

**Impact of the Amphibian Chytrid Fungus on
Sierra Nevada Yellow-legged Frogs in Yosemite National Park:
Insights from Population Resurveys and Frog Translocations**

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Summary

Amphibians are rapidly disappearing from habitats all around the world and a major cause of these declines is the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (“Bd”). The growth rate of Bd is temperature dependent and this relationship is described by a hump-shaped curve with maximum growth rate at 20° C and no growth below 4° C and above 30° C. Studies conducted in hot climates have shown that the relative impact of Bd on amphibians decreases with increasing temperature, with no impacts at temperatures above 30° C. The effect of temperature on Bd-amphibian interactions should change just as dramatically across the low end of the temperature scale, but virtually no research on this topic has been conducted in cool or cold climates. We studied the impact of Bd on the declining Sierra Nevada yellow-legged frog (*Rana sierrae*) across a wide elevational (i.e., temperature) gradient in Yosemite National Park, a region characterized by cool summers and cold winters. We used resurveys of all 285 *R. sierrae* populations to describe the landscape-scale patterns of Bd infection intensity, frog population size, and frog population persistence. Specifically, we tested the hypotheses that infection intensity should decrease with elevation, and consequently population size and persistence should both increase with elevation. Second, we conducted a frog translocation experiment to test the hypothesis that the thermal regime experienced by frogs influences frog survival via effects on infection intensity. In both the resurvey study and the translocation experiment, Bd infection intensity decreased with increasing elevation. Likely as a consequence of this effect, frog survival in the translocated populations and *R. sierrae* population size in the resurvey study were both positively correlated with elevation. *R. sierrae* were not detected at 38% of the sites where they formerly occurred, but the probability of frog population persistence was unrelated to elevation. The current study provides the most detailed analysis to date of how temperature influences the Bd-amphibian interaction in temperate montane regions, and provides important guidance for the development of conservation actions designed to ensure the long-term persistence of *R. sierrae* in Yosemite National Park.

Introduction

Amphibians are declining worldwide at an alarming rate and a recent study suggests that more than 40% of the world's 6000+ species are now threatened with extinction (Stuart et al. 2004). Causes of endangerment include the well-documented effects of habitat alteration (Dodd and Smith 2003) and nonnative species (Kats and Ferrer 2003), but a recently-described disease is increasingly considered a serious threat (Skerratt et al. 2007). Chytridiomycosis is an emerging infectious disease of amphibians caused by the fungal pathogen *Batrachochytrium dendrobatidis* ("Bd"). Diseases are rarely considered as a driving force of host extinctions but the extraordinary virulence of chytridiomycosis to amphibians has resulted in the decline or extinction of hundreds of species during the last several decades. This impact of chytridiomycosis on amphibians has been described as "the most spectacular loss of vertebrate biodiversity due to disease in recorded history" (Skerratt et al. 2007).

Bd is a waterborne pathogen and amphibians are its only known host. Bd is transmitted among host individuals via a free-swimming zoospore stage that infects keratinized tissues in the skin of post-metamorphic amphibians (Longcore et al. 1999) and the mouthparts of tadpoles (Knapp and Morgan 2006). Bd apparently kills amphibians by disrupting the normal functioning of the skin, including osmoregulation (Voyles et al. 2007). Bd was first described in the late 1990s (Berger et al. 1998, Longcore et al. 1999) and subsequent retrospective analyses of museum collections indicate its presence in South Africa in the 1930s (Weldon et al. 2004) and on all continents except Antarctica (which lacks amphibians) during subsequent decades. The genetic similarity of Bd from around the world (Morehouse et al. 2003, Morgan et al. 2007) likely indicates that the recent emergence of chytridiomycosis is the result of recent human-mediated spread into new geographic areas (Lips et al. 2006, Lips et al. 2008), but disease emergence could also be the result of environmental change facilitating the emergence of an endemic and already widespread pathogen (Pounds et al. 2006). The

role of climate warming as a cause of Bd emergence remains the topic of considerable debate (Lips et al. 2008, Rohr et al. 2008).

The impact of chytridiomycosis on amphibians varies markedly between species (Woodhams et al. 2007) and even between populations of the same species (Briggs et al. 2005), and differences in environmental conditions, in particular temperature, appear to play an important role in causing these differences in disease outcomes. Bd growth rate is strongly influenced by temperature, with high growth rates between 14 and 27° C and little to no growth below 4° C and above 30° C (Figure 1; Piotrowski et al. 2004, Woodhams et al. 2008). In agreement with this temperature sensitivity, the impact of chytridiomycosis is often reduced in warmer relative to cooler climates. For example, impacts have been widely reported as less severe at low versus high elevations (Woodhams and Alford 2005), low versus high latitudes (Kriger et al. 2007), and during summer versus winter (Berger et al. 2004, Kriger and Hero 2007). However, most field studies that investigated the effects of temperature on Bd-amphibian dynamics were conducted in tropical or subtropical climates, i.e., at the high end of the temperature range over which Bd is active. As such, these studies likely provide only a partial view of the effects of temperature in influencing disease dynamics. For example, in contrast to the general patterns reported in the literature, Muths et al. (2008) reported that in high latitude sites in the Rocky Mountains (USA) the probability of Bd occurrence was negatively correlated with elevation and positively correlated with temperature. Given the increasing ubiquity of Bd worldwide and its severe impacts on many amphibian species, understanding the influence of temperature on the Bd-amphibian interaction is critically important for the conservation of global amphibian biodiversity.

The mountain yellow-legged frog is a species complex composed of two closely-related taxa, the Sierra Nevada yellow-legged frog, *Rana sierrae*, and the Southern mountain yellow-legged frog, *Rana muscosa* (Vredenburg et al. 2007). Historically, these frogs were abundant across California's Sierra Nevada mountains (including in Yosemite National Park; Grinnell and Storer 1924) and in southern

California's Transverse Range. Both species have declined precipitously during the last century and are now absent from more than 90% of known localities (Vredenburg et al. 2007), including in Yosemite (Drost and Fellers 1996). The negative effects on mountain yellow-legged frogs of nonnative fish introductions into fishless lakes and streams is well documented (Bradford 1989, Knapp and Matthews 2000, Knapp 2005). However, during the past decade Bd has caused the extinction of hundreds of mountain yellow-legged frog populations from remaining fishless habitats across the Sierra Nevada (Rachowicz et al. 2006; Knapp, unpublished data), habitats in which until recently populations were thought to be secure.

To describe the status of amphibians in Yosemite National Park (hereafter, "Yosemite"), in 2000-2002 all lentic habitats ($n = 2655$) were surveyed for *R. sierrae* and all other amphibian species. Analysis of amphibian distributions clearly indicated the strong negative effect of introduced trout on *R. sierrae* (Knapp 2005). However, the complete absence or the presence of uncharacteristically small *R. sierrae* populations in even high-quality fishless habitats suggested that other factors in addition to introduced trout were influencing the distribution and dynamics of these frog populations (see also Drost and Fellers 1996). The documentation in 2001 of chytridiomycosis in Yosemite's *R. sierrae* populations (Fellers et al. 2001) and the relatively frequent observations of dead and dying *R. sierrae* made during the 2000-2002 Yosemite amphibian survey suggested that chytridiomycosis could be an important but poorly understood factor in the decline of *R. sierrae*.

The objectives of this study were two-fold. First, the fact that Bd is nearly ubiquitous in Yosemite's *R. sierrae* populations (see **Results**) allowed us to describe the landscape-scale patterns of Bd infection intensity, *R. sierrae* population size, and *R. sierrae* population persistence across a wide elevational (i.e., temperature) gradient. This was accomplished by revisiting all *R. sierrae* populations in Yosemite and quantifying Bd infection intensity and *R. sierrae* occurrence and population size. Based on the known temperature sensitivity of Bd and the relatively cool climate of Yosemite we hypothesized

that Bd infection intensities would be positively correlated with air and water temperatures (i.e., negatively correlated with elevation). Because Bd infection intensities in mountain yellow-legged frogs strongly influence survival (Vredenburg et al., unpublished data) we also hypothesized that this relationship between infection intensity and elevation would result in larger frog populations and a higher probability of population persistence at high versus low elevations. Our second objective was to test the hypothesis that the thermal regime experienced by frogs influences frog survival via effects on infection intensity. We tested this hypothesis using a replicated whole-lake experiment in which *R. sierrae* from a large source population in Yosemite were translocated to three nearby lakes that spanned a wide elevational gradient.

Methods

Study area description

Yosemite National Park encompasses 3027 km² of the central Sierra Nevada (37°30'–38°11'N, 119°120'–119°53'E; see Figure 1 in Knapp 2005). Precipitation in Yosemite falls mostly in the winter months and is primarily rain at the lowest elevations and snow at the higher elevations. Yosemite encompasses an ideal geography in which to conduct research related to our two study objectives because the broad elevational gradient produces a wide range of temperatures to which frogs and Bd are exposed. Most of Yosemite's more than 2600 lakes, ponds, and marshes range in elevation from approximately 2000 m to over 3600 m, with a median elevation of 2786 m. These lentic habitats are generally small (<10 ha) and range from relatively warm water bodies at lower elevations that are typically surrounded by forest and often contain abundant aquatic vegetation, to high elevation, cold, oligotrophic water bodies surrounded by alpine meadows and rock. During summer, average maximum littoral zone water temperatures range from approximately 24° C at 2000 m to 16° C at 3300 m (Knapp, unpublished data). All natural lentic habitats in Yosemite were historically fishless as a result of

numerous natural barriers on streams and rivers at low elevation. Several species of trout were widely introduced between 1870 and 1991, most commonly rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). All trout stocking was halted in 1991 but historic stocking resulted in the establishment of self-sustaining populations. Trout are currently found in 9% of all lentic water bodies and 56% of water bodies deeper than 4 m, the latter being the habitat type favored by *R. sierrae* (Knapp 2005).

Data Collection

Frog population resurvey. During the original survey of all lentic habitats in Yosemite conducted in 2000-2002, *R. sierrae* was detected at 285 sites (Knapp 2005). We resurveyed all of these sites during the summers of 2005, 2006, and/or 2007 (Figure 2). Sites were surveyed at most once per year and 1-3 times during the three-year study period (201 sites were surveyed once, 78 sites were surveyed twice, 6 sites were surveyed three times). For sites surveyed more than once only the results of the final survey were used in analyses. During the original surveys and resurveys the occupancy of sites by *R. sierrae* and the abundance of all life stages (tadpoles, juveniles, adults) was estimated using visual encounter surveys of the entire water body shoreline and the first 100 m of each inlet and outlet stream (Crump and Scott 1994, Knapp 2005). To ensure that Bd was not spread between frog populations by survey activities, we disinfected all field gear by immersion in 0.01% quaternary ammonia for five minutes (Johnson et al. 2003) whenever moving between water bodies.

Recent advances in occupancy modeling have made possible improved estimates of occupancy by incorporating detection probabilities. These study designs require both spatial and temporal replication (e.g., repeat surveys at multiple sites conducted during a relatively narrow time window; MacKenzie et al. 2002). Estimates of occupancy from single-site visits can be biased when detection probabilities are low, but improvements gained by incorporating detection probabilities are minimal

when detection probabilities are close to one (MacKenzie et al. 2002). We used a study design without temporal replication based on information that detection probabilities for *R. sierrae* are likely to be close to one. This allowed us to survey a much larger number of sites than would have been possible under a replicated sampling design, and results from these surveys are providing critical guidance to the ongoing development of site-specific amphibian restoration efforts in Yosemite National Park. Our suggestion that detection probabilities are close to one is based on several characteristics of *R. sierrae* and its habitat. First, during the day adults spend the majority of their time on shore immediately adjacent to water and tadpoles are found primarily in near-shore shallows, making both life stages highly visible during shoreline surveys. Second, tadpoles are present throughout the summer (and during all other seasons) due to the 2-3 year duration of this life stage in *R. sierrae* (Bradford 1983). Third, Yosemite's lakes and ponds are generally oligotrophic (Sickman et al. 2003) and have high water clarity. Despite our suggestion that *R. sierrae* detection probabilities are high, we acknowledge that our temporally unreplicated study design could introduce some bias into our estimates of occupancy. Such biases would only affect our study conclusions related to frog population persistence.

Water body elevation was obtained from U.S. Geological Survey 1:24000 topographic maps. Maximum lake depth was determined during the original survey by sounding with a weighted line. The presence/absence of trout was determined at each water body using visual encounter surveys or gill nets (Knapp and Matthews 2000). During the original survey, we used shoreline visual encounter surveys in shallow water bodies (<3 m deep) in which the entire bottom could be seen. In deeper water bodies, fish presence/absence was determined using both visual surveys and a single monofilament gill net set for 8–12 h (for gill netting details, see Knapp and Matthews 1998). During the resurvey only visual encounter surveys were used. Original and repeat surveys provided identical fish presence/absence results.

During the resurvey, we estimated Bd infection intensity on *R. sierrae* in each population using skin swabs collected from adults (≥ 40 mm snout-vent length (SVL)) and juveniles (< 40 mm SVL) and mouthpart swabs collected from tadpoles (Knapp and Morgan 2006, Hyatt et al. 2007). Swabs were analyzed for Bd amounts using quantitative (real-time) PCR (hereafter, “qPCR”). We attempted to swab 5-20 individual *R. sierrae* per site, with animals collected as available during or after surveys. Swabbed tadpoles were of at least Gosner stage 30 because tadpoles in earlier developmental stages are generally not yet infected (Knapp and Morgan 2006). For post-metamorphic frogs we swiped a nylon-tipped swab five times each across the left and right sides of the lower abdomen, left and right inner thighs, and left and right rear feet. On tadpoles, all 30 swipes were made across the mouthparts. Following collection, swabs were air-dried, placed into sterile micro-centrifuge tubes, and stored in the laboratory at room temperature. DNA extraction and qPCR protocols followed Boyle et al. (2004) and Hyatt et al. (2007) except that swab extracts were analyzed in singlicate instead of triplicate (Kriger et al. 2006). We calculated a measure of infection intensity (i.e., the number of Bd zoospores on each swab - “zoospore equivalents”) by multiplying the genomic equivalent values generated during the qPCR by 80; this multiplication accounts for the fact that DNA extracts from swabs were diluted 80-fold during extraction and qPCR. Swabs were considered Bd-positive when zoospore equivalents were ≥ 1 and Bd-negative when zoospore equivalents (ZE) were < 1 .

Frog translocation experiment. In this study frogs were collected from a single source population and moved to three lakes that spanned a wide elevational gradient, thereby allowing an experimental test of the effect of elevation and temperature on Bd infection intensity and frog survival. The source of frogs for the three translocations was a pond (“Conness Pond”) located near the eastern border of Yosemite (Figure 3) that harbored the largest *R. sierrae* population in the Park. This frog population has been intensively monitored since 2004 and in 2006 contained a population of adult *R. sierrae* estimated at

more than 1000 adult frogs (Knapp, unpublished data). This population was first sampled for Bd in 1998 and was found to be Bd-positive (Fellers et al. 2001). qPCR analysis of 563 swabs collected in 2004 and 2005 indicated that Bd prevalence was high (75% of frogs were infected) but that infection intensities were relatively low (median = 83 zoospore equivalents per swab), as is typical for *R. sierrae* populations in Yosemite (Knapp, unpublished data). In 2005 we initiated a capture-recapture study at Conness Pond to quantify frog survival. On July 25, 323 adult frogs were marked using 12 mm passive integrated transponder (PIT) tags. No additional frogs were tagged at this site until July 14, 2008 when an additional 71 adult frogs were PIT-tagged.

The three translocation sites (Figure 3; McGee Lake, “Tioga Pass Pond”, and Skelton Lake) were all relatively deep perennial water bodies characterized by excellent habitat for *R. sierrae* (Table 1; see also Knapp et al. 2003). Unpublished records for Tioga Pass Pond and Skelton Lake indicate the historical presence of *R. sierrae* at these two sites, but their historical presence at McGee Lake is uncertain (but likely given their historical presence in nearby lakes). McGee and Skelton Lakes were stocked with trout repeatedly between 1913 and 1951 (Elliot and Loughlin 1992), but all were fishless by the time of the 2000-2002 Yosemite amphibian survey. Based on repeat visual encounter surveys conducted at the translocation sites between 2001 and 2006, all sites lacked *R. sierrae* but two (McGee, Tioga Pass) contained breeding populations of the Pacific treefrog (*Pseudacris regilla*). Adult Yosemite toads (*Bufo canorus*) were occasionally seen at Tioga Pass Pond and Skelton Lake but breed in adjacent marshes and not in the lakes themselves. Frogs for translocation to McGee Lake were collected on July 17, 2006. Collected frogs were all untagged adults and were swabbed, measured, weighed, and tagged with 12 mm PIT tags. Following processing, frogs were held individually in wet cloth baths until their release. Frogs were held overnight and then transported 10 km (July 18) on foot to McGee Lake. Upon release, 43 of the frogs were active and seemingly healthy. The remaining seven frogs were dead for unknown reasons. In an attempt to reduce mortality in the remaining two translocations we made two changes to

the protocol. First, frogs translocated to Tioga Pass Pond and Skelton Lake were placed individually in small plastic containers that held a small amount of water and had air holes to ensure adequate ventilation. Second, frogs were transported to the two release sites on the same day they were collected. All other frog handling procedures were unchanged. Forty adult frogs were translocated to Skelton Lake and to Tioga Pass Pond on August 7 and August 8, 2006, respectively (80 frogs total). Thirty-nine of the frogs moved to Skelton Lake survived as did all frogs moved to Tioga Pass Pond. All surviving frogs were active and appeared healthy upon release.

Following translocation, we visited the source population and all three translocated populations approximately once per month in 2006 and 2007. In 2008 fewer revisits were made to McGee Lake and Tioga Pass Pond due to the apparent absence of frogs at these sites. During each visit we first conducted a shoreline visual encounter survey and then recaptured as many frogs as possible. Captured frogs were identified via their PIT tag and were swabbed, measured, and weighed. Frogs were released within five minutes of capture. The number of post-translocation visits to each site during the 2006-2008 study period is shown in Table 2. The amount of Bd on each swab was determined using qPCR and methods were identical to those used in the frog resurvey study except that swabs from 2006 and 2007 were run in triplicate.

Data Analysis

Frog population resurvey. Twenty-eight of the surveyed sites were immediately adjacent to at least one other surveyed site (e.g., ponds within marshes, marshes abutting lakes). To maximize the independence of survey data, data from every cluster of adjacent sites were combined into single sites (clusters contained 2-5 sites each). This produced 268 unique sites for analyses. Given that measuring air and water temperatures at each of these 268 sites throughout the summer was not feasible, in our analyses we used elevation as a proxy for the temperature regimes experienced by Bd and frogs. We

collected lake temperature data in 2006 at the four lakes used in the frog translocation experiment and in five other Yosemite study lakes in 2008. These lakes spanned a wide elevational gradient and resulting temperature data were used to verify that the 0.6° C per 100 m lapse rate for air temperature (Barry and Chorley 1992) also applies to water temperature. Water temperatures were measured hourly from July through September at 20 cm water depth using iButton loggers. Four (2006) or two (2008) loggers were deployed widely spaced in the littoral zone of each lake. Loggers were attached to a line anchored in 1 m of water and the loggers were kept at 20 cm depth by a surface float. The lake-specific average maximum daily water temperature was used in a linear regression analysis with elevation.

We used multivariate generalized additive models (GAM) to evaluate the strength of associations between elevation (i.e., temperature) and (1) Bd infection intensity, (2) *R. sierrae* population size, and (3) the probability of *R. sierrae* persistence between the original and repeat survey. GAMs are similar to generalized linear models, but relax the assumption that the relationships between the dependent variable (when transformed to a logit scale) and predictor variables are linear. Relaxation of this assumption is accomplished by estimating a nonparametric smooth function to describe the relationships between the dependent and predictor variables (Wood 2006). All analyses were conducted using the *mgcv* package in R (Wood 2006, R Development Core Team 2007), in which the underlying representation and estimation of the models is based on a penalized regression spline approach, with automatic smoothness selection. The significance of predictor variables was determined using Wald tests (Wood 2006). For each regression analysis, we first built a full model with all predictor variables and then, one by one, removed variables that did not significantly contribute to the model ($P > 0.05$; variable with highest P -value removed first) to derive a final model containing only significant variables. Removal of non-significant predictor variables had little or no effect on the significance of remaining predictor variables, indicating minimal variable collinearity. A Gaussian error model was used for continuous dependent variables (Bd infection intensity, frog population size) and a binomial error model

was used for the dichotomous dependent variable (population persistence: frogs detected versus not detected). Nonnative trout presence/absence was not included as a predictor variable in any of the analyses because trout were found at only seven of the 268 sites.

To test for an effect of elevation on Bd infection intensity, we used Bd infection intensity on an individual swab as the dependent variable and elevation, *R. sierrae* life stage (adult, juvenile, tadpole), day of the year, and water body depth as predictor variables. We were primarily interested in the effect of elevation but included covariates to reduce the chances of confounding effects caused by not including other variables with important effects on infection intensity. Infection intensity could differ between *R. sierrae* life stages because of differences in the amount of keratin (the substrate attacked by Bd) and in innate immune responses in frogs versus tadpoles (e.g., relative lack of antimicrobial skin peptides in tadpoles compared to adults; Wabnitz et al. 1998). We included day of the year (Day 1 = January 1) because of known effects of season and temperature on Bd growth rate (Berger et al. 2004, Kriger and Hero 2007). Maximum water depth was included because of its possible effects on Bd zoospore density.

In the analysis of the effect of elevation on *R. sierrae* population size, we used as the dependent variable the number of tadpoles counted during the resurvey. Only those sites in which *R. sierrae* were detected during the resurvey were included in the analysis. We used the number of tadpoles instead of the number of post-metamorphic frogs because tadpoles are unable to move between sites but adults can move widely over the course of the active season (Pope and Matthews 2001). This movement by adults could obscure an effect of habitat (e.g., site elevation) on population size. The predictor variables in this model were elevation, maximum water depth, and water body perimeter. Maximum water depth was included because *R. sierrae* are known to breed preferentially in water bodies deeper than 4 m (Knapp et al. 2003, Knapp 2005). This preference is likely a consequence of survivorship in the multi-year *R. sierrae* tadpole stage being higher in deeper water bodies due to a reduced probability of complete

water body freezing or drying. Water body perimeter was included to account for the possible effect of habitat size on population size.

To determine whether elevation influenced the probability of *R. sierrae* persistence between the original and repeat surveys, we used as the dependent variable the population status of *R. sierrae* during the resurvey (i.e., detection or non-detection of any *R. sierrae* life stage). Predictor variables included elevation, the number of tadpoles counted during the original survey, lake depth, and the number of years between the original and repeat survey. The population size during the original survey was included as a predictor variable because of the positive effect of population size on the probability of population persistence in many taxa (Soulé 1987). The survey interval was included because a longer survey interval might be associated with a lower probability of persistence. For our study sites, the survey interval ranged from 4 to 7 years (average = 5.7).

The distribution of continuous predictor and dependent variables were checked for non-normality and when non-normal, were log-transformed (when null values were present, we added 0.1 to all values). Transformed variables included Bd infection intensity, number of tadpoles counted during the original survey, number of tadpoles counted during the resurvey, and water depth. Following analysis, the fit of the Gaussian regression models was checked for non-normality of errors and non-constancy of variance. Neither problem was associated with the two models.

The relationships between the significant predictor variables and each dependent variable are shown graphically in separate plots. For the two models with continuous dependent variables, the plotted smooth terms are based on partial residuals. For the model with a binomial dependent variable, the y-axis represents the log-odds that *R. sierrae* was detected during the resurvey. In all plots, the y-axis is standardized to have an average value of zero.

Frog translocation experiment. Survivorship of *R. sierra* adults was estimated at the source and translocation sites using frog capture-recapture histories in program MARK to parameterize population

models (White and Burnham 1999). Recapture histories for individual frogs described whether or not a frog was captured during each of the survey occasions in 2006, 2007, and 2008. For each lake-specific capture-recapture data set, only those years in which at least one frog was recaptured were included. For each site we developed a set of candidate models that included models in which (1) both apparent survival (Φ) and probability of capture (p) were time-dependent, (2) Φ was time-dependent but p was not, (3) Φ was not time-dependent but p was, and (4) neither Φ nor p were time dependent. The much larger population of marked frogs at Conness Pond allowed us to also evaluate the effect of frog gender (g) and time on Φ . The best model was selected from the candidate set using AIC. To test for over-dispersion in the most parsimonious model we used the median \hat{c} procedure (White 2002). For this procedure, 100 simulations were performed at each of 12 \hat{c} values (upper and lower bounds plus 10 intermediate points).

To determine whether Bd infection intensity changed after translocation, we compared infection intensities on swabs collected from frogs the day prior to translocation (McGee Lake) or the day of translocation (Tioga Pass Pond, Skelton Lake) with infection intensities on all swabs collected following translocation (2006-2008). To provide a baseline for these comparisons we also compared infection intensities at the Conness Pond source population before and after frogs were collected for the translocations. The “before” swabs were those collected in 2006 as part of or prior to the translocations that concluded on August 8. The “after” swabs were those collected in 2006 after August 8 and in 2007 and 2008. Infection intensity data were not normally distributed and standard transformations were ineffective. Therefore, differences in infection intensities were analyzed using non-parametric Wilcoxon rank-sum tests.

Results

Frog population resurvey

Water temperature. Average maximum daily water temperature was negatively correlated with elevation and showed the same 0.6° C per 100 m lapse rate as shown by air temperature ($n = 9$, $F_{1,8} = 16.56$, $P = 0.004$, adjusted $R^2 = 0.63$; temperature = $38.1 - 0.006(\text{elevation})$). Therefore, elevation is an accurate proxy for littoral zone water temperatures in the study lakes.

Bd infection intensity. We were able to collect skin swabs from *R. sierrae* at 117 of the 166 sites at which *R. sierrae* were detected during the resurvey. At these sites, 1-42 *R. sierrae* were swabbed per site (average = 6). No swabs were collected at the remaining 49 sites due to an inability to capture any frogs or tadpoles. A total of 647 swabs were collected, 316 of which were from adults, 78 from juveniles, and 253 from tadpoles. For the 117 sites at which at least one swab was collected, 20 sites showed no evidence of Bd (ZE = 0), seven had maximum ZE values greater than zero and less than one (range = 0.02-0.81), and 90 had maximum ZE values greater than one (range = 2-103159). Therefore, at least 77% of these 117 sites were Bd-positive.

Results from regression modeling indicated that *R. sierrae* life stage, site elevation, and day of the year all had significant effects on Bd infection intensity (Table 3a). In the full model that also included water body depth, water depth did not approach statistical significance ($P = 0.19$). The plot of the estimated effect of each life stage showed that infection intensities were lower on adults than on juveniles and tadpoles and that loads on juveniles and tadpoles were similar (Figure 4a). As predicted, infection intensity decreased with elevation (Figure 4b). This relationship was significantly non-linear with infection intensities being highest at the lowest elevations, lower and relatively constant across a wide range of intermediate elevations, and lowest for the highest elevation populations. Infection intensities were generally highest in early summer and fall and lowest during mid-summer (Figure 4c). In

summary, after accounting for significant covariates, Bd infection intensity was inversely related to elevation.

Frog population size. At the 166 sites at which *R. sierrae* was detected during both the original survey and the resurvey there was no change in the number of adults ($\bar{X}_{\text{original}} = 5.5$, $\bar{X}_{\text{repeat}} = 6.5$; Wilcoxon signed-rank test, $P = 0.76$) or in the number of tadpoles ($\bar{X}_{\text{original}} = 116.2$, $\bar{X}_{\text{repeat}} = 151.0$; Wilcoxon signed-rank test, $P = 0.22$) but the number of juveniles declined significantly ($\bar{X}_{\text{original}} = 6.5$, $\bar{X}_{\text{repeat}} = 2.3$; Wilcoxon signed-rank test, $P < 0.0001$). The results from the regression modeling indicate that elevation and water body depth were significant predictors of *R. sierrae* population size, as measured by the number of tadpoles counted during the resurvey (Table 3b). In the full model that also included water body perimeter, perimeter did not approach statistical significance ($P = 0.32$). As predicted, population size generally increased with elevation (Figure 5a) and sites at which more than 200 tadpoles were counted were at significantly higher elevations than those at which 200 or fewer tadpoles were counted ($\bar{X}_{>200} = 3004$ m, $\bar{X}_{\leq 200} = 2673$ m; Wilcoxon rank-sum test, $P < 0.0001$; Figure 6). Population size also increased with water depth (Figure 5b), with the number of tadpoles increasing from 0 to 3 m depth ($\log[\text{water depth}] = -1$ to 0.5) and remaining high and relatively constant for water bodies deeper than 3 m ($\log[\text{water depth}] > 0.5$). Using the total number of post-metamorphic frogs as a measure of population size produced similar results. Therefore, after accounting for the effect of water depth, *R. sierrae* population size was directly related to elevation.

Frog population persistence. During the resurvey, *R. sierrae* were detected at 166 of the 268 sites at which they were detected during the original survey (Figure 7). Therefore, *R. sierrae* were not detected at 38% of the sites at which they were detected 4-7 years previously. Results from the regression modeling indicated that the number of tadpoles counted during the original survey and water body depth were both significant predictors of the probability of *R. sierrae* population persistence (Table 3c).

The probability of frog population persistence increased linearly with both the number of tadpoles counted during the original survey and water depth (both \log_{10} -transformed; Figure 8). Using the number of post-metamorphic *R. sierrae* counted during the original survey instead of the number of tadpoles as a predictor variable produced similar results. In the full model that also included survey interval and elevation, neither of these predictor variables approached statistical significance ($P = 0.24$ and $P = 0.13$, respectively). Rerunning the analysis without the predictor variable, population size during the original survey, had little effect on the significance of elevation. Therefore, the hypothesized positive relationship between elevation and population persistence was not supported.

Frog translocation experiment

Results from visual encounter surveys of the Conness Pond source population showed strong seasonal fluctuations in frog and tadpole numbers, with the highest annual counts always occurring in mid-summer or fall (Figure 9a). Annual maximum frog counts suggested a decrease in the population size of approximately 50% between 2006 and 2008 (977 frogs in 2006, 490 frogs in 2008), likely driven in part by the collection of frogs in 2006 for the translocation experiment and in 2008 for a separate translocation experiment (70 adult *R. sierrae*; Knapp, unpublished data). In contrast to frog numbers, tadpole numbers increased by nearly 100% during this same time period (Figure 9a; 1650 tadpoles in 2006, 3176 tadpoles in 2008).

Frog population counts at the translocation sites suggested highly disparate outcomes. At McGee Lake, the lowest elevation translocation site, only four of the 43 frogs that survived the translocation were recaptured in 2006 and no *R. sierrae* were ever seen during surveys in 2007 and 2008 (Figure 9b). No evidence of successful reproduction (i.e., egg masses, tadpoles) was ever observed. The Tioga Pass Pond frog population declined more slowly than did the McGee Lake population but declined to apparent extinction by 2008 (Figure 9c). At Tioga Pass Pond, 33 of the 40 released frogs were

recaptured in 2006 but only seven frogs were recaptured in 2007 and none were seen during surveys in 2008. Two dead frogs were found during the first survey in 2007 but were too decomposed to swab for Bd. There was no evidence of successful reproduction during the two years following the translocation. At Skelton Lake, the highest elevation translocation site, the frog population also declined following translocation but did so at a noticeably slower rate than did the McGee Lake and Tioga Pass Pond populations (Figure 9d). Thirty-four of the 39 released frogs were captured in 2006, 21 in 2007, and 11 in 2008. Successful reproduction occurred in both 2007 and 2008; first-year tadpoles were observed during the last survey in 2007 and both first and second-year tadpoles were observed in 2008 (Figure 9d). Second-year tadpoles began metamorphosing in late September: 22 were observed during the September 7 survey (Figure 9d). In summary, population size estimates based on visual surveys suggested that the survival of translocated frogs was positively correlated with site elevation.

Analysis of capture-recapture data was possible for all sites except McGee Lake (capture-recapture data were too sparse for the McGee Lake population). For the other three sites, results from the analysis of capture-recapture data indicated that the best model for each of the three data sets was one which included time dependence in the probability of capture but not in apparent survival ($\{\Phi(\cdot)p(t)\}$; Table 4). Therefore, capture probability varied through time but apparent survival did not. The median \hat{c} procedure indicated that median \hat{c} values were very close to one in all cases ($\bar{X} \pm 1$ SE; Conness Pond: 1.13 ± 0.01 ; Tioga Pass Pond: 0.94 ± 0.02 ; Skelton Lake: 0.98 ± 0.01). Therefore, no adjustments to AIC were made. In the Conness Pond analysis, the second-best model (apparent survival dependent on gender, time dependence in capture probability ($\{\Phi(g)p(t)\}$; Table 4a) had only 2.6 times less support than the best model. However, estimates of apparent survival between males and females were virtually identical and suggested that gender differences in survival were biologically unimportant. In the two translocated populations $\{\Phi(\cdot)p(t)\}$ was by far the best model, with the remaining models having essentially no support (Table 4b, c).

Apparent survival was relatively high in both the Conness Pond and Skelton Lake populations compared to that in the Tioga Pass Pond and McGee Lake populations. Annual survival increased with elevation (Figure 10a) and generally decreased with average maximum daily water temperature (Figure 10b). These differences in survival were consistent with changes in Bd infection intensity observed following translocation. In the Conness Pond source population, despite large sample sizes there was no significant difference in infection intensities before and after frogs were collected for the translocations (Figure 11a). In all three translocated populations, infection intensity increased significantly following translocation (Figure 11b-d) and the magnitude of change decreased with increasing elevation. Finally, frog survival was inversely correlated with the proportional change in infection intensity (Figure 12).

Discussion

Support for hypotheses. The curve describing the temperature-Bd growth rate relationship is hump-shaped with a peak at approximately 20° C (Piotrowski et al. 2004, Woodhams et al. 2008) and in combination with information on lake temperature regimes, led to several predictions about the effect of elevation and temperature on the interaction between Bd and *R. sierrae* in Yosemite. We predicted that Bd infection intensity would be reduced at high versus low elevations and that these differences would lead to increased frog survivorship, population size, and population persistence at high elevations compared to low elevations. Results from our *R. sierrae* resurvey study and translocation experiment provided support for most of these predictions.

Bd was detected at 77% of the *R. sierrae* populations in Yosemite. This is likely an underestimate of the true Bd distribution for two reasons. First, we used a cut-off of 1 ZE to categorize populations as Bd-positive or Bd-negative. However, seven of the *R. sierrae* populations had maximum ZE values greater than zero but less than one, indicating a very low infection intensity but still demonstrating the presence of Bd. Second, *R. sierrae* populations in Yosemite are generally small and as a consequence we

were able to swab only a relatively small number of *R. sierrae* from most sites. Given that Bd prevalence is typically less than 100%, this likely resulted in at least some populations being characterized as Bd-negative when Bd was actually present. Collectively, these results suggest that Bd is likely to be essentially ubiquitous in Yosemite's *R. sierrae* populations.

The prediction that Bd infection intensity should be negatively correlated with elevation was supported by regression results that indicated that Bd infection intensity on individual *R. sierrae* decreased significantly from low to high elevation. This non-linear relationship was driven primarily by infection intensities at the lowest and highest elevation sites, with infection intensities at the intermediate sites being relatively constant over a wide range of elevations. We suggest that the negative correlation between infection intensity and elevation is likely a result of slow Bd growth rates in the high elevation sites due to cold water temperatures. Temperatures in these lakes are 0-4° C from approximately November to July. Even during mid-summer temperatures rarely exceed 17° C and then only in shallow water and only for 1-2 hours per day. The relatively strong effect of elevation on infection intensity we observed contrasts with the lack of any such pattern in eastern Australia (Kriger and Hero 2008). This difference may be a consequence of the narrower altitudinal range encompassed by the Australian study (700 m) compared to our study (1350 m). *R. sierrae* life stage and day of the year were also significantly associated with infection intensity. Similar effects of life stage have been reported in previous studies, including a negative correlation between body size and both Bd prevalence and infection intensity (Kriger et al. 2007) and higher Bd prevalence in tadpoles compared to adults (Woodhams and Alford 2005). The mechanisms underlying this effect of life stage remain unknown and are deserving of additional study. The results of the translocation experiment provide additional support for the hypothesis of a negative correlation between infection intensity and elevation. Across the three translocated populations, the magnitude of increase in infection intensity was inversely correlated with the elevation of the translocation site. Although this result is based on a sample size of only three

populations and therefore its generality remains uncertain, this result is consistent with the negative correlation between infection intensity and elevation obtained from the *R. sierrae* resurvey study.

We hypothesized that the infection intensity-elevation relationship would result in a positive correlation between elevation and both *R. sierrae* survival and population size. Results from the translocation experiment and resurvey study were consistent with these predictions. In the four frog populations used in the translocation experiment, frog survival rate was positively correlated with elevation. Similarly, in the *R. sierrae* resurvey study, regression results indicated that population size increased with elevation. In the translocation experiment, evidence suggests that differences in survival between populations were likely driven at least in part by infection intensity because frog survival was negatively correlated with the magnitude of increase in infection intensity following translocation. In summary, frog populations at the higher elevations showed the smallest increases in infection intensity and had higher frog survival rates compared to lower elevation populations that had larger increases in infection intensity and lower frog survival. Finally, in the regression analysis the positive effect of the covariate, maximum water depth, on population size was expected given that deeper habitats are essential for successful overwintering of the multi-year *R. sierrae* tadpole stage (Knapp et al. 2003, Knapp 2005).

The hypothesis that higher frog survival rates and larger frog populations sizes at high elevations would result in a higher likelihood of frog population persistence at high versus low elevations was not supported by results from the resurvey study. In the regression analysis, elevation did not have a significant effect on whether *R. sierrae* were detected during the resurvey. However, population size during the original survey did have a significant positive effect on population persistence, as did water depth. We interpret this to mean that population size does influence population persistence, but this effect is independent of elevation. Extinction of populations is often an unpredictable process driven by many different factors, including Allee effects, demographic stochasticity, and environmental

stochasticity (Lande 1993, Courchamp et al. 1999). Obtaining additional insights into the effect, if any, of elevation and related factors on the probability of frog population persistence will likely require additional resurvey of the study populations after a longer time interval since the original survey than the 4-7 year interval used in the current study.

Potential confounding factors in the translocation experiment. Results from the resurvey study and translocation experiment were largely consistent with the predictions from our hypotheses related to the cascading effect of elevation on Bd infection intensity, frog survival, and population size. However, there are two caveats related to the translocation experiment that could have influenced the outcome of this study. Results on Bd infection intensity provided a compelling explanation for the between-population differences in frog survival that we observed, but two other factors may also have influenced survivorship. First, surveys conducted at the three translocation sites before the experiment was initiated indicated that the mountain garter snake, *Thamnophis elegans elegans*, was absent from Skelton Lake and Tioga Pass Pond, but present in low numbers at McGee Lake (none seen in 2001, two seen in early 2006). *T. e. elegans* (hereafter, “snake”) is a major predator on mountain yellow-legged frogs and its distribution is strongly affected by the presence of this prey (Matthews et al. 2002, Knapp 2005). During surveys conducted in 2006 following the translocations and in 2007 and 2008, snakes were never observed at Tioga Pass Pond and at Skelton Lake only a single snake was observed on two occasions (both in 2008). In contrast, in 2006 following the translocation we counted on average 12 snakes per survey at McGee Lake. Counts made during the 2007 and 2008 surveys indicated that snake numbers had returned to more typical levels, with an average of two snakes per survey. The high density of snakes observed at McGee Lake in 2006 following the translocation could have resulted in considerable predation on released frogs, perhaps sufficient to contribute to the rapid extinction of this frog population. Although this possible impact may have been completely independent of frog infection

intensities it is also possible that snake predation interacts with Bd infection. *R. sierrae* adults with high infection intensities (i.e., above 1000 ZE) are often noticeably lethargic (Knapp, unpublished data) and three of the swabs collected at McGee Lake had ZE values above 1000. Therefore, frogs at this site may have been more vulnerable to snake predation than if they were uninfected. Under this scenario, the snake-Bd interaction could contribute to the pattern seen in Yosemite of smaller frog populations and lower frog survival at low versus high elevation sites.

Frog survival at Tioga Pass Pond could have been affected by another unforeseen factor. Gill nets set in this pond in 2001 and 2006 (prior to the translocation) indicated the absence of nonnative trout. In addition, no trout were seen during visual surveys conducted throughout 2006. However, during a survey immediately following ice-out in 2007 (May 28) four brook trout were observed in the ephemeral outlet stream just below the pond. These fish likely took advantage of a brief runoff pulse to migrate approximately 2 km up an ephemeral stream from the perennial Dana Fork of the Tuolumne River. Three of the four fish were removed and the outlet stream dried a week later. However, during a visual survey in August 2007 a trout was observed in Tioga Pass Pond. Gill nets set for 10 days in September 2007 captured two brook trout and nets set for 15 days in June 2008 captured no fish. Therefore, two predatory brook trout co-occurred with *R. sierrae* adults in Tioga Pass Pond for more than three months and could have impacted frog survival. Two factors suggest that the pattern of frog population decline at this site was not strongly affected by the presence of trout. First, results from visual surveys at this site indicated that the rate of decline in 2006 following translocation was steep, despite the absence of trout at this site until 2007. As a result of the mortality that occurred in 2006, few frogs remained at Tioga Pass Pond by the time that fish invaded. Second, the best model from the analysis of capture-recapture data was one without time dependence in apparent survival. Therefore, rates of frog survival were not distinguishable between 2006 and 2007. Possible reasons for the apparent lack of a fish effect on frog survival was the relatively large size of the frogs in 2007 (average

SVL = 54 mm) and the abundance of invertebrate prey (T. Smith, unpublished data) due to the fishless condition of the pond in years prior to 2007.

Conclusions and management implications. Previous research has clearly shown that the growth rate of Bd is strongly temperature dependent, and this relationship is described by a hump-shaped curve with a maximum growth rate at approximately 20° C and little to no growth below 4° C and above 30° C (Piotrowski et al. 2004, Woodhams et al. 2007). Nearly all field studies published to date on the impact of Bd on amphibians across temperature gradients were conducted in warm or hot climates (primarily tropical and subtropical regions) and the patterns reported by these studies support predictions from the Bd growth rate-temperature relationship. In general, this body of research shows that the impact of Bd on amphibians is less severe in hotter climates, such as at low elevations, high latitudes, and during the summer (Woodhams and Alford 2005, Kriger and Hero 2007, Kriger et al. 2007). These patterns are likely driven largely by the fact that amphibians at low elevations or low latitudes are routinely exposed to temperatures above 30° C while those at high elevations or high latitudes experience lower temperatures that are more conducive of high Bd growth rates.

The hump-shaped relationship between temperature and Bd growth rate suggests that the effect of temperature on Bd-amphibian interactions should change just as dramatically across the low end of the temperature scale (e.g., 0-20° C) as across the high end (20-30° C). However, there have been virtually no studies conducted on the effect of temperature on the Bd-amphibian interaction in cool or cold temperate regions (with the exceptions being Muths et al. 2008 and the current study). Muths et al. (2008) reported that in the Rocky Mountains Bd was detected less often in amphibian populations at higher compared to lower elevations, and they suggested that the lower temperatures at high elevations may limit Bd growth rates. Our results support this interpretation. The current study provides the most detailed analysis to date of how temperature influences the Bd-amphibian interaction in

temperate montane regions, and our results are largely consistent with expectations based on the shape of the temperature-Bd growth curve. As such, these findings likely have considerable relevance to amphibian populations throughout the temperate zone, a region where Bd is widespread (Garner et al. 2005, Ouellet et al. 2005, Longcore et al. 2007, Pearl et al. 2007). The combination of our results with those from previous studies allows a more comprehensive framework for understanding how temperature influences the impact of Bd on amphibians.

In the Sierra Nevada, *R. sierrae* has disappeared from more than 90% of its historic range, including in Yosemite (Vredenburg et al. 2007). Although introduced trout are a key cause of this decline (Knapp 2005) our findings indicate that Bd is also a critically important limiting factor for the remaining populations. The results of the current study provide insights into the contribution of elevation and temperature in generating the spatial patterns of Bd infection intensity, frog survival, and frog population size in the Park. These insights suggest that efforts designed to restore *R. sierrae* populations, such as fish removal (Vredenburg 2004, Knapp et al. 2007) and frog translocation, might have an increased chance of success when conducted at higher elevations compared to lower elevations. While possibly the case, it is clear that temperature is only one of several factors that can alter the impact of Bd on amphibians. Other factors may include inter-strain differences in Bd virulence (Retallick and Verma 2007), differences between frog populations in susceptibility to Bd, and environmental conditions in addition to temperature such as precipitation (Puschendorf et al. 2009). The low-elevation populations of *R. sierrae* in Yosemite are characterized by small population sizes and, if the results from the translocation experiment can be generalized to the entire Park, relatively low frog survival. Over the long-term these characteristics could make these populations vulnerable to extinction. It is therefore of utmost importance that conservation measures be implemented across Yosemite, including at low elevations. In particular, removal of nonnative trout may increase frog population sizes

(Knapp et al. 2007) and improve the chances of frog populations persisting despite ongoing chytridiomycosis.

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Tables

Table 1. Habitat characteristics of the water bodies used in the *Rana sierrae* translocation experiment.

Lake name	Lake category	Location (lat.-long.)	Elevation (m)	Maximum depth (m)	Surface area (ha)	Water temperature ^a
Conness Pond	Source	37°58'14" N, 119°20'42" W	3176	3.0	0.4	18.1
McGee Lake	Translocation	37°54'08" N, 119°25'49" W	2471	4.0	2.2	21.8
Tioga Pass Pond	Translocation	37°54'29" N, 119°15'28" W	3018	4.0	0.8	20.4
Skelton Lake	Translocation	37°55'55" N, 119°18'18" W	3200	11.0	4.7	15.9

^a Average maximum daily water temperature (°C) measured 20 cm below the water surface in 1 m water depth. Measurements made between July 17 and September 27, 2006.

Table 2. Number of frog surveys conducted at the source and translocation lakes after frogs were moved from the source lake.

Lake name	2006	2007	2008
Conness Pond	5	3	5
McGee Lake	3	6	1
Tioga Pass Pond	5	6	2
Skelton Lake	5	6	5

Table 3. Description of the reduced generalized additive models showing the effect of elevation on (a) Bd infection intensity, (b) frog population size, and (c) frog population persistence (*R. sierrae* detected versus not detected during resurvey).

(a) Starting model: $\log(\text{zoospore equivalents}) \sim \text{life stage} + s(\text{elevation}) + s(\text{day of year}) + s(\log[\text{water depth}])$

Variable name	df	F	P
Life stage	3.0	97.3	$<1 \times 10^{-16}$
Elevation	5.4	7.6	2.5×10^{-7}
Day of year	7.2	6.0	7.0×10^{-7}

(b) Starting model: $\log(\# \text{ of tadpoles during resurvey}) \sim s(\text{elevation}) + s(\log[\text{water depth}]) + s(\log[\text{perimeter}])$

Variable name	df	F	P
Elevation	7.1	3.7	0.0009
Water depth	4.2	4.8	0.0010

(c) Starting model: $\text{population status} \sim \text{survey interval} + s(\text{elevation}) + s(\log[\# \text{ of tadpoles during original survey}]) + s(\log[\text{water depth}])$

Variable name	df	Chi-Sq	P
# of tadpoles	1.1	16.4	6.4×10^{-5}
Water depth	1.5	10.0	0.004

Table 4. Comparison of models for the three frog populations for which sufficient capture-recapture data were available.

(a) Conness Pond

Model	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	No. of parameters	Deviance
{ $\Phi(\cdot)p(t)$ }	2748.85	0.00	0.649	1.000	18	772.49
{ $\Phi(g)p(t)$ }	2750.73	1.88	0.253	0.390	19	772.27
{ $\Phi(t)p(t)$ }	2752.65	3.80	0.097	0.149	33	744.16
{ $\Phi(g^*t)p(t)$ }	2775.69	26.84	0.000	0.000	51	726.86
{ $\Phi(\cdot)p(\cdot)$ }	2850.72	101.87	0.000	0.000	2	907.26
{ $\Phi(g)p(t)$ }	2852.48	103.64	0.000	0.000	3	901.01
{ $\Phi(t)p(\cdot)$ }	2862.08	113.24	0.000	0.000	18	885.73
{ $\Phi(g^*t)p(t)$ }	2887.29	138.45	0.000	0.000	35	874.42

(b) Tioga Pass Pond

Model	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	No. of parameters	Deviance
{ $\Phi(\cdot)p(t)$ }	293.39	0.00	0.998	1.000	13	49.83
{ $\Phi(\cdot)p(\cdot)$ }	305.56	12.17	0.002	0.002	2	88.11
{ $\Phi(t)p(t)$ }	314.36	20.97	0.000	0.000	21	47.19
{ $\Phi(t)p(\cdot)$ }	316.92	23.53	0.000	0.000	13	73.36

(c) Skelton Lake

Model	AIC_c	ΔAIC_c	AIC_c weight	Model likelihood	No. of parameters	Deviance
{ $\Phi(\cdot)p(t)$ }	562.23	0.00	1.000	1.000	17	253.40
{ $\Phi(\cdot)p(\cdot)$ }	588.87	26.64	0.000	0.000	2	313.96
{ $\Phi(t)p(t)$ }	595.99	33.76	0.000	0.000	31	248.88
{ $\Phi(t)p(\cdot)$ }	617.44	55.21	0.000	0.000	17	308.61

Figures

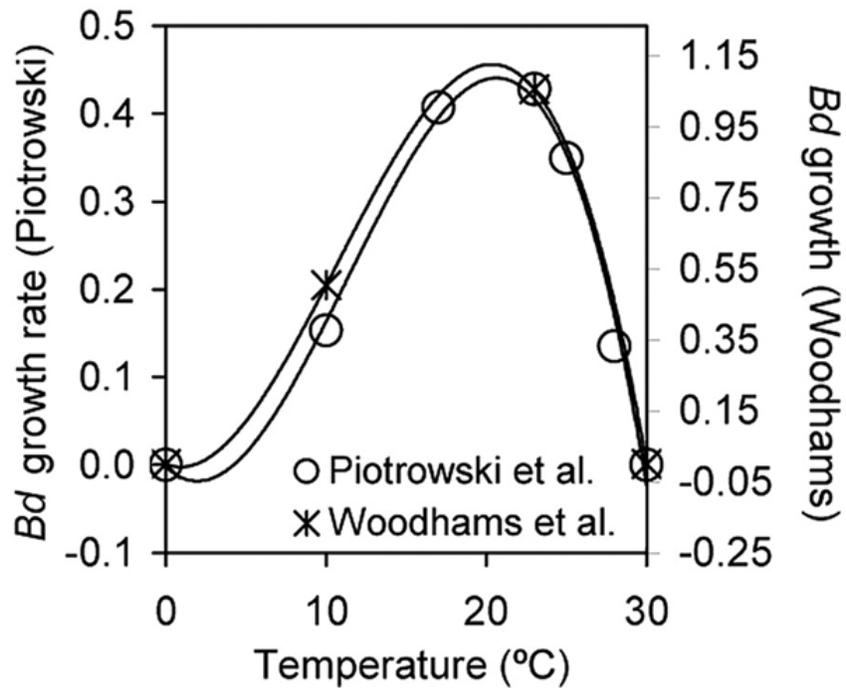


Figure 1. Relationship between daily growth rate of *Bd* in culture and temperature, based on the data of Piotrowski et al. (2004) and Woodhams et al. (2008). Figure is reprinted from Rohr et al. (2008).

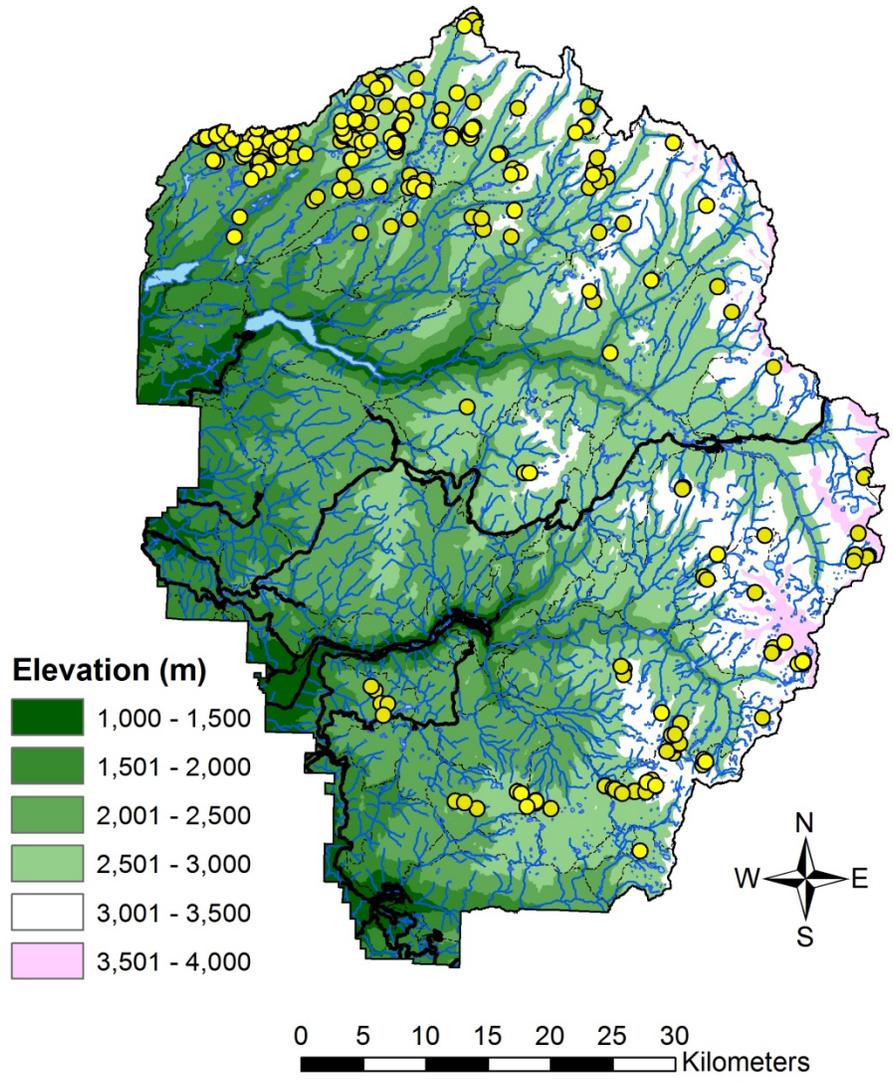


Figure 2. Map of Yosemite National Park showing *Rana sierrae* resurvey locations (yellow dots), elevation, streams and lakes (in blue), roads (solid black lines), and trails (dashed black lines).

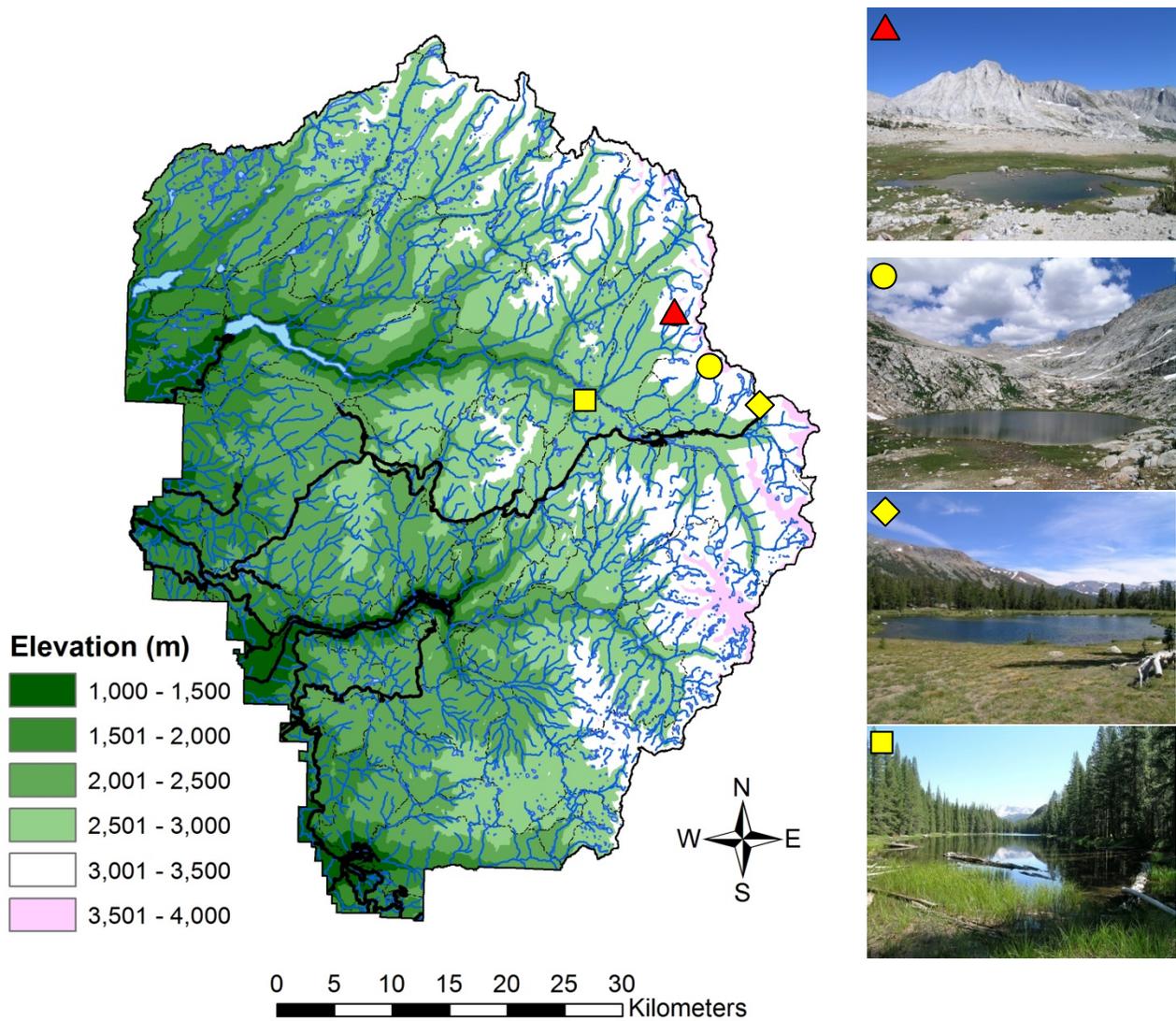


Figure 3. Map of Yosemite National Park showing the location of study sites used in the *Rana sierrae* translocation experiment, and photographs of each site. The Conness Pond source population is shown as a red triangle and three translocated populations are shown as yellow symbols (Skelton Lake: dot; Tioga Pass Pond: diamond; McGee Lake: square).

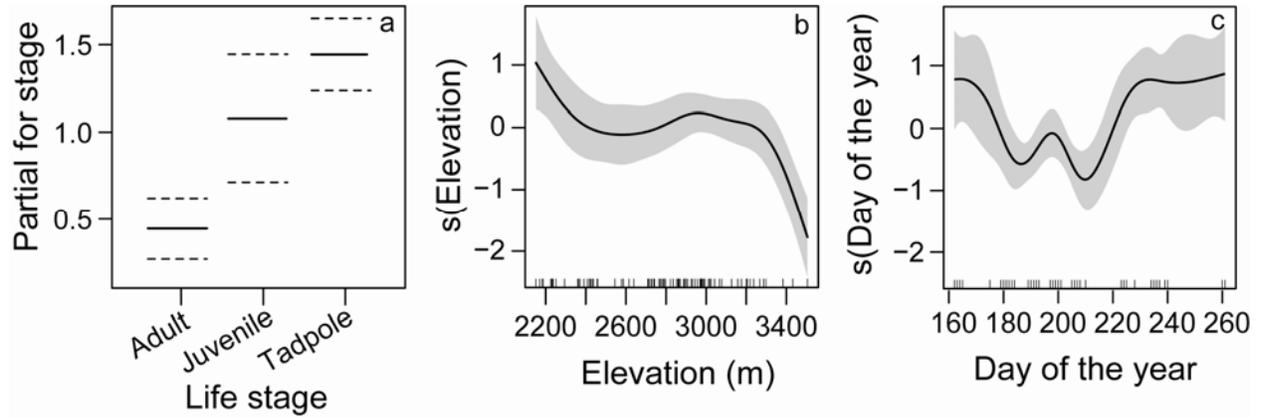


Figure 4. Plots showing the estimated effects (solid lines) on Bd infection intensity of (a) *Rana sierrae* life stage, (b) elevation, and (c) day of the year. Plots are arranged in order of the strength of their effect. Confidence intervals (95%) are shown as dashed lines for the parametric term (a) and gray shading for the smooth terms (b, c). Hatch marks above the x-axis in (b) and (c) indicate the observed values for each predictor variable.

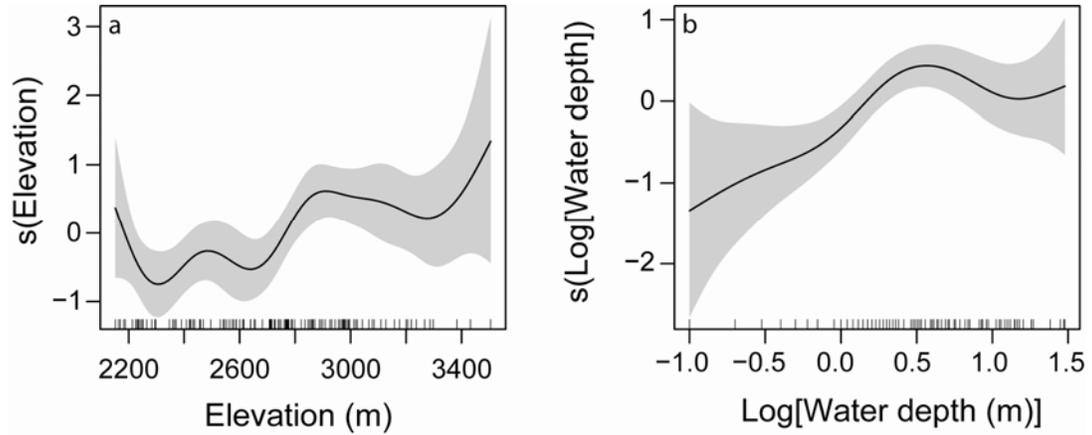


Figure 5. Plots showing the estimated effects (solid lines) on *Rana sierrae* population size of (a) elevation, and (b) maximum water depth. Plots are arranged in order of the strength of their effect. Confidence intervals (95%) are shown as gray shading. Hatch marks above the x-axes indicate the observed values for each predictor variable.

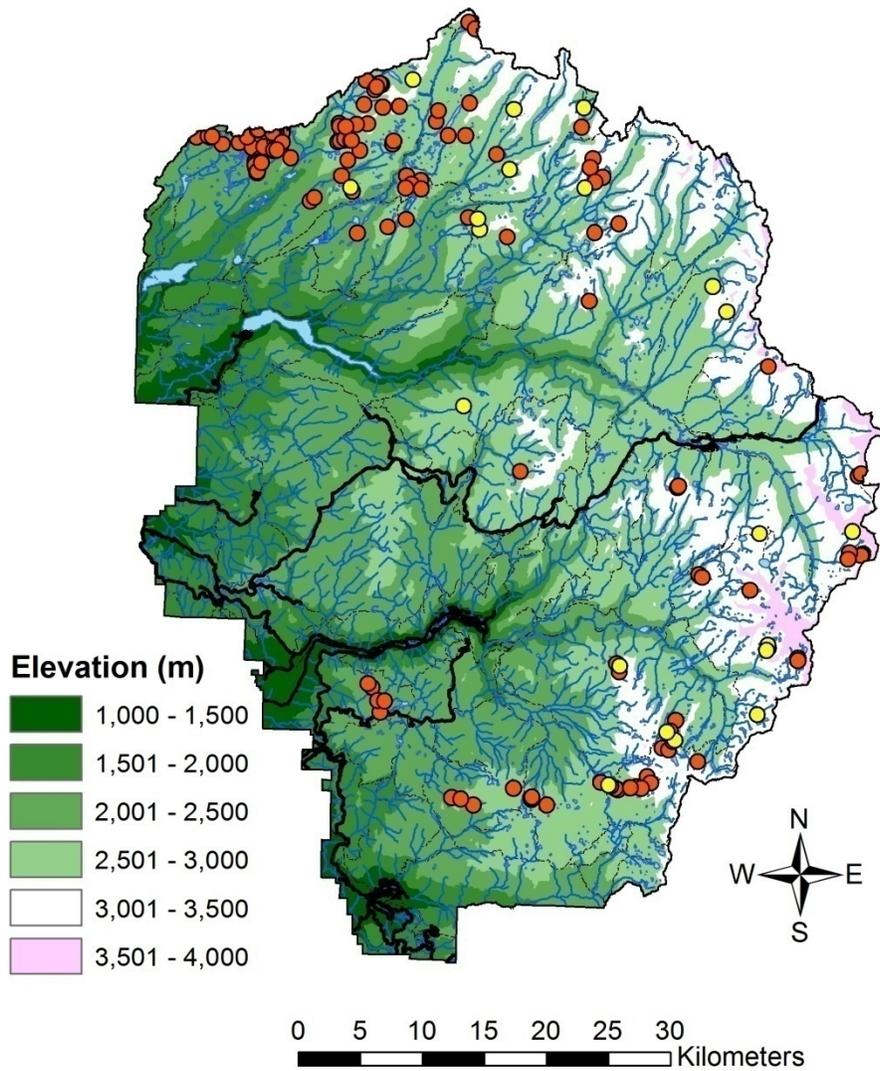


Figure 6. Map of Yosemite National Park showing *Rana sierrae* population sizes for populations that were extant during the resurvey. Yellow dots indicate populations at which >200 tadpoles were counted and red dots indicate populations at which ≤ 200 tadpoles were counted. The map also shows elevations, streams and lakes (in blue), roads (solid black lines), and trails (dashed black lines).

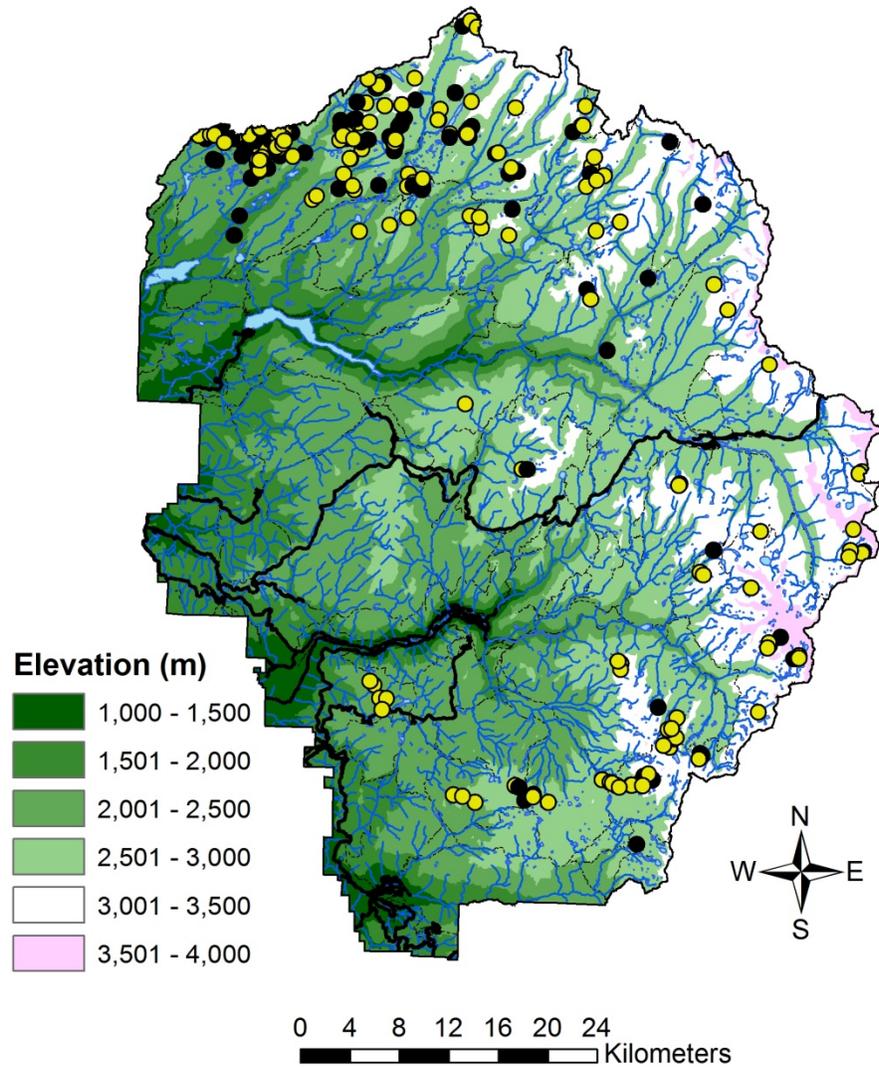


Figure 7. Map of Yosemite National Park showing the sites at which *Rana sierrae* were detected (yellow dots) or not detected (black dots) during the resurvey. The map also shows elevations, streams and lakes (in blue), roads (solid black lines), and trails (dashed black lines).

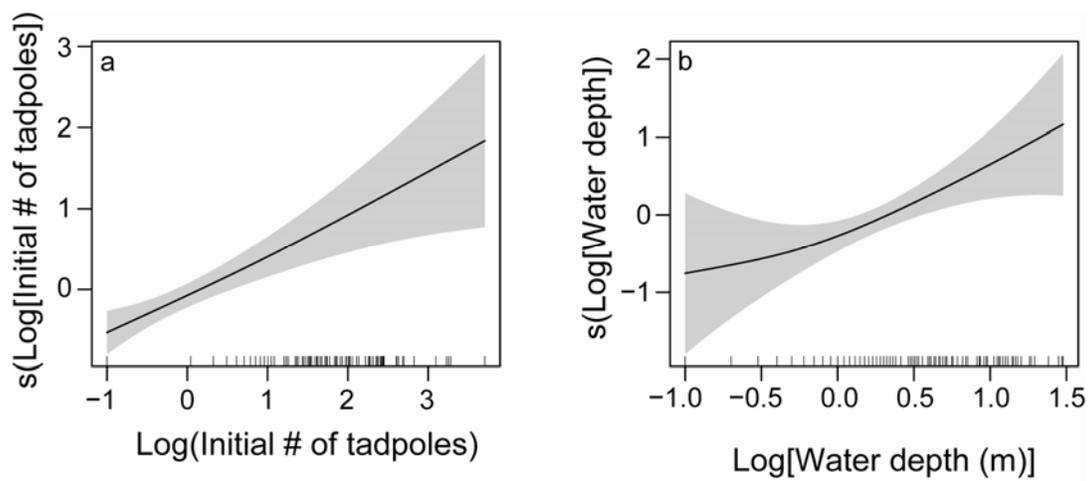


Figure 8. Plots showing the estimated effects (solid lines) on the probability of detecting *Rana sierrae* of (a) the number of tadpoles counted during the original survey, and (b) maximum water depth. Plots are arranged in order of the strength of their effect. Confidence intervals (95%) are shown as gray shading. Hatch marks above the x-axes indicate the observed values for each predictor variable.

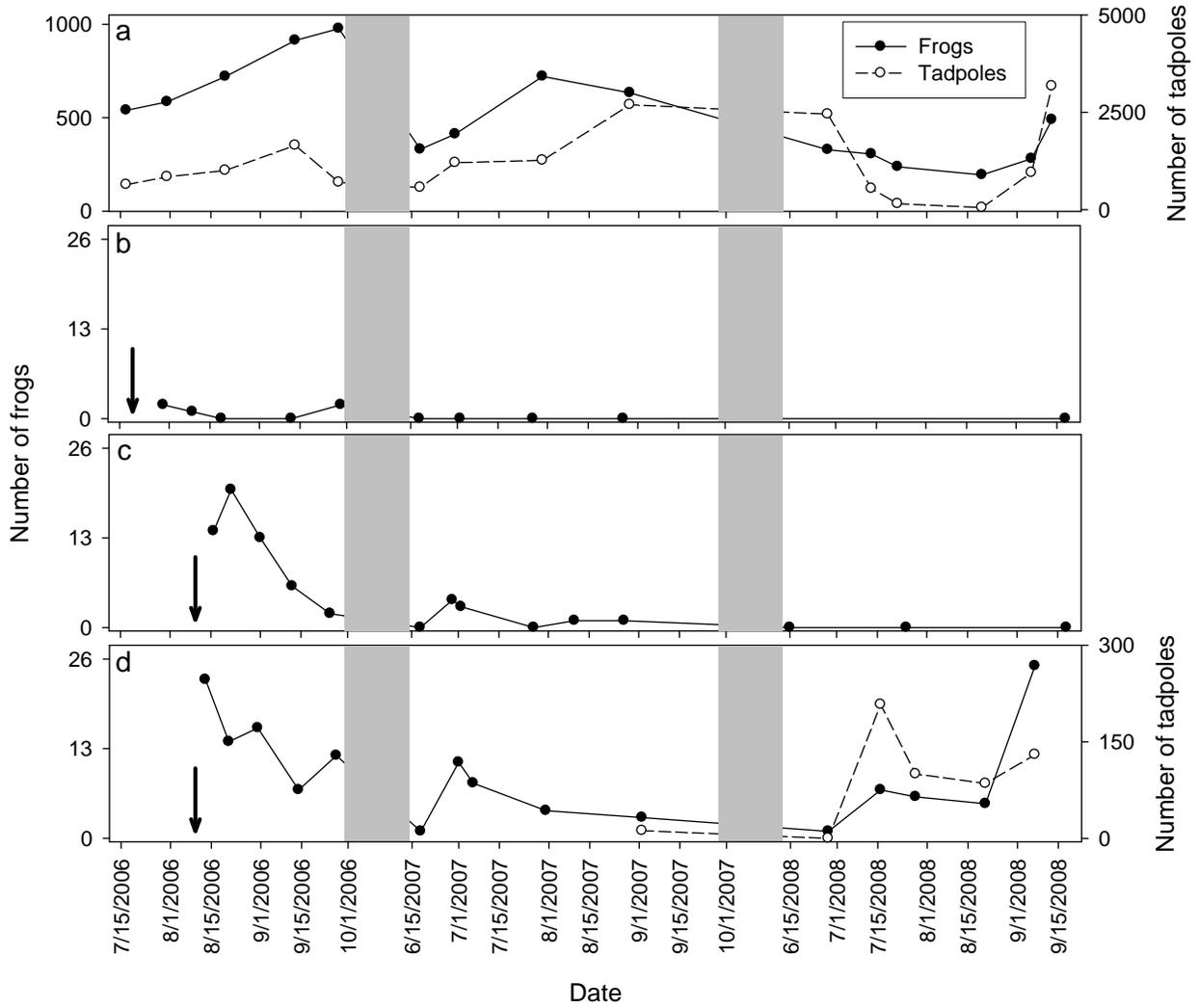


Figure 9. Counts of *Rana sierrae* frogs (adults + subadults) and tadpoles from 2006 through 2008 at the (a) Conness Pond source population and the three translocation sites: (b) McGee Lake, (c) Tioga Pass Pond, and (d) Skelton Lake. Gray bars indicate periods of winter ice-cover. Arrows in (b-d) show dates on which adult *R. sierrae* were moved to the site.

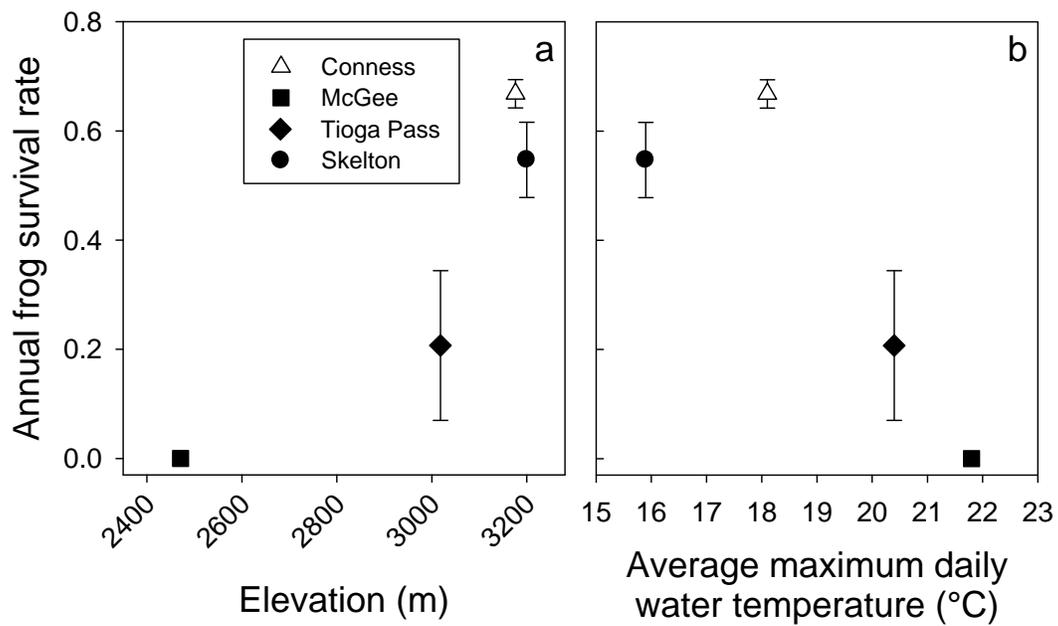


Figure 10. Annual survival ($\bar{X} \pm 1$ SE) of adult *Rana sierrae* in the source and translocated populations as a function of (a) site elevation and (b) average maximum daily water temperature.

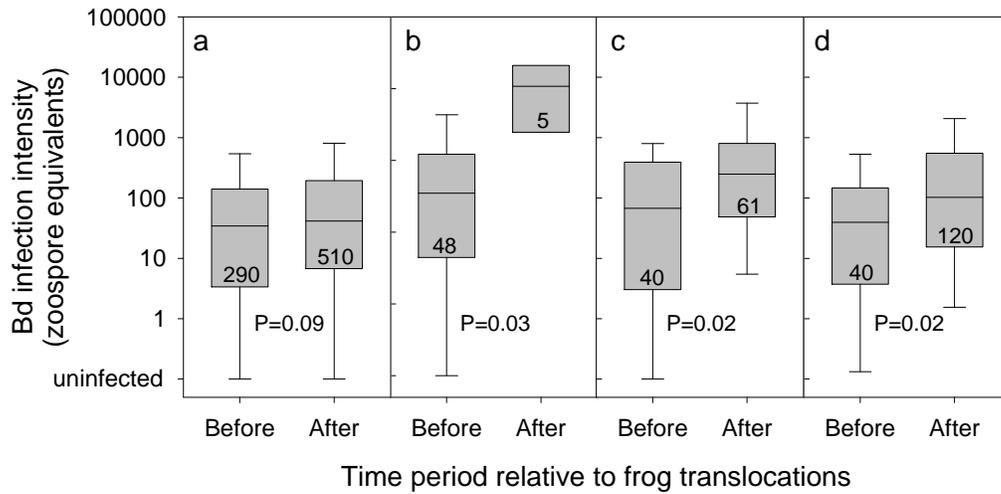


Figure 11. Box plots showing Bd infection intensities of adult *Rana sierrae* before and after translocation in (a) Conness Pond, (b) McGee Lake, (c) Tioga Pass Pond, and (d) Skelton Lake. The solid line within each box marks the median, the bottom and top of each box indicate the 25th and 75th percentiles, and the whiskers below and above each box indicate the 10th and 90th percentiles. Sample sizes are provided inside the base of each box and *P*-values are given below each pair of boxes. The y-axis is on a log scale and “uninfected” indicates a zoospore equivalent value of <1.

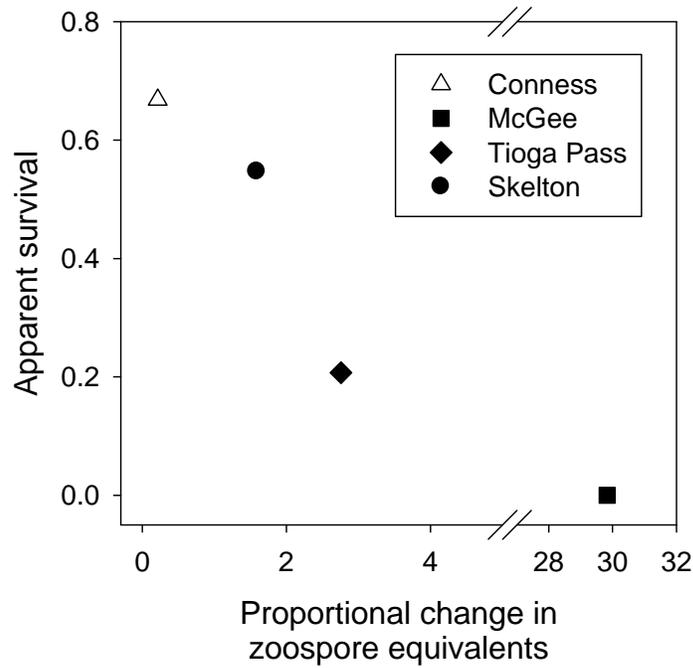


Figure 12. Apparent survival (Φ) in frog populations from the translocation experiment as a function of the change in infection intensity (zoospore equivalents) following translocation. Proportional change in zoospore equivalents is based on untransformed median values from swabs collected before and after frogs were moved ($[Z_{E_{after}} - Z_{E_{before}}] / Z_{E_{before}}$). Note that the x-axis is broken to accommodate the very high infection intensities of frogs in the McGee Lake population.