Population 1	0.41	0.29	29.9		
Population 2	−0.36	0.29	30.0		
Population 7	−0.41	0.38	22.3		
Population 8	0	—	—		
Week	0.10	0.02	63.7	30.8 (1)	<0.0001
Week <sup>2</sup>	−7.3e-3	3.6e-3	123.0	4.0 (1)	0.046

or climate change). Those examples that do exist are generally based on only a few populations (28–31) and provide important but limited insights into the potential for large-scale recovery. As such, the prospects for global amphibian conservation and recovery in the face of the unprecedented number of declining and increasingly imperiled species appear grim (5). In contrast, the results from our study indicate that the endangered *R. sierrae*, after decades of decline, is now increasing in abundance in Yosemite National Park, despite the ongoing presence of multiple stressors, including disease. These increases are occurring over a large landscape and across hundreds of populations, providing a rare example of amphibian recovery at an ecologically relevant spatial scale. That said, recovery of *R. sierrae* in Yosemite is far from complete and at a minimum, will require the continued removal of introduced fish from key habitats and translocation of frogs to reestablish populations in areas from which they are extirpated (21). However, our research in the Sierra Nevada—and complimentary work in Australia (30, 31)—suggests that amphibian declines may be at least partially reversible at a regional scale. Similar studies conducted in other systems and over large temporal and spatial scales will provide important insight and generality about the potential for recovery of endangered amphibians at a global scale.

## Materials and Methods

**Frog Surveys.** Surveys targeted all lentic water bodies in Yosemite as shown on US Geological Survey 7.5' topographic maps. Counts of *R. sierrae* life stages (adults, juveniles, and tadpoles) were made during diurnal visual encounter surveys of the entire water body shoreline and suitable habitat in inlet and outlet streams (16, 40) (survey details are in *SI Text*). During the 20-y study, a total of 8,976 frog population surveys were conducted at 3,164 water bodies. For our analyses, we excluded surveys from water bodies that were surveyed only once during the study period or conducted outside the time of peak frog activity (May 15 to September 20). We also excluded the few *R. sierrae* populations that were established recently via translocation (21). These restrictions resulted in 7,678 surveys conducted at 2,154 water bodies (average number of surveys per water body = 3.6) (Fig. S2).

**Trends in Frog Abundance.** In our primary analysis, we used generalized linear mixed models to describe the effect of year on frog counts after accounting for nonindependence of counts and potentially confounding factors that could influence frog counts. We developed a single best model from a set of models that differed in their underlying distribution; inclusion of random effects to account for spatial, temporal, and location dependence in counts (details are in *SI Text*); inclusion of fixed effects (in addition to year) to account for factors that could influence frog counts (day of the year and observer) (Table S1); and fixed effects for temporal trend in abundance (year, our primary interest). We specified that frog counts come from one of two distributions: (i) a negative binomial distribution because of its flexibility in specifying the error distribution for counts or (ii) a zero-inflated negative binomial distribution because of its flexibility to account for excess zeros from unoccupied water bodies.

The generalized linear mixed models were fit using R (41) and the INLA package (32); the INLA package fits hierarchical models with random effects using integrated nested Laplace approximations. We used default priors when fitting models. The response variable in all models was the number of *R. sierrae* counted at the *i*th site in the *t*th year,  $C_{it}$ , rather than actual abundance,  $N_{it}$  (details are in *SI Text*). We used a state space approach to modeling the count data, assuming a direct relationship between  $C_{it}$  and  $N_{it}$ . Observation error is incorporated by assuming that the count comes from a negative binomial distribution with a mean equal to the latent abundance at the site. This approach is consistent with standard methods for modeling count data under observational uncertainty (33, 42). A drawback of this approach is that we were not able to explicitly test whether observed trends could have been generated by nonstationarity in the detection process (e.g., resulting from different observers through time). However, the same

standardized protocol was used across all years, and surveys were conducted by only two teams of observers (led by G.M.F. or R.A.K.) whose survey periods broadly overlapped (1993–2012 and 2000–2012, respectively). We also included observer team (G.M.F. or R.A.K. led) as a fixed effect in models to account for potential observer effects on *R. sierrae* detection and resulting counts.

The base model that provided the best fit to the data was one using a zero-inflated negative binomial distribution and including the three random effects and the fixed effects year and day (but not observer). We fit separate models to counts of adults, juveniles, and tadpoles. All models produced similar results, and here, we focus primarily on the adult model. Using this “base” model, we then estimated the overall trend in frog abundance across the park during the 20-y study period. Trend was estimated as the slope of the relationship between counts and year for a model constrained using a log-link function (drawing a parallel between our statistical model and an exponential population growth model). The slope is equivalent to the intrinsic rate of increase,  $r$ . The base model was also used to map spatial variation in trends (using a conditional autoregressive random effect for the trend parameter) (43) and quantify how trend varied with respect to several environmental covariates, including water body depth, elevation, precipitation, fish, and watershed (Tables S1 and S2). Additional details on the modeling process are provided in *SI Text*. The dataset and R code used in the analysis are available at <http://dx.doi.org/10.5061/dryad.rm382>.

**Frog Susceptibility Experiment.** This laboratory experiment was conducted at San Francisco State University (SFSU) and approved by Institutional Animal Care and Use Committees at SFSU; University of California, Santa Barbara; and University of California, Berkeley. To provide frogs for the experiment, adult *R. sierrae* were collected from each of three persistent populations (including two in Yosemite) and three Bd-naïve populations (Table S4). Frogs from the persistent populations were Bd-positive (Table S4) and therefore, cleared of infection using itraconazole (44). Naïve frogs were uninfected but treated with itraconazole concurrently to ensure that all frogs were treated similarly. To start the experiment, two frogs, one from a persistent population and the other from a naïve population, were assigned at random to 1 of 16 replicate tanks (Fig. S3). Tanks were then inoculated with one of four strains of Bd: two cultured from persistent populations (including one from Yosemite) and two from populations that had experienced recent Bd-caused die offs (18). To quantify Bd load on frogs throughout the 15-wk experiment, skin swabs were collected from all frogs (18, 45) immediately before Bd exposure and weekly thereafter and analyzed using quantitative PCR (46) (details in *SI Text*).

We used a model selection strategy to examine the effects of frog type (collected from a persistent or naïve population), frog source (one of six populations), Bd type (collected from a persistent or die-off population), Bd source (one of four populations), and interactions of these effects on Bd load [ $\log_{10}(\text{zoospore equivalent [ZE]} + 1)$ ]. To select the best model, we followed the protocol by Zuur et al. (47). Additional details regarding the modeling steps are provided in *SI Text*. The dataset and SAS code used in the analysis are available at <http://dx.doi.org/10.5061/dryad.rm382>.

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