Dynamics of an emerging disease drive large-scale amphibian population extinctions

Vance T. Vredenburg[a,1], Roland A. Knapp[b], Tate S. Tunstall[c,d], and Cheryl J. Briggs[d]

[a]Department of Biology, San Francisco State University, San Francisco, CA 94132-1722; [b]Sierra Nevada Aquatic Research Laboratory, University of California, Mammoth Lakes, CA 93546; [c]Department of Integrative Biology, University of California, Berkeley, CA 94720-3140; and [d]Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA 93106-9610

Edited* by David B. Wake, University of California, Berkeley, CA, and approved March 25, 2010 (received for review December 6, 2009)

Epidemiological theory generally suggests that pathogens will not cause host extinctions because the pathogen should fade out when the host population is driven below some threshold density. An emerging infectious disease, chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) is directly linked to the recent extinction or serious decline of hundreds of amphibian species. Despite continued spread of this pathogen into uninfected areas, the dynamics of the host–pathogen interaction remain unknown. We use fine-scale spatiotemporal data to describe (*i*) the invasion and spread of Bd through three lake basins, each containing multiple populations of the mountain yellow-legged frog, and (*ii*) the accompanying host–pathogen dynamics. Despite intensive sampling, Bd was not detected on frogs in study basins until just before epidemics began. Following Bd arrival in a basin, the disease spread to neighboring populations at ≈700 m/yr in a wave-like pattern until all populations were infected. Within a population, infection prevalence rapidly reached 100% and infection intensity on individual frogs increased in parallel. Frog mass mortality began only when infection intensity reached a critical threshold and repeatedly led to extinction of populations. Our results indicate that the high growth rate and virulence of Bd allow the near-simultaneous infection and buildup of high infection intensities in all host individuals; subsequent host population crashes therefore occur before Bd is limited by density-dependent factors. Preventing infection intensities in host populations from reaching this threshold could provide an effective strategy to avoid the extinction of susceptible amphibian species in the wild.

amphibian declines | *Batrachochytrium dendrobatidis* | chytridiomycosis | emerging infectious disease | *Rana muscosa*

Earth's biodiversity is increasingly threatened with extinction. The majority of contemporary extinctions are typically attributed to anthropogenic changes such as habitat destruction, overexploitation, and species introductions. Disease is generally not considered a major driving force in extinctions, in part because simple epidemiological theory suggests that a pathogen will fade out when its host population is driven below some threshold density (1, 2). Class Amphibia provides one of the best-documented examples of contemporary biodiversity loss, with ≈43% of the more than 6,600 described species currently threatened with extinction (3). Remarkably, an emerging infectious disease, chytridiomycosis, is directly linked to the recent extinction or serious decline of hundreds of amphibian species (4). The effect of chytridiomycosis on amphibians has been described as the greatest loss of vertebrate biodiversity attributable to disease in recorded history (4), and although doubts about the importance of disease in driving global amphibian declines have been expressed (5), these have largely been overcome by weight of evidence (4, 6–8).

Chytridiomycosis is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), whose only known host is larval and adult amphibians. This pathogen was described in the late 1990s (6, 9) and is now known from six continents (4). The infective stage is a free-living flagellated zoospore that encysts in the skin of an amphibian and develops into a zoosporangium. Zoosporangia produce zoospores via asexual reproduction [it remains unclear whether sexual reproduction also occurs (10, 11)], and the zoospores are released into the environment through a discharge tube. Tadpoles are typically little affected by chytridiomycosis, but sublethal and lethal effects are known (12, 13). Effects of chytridiomycosis on frogs are highly variable, with frogs of some species dying from the disease within weeks and others experiencing few negative effects (4). Chytridiomycosis likely causes frog mortality by severely disrupting epidermal functions and causing osmotic imbalance (14, 15). However, it remains unknown how chytridiomycosis is able to cause the extinction of its amphibian hosts, an outcome that would require that Bd not be severely limited by density-dependent factors. The objective of our study was to describe frog–Bd dynamics by measuring both Bd prevalence in populations and infection intensity in individual frogs during chytridiomycosis epizootics (epidemics in nonhuman species) in naive frog metapopulations (we use the term "metapopulation" to mean a collection of populations connected by dispersal) (16, 17). In doing so, we reveal the heretofore unknown importance of infection intensity as a factor allowing Bd to drive amphibian populations to extinction. We also sought to describe the rate of spread by Bd through these metapopulations, which is information critical to understanding the potential vectors of this pathogen.

The rapid decline of California's mountain yellow-legged frog (a species complex consisting of *Rana muscosa* and *Rana sierrae*) (18) is emblematic of global amphibian declines (3). Historically, these two species inhabited thousands of lakes and ponds in California's Sierra Nevada (where this study took place) (19). Both of these closely related species are highly aquatic and have a multiyear tadpole stage that allows them to breed successfully in the cold water bodies typical of the high elevation portions of this mountain range. Despite the fact that the majority of their habitat is fully protected, these frogs have disappeared from >93% of their historic range during the past several decades (18). As a consequence of this decline, the mountain yellow-legged frog has gone from being one of the most common vertebrates in the Sierra Nevada to one classified as "critically endangered" (3). One of the earliest recorded cases of Bd infecting amphibians in western North America (1975) was in *R. muscosa* specimens from the Sierra Nevada (20); these specimens were originally identified as *Rana boylii*, but subsequent inspection by one of the authors (V.T.V.) indicated that they are actually *R. muscosa*. Since then, Bd has spread across this mountain

ECOLOGY

Author contributions: V.T.V., R.A.K., and C.J.B. designed research; V.T.V., R.A.K., T.S.T., and C.J.B. performed research; V.T.V., R.A.K., and C.J.B. analyzed data; and V.T.V., R.A.K., and C.J.B. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

[1]To whom correspondence should be addressed. E-mail: vancev@sfsu.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0914111107/DCSupplemental.

range, causing the extinction of hundreds of mountain yellow-legged frog populations (21, 22).

Our study area comprised three lake basins: Milestone, Sixty Lake, and Barrett Lakes in Sequoia–Kings Canyon National Park, CA (Fig. S1). The three basins were separated from each other by 20–50 km. At the beginning of our study, we found no evidence of chytridiomycosis in the frog populations in these lake basins, but all three basins were immediately adjacent to basins in which chytridiomycosis epizootics and subsequent frog population extinctions had recently occurred. At the inception of our study (1996–2000), the three study basins, Milestone, Sixty Lake, and Barrett Lakes, contained 13, 33, and 42 frog populations, respectively, and represented the most intact remaining metapopulations of these species. To quantify trends in population size before and after Bd-caused epizootics, we used repeat surveys of all 88 frog populations over a 9–13-year period. Frog surveys were conducted 1–5 times per year at each population in Milestone Basin (*R. muscosa*: 2000, 2003–2008), 1–12 times per year in Sixty Lake Basin (*R. muscosa*: 1996–2008), and once per year in Barrett Lakes Basin (*R. sierrae*: 1997, 2002–2008), for a total of 1,995 surveys (yearly average = 1.8 surveys × population^{-1}). We measured Bd prevalence and infection intensity (expressed as zoospore equivalents × swab^{-1}) using a real-time quantitative PCR assay (23) conducted on skin swabs (24) collected from frogs in 2004–2008 (n = 4,591). Before the availability of the PCR assay, tadpole mouthpart inspections (25) were used for assessments of Bd prevalence (2002–2005, n = 1,389).

Results

We detected Bd in Milestone Basin in June 2004, in Sixty Lake Basin in August 2004, and in Barrett Lakes Basin in July 2005 (Fig. 1). In the relatively small Milestone Basin, Bd spread to virtually all populations within a single year (Fig. 1 *A* and *B*). In the larger Sixty Lake and Barrett Lakes Basins, it took 3–5 years for Bd to spread to all frog populations (Fig. 1 *F–O*). Our most detailed within-season Bd occurrence data were collected in Sixty Lake Basin, and these data allowed us to quantify the pattern and rate of Bd spread. In Sixty Lake Basin, the distance from the original Bd outbreak site (Fig. 1*F*) to subsequently infected populations increased linearly with time (linear regression through the origin: R^2 = 0.85, P < 0.001), consistent with a wave-like pattern (Fig. 1 *F–J*). The slope of the regression line indicated an average rate of Bd spread (±1 SE) of 688 ± 64 m·yr^{-1}. The pattern and rate of spread in Barrett Lakes Basin (where we collected skin swabs only once per year; Fig. 1 *K–O*) were qualitatively similar to those measured in Sixty Lake Basin.

In 48 of the 88 frog populations, Bd assays (n = 1,341 swabs, 909 mouthpart inspections) were conducted before the beginning of Bd-caused epizootics. We used results from these assays to calculate the probability that Bd was present on frogs at these

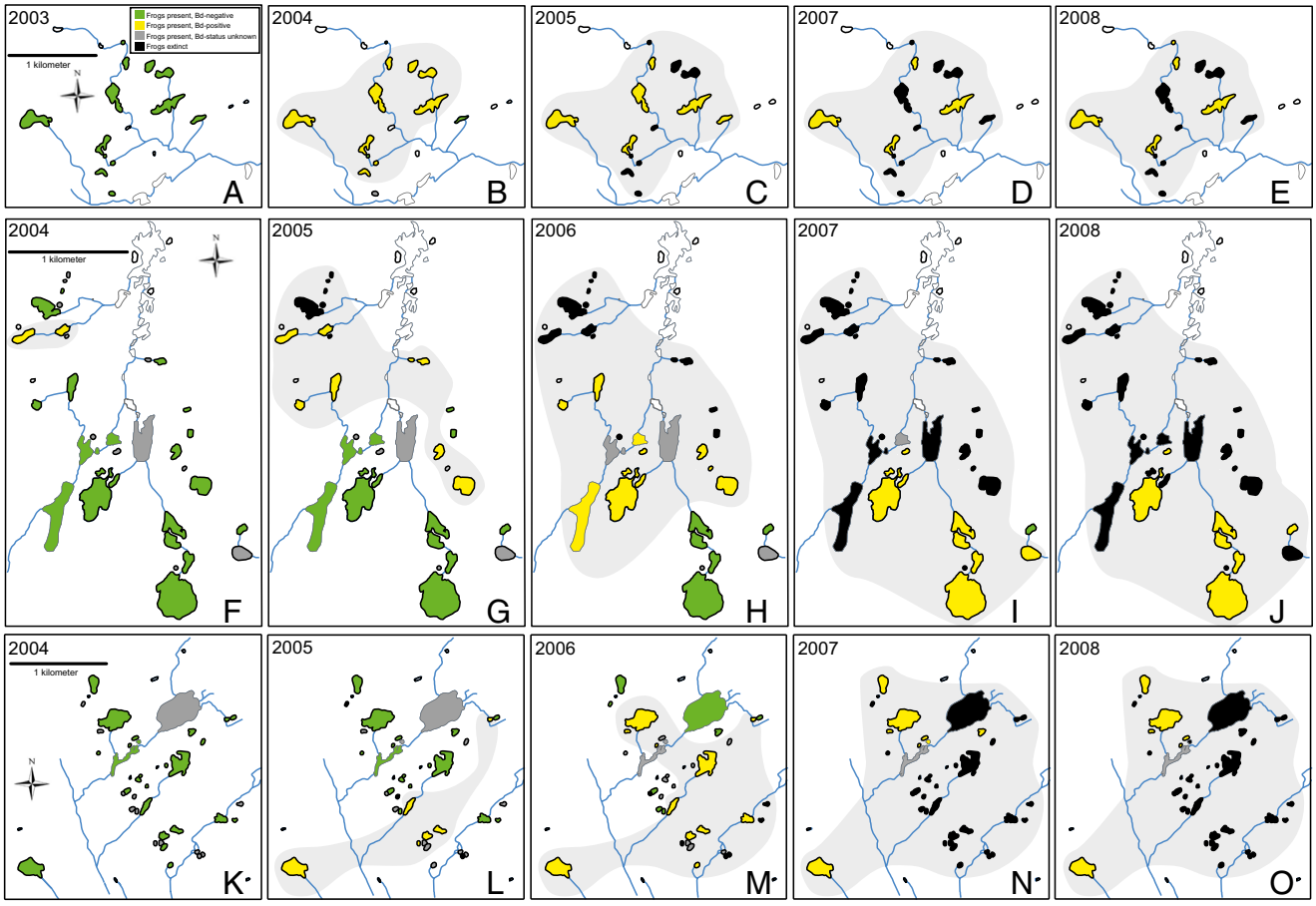

Fig. 1. Maps of the three study metapopulations showing the spread of Bd and frog population status (adults only) during a 4-year period following the initial detection of Bd. Depicted are Milestone Basin (*A–E*), Sixty Lake Basin (*F–J*), and Barrett Lakes Basin (*K–O*). Lake color (green, yellow, and black) shows the Bd infection and frog population status, and the light gray shaded region surrounds the area in which frog populations were Bd-positive in each year. Lakes shown with a thick black outline are fishless, and a thin gray outline indicates that nonnative fish were present (details on the historic fish distribution are presented in *SI Text*). The infection status of frog populations depicted in *A* and *K* is based on mouthpart surveys of 459 tadpoles. The infection status of frog populations in *B–J* and *L–O* is based on 4,591 skin swabs analyzed using a real-time PCR assay.

sites during the early part of our study but not detected (i.e., false-negative result). These calculations were based on the assumption that the true prevalence of Bd was 5%. For 33 (69%) of the 48 populations, the probability of false-negative results was less than 0.05 (median = 0.02; Table S1). For the best-sampled populations (22 populations for which >30 swabs or mouthpart inspections were collected before detection of Bd), the median probability of false-negative results was 1.6×10^{-3} (Table S1). These results strongly suggest that Bd was not present in frog populations in Milestone, Sixty Lake, and Barrett Lakes Basins in the early years of our study.

Soon after the detection of Bd, major declines in frog populations were observed in all three study basins (Fig. 2) and were coincident with observations of hundreds of dead and dying frogs (Fig. S2). By 2008, the number of adult frogs in Milestone Basin had declined from 1,680 (frog counts averaged over all surveys conducted before Bd arrival) to 22 (Fig. 2*A*), from 2,193 to 47 in Sixty Lake Basin (Fig. 2*B*), and from 5,588 to 436 in Barrett Lakes Basin (Fig. 2*C*). Similarly, by 2008, adult frogs were extinct from 9 of 13 populations in Milestone Basin, 27 of 33 populations in Sixty Lake Basin, and 33 of 42 populations in Barrett Lakes Basin (Fig. 1 *E*, *J*, and *O*). Based on high rates of population extinctions in nearby basins in the 10 years following Bd arrival, we expect that most, if not all, of the still-extant populations will also go extinct during the next 3 years as the remaining tadpoles metamorphose and succumb to chytridiomycosis (21).

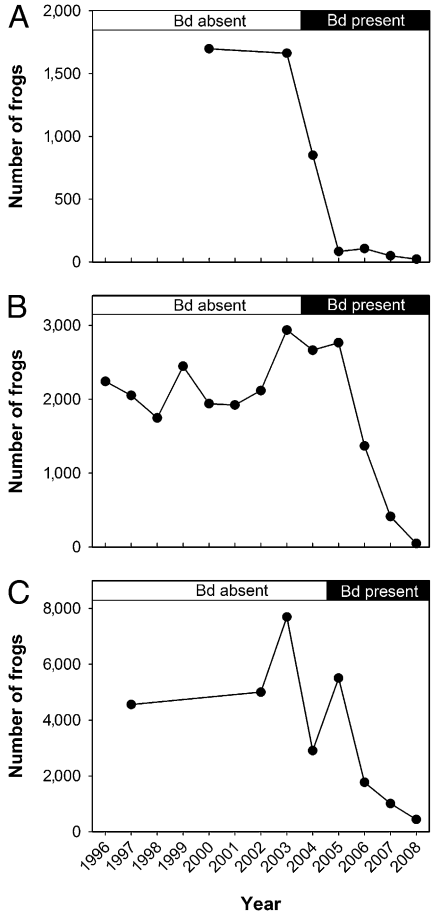

Fig. 2. Total number of adult and subadult frogs in the three study metapopulations during 1996–2008 before and after the detection of Bd: Milestone Basin (*A*), Sixty Lake Basin (*B*), and Barrett Lakes Basin (*C*).

To quantify the effect of Bd arrival on frog population growth rates, we compared population growth rates in (*i*) the years before Bd arrival, (*ii*) the year of Bd arrival, and (*iii*) the year after Bd arrival. There was a significant decrease in the frog population growth rate in the year of Bd arrival compared with the growth rate in the same populations before Bd arrival [Fig. 3; mean difference in growth rate ([before Bd arrival] – [year of Bd arrival]) = 1.8, paired *t* test: $t = 2.9$, df = 42, $P < 0.01$] and an even larger decrease in the year following *Bd* arrival [Fig. 3; mean difference in growth rate ([before Bd arrival] – [year after Bd arrival]) = 3.2, paired *t* test, $t = 7.5$, df = 36, $P << 0.01$]. Therefore, the decrease in the frog population growth rate began with the arrival of Bd and was clearly evident within 1 year after the detection of Bd.

We used detailed within-season data from the eight most intensively sampled populations in Milestone and Sixty Lake Basins to describe the frog–Bd dynamics during epizootics. Following the detection of Bd in these populations, adult frog populations invariably crashed to extinction ($n = 7$) or near-extinction ($n = 1$; Fig. 4*A*). On the date when Bd was detected, both prevalence and infection intensity were relatively low (prevalence: median = 0.42, range = 0.05–1; infection intensity: median = 13.4, range = 0.2–3,843.0). In all populations, Bd prevalence increased rapidly, and in all but one case, it reached 100% (97% in the remaining case), often in less than 50 days (Fig. 4*B*). Infection intensity increased exponentially; the within-year rate of increase ($\bar{x} \pm 1$ SE) was $0.15 \pm 0.02 \times \text{day}^{-1}$ (Fig. 4*C*). Declines in frog numbers were generally not evident until an average infection intensity of ≈10,000 zoospore equivalents per swab was reached [maximum infection intensity at time of population crash ($\bar{x} \pm 1$ SE) = 11,775 ± 5,851 zoospore equivalents × swab^{-1}; Fig. 4 *A* and *C*]. Exceeding this threshold consistently resulted in mass mortality and rapid population decline (Fig. 4*A*). Bd prevalence and infection intensity remained high even in the last surviving frogs following population crashes (Fig. 4 *B* and *C*). Frogs swabbed during the second summer after the outbreak (>300 days post-outbreak; Fig. 4 *B* and *C*) were all newly metamorphosed subadults (which had survived the winter as tadpoles). The fact that subadults have much higher infection intensities than do adults

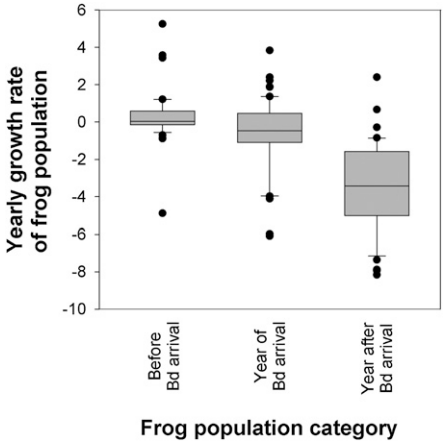

Fig. 3. Box plots showing the effect of Bd arrival on the yearly population growth rate (r_t) of three categories of frog populations: (*i*) populations before detection of Bd (r_t for each lake averaged over all years before Bd arrival), (*ii*) populations during the year in which *Bd* was detected, and (*iii*) populations 1 year after Bd was detected. In each case, $r_t = \ln(N_t) - \ln(N_{t-1})$, where N_t is the number of adult frogs in the lake in year *t*. Box plots display the median yearly frog population growth rate (horizontal line), 25th and 75th percentiles (gray boxes), 10th and 90th percentiles (whiskers), and all points that lie outside of the 10th and 90th percentiles (•). Data are from 88 frog populations located in all three study basins (1996–2008).

ECOLOGY

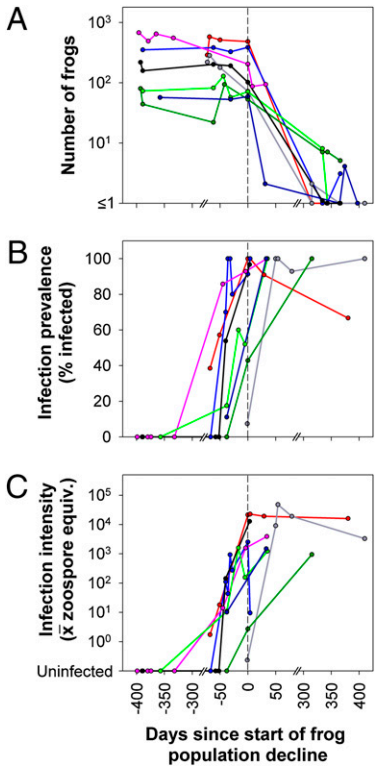

Fig. 4. Frog–Bd dynamics in eight intensively sampled populations in Milestone and Sixty Lake Basins before and after detection of Bd: frog counts (adults + subadults) from visual encounter surveys (*A*); infection prevalence, defined as the fraction of skin swabs collected from each population on each date positive for Bd (*B*); and infection intensity, defined as the average zoospore equivalents on swabs collected from each population on each date (*C*). Data are from frog populations that were sampled more than once per year, experienced >80% declines by the end of 2006, and for which the decline in the number of frogs was >10. This last criterion excluded populations that were very small before Bd arrival. Populations were aligned along the x axis such that "0" represents the date on which each frog population began to decline. This was calculated for each population by determining the date at which the number of postmetamorphic frogs dropped below 20% of the average population count before that point.

likely explains the high infection intensities even at the end of the epizootic when very few frogs remained (Fig. 4 *A* and *C*).

Discussion

Most of the frog study populations were sampled for Bd for at least 1 year before epizootics began. In all 48 of these frog populations, we found no evidence of Bd until just before the observed frog die-offs. Therefore, we suggest that Bd was absent from the three study metapopulations before 2004. Two studies in Central America (6, 7) also reported the absence of Bd until just before frog die-offs were observed. The apparent absence of Bd before frog die-offs is critically important in resolving the continued debate about whether Bd is a novel pathogen sweeping through naive host populations (7, 8, 26) or a widespread endemic pathogen that has emerged as a result of changing environmental conditions such as those caused by climate warming (Bd thermal optimum hypothesis) (27). Implicit in the Bd thermal optimum hypothesis is the presence of Bd in amphibian populations before chytridiomycosis epizootics (28). Our results indicate that Bd was likely not present on amphibians in our study populations until just before epizootics began. Therefore, our data do not support the Bd thermal optimum hypothesis but are consistent with Bd as a novel pathogen spreading through naive host populations.

Data from the intensively sampled Sixty Lake Basin metapopulation indicated that Bd spread as a distinct wave at a rate of 688 m·yr^{-1}, and rates of spread in Milestone and Barrett Lakes Basin were qualitatively similar. This rate of spread is much lower than rates reported for Bd in Central and South America and Australia (17–282 km·yr^{-1}) (8, 29), but it is unclear if these differences in rate of spread are real or are the result of different spatial scales of sampling used in our study compared with previous studies. In our study system, the observed pattern of Bd spread within a metapopulation is consistent with frog movement patterns, suggesting that frogs may be an important agent of dispersal at this scale (these frogs are known to move only several hundred meters between lakes in a single summer) (17, 30). However, the continuing between-basin spread of Bd and the lack of evidence for interbasin frog movement (17, 18) suggest the involvement of unknown additional vectors. Other possible between-basin dispersal agents include more vagile sympatric organisms, including amphibians (e.g., *Pseudacris regilla*), insects, or birds.

Before our study, the only data available on frog–Bd dynamics during disease outbreaks showed a temporal correlation between increases in Bd prevalence and amphibian population decline (7), but that study did not include any measurement of infection intensity. As a consequence, the dynamics of this disease were only partially described until now. Our quantification of infection intensity provided a key insight into how Bd causes host extinctions. Temporally intensive sampling at multiple frog populations showed that the very high growth rate and virulence of Bd in mountain yellow-legged frogs allowed the near-simultaneous infection and buildup of high infection intensities in all host individuals. Subsequent host population crashes therefore occurred before Bd could be limited by density dependence, host immune response, or other factors.

Chytridiomycosis is a major driver of an ongoing global mass extinction event (31) in amphibians, but field interventions designed to reduce disease impacts by altering Bd–host dynamics have only just begun. Our results show a primary role for infection intensity in driving the population extinctions that typically follow these epizootics. This suggests that interventions designed to prevent Bd infection intensity on frogs from reaching the critical lethal threshold could reduce the probability of population extinction. Interventions could include capturing frogs immediately in front of the Bd wave and releasing them back into the same habitat after the Bd wave has passed and pathogen pressure has declined following die-offs of resident frog populations or reducing the density of infective Bd zoospores by treating a large proportion of frogs during epizootics with antifungal drugs (32, 33) and releasing them back into the same habitat. In both cases, the goal of interventions would not be to eradicate the pathogen from the targeted habitats, because this would not be feasible, but, instead, to reduce pathogen transmission rates and thus increase host survivorship (34). Given a known rate of Bd spread in our study system and the resulting knowledge of exactly where the Bd front is within remaining frog metapopulations, the results of the current study create a unique opportunity to test these approaches, the results of which will be of critical importance to the global conservation of amphibians.

Methods

Study Area Description. The three study watersheds are in Sequoia–Kings Canyon National Park (milestone: 36°38′57″ N, 118°27′28″ W; Sixty Lake: 36°49′03″ N, 118°25′24″ W; Barrett Lakes: 37°04′52″ N, 118°31′35″ W; Fig. S1). Milestone and Sixty Lake Basins contain the southern mountain yellow-legged frog (*R. muscosa*), and Barrett Lakes Basin contains the closely related Sierra Nevada yellow-legged frog (*R. sierrae* (18). These basins are located in the subalpine and alpine zones and contain 13–42 oligotrophic lakes and ponds (elevation range: 3,030–3,790 m), all of which are naturally fishless.

Nonnative trout (primarily *Oncorhynchus mykiss* and *Salvelinus fontinalis*) have been introduced into many Sierra Nevada lakes to provide recreational fishing opportunities, and their negative impacts on mountain-yellow legged frogs are well known (35–37). The active season for frogs in the study basins is from early June to mid-October; the basins are typically covered by several meters of snow during the winter.

Frog Surveys. We used diurnal visual encounter surveys (38) of entire water body perimeters to describe the abundance of adult (≥40 mm snout–vent length) and subadult (<40 mm snout–vent length) mountain yellow-legged frogs at all water bodies in the study basins (36, 39). In these species, counts from surveys are highly correlated with estimates of population size obtained using mark-recapture techniques. These frogs' high detectability during visual surveys is a consequence of a diurnal habit, spending the majority of the active season at the water–land interface (30, 40), and not in terrestrial habitats (41), and occupying structurally simple habitats (e.g., subalpine lakes, alpine lakes) in which the lack of submerged logs or aquatic vegetation provides few places for frogs to hide.

Disease Prevalence and Infection Intensity. We used frog skin swabs and a real-time quantitative PCR assay to quantify Bd prevalence and infection intensity (23, 24). Swabs were stroked across a frog's skin in a standardized way: five strokes on each side of the abdominal midline, five strokes on the inner thighs of each hind leg, and five strokes on the foot webbing of each hind leg (total of 30 strokes $\times$ frog^{-1}). Swabs were air-dried in the field and stored individually in labeled microcentrifuge tubes before PCR analysis. We used standard Bd DNA extraction and real-time PCR methods (23, 24), except that swab extracts were analyzed singly instead of in triplicate (42). We defined infection intensity as the number of "zoospore equivalents" per swab. Zoospore equivalents were calculated by multiplying the genomic equivalent values generated during the real-time PCR assay by 80; this multiplication accounts for the fact that DNA extracts from swabs were diluted 80-fold during extraction and PCR. For calculations of Bd prevalence, swabs were categorized as Bd-positive when zoospore equivalents were ≥1 and as Bd-negative when zoospore equivalents were <1. Before the availability of the PCR assay, we determined the infection status (infected/uninfected) of frog populations using inspections of tadpole mouthparts (upper jaw sheaths). Tadpole mouthpart anomalies can have numerous causes, but in *R. muscosa* and *R. sierrae*, mouthpart anomalies are an accurate indicator of chytridiomycosis (25).

Bd Disinfection Procedures. To ensure that Bd was not spread between frog populations by field sampling activities, we disinfected all field gear by immersion in 1% sodium hypochlorite or 0.01% quaternary ammonia for 5 min (43). In Milestone and Barrett Lakes Basins, disinfection was performed whenever moving between frog populations. In Sixty Lake Basin, where the distribution of Bd was very well known during each summer, we divided the area into discrete units based on geography and Bd infection status (infected/uninfected) and disinfected gear when moving between units.

Rate of *Bd* Spread. Calculations of Bd spread rate in Sixty Lake Basin were based on the date of earliest Bd detection: August 22, 2004. For each newly infected frog population in this basin, we calculated (*i*) the minimum straight-line distance from the original outbreak sites (Fig. 1*F*) and (*ii*) the number of days between August 22, 2004 and the date on which Bd was detected. The slope from a linear regression model of distance as a function of time provided the rate of spread. The regression included only populations that became infected by the autumn of 2006. The intercept of the regression (±1 SE) was not significantly different from zero (189 ± 223 m); thus, the regression line was forced through the origin. Lakes that had not become infected by the autumn of 2006 were situated significantly further from the site of initial Bd detection than lakes that became infected (logistic regression: $P < 0.01$, df = 28).

ACKNOWLEDGMENTS. Research permits were provided by Sequoia–Kings Canyon National Park and the University of California, Berkeley; San Francisco State University; and University of California, Santa Barbara Institutional Animal Care and Use Committees. We thank the staff at Sequoia–Kings Canyon National Park for logistical support and many technicians for their help in collecting field data and running PCR assays. This work was funded by National Institutes of Health Grant R01ES12067 and National Science Foundation Grant EF-0723563 as part of the joint National Science Foundation–National Institutes of Health Ecology of Infectious Disease program.

1. Anderson RM, May RM (1979) Population biology of infectious diseases: Part I. *Nature* 280:361–367.
2. de Castro F, Bolker B (2005) Mechanisms of disease-induced extinction. *Ecol Lett* 8: 117–126.
3. Stuart SN, et al. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786.
4. Skerratt LF, et al. (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134.
5. McCallum H (2005) Inconclusiveness of chytridiomycosis as the agent in widespread frog declines. *Conserv Biol* 19:1421–1430.
6. Berger L, et al. (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc Natl Acad Sci USA* 95:9031–9036.
7. Lips KR, et al. (2006) Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proc Natl Acad Sci USA* 103:3165–3170.
8. Lips KR, Diffendorfer J, Mendelson JR, Sears MW (2008) Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *PLoS Biol* 6:e72.
9. Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219–227.
10. James TY, et al. (2009) Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. *PLoS Pathog* 5:e1000458.
11. Morgan JAT, et al. (2007) Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*. *Proc Natl Acad Sci USA* 104:13845–13850.
12. Blaustein AR, et al. (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. *Conserv Biol* 19:1460–1468.
13. Parris MJ, Cornelius TO (2004) Fungal pathogen causes competitive and developmental stress in larval amphibian communities. *Ecology* 85:3385–3395.
14. Voyles J, et al. (2007) Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. *Dis Aquat Org* 77:113–118.
15. Voyles J, et al. (2009) Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* 326:582–585.
16. Hanski I, Gilpin ME (1997) *Metapopulation Biology: Ecology, Genetics, and Evolution* (Academic, San Diego).
17. Bingham RE (2007) Differentiation across multiple spatial scales in three Californian amphibians. PhD dissertation (UnivERSITY of California, Berkeley).
18. Vredenburg VT, et al. (2007) Concordant molecular and phenotypic data delineate new taxonomy and conservation priorities for the endangered mountain yellow-legged frog. *J Zool* 271:361–374.
19. Grinnell J, Storer TI (1924) *Animal Life in the Yosemite* (Univ of California Press, Berkeley).
20. Ouellet M, Mikaelian I, Pauli BD, Rodrigue J, Green DM (2005) Historical evidence of widespread chytrid infection in North American amphibian populations. *Conserv Biol* 19:1431–1440.
21. Rachowicz LJ, et al. (2006) Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87:1671–1683.
22. Briggs CJ, Vredenburg VT, Knapp RA, Rachowicz LJ (2005) Investigating the population-level effects of chytridiomycosis: an emerging infectious disease of amphibians. *Ecology* 86:3149–3159.
23. Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis Aquat Org* 60:141–148.
24. Hyatt AD, et al. (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Dis Aquat Org* 73:175–192.
25. Knapp RA, Morgan JAT (2006) Tadpole mouthpart depigmentation as an accurate indicator of chytridiomycosis, an emerging disease of amphibians. *Copeia* 2006:188–197.
26. Rohr JR, Raffel TR, Romansic JM, McCallum H, Hudson PJ (2008) Evaluating the links between climate, disease spread, and amphibian declines. *Proc Natl Acad Sci USA* 105: 17436–17441.
27. Pounds JA, et al. (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439:161–167.
28. Rachowicz LJ, et al. (2005) The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. *Conserv Biol* 19:1441–1448.
29. Laurance WF, McDonald KR, Speare R (1996) Epidemic disease and the catastrophic decline of Australian rain forest frogs. *Conserv Biol* 10:406–413.
30. Pope KL, Matthews KR (2001) Movement ecology and seasonal distribution of mountain yellow-legged frogs, *Rana muscosa*, in a high-elevation Sierra Nevada basin. *Copeia* 2001:787–793.
31. Wake DB, Vredenburg VT (2008) Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc Natl Acad Sci USA* 105:11466–11473.
32. Parker JM, Mikaelian I, Hahn N, Diggs HE (2002) Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). *Comp Med* 52:265–268.
33. Garner TWJ, Garcia G, Carroll B, Fisher MC (2008) Using itraconazole to clear *Batrachochytrium dendrobatidis* infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. *Dis Aquat Org* 83:257–260.
34. Briggs CJ, Knapp RA, Vredenburg VT (In review) Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc Natl Acad Sci USA,* 1079695–9700.
35. Knapp RA, Matthews KR (2000) Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. *Conserv Biol* 14:428–438.
36. Vredenburg VT (2004) Reversing introduced species effects: Experimental removal of introduced fish leads to rapid recovery of a declining frog. *Proc Natl Acad Sci USA* 101: 7646–7650.

37. Knapp RA, Boiano DM, Vredenburg VT (2007) Removal of nonnative fish results in population expansion of a declining amphibian (mountain yellow-legged frog, *Rana muscosa*). *Biol Conserv* 135:11–20.
38. Crump ML, Scott NJ, Jr (1994) *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*, eds Heyer WR, Donnelly MA, McDiarmid RW, Hayek L- AC, Foster MS (Smithsonian, Washington, DC), pp 84–91.
39. Knapp RA, Matthews KR, Preisler HK, Jellison R (2003) Developing probabilistic models to predict amphibian site occupancy in a patchy landscape. *Ecol Appl* 13: 1069–1082.
40. Bradford DF (1984) Temperature modulation in a high-elevation amphibian, *Rana muscosa*. *Copeia* 1984:966–976.
41. Vredenburg VT, Fellers GM, Davidson C (2005) *Status and Conservation of US Amphibians*, ed Lannoo MJ (Univ of California Press, Berkeley), pp 563–566.
42. Kriger KM, Hero J-M, Ashton KJ (2006) Cost efficiency in the detection of chytridiomycosis using PCR assay. *Dis Aquat Org* 71:149–154.
43. Johnson ML, Berger L, Philips L, Speare R (2003) Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. *Dis Aquat Org* 57:255–260.

Supporting Information

Vredenburg et al. 10.1073/pnas.0914111107

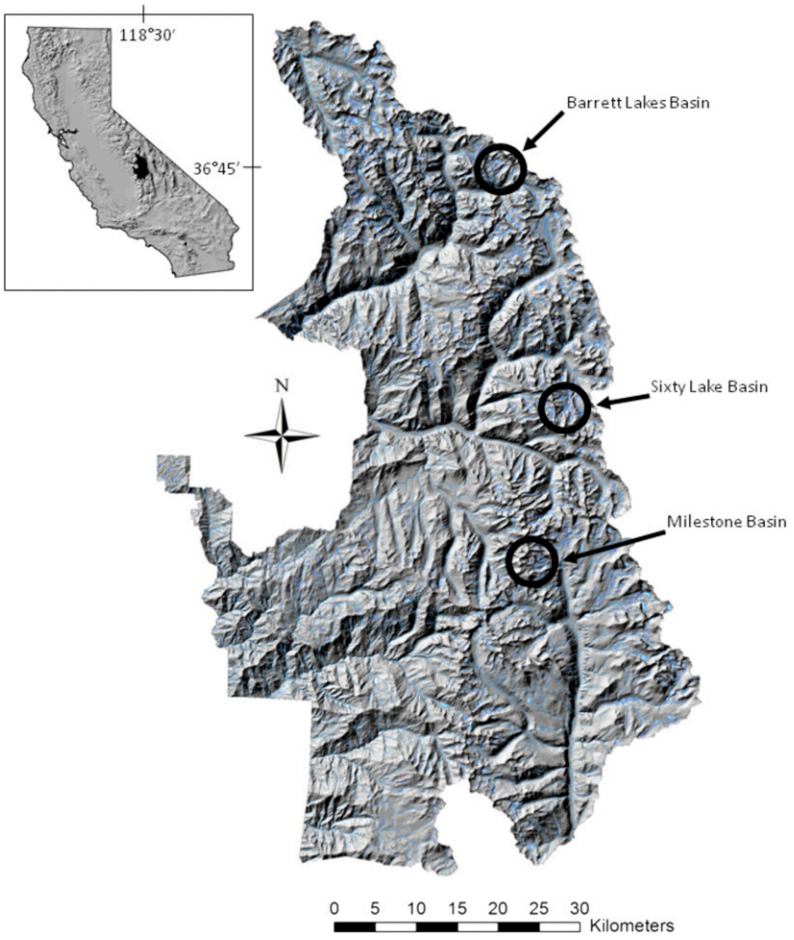

Fig. S1. Map showing the locations of the three study basins (Milestone, Sixty Lake, and Barrett Lakes) within Sequoia–Kings Canyon National Park. Milestone, Sixty Lake, and Barrett Lakes Basins are located in the headwaters of the Kern River, South Fork Kings River, and Middle Fork Kings River, respectively. The inset map locates Sequoia–Kings Canyon National Park (black polygon) within California.

Fig. S2. Dead frogs resulting from a chytridiomycosis epidemic in Sixty Lake Basin, Kings Canyon National Park, CA (July 15, 2008; photograph provided by J. Sartore, National Geographic Society).

Table S1. Probability of false-negative results associated with Bd assays (PCR on skin swabs or tadpole mouthpart inspections) for frog populations that were sampled for Bd before frog die-offs

Lake identification no.	Basin name	Date of earliest Bd detection	Average no. frogs (adults + subadults) before Bd arrival	No. swabs before Bd arrival (S)	No. mouthpart inspections before Bd arrival (M)	Probability of false-negative result
20192	Milestone	6/27/2004	60	0	13	0.11
20193	Milestone	8/9/2006	89	0	1	0.84
20195	Milestone	7/18/2005	6	0	17	0.05
20196	Milestone	6/28/2004	73	0	11	0.15
20197	Milestone	9/13/2004	16	0	9	0.21
20198	Milestone	6/27/2004	467	0	19	0.04
20199	Milestone	7/20/2004	188	0	22	0.02
21081	Milestone	7/20/2004	315	0	21	0.03
21085	Milestone	7/4/2004	13	0	6	0.36
21086	Milestone	7/4/2004	14	0	26	0.01
10406	Sixty Lake	8/23/2004	10	0	10	0.18
10407	Sixty Lake	7/28/2005	88	0	35	2.4×10^{-3}
10408	Sixty Lake	7/21/2005	103	2	34	2.6×10^{-3}
10411	Sixty Lake	8/6/2005	364	70	34	8.0×10^{-5}
10413	Sixty Lake	7/6/2006	15	19	0	0.38
10414	Sixty Lake	8/12/2006	17	26	0	0.26
10416	Sixty Lake	9/15/2005	59	20	30	2.1×10^{-3}
10417	Sixty Lake	9/15/2005	136	55	28	4.8×10^{-4}
10418	Sixty Lake	7/1/2006	176	46	42	6.9×10^{-5}
10419	Sixty Lake	7/1/2006	5	71	1	0.02
10420	Sixty Lake	7/1/2006	136	79	17	9.3×10^{-4}
10421	Sixty Lake	6/21/2007	196	85	31	6.2×10^{-5}
10422	Sixty Lake	6/21/2007	523	243	35	9.4×10^{-9}
10423	Sixty Lake	6/21/2007	934	95	34	2.2×10^{-5}
11064	Sixty Lake	7/11/2007	293	222	0	1.1×10^{-5}
11065	Sixty Lake	7/5/2006	15	3	0	0.86
11068	Sixty Lake	7/1/2006	77	21	24	5.5×10^{-3}
11069	Sixty Lake	7/1/2006	65	0	33	3.4×10^{-3}
11070	Sixty Lake	7/7/2006	130	47	9	0.02
11074	Sixty Lake	7/8/2005	27	4	35	2.0×10^{-3}
11075	Sixty Lake	7/8/2005	52	0	10	0.18
11076	Sixty Lake	9/17/2005	0	11	3	0.34
11081	Sixty Lake	7/3/2006	2	0	20	0.03
11096	Sixty Lake	8/6/2005	175	164	0	2.2×10^{-4}
12615	Sixty Lake	7/6/2006	32	0	7	0.30
10206	Barrett Lakes	9/15/2005	706	0	20	0.03
10222	Barrett Lakes	6/27/2007	438	48	30	4.9×10^{-4}
10223	Barrett Lakes	8/12/2006	1373	0	32	4.1×10^{-3}
10225	Barrett Lakes	8/13/2006	1133	0	26	0.01
10227	Barrett Lakes	8/13/2006	201	0	39	1.2×10^{-3}
10228	Barrett Lakes	8/14/2006	114	0	35	2.4×10^{-3}
11468	Barrett Lakes	8/14/2006	3	0	5	0.42
11469	Barrett Lakes	9/15/2005	162	0	20	0.03
11470	Barrett Lakes	8/5/2005	313	0	11	0.15
11493	Barrett Lakes	8/14/2006	18	0	26	0.01
11502	Barrett Lakes	6/29/2007	53	10	26	6.8×10^{-3}
12495	Barrett Lakes	8/12/2006	20	0	1	0.84
12498	Barrett Lakes	8/4/2005	102	0	21	0.03

For each population, we list the lake identification number, name of the basin in which the population was located, date on which Bd was detected, average number of frogs present before Bd arrival, number of collected swabs and tadpole mouthpart inspections before Bd arrival, and probability of a false-negative result. The date of Bd arrival is the date on which at least one collected swab had a zoospore-equivalent value of $\geq$1.0 (this includes swabs collected from any life stage: tadpoles, subadults, and adults). "Probability of false-negative result" is the probability of observing S Bd-negative swabs and M Bd-negative tadpole mouthpart inspections if the true Bd prevalence in the population is 5%. Smaller probabilities indicate a higher confidence that a water body sampled on a particular date was uninfected, or infected at a very low prevalence. This probability was calculated as $(1 \text{ to } 0.05)^S\ [0.05 \times FN + (1 \text{ to } 0.05) \times TN]^M$, where FN is the probability of false-negative results using tadpole mouthpart inspections (0.12; ref. 1) and TN is the probability that a mouthpart inspection accurately assigns a negative diagnosis to a tadpole that truly is Bd-negative (0.88; ref. 1). We assumed that the real-time PCR assay, the most sensitive assay currently available, was 100% accurate, and therefore gives no false-negative results (2), and that all swabs were independent random samples of the frog population at a site. These assumptions were probably not strictly met; thus, the probabilities presented should serve only as an approximation of the true probabilities.

1. Knapp RA, Morgan JAT (2006) Tadpole mouthpart depigmentation as an accurate indicator of chytridiomycosis, an emerging disease of amphibians. *Copeia* 2006:188–197.
2. Hyatt AD, et al. (2007) Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. *Dis Aquat Org* 73:175–192.

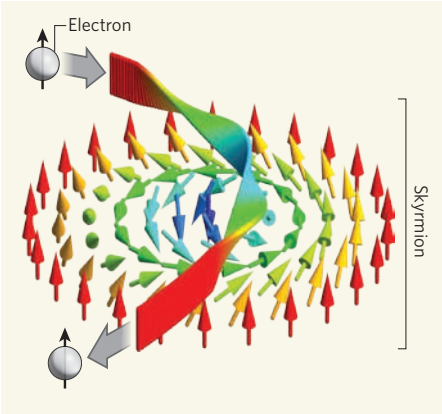

Figure 1 | Roaming through a skyrmion. When an electron moves through a special type of magnetic texture called a skyrmion, its magnetic moment (spin) twists to adjust to the skyrmion's local spin structure (ribbon-like pattern). This twisting changes the electron's direction of travel and pushes the electron and the skyrmion in opposite directions (not shown).

untied as long as the magnetization remains smooth and finite. Skyrmions are therefore classified as topologically stable — a characteristic drawn from the mathematical discipline of topology, which classifies geometric configurations according to properties, such as the winding number, that are robust against all small distortions or perturbations.

In the field of nanomagnetism, magnetic whirls that share similarities with skyrmions have long been known to exist at interfaces between magnetic domains within ferromagnets or in specially tailored magnetic nanosystems[3]. But the skyrmions now discovered by Yu *et al.* arise from a completely different microscopic mechanism: from magnetic interactions that have a unique handedness in materials that lack a centre of inversion symmetry. These 'chiral' magnetic interactions, also known as Dzyaloshinsky–Moriya interactions, favour the twisting of the magnetization and thus lead to the formation of structures such as spirals or skyrmions.

Yu and colleagues' study[1] was inspired by recent neutron-scattering experiments and related theoretical work that established that lattices of skyrmions form spontaneously in a class of chiral magnet in a tiny temperature window and in the presence of a weak magnetic field[4,5]. But because neutron scattering can detect only periodic structures such as lattices, it didn't allow the direct identification of individual skyrmions. To observe individual skyrmions, Yu *et al.* studied thin samples (just several tenths of a nanometre thick) of the chiral magnet $Fe_{0.5}Co_{0.5}Si$ in a weak magnetic field.

They found that the formation of skyrmions is strongly favoured if the distance over which the winding caused by the chiral interactions takes place is larger than the sample's thickness — an observation that is in accord with numerical simulations devised by the authors[1]. To image the magnetic structure, they used Lorentz force microscopy, in which they observed the component of the magnetization that is parallel to the sample with a resolution much smaller than the typical 90-nanometre radius of the skyrmions[6]. Depending on the strength of the magnetic field and the temperature, they observed either perfect periodic arrangements of skyrmions, lattices with defects, glassy and amorphous configurations or even single skyrmions. As conjectured previously[4,5], because of the chiral interactions, all of these skyrmions wind in the same direction.

As exciting as the observation of single skyrmions is, the real excitement lies in the prospect of using them for a very efficient coupling of an electric or spin current to a magnetic structure. Such a coupling would underlie most potential applications of skyrmions, such as for magnetic storage devices or perhaps even transistors.

To illustrate the nature of this coupling, consider an electron traversing a skyrmion (Fig. 1). As the electron travels through the skyrmion, its spin orientation is twisted back and forth to adjust to the direction of the skyrmion's local spin structure. While doing so, the electron acquires a quantum-mechanical phase known as the Berry phase. From the viewpoint of the electron, the net effect of the change in its spin orientation is an effective force perpendicular to its motion that is similar to the Lorentz force it experiences in the presence of a magnetic field. This 'topological' force is directly proportional to the winding that characterizes the skyrmion and has recently been observed in measurements of the Hall effect in a skyrmion lattice[7]. But as the electron feels the topological force, it must also counteract it by exerting a force on the skyrmion. This force could be exploited to manipulate skyrmions with electrons.

Yu and colleagues' work[1] shows that skyrmions in chiral magnets can be created either in regular lattices or as topologically stable 'stand-alone' particles. These structures could provide the building blocks for new complex textures, which could then be either manipulated with electric or spin currents or, conversely, used to direct the motion of spins and charges. Taken together with the realization that the chiral magnetic interactions that underlie skyrmions are a general feature of essentially all systems that lack inversion symmetry, in particular surfaces and interfaces[8], Yu and colleagues' study pushes the door wide open for many applications. ■

Christian Pfleiderer is at the Lehrstuhl für Experimentalphysik E21, Technische Universität München, D-85748 Garching, Germany. Achim Rosch is at the Institute for Theoretical Physics, Universität zu Köln, D-50937 Köln, Germany.
e-mails: christian.pfleiderer@frm2.tum.de; rosch@thp.uni-koeln.de

1. Yu, X. Z. *et al. Nature* **465,** 901–904 (2010).
2. Skyrme, T. H. R. *Nucl. Phys.* **31,** 556–569 (1962).
3. Bader, S. D. *Rev. Mod. Phys.* **78,** 1–15 (2006).
4. Mühlbauer, S. *et al. Science* **323,** 915–919 (2009).
5. Münzer, W. *et al. Phys. Rev. B* **81,** 041203 (2010).
6. Uchida, M., Onose, Y., Matsui, Y. & Tokura, Y. *Science* **311,** 359–361 (2006).
7. Neubauer, A. *et al. Phys. Rev. Lett.* **102**, 186602 (2009).
8. Bode, M. *et al. Nature* **447,** 190–193 (2007).

See also Editorial, page 846.

CONSERVATION BIOLOGY

When an infection turns lethal

Andrew R. Blaustein and Pieter T. J. Johnson

Losses in biodiversity and the emergence of new infectious diseases are among the greatest threats to life on the planet. The declines in amphibian populations lie at the interface between these issues.

It is estimated[1] that roughly one-third of amphibian species are under threat of extinction and that more than 100 species may have become extinct since 1980. The reasons for the decline and extinction of amphibian populations are probably complex and multifactorial[2]. But growing evidence[3] indicates that, in many cases, infectious disease is driving amphibian losses. In particular, the pathogenic fungus *Batrachochytrium dendrobatidis* has been linked to the decline of amphibian populations throughout the world. Two related papers published in *Proceedings of the National Academy of Sciences*, by Vredenburg *et al.*[4] and Briggs *et al.*[5], considerably improve our understanding of the dynamics of *B. dendrobatidis* infection.

This pathogen causes the often-lethal disease chytridiomycosis, which disrupts the function of epidermal structures such as the skin and teeth and the regulation of osmosis[6] to varying degrees, depending on the amphibian species and its life stage[7]. Since its description in the late 1990s, *B. dendrobatidis* has been the subject of hundreds of studies by researchers from various disciplines. Nonetheless, many ecological questions remain. Why does *B. dendrobatidis* cause extinction of the host population (through inducing an epidemic) in some regions but persist in the population in an endemic state in other regions? In addition, how does this pathogen induce host losses without a concomitant decrease in its transmission (as would be expected to occur for a density-dependent parasite)? After all, as infected hosts die, one would expect the disease to decline in prevalence as well.

Vredenburg and colleagues[4] and Briggs *et al.*[5] carried out long-term, large-scale monitoring and sampling of amphibian populations in the Sierra Nevada in California, focusing on yellow-legged frogs — *Rana muscosa* and *Rana sierrae* — the populations of which have declined in recent decades. Previous studies focused exclusively on the prevalence of infection (that is, the proportion of infected hosts), ignoring the role of infection intensity (the amount of infection per individual host) in controlling host-population losses. Instead of simply cataloguing the presence or absence of *B. dendrobatidis* and its spread among host populations, these investigators[4,5] identify a 'lethal threshold' of pathogen infection intensity, which may be the key to understanding how *B. dendrobatidis* epidemics can be controlled.

Vredenburg *et al.*[4] carried out intensive sampling of 88 frog populations over 9–13 years. Among the lakes they studied, they found that, within three years of its arrival, *B. dendrobatidis* had spread in a wave-like pattern — that is, the area covered by the pathogen increased steadily in size over time — until nearly all of the frog populations at the lake were infected. The amphibian populations did not, however, collapse until a lethal threshold of about 10,000 zoospores of the fungus per frog was reached.

The existence of such an intensity threshold may help to explain how *B. dendrobatidis* causes almost complete losses of amphibian hosts. Because of this threshold, there is a time lag between exposure and mortality, so the pathogen can spread through much of the amphibian population before disease-driven reductions in host density negatively affect the transmission of *B. dendrobatidis*. Consequently, the pathogen can cause the loss and extinction of its host population, unlike the many other pathogens that disappear as their hosts decline in numbers.

Briggs *et al.*[5] combine long-term field data with modelling analysis to investigate how some amphibian populations persist even though *B. dendrobatidis* is present in their habitat. The authors' intensive data — involving marking the animals and later recapturing them — show that, in populations that survive, infected yellow-legged frogs have fungal loads well below the lethal intensity threshold, and that these frogs have cleared fungal infection and become reinfected over the course of years, with no effect on their survival.

Previous studies suggested that genetic changes that alter host tolerance of the pathogen or pathogen virulence might explain how some amphibian populations persist in the presence of *B. dendrobatidis*. Briggs and colleagues' modelling efforts, however, hint that simple decreases in host density and the resultant reduction in pathogen transmission could account for such an outcome. This is particularly true when there are environmental reservoirs of *B. dendrobatidis*, including amphibian species or life stages (such as tadpoles) that can persist with the infection for long periods and spread it to more sensitive hosts.

This modelling work[5], which was based on a variety of biological scenarios, offers insight into both the epidemic and endemic aspects of *B. dendrobatidis* dynamics. For instance, the study predicts that infection intensity builds up rapidly when frog populations are dense, as well as under conditions that promote reinfection. If *B. dendrobatidis* reaches its intensity threshold, the infected amphibian population can become extinct. By contrast, if some members of the host population survive, then a new endemic state develops, with persistent infection in the remaining frogs.

Intriguingly, both studies[4,5] indicate that the traditional dichotomous classification of pathogens as either microparasites or macroparasites may be overly simplistic, as the dynamics of infection with *B. dendrobatidis* — a microparasite — strongly depend on infection intensity (which is usually considered only for macroparasites). This finding suggests that incorporating infection intensity into other microparasite disease models could provide insight into other host–pathogen systems.

The new papers[4,5] markedly increase the understanding of a disease that affects many amphibian populations. In particular, the types of data presented — based on long-term, extensive monitoring that generates detailed records — are largely unprecedented for analyses of many wildlife disease systems.

Nevertheless, large gaps remain in the knowledge of *B. dendrobatidis* and in how the dynamics of chytridiomycosis vary between geographical regions. The populations that these researchers[4,5] studied are from montane ecosystems that have low species diversity and relatively harsh winter conditions. Will the reported dynamics for *B. dendrobatidis* in this system explain the spread of this pathogen in, for example, lowland regions of Europe or in the tropics, where host-species density is substantially higher?

Moreover, it is still not clear precisely which vectors spread the infection, in which systems it is endemic and in which ones it is epidemic, and whether environmental changes can trigger the emergence of this pathogen. By focusing on infection intensity and the differences between epidemic and endemic states of *B. dendrobatidis* infection, Vredenburg *et al.* and Briggs *et al.* lay a valuable foundation for addressing questions such as how the intensity threshold of *B. dendrobatidis* varies across species or with environmental conditions, and what part is played by environmental cofactors such as climate change[8] in affecting the dynamics of endemic infection.

How can this information be applied so as to slow, or even prevent, population declines? As the authors of both papers propose, interventions designed to prevent *B. dendrobatidis* infection from reaching the lethal-intensity threshold could reduce extinction events. Because it is unlikely that the pathogen will be completely eradicated, the only realistic option may be to manage sensitive amphibian populations in such a way as to create an endemic state of infection. For instance, as described in a News Feature in these pages last week[9], reducing the density of susceptible frogs by capturing them before the infection wave, or by treating a subset of individuals with an antifungal agent, could reduce transmission of *B. dendrobatidis* and prevent infection intensities from becoming lethal. ■

Andrew R. Blaustein is in the Department of Zoology, 3029 Cordley Hall, Oregon State University, Corvallis, Oregon 97331-2914, USA. Pieter T. J. Johnson is in the Department of Ecology and Evolutionary Biology, N122, CB334 University of Colorado, Boulder, Colorado 80309-0334, USA.
e-mails: blaustea@science.oregonstate.edu; pieter.johnson@colorado.edu

1. Stuart, S. N. *et al. Science* **306,** 1783–1786 (2004).
2. Blaustein, A. R. & Kiesecker, J. M. *Ecol. Lett.* **5,** 597–608 (2002).
3. Daszak, P., Cunningham, A. A. & Hyatt, A. D. *Divers. Distrib.* **9,** 141–150 (2003).
4. Vredenburg, V. T., Knapp, R. A., Tunstall, T. S. & Briggs, C. J. *Proc. Natl Acad. Sci. USA* **107,** 9689–9694 (2010).
5. Briggs, C. J., Knapp, R. A. & Vredenburg, V. T. *Proc. Natl Acad. Sci. USA* **107,** 9695–9700 (2010).
6. Voyles, J. *et al. Science* **326,** 582–585 (2009).
7. Blaustein, A. R. *et al. Conserv. Biol.* **19,** 1460–1468 (2005).
8. Pounds, J. A. *et al. Nature* **439,** 161–167 (2006).
9. Lubick, N. *Nature* **465,** 680–681 (2010).

STRUCTURAL BIOLOGY

Immunity takes a heavy Toll

Steven A. Wasserman

Toll receptors trigger immune responses through adaptor proteins and kinase enzymes. Structural studies reveal that hierarchical assembly of these proteins into a helical tower initiates downstream signalling events.

Communication within cells often involves a series of molecular handshakes, each protein contacting the next and modifying its activity. An accessory protein may serve as a matchmaker, holding components together for the exchange of information. Until now, this model fitted well with what was known about signalling in mammalian Toll pathways, which activate innate immune defences[1]. Three proteins — MyD88 and two members of the IRAK

Nature Reviews Microbiology | AOP, published online 7 June 2010; doi:10.1038/nrmicro2388

DIGITALVISION

EPIDEMIOLOGY

It's not easy being green

> "intensity of *B. dendrobatidis* infection may be the key difference between frog populations heading for extinction and those that survive"

Chytridiomycosis, a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis*, has been directly linked to the decline and extinction of hundreds of amphibian species worldwide. Now, two long-term monitoring studies published in the *Proceedings of the National Academy of Sciences USA* describe the dynamics of disease spread and persistence in Californian frog populations.

B. dendrobatidis infects adult and larval amphibians as a zoospore and encysts in the skin, where it develops into a zoosporangium. The zoosporangium then produces and releases zoospores, which start a new infection cycle at a different site on the skin. The outcome of infection varies between species and even among populations in a species. In most frogs, infection of the tadpole is not lethal, whereas infection of adults can lead to death within weeks.

B. dendrobatidis infection has had a devastating effect on California's mountain yellow-legged frog population (a species complex consisting of *Rana muscosa* and *Rana sierrae*), which has disappeared from 93% of its Sierra Nevada mountain lake habitat. In the first of the two studies, Vredenburg *et al.* used quantitative PCR to monitor the spread of *B. dendrobatidis* into previously uninfected frog populations in three lake basins. After the fungus was first detected, the infection spread to almost all of the frog populations in each basin in 1–5 years, and frog numbers rapidly declined, with extinction occurring in most populations. The decline in frog numbers was not evident until a threshold value of 10,000 zoospore equivalents per swab had been reached, indicating that infection intensity probably has a primary role in driving population extinction.

In the second study, Briggs *et al.* used a mark-and-recapture approach to investigate three sites in which persistent *B. dendrobatidis* infection had decreased the frog population without causing extinction. They observed that frogs at these sites frequently lost and gained *B. dendrobatidis* infections and that the average infection intensity (220 zoospore equivalents per swab) was much lower than that observed by Vredenburg *et al.* in the rapidly declining populations. Such loss and gain of infection suggests that there is a fine balance between infected and uninfected states, such that the rate of infection by zoospores must equal the rate of zoosporangia loss to maintain the pathogen population and must exceed the rate of zoosporangia loss to allow the pathogen population to grow. The authors developed a model that suggests that long-term persistence of the pathogen is probably achieved by the presence of a reservoir of zoospores that can maintain pathogen levels when the frog population declines. Curiously, it may be the infected tadpoles that provide the source of zoospores which prevent the normal boom-and-bust cycle, allowing the pathogen to persist in these sites.

Taken together, these papers suggest that the intensity of *B. dendrobatidis* infection may be the key difference between frog populations heading for extinction and those that survive, a fact that might be exploited in strategies aiming to halt the spread of this devastating disease.

Andrew Jermy

ORIGINAL RESEARCH PAPER Vredenburg, V. T., Knapp, R. A., Tunstall, T. S. & Briggs, C. J. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proc. Natl Acad. Sci. USA*, 10 May 2010 (doi:10.1073/pnas.0914111107) | Briggs, C. J., Knapp, R. A. & Vredenburg, V. T. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc. Natl Acad. Sci. USA*, 10 May 2010 (doi:10.1073/pnas.0912886107)