Supporting Information

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SI Text

Frog Survey Methods, Analysis, and Results.

Study area. Yosemite National Park is located in the central Sierra Nevada, California, United States (37° 53.984′ N, 119° 31.762′ W). Nearly the entire park is within the range of *Rana sierrae*, including water bodies along an elevation gradient from the lower montane zone (~1,650 m) to the alpine zone (3,200–3,500 m). In Yosemite, the active season for *R. sierrae* is from May to October. During the remainder of the year, the landscape is generally snow-covered, and during this time, *R. sierrae* overwinter in ice-covered perennial water bodies.

Frog counts. Counts of *R. sierrae* made during diurnal visual encounter surveys of water body shorelines were used as a proxy for abundance. The degree to which counts accurately reflect abundance depends largely on animal detectability, and *R. sierrae* are highly detectable. Counts from visual encounter surveys conducted 6–12 d after reintroductions of 32–50 adult *R. sierrae* into seven lakes in Yosemite National Park indicated an average proportion counted of 0.36 (range: 0.20–0.62). This relatively high detectability results from several characteristics of *R. sierrae* and its habitat. First, during the day, *R. sierrae* adults, juveniles, and tadpoles bask in near-shore shallows, making all life stages highly visible during diurnal shoreline surveys. Second, the oligotrophic water bodies generally inhabited by *R. sierrae* have high water clarity with little or no aquatic vegetation. Third, tadpoles are present throughout the summer (and during all other seasons) because of the 2–3 y duration of this life stage in *R. sierrae*.

Trends in frog abundance. The following provides additional details regarding all steps in the modeling process that was used to quantify trends in *R. sierrae* abundance from surveys conducted across Yosemite National Park during the study period. The first step was to choose the best distribution and random effect structure. We did this by comparing four models, each based on one of two distributions (negative binomial or zero-inflated negative binomial) and with or without three random effects. The three random effects were as follows.

i) A conditional autoregressive structure (CAR) to account for spatial autocorrelation (43) in frog counts among sites. To apply this structure, we divided the study area into an array of hexagonal grid cells with centroids that were located 6,500 m apart in the east–west direction and 5,629 m apart in the north–south direction (Fig. 3).

ii) A first-order temporal autocorrelation structure to account for temporal dependence in repeated counts at the same water body.

iii) A random effect for variation in mean counts among all water bodies. This random effect accounted for variability in mean densities of frogs among water bodies and helped control for among-site variation when dealing with missing data (i.e., combinations of water body and year when a survey did not occur).

At this step, we also included two fixed effects in all models: day of the year on which a survey was conducted (day; as a continuous variable) (Table S1) because of its potential to confound frog counts (for example, if counts are lower in early summer compared with mid- or late summer) and survey year (year; as a continuous variable) (Table S1) to account for temporal trend, our primary interest. The deviance information criterion (DIC) was used as a measure of model fit, and we used the model with the lowest DIC value in subsequent analyses.

In the second step, we tested for evidence of differences among survey teams in mean counts and/or trend in counts across years. We compared three models: no observer effects, observer differences in mean count, and observer differences in both mean count and trend. Again, we used DIC to choose among models, and we used the model structure with the lowest DIC value in subsequent analyses. We refer to this best model as the base model.

In the third step, we used the base model to estimate an overall trend in *R. sierrae* abundance across the park over the 20-y study period. In addition, we fit an equivalent model to data for counts of juveniles and tadpoles to determine whether trends were consistent across all three life stages.

To explore whether environmental conditions influenced counts and/or trends across the park, our next step was to add five environmental covariates (fixed effects) to the base model (Table S1). Four of the covariates were chosen because they could be related to spatial variation in abundance of *R. sierrae*: water body elevation, water body depth, presence/absence of introduced fish, and watershed in which the water body was located. These variables differ between sites but not years. The fifth covariate that we considered was precipitation during the previous year, which we predicted could explain some of the year to year variation in the overall rate of population increase. This variable differed between years but within a year, was constant for all sites. Given the focus of the analysis on trends in *R. sierrae* abundance, we were particularly interested in whether the average rate of increase in abundance depended on each of four spatial covariates, which we evaluated by examining the interaction between year (i.e., our measure of trend) and each of four covariates (Table S2). The temporal covariate × year interactions would have a completely different meaning and would be difficult to interpret. As such, the precipitation × year and survey date × year interactions were not included in the model. In addition, no Bd-specific covariates, such as the year of Bd arrival at a site, were included, because little historical Bd data exist for Yosemite's *R. sierrae* populations that could be used to describe the initial arrival and spread of Bd in this area.

Continuous covariates were standardized to have a mean = 0 and an SD = 1 to allow parameter effect sizes to be interpreted as the effect of a 1-SD change in the covariate value. The full fixed effect structure included an effect for year (i.e., trend), the five covariates, and the interaction between year and each of the spatial covariates. To examine effect sizes of all covariates, we fit the full model and made inferences about the importance of covariates based on parameter estimates and 95% CIs. Because collinearity between covariates could complicate interpretation of results, we evaluated the degree of correlation between the covariates, focusing specifically on the spatial covariates, because they were measured across the same set of sample units. Collinearity was low for all pairwise comparisons (Table S3) and would not have affected the results.

The final step was to visualize spatial variation in trends in *R. sierrae* abundance across the park. We accomplished this by fitting a spatially explicit model for the trend parameter, again using a CAR structure for the random effect. The random trend parameter allowed us to map variability in trends across the park while accounting for spatial autocorrelation among adjacent cells when making predictions.

Sensitivity of results. We conducted several analyses using data subsets to assess the sensitivity of results to assumptions made in the primary analysis. The first assumption is that the number of surveys conducted per water body did not influence estimates of population

trend. The number of surveys per water body ranged from 2 to 55, with an average of 3.6 (Fig. S2). To determine how robust the results are to the number of times that a site was surveyed, we reanalyzed the dataset, this time restricting the analysis to only those sites where at least five surveys were conducted and those surveys occurred over at least a 15-y period. Based on this analysis, the estimated trend (r) for the number of adult *R. sierrae* is 0.066 (95% CI = 0.037–0.095), equivalent to a 6.8% annual increase over the 20-y study. This estimate is somewhat lower but still quite similar to the estimated trend based on the full dataset ($r = 0.105$; 95% CI = 0.075–0.134; 11.0% annual increase). The estimates for juveniles and tadpoles were also somewhat lower than for the full dataset (juveniles: $r = 0.079$; 95% CI = 0.039–0.120; 8.1% annual increase; tadpoles: $r = 0.111$; 95% CI = 0.055–0.168; 11.7% annual increase). The sites included in the data subset had a higher average abundance of adult *R. sierrae* than those in the full dataset, likely because these larger populations were of particular interest and therefore, surveyed more frequently than small populations. As such, the somewhat lower rate of increase for the data subset may be reflective of trends in water bodies that contained larger frog populations in the early years of the study, populations that a priori might be expected to grow more slowly than those that were relatively small at the start of the 20-y study.

The second assumption that could have affected the results is that the inclusion of water bodies in which adult *R. sierrae* were never detected (but other life stages may have been observed) did not affect estimates of population trend. To determine the sensitivity of the results to this assumption, we excluded these sites from the data subset described above and reran the analysis. Based on this analysis, the estimated trend (r) for the number of adult *R. sierrae* is 0.066 (95% CI = 0.037–0.094), equivalent to a 6.8% annual increase over the 20-y study. The estimates for juveniles and tadpoles were as follows: juveniles, $r = 0.079$; 95% CI = 0.039–0.123; 8.1% annual increase; tadpoles: 0.116; 95% CI = 0.056–0.178; 12.3% annual increase. These estimates of trend are virtually unchanged from those obtained using a dataset that included all water bodies, regardless of whether adult *R. sierrae* were detected (see above), and were somewhat lower than for the full dataset.

We expected frog population trends to exhibit strong temporal and spatial autocorrelation, and we accounted for these effects using temporal and spatial random effect terms. As part of our analysis, we evaluated the fit of models with and without these terms. Because of space limitations, these results are not presented in the text and are described here instead. Compared with the "full model" presented in the text, the fit of a model lacking the temporal and spatial random effect terms is much poorer (ΔDIC = 4,455). The trend estimate based on this model is 0.193 (95% CI = 0.170–0.215) (Fig. S1), much higher than the estimate from the full model of 0.10 (Fig. 1). We conclude that including the spatial and temporal effects in the model provides a much better fit to the count data and results in a more conservative estimate of trend in frog abundance.

Methods in Frog Susceptibility Experiment and Analysis. To provide frogs for the experiment, adult *R. sierrae* were collected from each of three persistent populations and three Bd-naïve populations (22) (Table S4). At the time of the collections, the persistent populations had been Bd-positive for at least 10 y (and probably substantially longer) and are characterized by high Bd prevalence but low to moderate loads and relatively small populations that are stable or expanding (Table S4). The Bd-naïve populations are located ahead of the Bd "wave" that is currently spreading across Kings Canyon National Park and its vicinity (18). These six populations have been surveyed repeatedly since at least 1997, are considerably larger than the persistent populations, and through 2009, when this experiment was conducted, were Bd-negative (Table S4). Two of the persistent populations are located in Yosemite, and the third population is immediately north of the park. For three naïve populations, two are located in northern Kings Canyon National Park, and one is immediately north of the park (Table S4).

Frogs collected for the experiment 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. Unexpectedly, frog mortality occurred during the treatment and resulted in the loss of 58% of the frogs, a much higher mortality rate than was observed during previous or subsequent treatments of *R. sierrae* using the same itraconazole dose (0.01% bath applied for 5 min daily for 11 d). The cause of this mortality event is, therefore, uncertain. All surviving frogs tested negative for Bd during 2 consecutive weeks after the treatment. 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 (standard 10-L plastic rat containers with filtered lids that allow airflow). Two of the naïve and two of the persistent populations provided five frogs each, and the remaining naïve population and persistent population provided six frogs each (Fig. S3).

The 16 tanks were inoculated with one of four strains of Bd (selected at random). These Bd strains were cultured from two persistent frog populations (one in Yosemite) and two frog populations that had experienced recent Bd-caused die offs (18). Each tank was inoculated with a single Bd strain, and each of four strains was used to inoculate four tanks (Fig. S3). An additional eight tanks, each containing a pair of frogs, served as unexposed controls.

To quantify Bd load on frogs throughout the 15-wk experiment, skin swabs were collected from all frogs immediately before Bd exposure and weekly thereafter. Swabbing of frogs was conducted using a standardized method, with a total of 30 strokes made across the ventral portion of each animal (5 strokes each on the left and the right sides of the abdomen, left and right thighs, and left and right foot webbing) (18). After collection, swabs were air-dried, stored at −80 °C, and analyzed within 7 d of collection using a real-time quantitative PCR (qPCR) assay (46). Two weeks after the initial Bd inoculation, both frogs in 4 of 16 tanks remained uninfected. These tanks were reinoculated using the original Bd strain and the same methods as in the original inoculation. This reinoculation resulted in all frogs becoming infected. To account for the different infection timeframes between tanks, in which frogs became infected after the first vs. the second inoculation, the time course for each tank is expressed as weeks after the last inoculation.

After 6 wk, Bd infection had reached moderate levels; frogs in randomly selected 12 of 24 tanks (including 4 of 8 control tanks) were killed, and their gene expression profiles were analyzed using microarrays (these results are presented elsewhere). Frogs in the remaining tanks were followed for the full 15-wk experiment. Throughout the experiment, water in tanks (~1.5 L carbon-filtered water) was changed weekly, and each frog was fed approximately five crickets twice weekly. At the conclusion of the experiment, all frogs were cleared of Bd infection using itraconazole (using the methods described previously) and held in captivity for use in other studies. No frog mortality occurred during this treatment period.

We used a model selection strategy recommended in ref. 47 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. We also included the individual-level variables of snout-vent length (SVL; centered by subtracting the mean SVL) and sex. The response variable was Bd load as measured by $\log_{10}(ZE + 1)$; ZE was measured from qPCR, on each frog at each of the weekly time points. Results from frogs in all 16 Bd-inoculated tanks were included for weeks 1–6, tanks remaining after euthanasia of frogs

from one-half of the tanks (at week 6) were included for weeks 7–15, and the analysis accounted for the change in sample size after week 6.

We first determined the best structure for the random components of the model, including all possible fixed effects, using Restricted Maximum Likelihood (REML) estimation and compared random effect models using the Akaike Information Criterion (AIC). We included correlated errors among the multiple temporal measurements on individual frogs using the REPEATED statement in PROC MIXED, with frogid as the subject and a first-order temporal autoregressive model [AR(1)]. Other temporal correlation structures were investigated, but none improved the fit. The errors differed between Bd type, and therefore, we included an error structure that allowed for different error parameters in these two groups. We investigated more complicated random effects models, including random effects of tanks, random slopes for individual frogs, random effects of frog source nested within frog type, or random effects of Bd source nested within Bd type; however, none of these improved the model fit. Satterthwaite dfs were used throughout to deal with the complex error structures. After we arrived at the best fit random effects structure, we used it in the model to compare alternative fixed effects models. Fixed effects models were fit using maximum likelihood estimation and compared using AIC. Because frog source is perfectly correlated with frog type, we could not include both of these predictor variables in the model. Likewise, we could not include both Bd source and Bd type in the model. Therefore, we compared the fit of the models that included either one or the other of the alternative frog and Bd groupings. In addition to model comparison using AIC, the significance of each fixed effect was determined by likelihood ratio tests comparing the likelihood of the full model and a model with that term of interest removed. Final parameter estimates were obtained using REML estimation (Table 1).

The best fit random effects component of the model included temporal autocorrelation in the errors for the repeated measurements of Bd load on individual frogs and different error parameters for the frogs in the Bd type groups (Table S5). For the fixed effects component of the model, the effects of week after Bd inoculation and week^2 (each centered by subtracting the mean) were always significant, whereas the individual-level variables of SVL and sex were not significant. Models with the alternative frog and Bd groupings were compared using AIC, and the overall best fit model included the variables frog type and Bd source (Table S6). The interactions between frog grouping (either frog source or frog type) and Bd grouping (either Bd source or Bd type) were never significant. Frog type and Bd source both had highly significant effects on Bd load, with frogs from naïve populations having significantly higher Bd loads than frogs from persistent populations (Table 1). Frogs exposed to the different Bd sources differed in their loads, but loads did not consistently differ between Bd from persistent and die-off sites. To further illustrate this point, we compared the model including frog source and Bd source with the model including frog type and Bd type. In the first of these two models, both frog source and Bd source are highly significant; however, in the second model, frog type is significant, but Bd type is not.

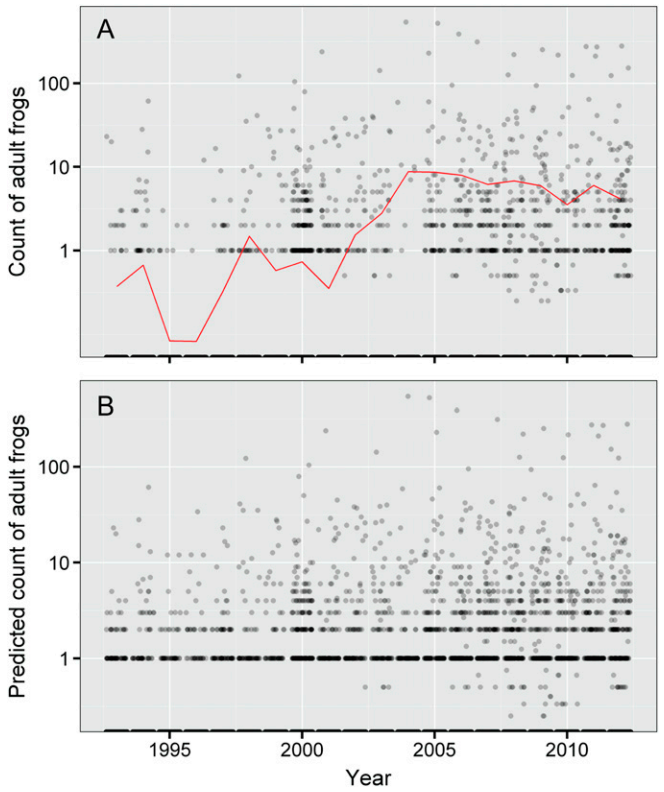

Fig. S1. Trends in frog abundance during the 20-y study showing results used to generate Fig. 1: (*A*) actual counts of adult *R. sierrae* for all sites sampled in each year (points) and average annual counts (red line) and (*B*) actual counts for sampled water bodies and predicted counts for unsampled water bodies based on the estimated distribution in the trend model. In *A* and *B*, when more than one survey was conducted per year, results were averaged by year. *Y* axes are shown on a log scale. Although only a subset of sites was surveyed in any 1 y (shown in *A*), the general distribution of points in *A* is similar to that in *B*, which is based on actual and predicted counts at all water bodies. Average values from *B* were used to create the trend line and 95% CI in Fig. 1.

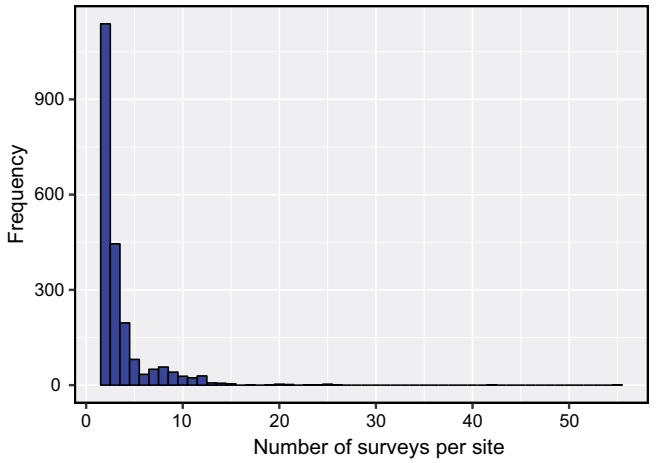

Fig. S2. Frequency histogram showing the number of surveys conducted per site over the 20-y study period.

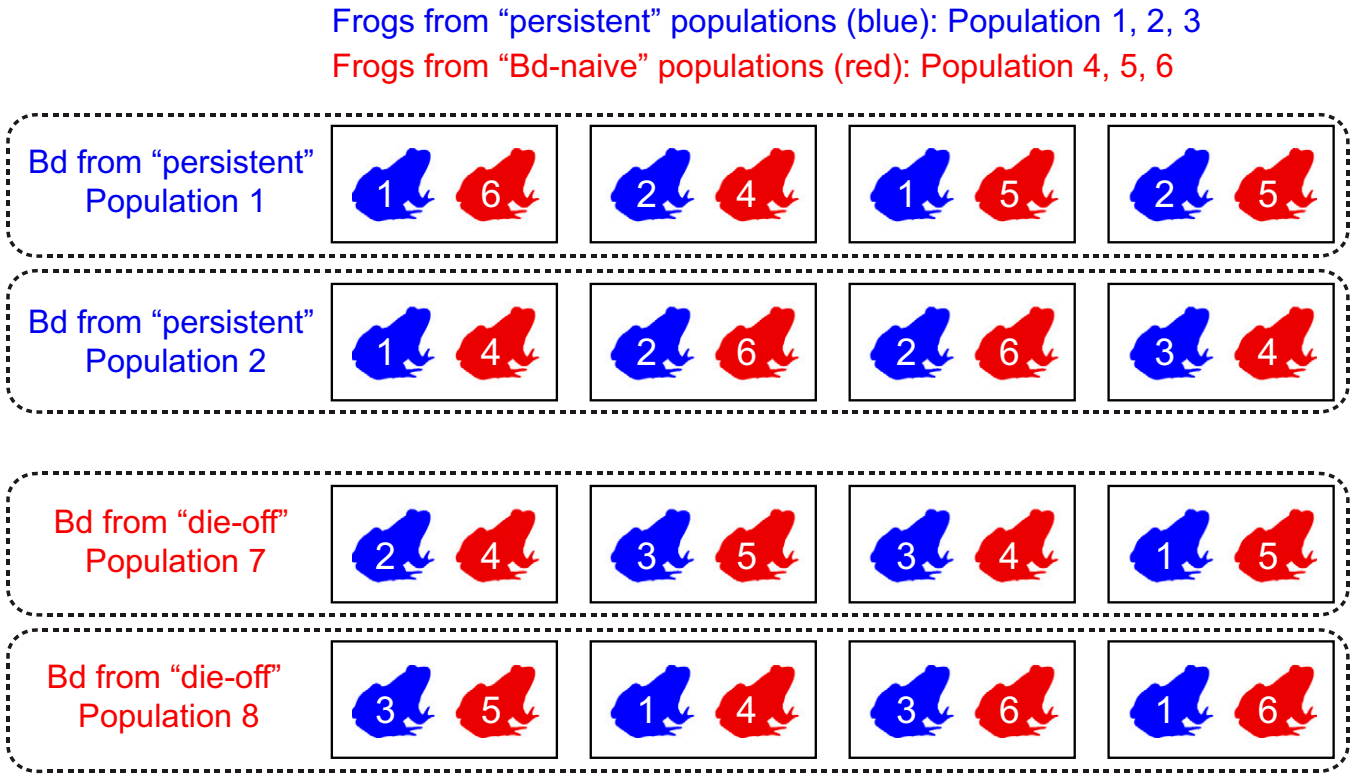

Fig. S3. Design of the laboratory *R. sierrae* susceptibility experiment. Numbers associated with frogs indicate the population from which each frog was collected (listed at the top and described in Table S4). Eight control tanks that were not exposed to Bd are not shown.

Table S1. Description of predictor variables in the linear mixed models used to describe trends in *R. sierrae* abundance

Variable name	Description
Year	Year in which survey was conducted
Day	Day of year on which survey was conducted
Water body depth	Maximum depth of water body (in meters) as estimated for deeper water bodies by sounding with a weighted line or visually for shallower water bodies (16)
Water body elevation	Elevation of the water body (meters above sea level) as estimated from 1:24,000 US Geological Survey topographic maps
Precipitation	Annual sum of monthly Merced River discharge in a given year divided by the annual sum of average monthly discharge during 1951–2000; provides a measure of the snowfall amount during the previous winter
Fish	Presence or absence of one or more species of nonnative trout as determined by gill netting for deeper water bodies or visually for shallower water bodies (16)
Watershed	Watershed in which the water body is located: Merced River or Tuolumne River
Observer	Leader of the team conducting the survey: G.M.F. or R.A.K.

Table S2. Parameter estimates for the fixed effects in the full generalized linear mixed effects model used to describe trends in frog populations during 1993–2012

Fixed effect	Estimate	SE	Lower 95% CI*	Upper 95% CI*
Intercept	−6.562	0.534	−7.662	−5.563
Year†	0.101	0.027	0.049	0.155
Day†	0.091	0.041	0.011	0.172
Fish†	0.281	0.136	0.016	0.549
Water depth†	−3.782	0.649	−5.102	−2.553
Elevation†	0.597	0.105	0.390	0.803
Watershed†	0.560	0.291	0.002	1.146
Precipitation	0.344	0.696	−1.020	1.717
Fish × year†	0.111	0.038	0.038	0.187
Depth × year	−0.005	0.006	−0.018	0.007
Elevation × year	−0.004	0.020	−0.044	0.036
Watershed × year	−0.029	0.045	−0.118	0.059

*The importance of predictor variables in affecting frog abundance is determined based on whether 95% CIs include zero.
†The 95% CIs of variables with important effects do not include zero.

Table S3. Correlation matrix for four environmental covariates included in the full model (all differ between sites but not between years)

	Depth	Elevation	Fish	Watershed
Depth	1.000	0.035	0.295	−0.065
Elevation		1.000	−0.042	0.088
Fish			1.000	0.058
Watershed				1.000

Table S4. Characteristics of the *R. sierrae* populations from which frogs were collected for the frog susceptibility experiment

Population identifier*	Location	Population type	Adult frog count (2009)	Bd prevalence	Average Bd load (1 SE)
1	Humboldt–Toiyabe NF	Persistent	76	0.83	45.2 (18.7)
2	Yosemite NP	Persistent	280	1.00	693.8 (173.8)
3	Yosemite NP	Persistent	46	0.44	8.0 (3.0)
4	Inyo NF	Bd naïve	993	0.00	0 (0)
5	Kings Canyon NP	Bd naïve	694	0.00	0 (0)
6	Kings Canyon NP	Bd naïve	3,531	0.00	0 (0)

NF, National Forest; NP, National Park.
*Population identifiers are the same as those used in Fig. S3.

Table S5. Covariance parameter estimates for the repeated measures model of Bd load

Covariance parameter	Subject	Group	Estimate	SE	*Z* value	*P* value
Variance	Frogid	Bd type = die off	1.45	0.270	5.40	<0.0001
AR(1)	Frogid	Bd type = die off	0.73	0.051	14.41	<0.0001
Variance	Frogid	Bd type = persistent	0.56	0.073	7.72	<0.0001
AR(1)	Frogid	Bd type = persistent	0.34	0.085	4.03	<0.0001

Table S6. Comparison of model fits including the alternative frog and Bd grouping variables

Fixed effects*	−2 Log likelihood	No. of parameters	AIC	BIC†
Frog type + Bd source + week + week^2	774.4	10	794.4	809.0
Frog source + Bd source + week + week^2	768.5	14	796.5	817.0
Frog type + Bd type + week + week^2	793.4	8	809.4	821.2
Frog source + Bd type + week + week^2	791.7	12	815.7	833.2

*The frog source and Bd source variables indicate the identities of the source lakes for the frogs and Bd, whereas the frog type and Bd type variables indicate only whether the frogs and Bd came from one of the persistent populations.

†Bayesian information criterion.