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HIGH PREVALENCE AND SEASONAL PERSISTENCE OF AMPHIBIAN CHYTRID FUNGUS INFECTIONS IN THE DESERT-DWELLING AMARGOSA TOAD, *ANAXYRUS NELSONI*

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***Abstract.*—Hot summer conditions in the Mojave Desert of southern Nevada, USA, would not appear to favor the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which has a thermal limit around 28° C. Within this region, however, we detected *Bd* in an amphibian of conservation concern, the Amargosa Toad (*Anaxyrus nelsoni*). We assessed *Bd* prevalence and infection intensity (*Bd* load) across spring and summer in *A. nelsoni* at two sites over two years, and in the sympatric American Bullfrog, *Lithobates catesbeianus*, at one site during one year. We observed high overall *Bd* prevalence in both *A. nelsoni* (48%) and *L. catesbeianus* (74%), with *Bd* loads in *A. nelsoni* reaching 404,000 copies of ribosomal RNA internal transcribed spacer 1 (ITS 1 copy number; CN). Prevalence remained high in mid-summer when air temperatures during sampling were 23.7–32.5° C and daily highs reached 36.1–37.8° C. We observed trends toward lower *Bd* prevalence in *A. nelsoni* during late summer, but even then infection prevalence was at least 26%, with *Bd* loads reaching 69,100 CN. The high levels of infection during summer months may be explained by the actual conditions experienced by these amphibians. Water temperatures associated with captures (13.2–27.8° C) and body temperatures of *A. nelsoni* (11.1–26.6° C) remained predominately favorable to *Bd* throughout summer sampling periods. The mostly nocturnal behavior of *A. nelsoni* also likely limited temperature extremes. Recapture data showed that *A. nelsoni* can clear *Bd* infection, and we observed no individuals with obvious symptoms of disease, indicating potential resistance to, or tolerance of, the pathogen in this species.**

Key Words.—Batrachochytrium dendrobatidis; Bufo; chytridiomycosis; disease; Mojave Desert; pathogen

INTRODUCTION

The Amargosa Toad, *Anaxyrus nelsoni* (= *Bufo nelsoni*; Stejneger 1893), is a narrowly distributed species confined to the Amargosa River and nearby spring systems within Oasis Valley in southern Nevada, USA (Altig and Dodd 1987; Goebel et al. 2005). Currently, *A. nelsoni* is the focus of a voluntary conservation agreement and strategy among local, state, and federal entities (Nevada Department of Wildlife. 2000. Conservation agreement for the Amargosa Toad [*Bufo nelsoni*] and co-occurring sensitive species in Oasis Valley, Nye County, Nevada. Nevada Department of Wildlife, Reno, Nevada, USA). This toad has long been of conservation concern and was considered a candidate species for listing under the Endangered Species Act (ESA) as early as 1977 (Goebel et al. 2005). In 1994 and again in 2008, the U.S. Fish and Wildlife Service was petitioned to emergency list *A. nelsoni* under the ESA, but in both cases the perceived threats were considered insufficient to warrant listing (U.S. Fish and Wildlife Servive 1996, 2010). Importantly, amphibian chytridiomycosis, an emergent disease caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*; Skerrat et al. 2007), was not considered a substantial threat informing these decisions, despite detection of *Bd* in American Bullfrog, *Lithobates catesbeianus* (= *Rana catesbeiana*) from the Oasis Valley in 2005 (D. E. Green. 2015. Global *Bd*-mapping project. Available from http://www.bd-maps.net [Accessed 13 January 2015]). Introduced *L. catesbeianus* co-occurs with *A. nelsoni* and can act as a vector for *Bd* (Daszak et al. 2004; Garner et al. 2006). Given the potential negative impacts from chytridiomycosis, we initiated an assessment of *Bd* infection prevalence in *A. nelsoni* and *L. catesbeianus* at the site (Boiling Pot Ranch) in the Oasis Valley where *Bd* had been detected previously.

There were reasons to believe that *A. nelsoni* was susceptible to *Bd* infection and subsequent disease. The species is a member of the Western Toad (*Anaxyrus boreas*) species group (Goebel et al. 2009) and studies have shown that these toads are susceptible to chytridiomycosis (Carey et al. 2006). For example, declines in populations of *A. boreas* in the Rocky Mountains and *Anaxyrus canorus* (another member of

TABLE 1. Observed patterns of *Bd* infection by species (*Anaxyrus nelsoni*; *Lithobates catesbeianus*), sample site (Boiling Pot Ranch; Amargosa River), and date. When *A. nelsoni* were recaptured, only the data from the last capture were included. Average and median *Bd* loads (ITS 1 copy number) calculated using infected animals only.

Species	Site	n	Month/Year	Avg. Animal Temp. (° C)	Avg. Air Temp. (° C)	Infection Prevalence (%)	Avg. *Bd* Load	Median *Bd* Load	Max. *Bd* Load
A. nelsoni	Ranch	35	May 2011	11.3	14.7	0.43	21,078	680	208,000
	Ranch	60	July 2011	19.0	29.4	0.48	11,992	1,290	129,200
	Ranch	36	Sept. 2011	18.5	24.8	0.42	23,087	1,920	171,000
	Ranch	28	May 2012	13.8	20.5	0.61	1,236	71	14,400
	River	24	July 2011	20.0	23.7	0.63	29,103	1,320	404,000
	River	22	Sept. 2011	15.8	25.9	0.41	339	81	1,200
	River	24	May 2012	13.5	21.6	0.71	4,533	829	41,000
	River	27	Aug. 2012	22.9	30.8	0.26	10,518	1,150	69,100
L. catesbeianus	Ranch	27	May 2011	19.0	18.9	0.81	7,141	1,149	111,700
	Ranch	19	July 2011	20.5	32.1	0.63	13,160	1,864	106,600

the species group) in the Sierra Nevada have been attributed to *Bd* infections (Green and Kagarise Sherman 2001; Muths et al. 2003; Pilliod et al. 2010). More directly, chytridiomycosis was identified *post hoc* as the cause of death in captive *A. nelsoni*, although the causative agent was originally misidentified as "fungal-like protists" (Nichols et al. 1998). Our expectation, however, was that high ambient temperatures often present in the Oasis Valley would constrain *Bd*.

The Oasis Valley is at relatively low elevations (about 1000 m) in the Mojave Desert, where summer temperatures are generally high (average maximum in July about 37° C) and conditions are arid (average precipitation about 145 mm/year). Growth of *Bd* in culture occurs at temperatures between 4–25° C, but growth slows at higher temperatures, with an upper thermal limit ≥ 28° C (Piotrowski et al. 2004). Exposure to high temperatures (27–37° C) has been shown to limit pathogenicity or clear *Bd* infection in some amphibians (Berger et al. 2004; Chatfield and Richards-Zawacki 2011; Woodhams et al. 2003). Temperature is also strongly associated with the amphibian immune system (Rollins-Smith et al. 2011), and at warmer temperatures amphibians may be less susceptible to *Bd* (Raffel et al. 2006; Andre et al. 2008). Inducement of behavioral fever in response to *Bd* infection has also been shown in some amphibians, including captive *A. boreas* (Richards-Zawacki 2010; Murphy et al. 2011).

In habitats and regions where temperatures commonly occur above the thermal optimum for *Bd* growth, the pathogen should be absent or its abundance substantially limited (Piotrowski et al. 2004; Puschendorf et al. 2009). There is considerable support for this hypothesis in that the occurrence and impact of *Bd* tends to be limited in regions and during seasons that are characterized by high temperatures (Berger et al. 2004; Drew et al. 2006; Kriger et al. 2007; Savage et al. 2011; Whitfield et al. 2012). Elevated water temperature also appears to provide frogs refuge from *Bd* in the wild (Forrest and Schlaepfer 2011). Yet our initial sampling efforts in the Oasis Valley revealed surprisingly high prevalence of *Bd* infections in both of our targeted species. Consequently, we expanded our sampling to assess whether seasonal change towards higher temperatures would influence infection patterns, and to include a second population segment of *A. nelsoni* along the Amargosa River.

MATERIALS AND METHODS

We sampled *A. nelsoni* for *Bd* infection at two sites, Boiling Pot Ranch (36.998°N, 116.718°W) about 10 km north of Beatty, Nevada, USA, and along a short stretch of the Amargosa River within Beatty (36.907°N, 116.753°W). Our sampling periods represented spring, summer, and late summer. At Boiling Pot Ranch, we sampled 171 *A. nelsoni* over four events in May, July, and September 2011, and May 2012. At the Amargosa River, we sampled 111 *A. nelsoni* over four events in July and September 2011, and May and August 2012. We also sampled 46 *L. catesbeianus* at Boiling Pot Ranch during May and July 2011 (Table 1).

Our sample sites were in areas where the Nevada Department of Wildlife (NDOW) was conducting long-term monitoring of *A. nelsoni* and many of the adult toads that we sampled were previously PIT tagged (Destron Fearing Co., South St. Paul, Minnesota, USA). We scanned toads for tags at time of capture and, for unmarked individuals > 50 mm snout-to-vent length (SVL), we inserted an 8 or 12 mm PIT tag subcutaneously under the dorsal skin. In total, we recaptured 24 toads during subsequent sampling events (one toad was recaptured twice), thereby providing information on *Bd* infections in individuals over time.

We captured animals by hand using sterile techniques (e.g., Brem et al. 2007). We recorded ambient air temperature at the beginning of capture events, and used a digital pen thermometer (accuracy ± 0.4° C) to measure water temperature for animals captured in or near water. We obtained body temperatures of toads captured away from water within seconds of capture by pressing the thermometer tip against the body and under a folded rear leg. While this method may not be as accurate as taking internal temperatures (e.g., via the cloaca), we felt that it was less invasive, while providing acceptable measurements for our comparisons. We used sterile swabs (Whatman® Omni Swab, Medical Wire and Equipment Co., Corsham, Wiltshire, England) to sample skin for *Bd* from rear feet, ventral surface of thighs, and abdominal surface (Brem et al. 2007). We individually stored each swab in 70% ethanol at 4° C to ensure integrity of *Bd* DNA until testing. Following swabbing, we measured SVL and weight of each animal, and determined sex of adult animals.

We sent samples to a commercial laboratory (Pisces Molecular, Boulder, Colorado, USA) within one month of collection to assay for *Bd* using quantitative real-time polymerase chain reaction (qPCR). DNA extraction followed the protocol outlined by Forzán et al. (2010). The assay targeted a 97 base pair fragment of *Bd* ribosomal RNA internal transcribed spacer 1 (ITS1; Kirshtein et al. 2007) using a 45-cycle qPCR protocol on a Stratagene MX4000 Multiplex Quantitative PCR Cycler. Each reaction contained an internal positive control (TaqMan Exogenous Internal Positive Control, Life Technologies, Grand Island, New York, USA), with a standard curve developed by serial ten-fold dilutions of linearized plasmid DNA containing one copy of the *Bd* ribosomal RNA region (thus, representing a single copy of ITS1). Detection sensitivity of the assay was three target sequence molecules. The number of *Bd* ribosomal RNA copies varies by *Bd* strain (Longo et al. 2013) and we did not identify the strain present in our system; thus, we quantified infection intensity (*Bd* load) as the number of ITS1 copies detected in the sample.

For statistical analyses, we used SYSTAT (ver. 12, Systat Software Inc., San Jose, California, USA), with data examined for homogeneity of variances and normality prior to evaluation. We only used data from the last recapture event for toads captured more than once. We did not conduct an overall multi-factor analysis (i.e., multi-factor logistic regression or multi-factor analysis of covariance) because our sampling was non-orthogonal for various reasons associated with the *ad hoc* development of the study design. Instead, we parsed our data by site and then by year to create orthogonal combinations, and ran independent assessments for each potential explanatory variable. We used a modified false discovery rate controlling procedure when running multiple tests of related hypotheses to compensate for the potential compounding of Type I error (false positives), while minimizing over-correcting that might increase Type II error (false negatives). Following this procedure, we considered our overall significance criteria to be 0.01. We conducted a *post hoc* assessment of overall difference in *Bd* prevalence in *A. nelsoni* between sexes using a binomial test of data combined across sites and years.

We used independent logistic regressions to examine the effects of potential explanatory variables on *Bd* infection status (positive or negative), and calculated odds ratios to compare the relative chance of infection among different levels of each variable. Specifically, for *A. nelsoni*, we examined whether infection prevalence differed among months (May vs. July vs. August-September) and years (2011 vs. 2012) at each site, or whether it varied with air temperature, animal temperature (i.e., body temperature or water temperature at point of capture), sex, or SVL. For *L. catesbeianus*, we used logistic regressions to examine whether infection prevalence differed between collection season (May vs. July), or whether it varied with air temperature, water temperature (at point of capture), or SVL. We also examined local temperature patterns from the day of sampling, as well as average maximum and minimum temperatures for three days and one week prior to sampling, using data from Weather Underground (Available from http://www.wunderground.com [Accessed 13 January 2015]). A preliminary correlation analyses indicated that all these temperature data and those we collected in the field were highly correlated (data not shown); therefore, we only used animal and air temperatures from field measurements in subsequent analyses.

We used Kruskal Wallis tests to examine the effects of year (2011 vs. 2012) and month on median *Bd* load in *A. nelsoni*. We used independent linear regressions to examine the relationships between *Bd* load and animal temperature, air temperature, sex, and SVL. We conducted these analyses only on infected individuals after log transformation of the data to correct for skewness and unequal variances. For recaptured toads, we used a binomial test to examine whether the infection status changed between recaptures (setting infection prevalence at time of initial captures as the expected). We used a logistic regression to see if there was any relationship between *Bd* load at initial capture and whether toads cleared infections. We used a paired t-test to compare *Bd* loads of individuals between capture events. For *L. catesbeianus*, we compared median *Bd* loads between months (May vs. July) using a Kruskal Wallis test, and used independent linear regressions to assess the relationships between *Bd* load and water temperature, air temperature, sex, and SVL.

TABLE 2. Results from logistic regressions of various potential explanatory variables on *Bd* infection prevalence for *Anaxyrus nelsoni* at Boiling Pot Ranch and Amargosa River. *P*-values are uncorrected and boldface type denotes effects considered significant ($P \leq 0.01$) for that variable.

Site	Variable	Contrast	*P*-value	Odds Ratio
Ranch	Year	2011 vs. 2012	0.14	0.53
	Sex	Females vs. Males	0.31	0.65
	Animal Temp		0.38	1.03
	Air temp		0.89	1.00
	SVL		0.33	0.99
	Month (2011)	May vs. Sept.	0.64	1.33
		July vs. Sept.	0.44	1.53
		May vs. July	0.81	0.87
	Sex (2011)	Females vs. Males	0.36	0.64
	Animal Temp. (2011)		0.15	1.05
	Air Temp. (2011)		0.61	1.01
	SVL (2011)		0.14	0.99
	Sex (2012)	Females vs. Males	0.45	0.49
	Animal Temp. (2012)		0.99	1.00
	Air Temp. (2012)		0.70	1.35
	SVL (2012)		0.68	0.99
River	Year	2011 vs. 2012	0.62	1.23
	Sex	Females vs. Males	0.02	0.38
	Animal Temp.		0.04	0.91
	Air Temp.		**0.01**	0.82
	SVL		0.04	0.96
	Month (2011)	July vs. Sept.	0.15	2.41
	Sex (2011)	Females vs. Males	0.15	0.41
	Animal Temp. (2011)		0.76	1.03
	Air Temp. (2011)		0.15	0.67
	SVL (2011)		0.47	1.02
	Month (2012)	May vs. Aug.	**0.01**	6.94
	Sex (2012)	Females vs. Males	0.09	0.36
	Animal Temp. (2012)		**0.01**	0.84
	Air Temp. (2012)		**0.01**	0.81
	SVL (2012)		0.03	0.94

RESULTS

We observed high overall prevalence of *Bd* infection in both *A. nelsoni* and *L. catesbeianus* within the Oasis Valley. Specifically, 48% (124 of 256) of all *A. nelsoni* and 74% (34 of 46) of all *L. catesbeianus* sampled were *Bd* positive (Table 1). Qualitatively, there was some variation among seasons in overall *Bd* infection prevalence in *A. nelsoni* (Table 1), with high infection prevalence in May (56% across sites and years) and July (52% across sites and years), but lower prevalence late in the summer (36% across sites and years). We observed a higher prevalence of infection in *L. catesbeianus* in May (81%) than in July (63%).

We found no significant relationships between *Bd* prevalence in *A. nelsoni* at Boiling Pot Ranch and any of the potential explanatory variables considered (Table 2). Similarly, we found no significant relationships between *Bd* prevalence and these variables in *L. catesbeianus* at this site (Table 3). In contrast, along the Amargosa River, we found that overall infection prevalence in *A. nelsoni* was significantly related to air temperature, with increasing temperatures associated with lower infection prevalence (Table 2). Animal temperature showed a similar trend. The negative relationship between temperature and occurrence of *Bd* infection at the Amargosa River was primarily driven by low infection prevalence (26%) in August 2012 (Table 1; at that time we were no longer sampling at Boiling Pot Ranch). Infection prevalence at this site was significantly different between May and August 2012, and related to both air and animal temperatures (Table 2).

The median *Bd* load of infected animals was 862 ITS1 copies (copy number, CN) in *A. nelsoni* and 1,412 CN in *L. catesbeianus*. We observed 19 *A. nelsoni* and five *L. catesbeianus* with *Bd* loads > 10,000 CN, of which five *A. nelsoni* and two *L. catesbeianus* had loads > 100,000 CN (infection intensity has been associated with mortality in some species, including *A. boreas*; Carey et al. 2006; Vredenburg et al. 2010). We did not, however, observe clinical signs of disease in any of these infected animals, nor did we detect strong trends for *Bd* load among the potential explanatory variables assessed in either species. Neither air nor animal temperatures were

TABLE 3. Results from logistic regressions of potential explanatory variables on *Bd* infection prevalence for *Lithobates catesbeianus* collected at Boiling Pot Ranch.

Variable	Contrast	*P*-value	Odds Ratio
Month	May vs. July 2011	0.17	2.56
SVL		0.97	1.01
Water temp.		0.15	0.56
Air temp.		0.21	0.65

significantly related to *Bd* load in *A. nelsoni*, and there were no significant relationships between *Bd* load in *L. catesbeianus* and any of the variables assessed.

We observed a significant ($U_{15,17}$ = 63, P = 0.006) temporal difference in median *Bd* load in *A. nelsoni* at Boiling Point Ranch between May 2011 (*Md* = 680 CN) and May 2012 (*Md* = 71 CN). The sampling in 2011 occurred following several days of relatively cool weather (average maximum temperature on sampling date = 20.6° C), whereas sampling in 2012 followed a prolonged warm period (average maximum temperatures on sampling dates = 30.9° C). We did not, however, detect significant differences in air or animal temperatures between these sampling periods. Furthermore, median *Bd* load in *A. nelsoni* at the Amargosa River in May 2012 was not reflective of the low value observed at Boiling Pot Ranch at that time (*Md* = 829 vs. 71 CN, respectively; Table 1).

At the Amargosa River, *Bd* infection intensity was not significantly related to our potential explanatory variables, with the sole exception of a significant ($U_{15,9}$ = 115, P = 0.005) decrease in median *Bd* load between July (*Md* = 1,320 CN) and September 2011 (*Md* = 81 CN). Again, we did not detect significant differences in air or animal temperatures between these periods, and a review of ambient temperatures revealed no obvious differences between these sampling periods; indeed, maximum daily temperatures during sampling dates in July and September 2011 were identical (36.1° C).

Male *A. nelsoni* were significantly more likely to be infected than females when assessed across sites and seasons (P < 0.001; 47 of 82 males infected; 46 of 116 females infected). We detected some evidence for the sex bias in overall infection prevalence at the Amargosa River, but not at Boiling Pot Ranch (Table 2). At Boiling Pot Ranch, however, we documented significantly higher overall *Bd* loads in males than females ($U_{17,29}$ = 160, P = 0.05, *Md* values = 880 vs. 348 CN, respectively), but this was not evident at the Amargosa River ($U_{30,17}$ = 249, P = 0.89, *Md* values = 706 vs. 774 CN, respectively). We found that larger individuals of *A. nelsoni* had significantly lower *Bd* loads than smaller individuals at Boiling Pot Ranch (*Bd* load = 10.37 − [0.05 × SVL], P = 0.001; explaining 18% of the variation) where juveniles (≤ 55 mm SVL) were common, but we did not detect significance in this pattern at the Amargosa River, where we observed few juvenile toads and sampled none < 50 mm SVL.

When individual *A. nelsoni* were recaptured, the average time between resampling events was 200 d (range: 44–397 d). The *Bd* load of infected toads ranged from 15–5,360 CN. We did not detect a significant change in *Bd* infection intensity between recapture events (t_{23} = 1.93, P = 0.066); however, over these sampling events, 33% (n = 8) of the recaptured *A. nelsoni* lost their infection status, 17% (n = 4) were consistently infected, 25% (n = 6) had become infected, and 25% (n = 6) showed no sign of infection. We found no relationship (P = 0.98) between *Bd* load at time of first capture and whether toads cleared infection by subsequent capture, and the initial median *Bd* loads of these two groups were similar (*Md* of toads that cleared = 1,140 CN; *Md* of toads that did not = 1,845 CN).

DISCUSSION

We anticipated that hot ambient temperatures common during summer in the Mojave Desert would greatly limit *Bd* infection prevalence and intensities in anurans, as has been generally hypothesized for areas of high temperature (Piotrowski et al. 2004; Puschendorf et al. 2009). Yet, we observed overall high rates of *Bd* infections in both *A. nelsoni* and *L. catesbeianus* from this region during sampling events across spring and summer. In July, when ambient temperatures during our evening sampling events were 23.7–32.5° C and maximum daily temperatures were considerably warmer (36.1–37.8° C), infection prevalence in *A. nelsoni* and *L. catesbeianus* remained around 50%, with individual *Bd* loads as high as 404,000 CN. The pattern of high infection prevalence in *A. nelsoni* was consistent across two years and two sample sites. We observed some mitigation of *Bd* infection prevalence during late summer sampling, but only documented a significant reduction associated with our sampling event on 20 August 2012 at the Amargosa River. The ambient temperature during sampling that night was 30.8° C and water temperatures averaged 25.9° C. Even then, however, we found that 26% of the toads sampled were infected, with *Bd* loads as high as 69,100 CN. Infection intensity showed some significant influences related to temperatures, but these patterns were not consistent across time or sites.

The observed discrepancy from our hypothesized expectation likely resulted from actual temperatures and conditions experienced by *A. nelsoni*. Our measurements of body temperatures when we captured animals away from water indicated that *A. nelsoni* maintained temperatures favorable to *Bd* at night during summer months (range: 11.1–26.6° C; n = 74), likely associated with evaporative cooling. Adult *A. nelsoni*

are generally nocturnal, and the burrows and other shelters that these toads occupy during daylight hours probably limit exposure to diurnal temperature extremes and restrict drying. Water temperatures associated with our captures occasionally approached the thermal limit for *Bd*, but averaged only 21.0° C during summer months (range: 13.2–27.8° C; n = 141). Much of the waters used by *A. nelsoni* were also shared with *L. catesbeianus*, Baja California Treefrog (*Pseudacris hypochondriaca;* Recuero et al. 2006), and introduced Red Swamp Crayfish (*Procambarus clarkii*), species that are known or suspected vectors for *Bd* (Daszak et al. 2004; Reeder et al. 2012; McMahon et al. 2013). Once introduced, *Bd* has been shown to remain infective in aquatic systems for weeks (Johnson and Speare 2003). Therefore, we speculate that the pathogen may have been recurrently transmitted to individuals seeking hydration.

Our sampling was not explicitly designed to detect demographic (sex and size) variation in *Bd* infection, but the observation that male *A. nelsoni* were more likely to be infected than females was not unexpected. Greater exposure of males to the pathogen has been hypothesized to explain a similar pattern in *A. boreas* in the Rocky Mountains, where males tend to congregate and spend considerably more time in aquatic systems during breeding than females, thus presumably increasing their probability of infection (Carey et al. 2006; Hossack et al. 2013). Breeding behavior in *A. nelsoni* appears to be similar. Our assessment also provided some evidence that smaller toads were more likely to be infected than larger toads. Such a pattern has been hypothesized to result from either energetic trade-offs between development (growth) and the functioning of a challenged immune system, or age differences in development of immune defenses (e.g., Kriger et al. 2007). Our assessment of size (thus age) differences was limited by the lack of small, juvenile toads in our sample from the Amargosa River. Juveniles were common along other stretches of the river, but dense emergent vegetation and an abundance of crayfish may have limited successful reproduction or allowed juveniles to hide more efficiently along the short stretch of river that we sampled.

The potential impact of *Bd* infection on *A. nelsoni* has not been investigated, nor is the timing of the arrival of *Bd* in the Oasis Valley known, other than it was detected in 2005. Early accounts of limited population numbers of *A. nelsoni* were based on anecdotal observations (Goebel et al. 2005), and we know of no observations of large-scale mortality events. More systematic monitoring indicates a robust population of *A. nelsoni* given its limited range (Goebel et al. 2005; NDOW, unpubl. data). In the current population of *A. nelsoni*, *Bd* appears to be persistent, and while not assessed, it is possible that the pathogen may cause sub-lethal effects, including a decrease in survivorship. Persistent *Bd* infection in populations of *A. boreas* in the Rocky Mountains was found to reduce survivorship and cause slow population declines (Pilliod et al. 2010). The outcome of *Bd* infection in *A. boreas* appears to be environmentally influenced. In a laboratory experiment, intermittent clearing of *Bd* in *A. boreas* through periodic drying or warming increased survivorship (Murphy et al. 2011). Such previous exposure appears to stimulate immunological resistance to the pathogen in some species (Murphy et al. 2011; McMahon et al. 2014), with resistance potentially mediated by innate or acquired immunogenetic responses (Richmond et al. 2009; Savage and Zamudio 2011; Ellison et al. 2015).

Our recapture data showed that *A. nelsoni* can clear *Bd* infection in the wild, or at least reduce it to the point of non-detection, indicating potential resistance to the pathogen. We also observed toads with high *Bd* loads (up to 404,000 CN) but with no obvious clinical symptoms of disease, indicating potential tolerance (Savage and Zamudio 2011). Three scenarios might explain our observations. Possibly, *A. nelsoni* may never have been highly susceptible to chytridiomycosis, particularly given the seasonally warm environmental conditions that allow individuals to clear the infection, potentially repeatedly. Alternatively, the current population may be descended from individuals that survived a previous epizootic and now have evolved greater resistance to the pathogen; population recovery following *Bd* decline has been documented in other species (e.g., Scheele et al. 2014). Finally, given differences in pathogenicity, the *Bd* strain currently infecting the region may not be highly virulent (Piovia-Scott et al. 2014). Although we are uncertain which of these scenarios is most plausible, the first appears least likely because chytridiomycosis was identified as the cause of death in captive *A. nelsoni*.

Our assessment of *Bd* in *A. nelsoni* was intended to inform conservation efforts, and these preliminary findings have influenced management strategy. For example, enhanced protocols have been implemented to minimize cross contamination between the large numbers of toads handled during population monitoring. Such actions seem prudent given limited knowledge concerning the mechanisms by which *Bd*, and many other amphibian pathogens (e.g., ranaviruses), are spread in the wild. While we observed high levels of *Bd* infection in *A. nelsoni*, we did not detect any obvious negative impacts of disease. Our study, however, was not designed to assess more subtle influences of the pathogen on overall survivorship or reproduction. Future monitoring efforts could be modified to assess the potential impacts of *Bd* infection on the long-term population trend in this narrowly endemic species.

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LITERATURE CITED

Altig, R., and C.K. Dodd, Jr. 1987. The status of the Amargosa Toad (*Bufo nelsoni*) in the Amargosa River drainage of Nevada. Southwestern Naturalist 32:276–278.

Andre, S.E., J. Parker, and C.J. Briggs. 2008. Effect of temperature on host response to *Batrachochytrium dendrobatidis* infection in the Mountain Yellow-legged Frog (*Rana muscosa*). Journal of Wildlife Diseases 44:716–720.

Berger, L.R., R. Speare, H.B. Hines, G. Marantelli, A.D. Hyatt, K.R. McDonald, L.F. Skerratt, V. Olsen, J.M. Clark, G. Gillespie, et al. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. Australian Veterinary Journal 82:31–36.

Brem, F., J.R. Mendelson III, and K.R. Lips. 2007. Field-sampling protocol for *Batrachochytrium dendrobatidis* from living amphibians, using alcohol preserved swabs. Version 1.0 (18 July 2007). Conservation International, Arlington, Virginia, USA.

Carey, C., J.E. Bruzgul, L.J. Livo, M.L. Walling, K.A. Kuehl, B.F. Dixon, A.P. Pessier, R.A. Alford, and K.B. Rogers. 2006. Experimental exposures of Boreal Toads (*Bufo boreas*) to a pathogenic chytrid fungus. EcoHealth 3:5–21.

Chatfield, M.W.H., and C.L. Richards-Zawacki. 2011. Elevated temperatures as a treatment for *Batrachochytrium dendrobatidis* infection in captive frogs. Diseases of Aquatic Organisms 94:235–238.

Daszak, P., A. Strieby, A.A. Cunningham, J.E. Longcore, C.C. Brown, and D. Porter. 2004. Experimental evidence that the Bullfrog (*Rana catesbeiana*) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herpetological Journal 14:201–207.

Drew, A., E.J. Allen, and L.J. Allen. 2006. Analysis of climatic and geographic factors affecting the presence of chytridiomycosis in Australia. Diseases of Aquatic Organisms 68:245–250.

Ellison, A.R., T. Tunstall, G.V. DiRenzo, M.C. Hughey, E.A. Rebollar, L.K. Belden, R.N. Harris, R. Ibáñez, K.R. Lips, and K.R. Zamudio. 2015. More than skin deep: functional genomic basis for resistance to amphibian chytridiomycosis. Genome Biology and Evolution 7:286–298.

Forrest, M.J., and M.A. Schlaepfer. 2011. Nothing a hot bath won't cure: infection rates of amphibian chytrid fungus correlate negatively with water temperature under natural field settings. PLoS ONE 6(12):e28444.

Forzán, M.J., R. Vanderstichel, N.S. Hogan, K. Teather, and J. Wood. 2010. Prevalence of *Batrachochytrium dendrobatidis* in three species of wild frogs on Prince Edward Island, Canada. Diseases of Aquatic Organisms 91:91–96.

Garner, T.W., M.W. Perkins, P. Govindarajulu, D. Seglie, S. Walker, A.A. Cunningham, and M.C. Fisher. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American Bullfrog, *Rana catesbeiana*. Biology Letters 2:455–459.

Goebel, A.M., T.A. Ranker, P.S. Corn, and R.G. Olmstead. 2009. Mitochondrial DNA evolution in the *Anaxyrus boreas* species group. Molecular Phylogenetics and Evolution 50:209–225.

Goebel, A., H.M. Smith, R.W. Murphy, and D.J. Morafka. 2005. *Bufo nelsoni* Stejneger, 1893 Amargosa toad. Pp. 427–430 *In* Amphibian Declines: The Conservation Status of United States Species. Lannoo, M.J. (Ed.). University of California Press, Berkeley, California, USA.

Green, D.E., and C. Kagarise Sherman. 2001. Diagnostic histological findings in Yosemite Toads (*Bufo canorus*) from a die-off in the 1970's. Journal of Herpetology 35:92–103.

Hossack, B.R., W.H. Lowe, J.L.Ware, and P.S. Corn. 2013. Disease in a dynamic landscape: host behavior and wildfire reduce amphibian chytrid infection. Biological Conservation 157:293–299.

Johnson, M.L., and R. Speare. 2003. Survival of *Batrachochytrium dendrobatidis* in water: quarantine and disease control implications. Emerging Infectious Diseases 9:922–925.

Kirshtein, J.D., C.W. Anderson, J.S. Wood, J.E. Longcore, and M.A. Voytek. 2007. Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. Diseases of Aquatic Organisms 77:11–15.

Kriger, K.M., F. Pereoglou, and J.M. Hero. 2007. Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. Conservation Biology 21:1280–1290.

Longo, A.V., D. Rodriguez, D. da Silva Leite, L.F. Toledo, C. Mendoza Almeralla, P.A. Burrows, and

K.R. Zamudio. 2013. ITS1 copy number varies among *Batrachochytrium dendrobatidis* strains: implications for qPCR estimates of infection intensity from field-collected amphibian skin swabs. PLoS One 8(3):e59499.

McMahon, T.A., L.A. Brannelly, M.W.H. Chatfield, P.T.J. Johnson, M.B. Joseph, V.J. Mckenzie, C.L. Richards-Zawacki, M.D. Venesky, and J.R. Rohr. 2013. Chytrid fungus *Batrachochytrium dendrobatidis* has non-amphibian hosts and releases chemicals that cause pathology in the absence of infection. Proceedings of the National Academy of Sciences USA 110:210–215.

McMahon, T.A., B.F. Sears, M.D. Venesky, S.M. Bessler, J.M. Brown, K. Deutsch, N.T. Halstead, G. Lentz, N. Tenouri, S. Young, et al. 2014. Amphibians acquire resistance to live and dead fungus overcoming fungal immunosuppression. Nature 511:224–227.

Murphy, P.J., S. St-Hilaire, and P.S. Corn. 2011. Temperature, hydric environment, and prior pathogen exposure alter the experimental severity of chytridiomycosis in Boreal Toads. Diseases of Aquatic Organisms 95:31–42.

Muths, E., P.S. Corn, A.P. Pessier, and D.E. Green. 2003. Evidence for disease-related amphibian decline in Colorado. Biological Conservation 110:357–365.

Nichols, D.K., A.P. Pessier, and J.E. Longcore. 1998. Cutaneous chytridiomycosis in amphibians: an emerging disease? Pp. 269–271 *In* Proceedings of American Association of Zoo Veterinarians and American Association of Wildlife Veterinarians Joint Conference. American Association of Zoo Veterinarians, Yulee, Florida, USA.

Pilliod, D.S., E. Muths, R.D. Scherer, P.E. Bartelt, P.S. Corn, B.R. Hossack, B.A. Lambert, R. McCaffery, and C. Gaughan. 2010. Effects of amphibian chytrid fungus on individual survival probability in wild Boreal Toads. Conservation Biology 24:1259–1267.

Piotrowski, J.S., S.L. Annis, and J.E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15.

Piovia-Scott J., K. Pope, S.J. Worth, E.B. Rosenblum, T. Poorten, J. Refsnider, L.A. Rollins-Smith, L.K. Reinert, H.L. Wells, D. Rejmanek, et al. 2014. Correlates of virulence in a frog-killing fungal pathogen: evidence from a California amphibian decline. International Society for Microbial Ecology Journal 9:1570–1578.

Puschendorf, R., A.C. Carnaval, J. VanDerWal, H. Zumbado-Ulate, G. Chaves, F. Bolanos, and R.A. Alford. 2009. Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica. Diversity and Distributions 15:401–408.

Raffel, T., J.R. Rohr, J.M. Kiesecker, and P.J. Hudson. 2006. Negative effects of changing temperature on amphibian immunity under field conditions. Functional Ecology 20:819–828.

Recuero, E., Í. Martínez-Solano, G. Parra-Olea, and M. García-París. 2006. Phylogeography of *Pseudacris regilla* (Anura: Hylidae) in western North America, with a proposal for a new taxonomic rearrangement. Molecular Phylogenetics and Evolution 39:293–304.

Reeder, N.M.M., A.P. Pessier, and V.T. Vredenburg. 2012. A reservoir species for the emerging amphibian pathogen *Batrachochytrium dendrobatidis* thrives in a landscape decimated by disease. PLoS ONE 7:e33567.

Richards-Zawacki, C.L. 2010. Thermoregulatory behavior affects prevalence of chytrid fungal infection in a wild population of Panamanian Golden Frogs. Proceedings of the Royal Society B 277:519–528.

Richmond, J.Q., A.E. Savage, K.R. Zamudio, and E.B. Rosenblum. 2009. Towards immunogenetic studies of amphibian chytridiomycosis: linking innate and acquired immunity. BioScience 59:311–320.

Rollins-Smith, L.A., J.P. Ramsey, J.E. Pask, L.K. Reinert, and D.C. Woodhams. 2011. Amphibian immune defenses against chytridiomycosis: impacts of changing environments. Integrative and Comparative Biology 51:552–562.

Savage, A.E., and K.R. Zamudio. 2011. MHC genotypes associate with resistance to a frog-killing fungus. Proceeding of the National Academy Sciences 108:16705–16710.

Savage, A.E., M.J. Sredl, and K.R. Zamudio. 2011. Disease dynamics vary spatially and temporally in a North American amphibian. Biological Conservation 144:1910–1915.

Scheele, B.C., F. Guarino, W. Osborne, D.A. Hunter, L.F. Skerratt, and D.A. Driscoll. 2014. Decline and re-expansion of an amphibian with high prevalence of chytrid fungus. Biological Conservation 170:86–91.

Skerrat, L.F., L. Berger, R. Speare, S. Cashins, K.R. McDonald, A.D. Phillott, H.B. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

Stejneger, L. 1893. Annotated list of the reptiles and batrachians collected by the Death Valley expedition in 1891 with descriptions of new species. North American Fauna 7:220.

U.S. Fish and Wildlife Service. 1996. Endangered and threatened wildlife and plants: 12-month finding for a petition to list the Amargosa Toad (*Bufo nelsoni*) as endangered. Federal Register 61:42 (1 March 1996):0812–0819.

U.S. Fish and Wildlife Service. 2010. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the Amargosa Toad as threatened or endangered. Federal Register 75:138 (20 July 2010):42040–42054.

Vredenburg, V.T., R.A. Knapp, T.S. Tunstall, and C.J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Sciences USA 107:9689–9694.

Whitfield, S.M., J. Kerby, L.R. Gentry, and M.A. Donnelly. 2012. Temporal variation in infection prevalence by the amphibian chytrid fungus in three species of frogs at La Selva, Costa Rica. Biotropica 44:779–784.

Woodhams, D.C., R.A. Alford, and G. Marantelli. 2003. Emerging disease of amphibians cured by elevated body temperature. Diseases of Aquatic Organisms 55:65–67.

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