



## Short communication

## Disease dynamics vary spatially and temporally in a North American amphibian

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## ABSTRACT

Local environmental conditions are a primary factor influencing chytridiomycosis, an emerging disease caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) that has affected over 200 amphibian species worldwide. In North America, seasonality and regional habitat differences predict considerable spatial and temporal disease variability, yet the sparse and opportunistic nature of most studies have provided insufficient data for understanding regional *Bd* epidemiology. We present a five-year field study that reveals spatial and temporal *Bd* dynamics across *Lithobates yavapaiensis* populations in Arizona, USA. Two populations showed no *Bd* infection or mortality, ten populations showed winter *Bd* infection, and five populations experienced winter mortality. Infection intensity decreased over winter sampling seasons, whereas mortality and infection prevalence did not change over time. Frogs dying from chytridiomycosis were significantly larger and had significantly higher infection intensities than survivors. We conclude that conserving *L. yavapaiensis* and other native amphibians requires protection and management of riparian ecosystems to promote populations large enough to survive winter cycles of chytridiomycosis.

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## 1. Introduction

*Batrachochytrium dendrobatidis* (*Bd*) is a fungal pathogen that has caused declines or extinction in over 200 amphibian species worldwide (Lips et al., 2006; Stuart et al., 2004). The consequences of *Bd* infection vary tremendously with local climatic conditions (Crawford et al., 2010; Kriger and Hero, 2007; Longo et al., 2010). In upland tropical regions that experience little temperature variation across seasons, *Bd* infection often causes rapid declines in host amphibians from epidemics of the skin disease chytridiomycosis (Berger et al., 1998; La Marca et al., 2005). In contrast, amphibians in temperate regions with larger seasonal temperature fluctuations show variable responses to *Bd*, ranging from no observed effects (Garner et al., 2006; Longcore et al., 2007), to sub-lethal fitness costs (Retallick and Miera, 2007), to episodic chytridiomycosis outbreaks and concomitant population declines (Kriger and Hero, 2006; McDonald et al., 2005). These punctuated chytridiomycosis outbreaks usually occur in cool months, suggesting that cooler temperatures promote *Bd* growth, host susceptibility, or both.

With the exception of a few well-studied regions in the Sierra Nevada mountains (Briggs et al., 2010; Vredenburg et al., 2010), studies of local climatic determinants of chytridiomycosis in North

America are patchy and conflicting. One retrospective study of North American museum specimens detected lower *Bd* prevalence in warmer months (Oullet et al., 2005), while a similar contemporary study (Green et al., 2002) found chytridiomycosis outbreaks beginning in warm and cool months. In Canada, *Lithobates pipiens* *Bd* prevalence decreased as mean monthly temperatures increased (Voordouw et al., 2010), but survival did not differ among infected and uninfected individuals. In Mexico, colder temperatures were associated with higher chytridiomycosis severity in four frog species (Hale et al., 2005). In contrast, Lips et al. (2004) documented chytridiomycosis mortalities in upland regions of Mexico during summer. These inconsistent patterns highlight the need to clarify the relationship between *Bd* infection, climate, local environmental conditions, and development of chytridiomycosis in North American amphibians.

Here, we present a five-year field study of *Bd* infection dynamics in the lowland leopard frog (*Lithobates* [*Rana*] *yavapaiensis*), a stream-dwelling species inhabiting southwestern desert regions that has experienced chytridiomycosis die-offs since at least 1992 (Bradley et al., 2002). Documented chytridiomycosis outbreaks in *L. yavapaiensis* occur only in cooler months, but population surveys have been opportunistic (Sredl, 2003). We conducted standardized, repeated surveys of twelve populations and measured *Bd* prevalence, intensity, and mortality in summer and winter. We used these data to determine whether (1) *Bd* infection and mortality vary among populations; (2) *Bd* infection and mortality vary seasonally; (3) *Bd* infection and mortality vary across

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years; and (4) *Bd* infection intensity, prevalence, and mortality co-vary within and across seasons, years and populations. Finally, we exploited the unique thermal dynamics in one region of Arizona to directly evaluate the relationship between water temperature, *Bd* infection, and chytridiomycosis.

## 2. Methods

We surveyed twelve *L. yavapaiensis* population localities in Arizona, USA, in summer (July–August) and winter (January–February) from 2006 to 2010 (Fig. S1). All populations were surveyed within three weeks to limit within-season variation due to local environmental conditions. We gave equal survey time and effort across seasons, years, and localities, and used diurnal visual encounter surveys (Crump and Scott, 1994) to measure the abundance of adult and sub-adult *L. yavapaiensis* individuals. We toe-clipped and swabbed the epidermis of each individual using sterile fine-tip swabs (Medical Wire & Equipment Co. MW113) following standardized protocols (Hyatt et al., 2007). Frogs were handled with unused latex gloves to prevent pathogen transmission. Recapture rates were low (mean = 0.4%), thus we treated each sampling season as independent.

We used a Taylor 9842 temperature logger to record water temperatures. Within each locality, we took three mid-afternoon readings and retained the maximum recorded temperature. We separated Muleshoe Ranch (MR) into three sub-regions based on mean temperature variation across microhabitats: (1) MR<sub>HS</sub> is a thermal spring >50 °C at the source and >30 °C throughout the frog sampling region, (2) MR<sub>SS</sub> is a pond 0.5 km away fed by a thermal spring >30 °C at the source and 20–30 °C throughout the frog sampling region, and (3) MR<sub>BC</sub> is a canyon stream 1.4 km away that has average winter water temperatures of 10 °C. To evaluate the role of water temperature, we separated frog populations into thermal spring localities (MR<sub>HS</sub> and MR<sub>SS</sub>) and non-thermal spring localities (all other populations).

Eight of our 12 localities are Nature Conservancy properties with continual monitoring since the 1980s by on-site staff (MR<sub>SS</sub>, MR<sub>HS</sub>, MR<sub>BC</sub>, AC, HR), or sites that have been surveyed by Tucson Herpetological Society volunteers since the 1990s (AS, TV, CIC; Table S1). For each dead and dying frog, we recorded the date of death as the first date that our team, a volunteer, or a site manager observed the mortality event. We collected individuals with signs of chytridiomycosis (i.e., skin redness, lethargy, failure to seek cover, and loss of righting ability) for overnight observation; if death occurred within 24 h and the individual tested positive for *Bd*, we categorized these frogs as chytridiomycosis mortalities. Individuals found dead during winter without other apparent causes of death (i.e., predation or injury) and testing positive for *Bd* were also considered chytridiomycosis mortalities.

We extracted DNA from swabs using PrepMan Ultra (Applied Biosystems; Hyatt et al., 2007). Infection prevalence and intensity were determined using quantitative PCR (Boyle et al., 2004). Infection intensity was measured as the number of zoospore equivalents per swab, and individuals were considered *Bd*-positive at  $\geq 1$  zoospore equivalents. Values were not obtained for the January 2007 season. We calculated *Bd* infection as the number of positives divided by the total number of frogs swabbed and *Bd* mortality as the number of dead frogs divided by the total number of frogs observed. We calculated 95% Clopper–Pearson binomial confidence intervals from observed proportions of infection and mortality. We compared *Bd* infection, chytridiomycosis mortality, numbers of frogs, and water temperatures using two-tailed paired sample Wilcoxon signed-rank tests and rank sum tests. We compared *Bd* infection intensity among dead, dying and asymptomatic individuals using two-tailed Student's *t*-tests assuming unequal variances.

## 3. Results

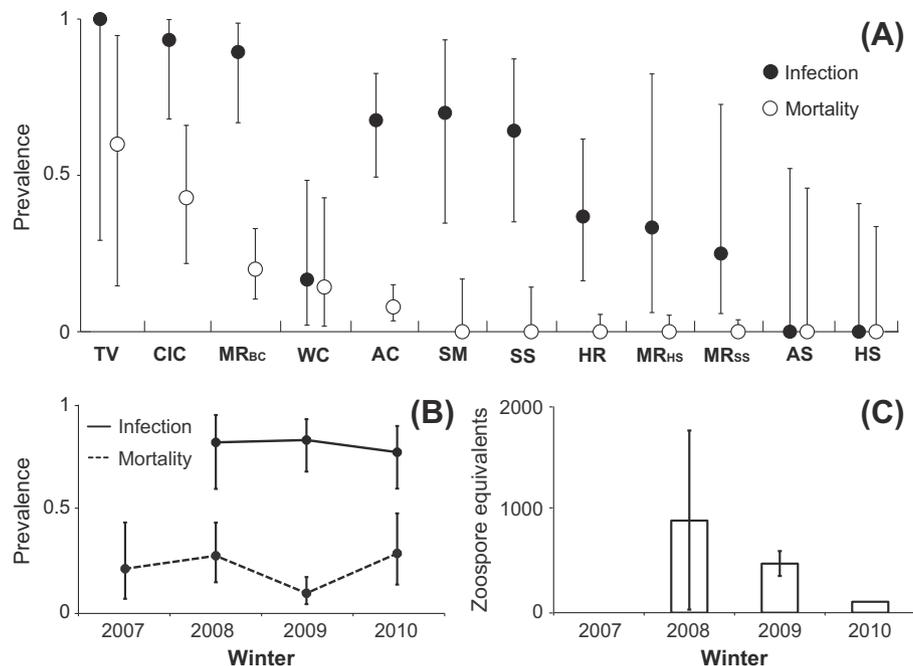
We sampled 692 *L. yavapaiensis* individuals from 12 localities in Arizona, USA, with a mean pairwise population distance of 198 km (Table S1). No mortality and low *Bd* prevalence (1.6%) occurred in summer. In contrast, all populations were infected with *Bd* in winter except for House Spring (HS) and Aliso Spring (AS; Fig. 1A), populations separated by 265 km. Of the ten infected populations, five experienced winter mortalities, and observed levels of *Bd* infection and mortality varied among localities (Fig. 1A). *Bd* infection ( $P = 0.005$ ) and mortality ( $P = 0.02$ ) were significantly lower in summer compared to winter. *Bd* infection was significantly higher than mortality in summer ( $P = 0.03$ ) and winter ( $P = 0.008$ ).

Winter *Bd* infection prevalence did not significantly change across sampling years, nor did winter *Bd* mortality (Fig. 1B). In contrast, infection intensity significantly decreased from 2008 to 2010 ( $P = 0.02$ ; Fig. 1C). Across all years, *Bd* infection intensity limits were 0–4040 zoospore equivalents among the 139 individuals sampled in winter months. Notably, we detected little *Bd* on individuals found dead; mean infection intensity was 1280.6 for dying individuals, 276.3 for healthy individuals, and 15.6 for individuals found dead. Dead individuals laid out for a mean of 5.3 days (range: 0–16) without predation before we collected them. Individuals found alive but showing signs of chytridiomycosis all died within 24 h, and these moribund individuals had significantly higher mean infection intensity compared to individuals found dead ( $P = 0.02$ ). Individuals sampled from populations with compared to without mortality did not differ in mean infection intensity ( $P = 0.89$ ). Among individuals sampled in winter, mean body mass was significantly higher for dead (28.5 g) and dying (33.7 g) individuals compared to asymptomatic individuals (18.2 g;  $P = 0.01$ ). *Bd* infection prevalence was highly seasonal, increasing in winter and decreasing in summer for all populations across the five-year sampling period (Fig. 2A). Across all localities, the mean number of frogs observed was significantly higher in summers compared to winters ( $P = 0.001$ ). At Tanque Verde Canyon (TV), the locality with the highest infection and mortality prevalence (Fig. 1A), we did not observe any frogs after summer 2009.

We examined the effect of water temperature on *Bd* infection and chytridiomycosis mortality by comparing thermal springs to other localities. Among non-thermal spring localities, maximum water temperature was significantly higher in summer versus winter ( $P = 0.002$ ), whereas maximum water temperature at thermal springs did not differ significantly in summer versus winter ( $P = 0.18$ ; Fig. 2B). In summer, maximum water temperature was not significantly different at thermal versus non-thermal spring sites ( $P = 0.166$ ). In contrast, maximum winter water temperature was significantly higher at thermal versus non-thermal spring sites ( $P = 0.03$ ; Fig. 2B). Concordantly, chytridiomycosis mortality was significantly higher at non-thermal versus thermal spring localities in winter ( $P = 0.05$ ). However, *Bd* infection did not differ significantly at non-thermal spring versus thermal spring localities in winter ( $P = 0.28$ ) or summer ( $P = 0.39$ ).

## 4. Discussion

Seasonal and spatial variation in disease prevalence are common in human and wildlife systems (Hosseini et al., 2004; Pascual and Dobson, 2005) and can arise from numerous factors, such as pathogen thermal requirements (Van Riper et al., 1986), host immunity changes over seasons or temperatures (Cheng et al., 2009), and variable host population genetic diversity (Pearman and Garner, 2005). However, *Bd* modeling suggests that intraspecific variation in infection outcomes do not require differences in susceptibility, virulence, or environment, but can instead result



**Fig. 1.** (A) Observed prevalence of winter *Lithobates yavapaiensis* Bd infection (closed symbols) and chytridiomycosis mortality (open symbols) across populations and years, with 95% Clopper–Pearson binomial confidence intervals indicated. Infection prevalence was measured as the proportion of swabbed individuals that harbored *Bd* infections, and chytridiomycosis mortality prevalence was measured as the proportion of frogs observed in winter that died with signs of chytridiomycosis. (B) Winter *Lithobates yavapaiensis* mean *Bd* infection prevalence (solid line) and chytridiomycosis mortality prevalence (dashed line) across sampling winters. Error bars show 95% Clopper–Pearson binomial confidence intervals. (C) Mean *Bd* infection intensity (measured as zoospore genome equivalents) across sampling winters. Error bars show standard deviations.

from epidemic versus endemic dynamics of the same host–pathogen interaction (Briggs et al., 2010). Understanding the intraspecific dynamics of *Bd* in *L. yavapaiensis*, a species that continues to persist despite high chytridiomycosis susceptibility, may provide critical knowledge for the recovery of other, more susceptible species.

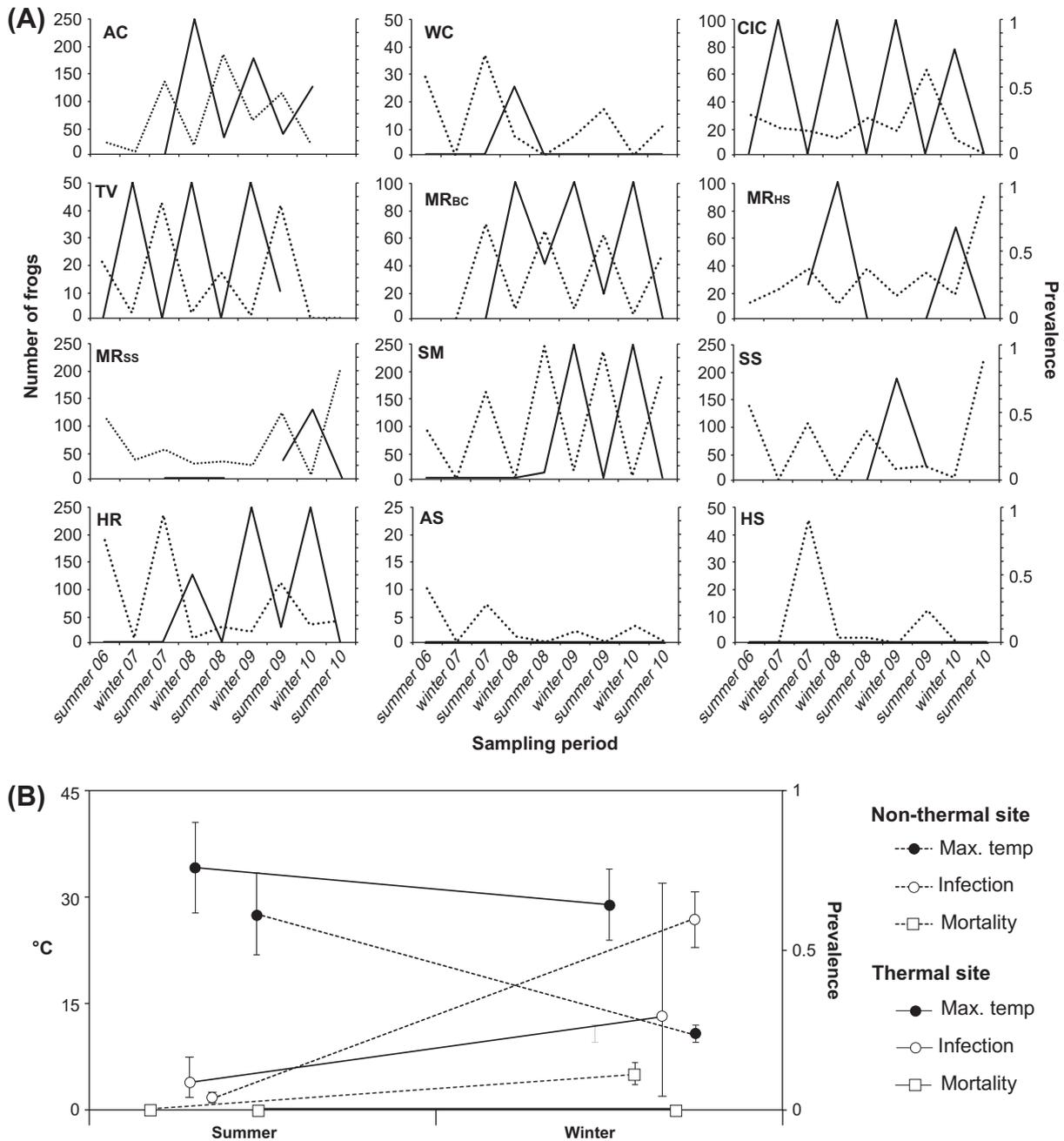
The higher infection and disease prevalence in cooler seasons we detected in *L. yavapaiensis* is consistent with other studies of *Bd* in natural amphibian populations (Berger et al., 2004; Kriger and Hero, 2006). Schlaepfer and colleagues (2007) estimated *Bd* infection prevalence in Arizona *L. yavapaiensis* from March through October and found no seasonal variation, no infection at three localities, and concluded *Bd* was likely excluded from these localities. We sampled two of the same three localities (MR<sub>HS</sub> and HR) and found 33% and 37% winter *Bd* prevalence, respectively. This finding highlights the need for sampling protocols to cover the range of seasons and temperatures to assure that prevalence estimates are not biased.

Our study is the first to report *Bd* infection intensity in an amphibian of the southwestern United States, and we recovered two surprising patterns. First, we found that chytridiomycosis susceptible individuals were significantly larger and had significantly higher infection intensities than individuals that survived winters, contradicting other studies of *Bd* across age and size classes. For example, juvenile *Dendrobates tinctorius* were more likely to die from chytridiomycosis than adults (Lamirande and Nichols, 2002), lower mass was associated with higher mortality in *Alytes obstetricans* (Garner et al., 2009), and metamorphs had significantly higher fungal loads than adults in *Lithobates sierrae* (Briggs et al., 2010). Very few *L. yavapaiensis* juveniles are captured in winter, thus our demographic infection patterns may result from the seasonal comparison of different sizes of adult frogs. In contrast, we saw no difference in infection between juveniles and adults during summer, thus we can exclude the possibility that juveniles emerging in the summer uninfected was skewing our summer prevalence

estimates. The second surprising pattern was that we found low *Bd* intensities on frogs found dead, whereas dying frogs swabbed within 24 h of death had the highest *Bd* loads. This pattern suggests that other microbes displace *Bd* or that *Bd* zoospores disperse from frog carcasses after death, but to our knowledge these phenomena are undocumented in the literature. Alternately, some of the dead frogs we found may have died from other causes; however, the fact that they were laying out in the open without signs of predation at localities where other frogs were dying of chytridiomycosis makes this explanation unlikely.

We found invariant *Bd* dynamics at thermal spring sites, where water perennially averages  $>30^{\circ}\text{C}$ , suggesting that seasonal chytridiomycosis differences at all other Arizona localities result from cooler winter water temperatures. Analysis of disease dynamics throughout Australia found strong negative effects on *Bd* when air temperatures averaged  $>30^{\circ}\text{C}$  (Drew et al., 2006), laboratory studies identify  $30^{\circ}\text{C}$  as the upper threshold for *Bd* viability (Piotrowski et al., 2004), and water temperature was negatively correlated with *Bd* infection intensity in North American newt populations (Raffel et al., 2011). Our study corroborates that temperature contributes to *Bd* infection outcomes, but we cannot confirm whether this pattern results from effects on the host, the pathogen, or both. Host immunity may play a role, as amphibian immune defenses decrease with temperature (Raffel et al., 2006) independent of season (Jozkovicz and Plytycz, 1998), and in the laboratory, innate immune defenses against *Bd* occur at  $26^{\circ}\text{C}$  but not  $18^{\circ}\text{C}$  (Ribas et al., 2009). What remains uncertain is the relative importance of increased pathogen virulence compared to decreased host immune defenses under cooler environmental conditions.

In the United States, *Bd* infects over 50 amphibian species in the Southeast (Chatfield et al., 2009), Northeast (Hossack et al., 2010), Northwest (Pearl et al., 2007), and Rocky Mountains (Muths et al., 2008) with no reports of chytridiomycosis outbreaks. However, if die-offs occur during winter months when temperate-zone



**Fig. 2.** (A) Observed number of frogs (dashed line) and *Bd* infection prevalence (solid line) within each *Lithobates yavapaiensis* population in summer and winter from 2006 through 2010. (B) Mean maximum water temperature (filled circles), *Bd* infection prevalence (open circles), and chytridiomycosis mortality prevalence (open squares) for thermal spring (solid line; N = 2) and non-thermal spring (dashed line; N = 10) populations in winter versus summer.

amphibians are inconspicuous and rarely monitored, chytridiomycosis declines may have gone unnoticed. The extreme seasonality of chytridiomycosis observed in *L. yavapaiensis* suggests that similar *Bd* dynamics may be occurring throughout North America. Consequently, better monitoring efforts should be in place for temperate-zone amphibians that have experienced enigmatic declines, such as *Lithobates pipiens* (Lannoo, 2005).

Amphibians of southwestern North America face a multitude of environmental threats (Witte et al., 2008). In Arizona and Mexico at least 13 native species are infected with *Bd* (Hale et al., 2005; Sredl, 2003), and 36% of 324 known ranid frog populations became extirpated from Arizona during 1986–2001 (Witte et al., 2008). We focused on *L. yavapaiensis* because it remains widespread compared to congeners such as *L. chiricahuensis*, which has declined

precipitously (Clarkson and Rorabaugh, 1989), and *L. tarahumarae*, which has been extirpated from the United States (Hale and Jarchow, 1988). However, chytridiomycosis continues to exert a toll on *L. yavapaiensis* populations, and habitat destruction and introductions of non-native animals present additional threats (Witte et al., 2008). Our study population with the highest measures of winter mortality (TV) was possibly extirpated, highlighting the fragility of these populations in the face of disease. *Bd* infects numerous amphibian species and may survive in temperate aquatic systems outside of amphibian hosts (Walker et al., 2007) making pathogen eradication a difficult solution. Management for *Bd* should thus be added to eliminating other threats, such as bullfrogs, crayfish, non-native fish, and land use practices that increase erosion and alter pools required by *L. yavapaiensis* (Wallace et al.,

2010), in order to promote populations large enough to persist through winter cycles of chytridiomycosis.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2011.03.018.

## References

- Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocumbe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Nat. Acad. Sci. USA* 95, 9031–9036.
- Berger, L., Speare, R., Hines, H.B., Marantelli, G., Hyatt, A.D., McDonald, K.R., Skerratt, L.F., Olsen, V., Clarke, J.M., Gillespie, G., Mahony, M., Sheppard, N., Williams, C., Tyler, M.J., 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Aust. Vet. J.* 82, 31–36.
- Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T., Hyatt, A.D., 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Dis. Aquat. Org.* 60, 141–148.
- Briggs, C.J., Knapp, R.A., Vredenburg, V.T., 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *Proc. Nat. Acad. Sci. USA* 107, 9695–9700.
- Bradley, G.A., Rosen, P.C., Sredl, M.J., Jones, T.R., Longcore, J.E., 2002. Chytridiomycosis in native Arizona frogs. *J. Wildl. Dis.* 38, 206–212.
- Chatfield, M.W., Rothermel, B.B., Brooks, C.S., Kay, J.B., 2009. Detection of *Batrachochytrium dendrobatidis* in amphibians from the Great Smoky Mountains of North Carolina and Tennessee. *USA. Herp. Rev.* 40, 176–179.
- Cheng, A.C., Cheng, S.A., Chen, Y.Y., Chen, J.C., 2009. Effects of temperature change on the innate cellular and humoral immune responses of orange-spotted grouper *Epinephelus coioides* and its susceptibility to *Vibrio alginolyticus*. *Fish Shellfish Immunol.* 26, 768–772.
- Clarkson, R.W., Rorabaugh, J.C., 1989. Status of leopard frogs (*Rana pipiens* complex: Ranidae) in Arizona and Southeastern California. *Southwest. Nat.* 34, 531–538.
- Crawford, A.J., Lips, K.R., Bermingham, E., 2010. Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *Proc. Natl. Acad. Sci. USA* 107, 13777–13782.
- Crump, M.L., Scott, N.J., 1994. Visual encounter surveys. In: Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C., Foster, M.S. (Eds.), *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Smithsonian Institution Press, Washington, DC, pp. 84–92.
- Drew, A., Allen, E.J., Allen, L.J.S., 2006. Analysis of climatic and geographic factors affecting the presence of chytridiomycosis in Australia. *Dis. Aquat. Org.* 68, 245–250.
- Garner, T.W.J., Walker, S., Bosch, J., Leech, S., Rowcliffe, J.M., Cunningham, A.A., Fisher, M.C., 2009. Life history trade-offs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. *Oikos* 118, 783–791.
- Garner, T.W.J., Perkins, M., Govindarajulu, P., Seglie, D., Walker, S.J., Cunningham, A.A., Fisher, M.C., 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. *Biol. Lett.* 2, 455–459.
- Green, D.E., Converse, K.A., Schrader, A.K., 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. *Ann. NY Acad. Sci.* 969, 323–339.
- Hale, S.F., Rosen, P.C., Jarchow, J.L., Bradley, G.A., 2005. Effects of the chytrid fungus on the Tarahumara frog (*Rana tarahumarae*) in Arizona and Sonora, Mexico. In: *USDA Forest Service Proceedings RMRS-P-36*, pp. 407–411.
- Hale, S.F., Jarchow, R.J.D., 1988. The status of the Tarahumara frog (*Rana tarahumarae*) in the United States and Mexico Part II. Arizona Game and Fish Department and Region. Office of Endangered Species. U.S. Fish & Wildlife Service.
- Hossack, B.R., Adams, M.J., Campbell Grant, E.H., Pearl, C.A., Bettaso, J.B., Barichivich, W.J., Lowe, W.H., True, K., Ware, J.L., Corn, P.S., 2010. Low prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*) in amphibians of U.S. headwater streams. *J. Herp.* 44, 253–260.
- Hosseini, P.R., Dhondt, A.A., Dobson, A., 2004. Seasonality and wildlife disease: how seasonal birth, aggregation and variation in immunity affect the dynamics of *Mycoplasma gallisepticum* in house finches. *Proc. Roy. Soc. Lond. B* 271, 2569–2577.
- Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, R., Gleason, F., Colling, 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Dis. Aquat. Org.* 73, 175–192.
- Jozkovicz, A., Plytycz, B., 1998. Temperature but not season affects the transplantation immunity of anuran amphibians. *J. Exp. Zool.* 28, 58–64.
- Kruger, K.M., Hero, J.M., 2006. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *J. Zool.* 271, 352–359.
- Kruger, K.M., Hero, J.M., 2007. The chytrid fungus *Batrachochytrium dendrobatidis* is non-randomly distributed across amphibian breeding habitats. *Divers. Distrib.* 13, 781–788.
- La Marca, E. et al., 2005. Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37, 190–201.
- Lamirande, E.W., Nichols, D.K., 2002. Effects of host age on susceptibility to cutaneous chytridiomycosis in blue and yellow poison dart frogs (*Dendrobates tinctorius*). In: McKinnell, R.G., Carlson, D.L. (Eds.), *Proceedings of the Sixth International Symposium on the Pathology of Reptiles and Amphibians*. Saint Paul, Minnesota, pp. 3–13.
- Lannoo, M. (Ed.), 2005. *Amphibian Declines: The Conservation Status of United States Species*. University of California Press, Berkeley, California.
- Lips, K.R., Mendelson III, J.R., Muñoz-Alonso, A., Canseco-Márquez, L., Mulcahy, D.G., 2004. Amphibian population declines in montane Southern Mexico: resurveys of historical localities. *Biol. Conserv.* 119, 555–564.
- Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P., Collins, J.P., 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc. Nat. Acad. Sci. USA* 103, 3165–3170.
- Longcore, J.R., Longcore, J.E., Pessier, A.P., Halteman, W.A., 2007. Chytridiomycosis widespread in anurans of Northeastern United States. *J. Wildl. Manage.* 71, 435–444.
- Longo, A.V., Burrows, P.A., Jogle, R.L., 2010. Seasonality of *Batrachochytrium dendrobatidis* infection in direct-developing frogs suggests a mechanism for persistence. *Dis. Aquat. Org.* 92, 253–260.
- McDonald, K.R., Mendez, D., Muller, R., Freeman, A.B., Speare, R., 2005. Decline in the prevalence of chytridiomycosis in frog populations in North Queensland. *Australia. Pac. Conserv. Biol.* 11, 114–120.
- Muths, E., Pilliod, D.S., Livo, L.J., 2008. Distribution and environmental limitations of an amphibian pathogen in the rocky mountains. *USA. Biol. Conserv.* 141, 1484–1492.
- Oullet, M., Mikaelian, I., Pauli, B.D., Rodrigue, J., Green, D.M., 2005. Historical evidence of widespread chytrid infection in North American amphibian populations. *Conserv. Biol.* 19, 1431–1440.
- Pascual, M., Dobson, A., 2005. Seasonal patterns of infectious diseases. *PLOS Med.* 2, 18–20.
- Piotrowski, J.S., Annis, S.L., Longcore, J.E., 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96, 9–15.
- Pearl, C.A., Bull, E.L., Green, D.E., Bowerman, J., Adams, M.J., Hyatt, A., Wente, W.H., 2007. Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. *J. Herp.* 41, 145–149.
- Pearman, P.B., Garner, T.W.J., 2005. Susceptibility of Italian agile frog populations to an emerging strain of Ranavirus parallels population genetic diversity. *Ecol. Lett.* 8, 401–408.
- Raffel, T.R., Michel, P.J., Sites, E.W., Rohr, J.R., 2011. What drives chytrid infections in new populations? Associations with substrate, temperature, and shade. *EcoHealth*. doi:10.1007/s10393-010-0358-2.
- Raffel, T.R., Rohr, J.R., Kiesecker, J.M., Hudson, P.J., 2006. Negative effects of changing temperature on amphibian immunity under field conditions. *Funct. Ecol.* 20, 819–828.
- Retallick, R.W.R., Miera, V., 2007. Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. *Dis. Aquat. Org.* 75, 201–207.
- Ribas, L., Li, M.S., Doddington, B.J., Robert, J., Seidel, J.A., Kroll, J.S., Zimmerman, L.B., Grassly, N.C., Garner, T.W., Fisher, M.C., 2009. Expression profiling the temperature-dependent amphibian response to infection by *Batrachochytrium dendrobatidis*. *PLoS ONE* 4, e8408.
- Schlaepfer, M.A., Sredl, M.J., Rosen, P.C., Ryan, M.J., 2007. High prevalence of *Batrachochytrium dendrobatidis* in wild populations of lowland leopard frogs *Rana yavapaiensis* in Arizona. *EcoHealth* 4, 421–427.
- Sredl, M.J., 2003. Understanding and mitigating effects of chytrid fungus to amphibian populations in Arizona. Technical Report No. 208. In: Sredl, M.J. (Ed.), *Ranid frog conservation and management, Nongame and Endangered Wildlife Program*. Arizona Game and Fish Department, Phoenix, Arizona, pp. 1–20.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786.
- Van Riper, C., Van Riper, S.G., Goff, L.M., Laird, M., 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecol. Monogr.* 56, 327–344.
- Voordouw, M.J., Adama, D., Houston, B., Govindarajulu, P., Robinson, J., 2010. Prevalence of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, in an endangered population of northern leopard frogs, *Rana pipiens*. *BMC Ecol.* 10, 6.
- Vredenburg, V.T., Roland, A., Tate, S., Briggs, C.J., 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proc. Nat. Acad. Sci. USA* 107, 9689–9694.
- Walker, S.F., Salas, M.B., Jenkins, D., Garner, T.W.J., Cunningham, A.A., Hyatt, A.D., Bosch, J., Fisher, M.C., 2007. Environmental detection of *Batrachochytrium dendrobatidis* in a temperate climate. *Dis. Aquat. Org.* 77, 105–112.

- Wallace, J.E., Steidl, R.J., Swann, D.E., 2010. Habitat characteristics of lowland leopard frogs in mountain canyons of Southeastern Arizona. *J. Wild. Manage.* 74, 808–815.
- Witte, C.L., Sredl, M.J., Kane, A.S., Hungerford, L.L., 2008. Epidemiologic analysis of factors associated with local disappearances of native ranid frogs in Arizona. *Conserv. Biol.* 22, 375–383.