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Project Title:

Effects of *Batrachochytrium dendrobatidis* on amphibian communities in New Mexico

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Start: 1 March 2014; End 31 December 2014

Project Summary

The disease chytridiomycosis, caused by pathogen *Batrachochytrium dendrobatidis* (Bd), has been implicated in the decline and extirpation of amphibians around the world. Responding to chytridiomycosis is a challenge for conservation programs and wildlife management agencies because species responses to Bd-infection can differ dramatically. Additionally, Bd is an environmentally sensitive pathogen and disease dynamics can vary across biogeographic zones. Understanding differences in susceptibility among species and the suitability of different habitats for pathogen proliferation is critical for implementing effective management plans for threatened and near threatened species. In New Mexico, the threat of chytridiomycosis to New Mexico's amphibian species has not yet been fully resolved because not all species have been systematically sampled. The Boreal Chorus Frog (*Pseudacris maculata*) is an ideal species for Bd investigation due to its distribution in multiple aquatic eco-regions and co-occurrence with a high number of other native and endangered anuran species. To determine Bd disease dynamics within the anuran communities of New Mexico, we used historic distribution data, conducted field surveys to determine the prevalence of Bd in the Boreal Chorus Frog and the sympatric anuran species in northern and central New Mexico. Because Bd is an environmentally sensitive pathogen, we also collected microhabitat temperatures, amphibian body temperatures as well as environmental data at each site to determine the relationship between temperature and disease patterns. Results from our surveys indicated that Boreal Chorus Frog populations are found in only 26% of historical sites (16 of 61 sampled sites). At the 16 occupied sites we swabbed a total of 139 anurans and found an overall low prevalence for Bd of 25.9%. Our surveys revealed differences in prevalence of Bd across biogeographic zones (ranging from 8% to 100% prevalence). Bd prevalence and intensity of infection is relatively low in Boreal Chorus Frogs compared to other susceptible species. Additionally, we found no evidence of disease or disease-related mortality in this species. Although we cannot rule out the possibility that Bd played a role in past declines, we suggest that chytridiomycosis may not currently be the most significant threat to Boreal Chorus Frogs. Here we make recommendations for additional investigations to understand the cause(s) of declines in this species.

Key Findings

- Boreal Chorus frogs were detected in only 30% of historical range and, most surprisingly, were not detected from historical locales in the Rio Grande Valley
- Detectability of Boreal Chorus Frogs was improved when we conducted surveys under specific, optimal conditions
- Boreal Chorus Frogs may not be as susceptible to Bd as other New Mexico species
- Bd prevalence varied from site to site (10%-75%) and Bd was detected in all areas where amphibians were found

Amphibian Declines and Chytridiomycosis

The world is currently experiencing a global biodiversity crisis (Wake et al. 2008). Amphibians are at the forefront of biodiversity crisis with approximately 41% of all amphibian species threatened with extinction (Monastersky 2014). The causes of amphibian declines are multifaceted and include climate change, habitat loss, and disease as the three primary factors (Hof et al. 2011), among many others (Wake et al. 2008). One of the most critical factors in the disappearance of many amphibian species is chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). Chytridiomycosis has devastated global amphibian populations and has been implicated in the extirpation of thousands of populations, and the extinction of more than 100 amphibian species (Skerratt et al. 2007). Within New Mexico, Bd has been implicated in the declines of the Boreal Toad, Chiricahua Leopard Frog, Northern Leopard Frog, and Lowland Leopard Frog, while other New Mexico species have not been reported to experience disease related declines (Michelle Christman- *pers comm*). Understanding species differences in susceptibility and environmental factors that contribute to disease dynamics is essential for assessing the impact and potential threat of chytridiomycosis for the amphibians of New Mexico.

Differences in species susceptibility — In disease declines that have been well documented in other parts of the world, the initial outbreaks of chytridiomycosis are characterized by mass-mortality events that can occur rapidly and affect multiple species simultaneously (Lips et al. 2006; Woodhams et al. 2008). During a Bd outbreak all species exhibit mortality and initial declines, but these are high levels of interspecies responses with some species disappearing quickly (Ryan et al 2008) and other species persisting (McCaffery & Lips 2012). However, in the months following initial outbreaks, the effects of Bd-infection differ among amphibian populations and species. For example, in Australia, where chytridiomycosis has been studied for many decades, some species have gone extinct [e.g., the Sharp Snouted Day Frog (*Taudactylus acutirostris*, Schloegel et al. 2006)], some persist with severely depressed populations [e.g., the Armored Mist Frog (*Litoria lorica*; Puschendorf et al. 2011)] and other species show population recovery despite the ongoing presence of Bd [e.g., the Green-eyed Tree Frog (*Litoria genimaculata*); McDonald et al. 2005].

Similar patterns of interspecific differences occur in New Mexico where Bd-related declines have been reported for the Northern Leopard Frog complexes and the Boreal Toad, while other species appear to be less impacted, such as the Canyon Tree Frog and Tiger Salamander (Michelle Christman- *pers comm*). Wide variation in species responses to Bd-infection presents a central challenge for conservation programs and wildlife management because it is difficult to identify those species that are most vulnerable and require conservation action. An additional concern is that species that recover and become tolerant of Bd infection may act as Bd reservoirs or spreaders for more susceptible species. Thus, understanding differences in interspecific susceptibility and pathogen tolerance is critical for implementing effective management of threatened species in New Mexico.

Environmental factors — One key aspect of chytridiomycosis dynamics is that Bd is an environmentally sensitive pathogen; its optimal reproduction occurs within a narrow thermal range of (~17°C - 25°C; Piotrowski et al., 2002, Woodhams et al., 2008). This thermal optimum of Bd has a profound effect on the disease dynamics. For example, chytridiomycosis outbreaks in tropical regions have predominantly occurred in cooler high elevation sites and when temperatures are at seasonal lows (Woodhams & Alford 2005, Berger et al., 2004). In contrast, host populations in temperate regions frequently experience a wider range of temperatures and still experience dramatic declines despite the fact that host species spend significant amount of time in temperatures that are lower than Bd's thermal optimum (Knapp et al., 2011, Lannoo et al. 2011). In North American temperate regions, preliminary surveys suggest that Bd-prevalence is highest in the spring. Seasonal fluctuations in Bd-prevalence could be directly linked to environmental temperatures or changes in host densities (e.g., when amphibians aggregate for breeding) or both. Therefore, resolving the ecological variables that are at play in a given environment is essential for understanding chytridiomycosis dynamics and the threat of Bd to amphibian hosts and for devising science-based management strategies.

Chytridiomycosis in New Mexico

Chytridiomycosis occurs in New Mexico and has been implicated in the declines of numerous species, with some species now at critically low levels (Bradley et al. 2002; Muths et al. 2003; Scherer et al. 2005, Lannoo et al. 2011). Many of these same species have also declined in neighboring states of Arizona and Colorado (Muths et al. 2003). Species of concern include the Chiricahua Leopard Frog (*Lithobates* (*Rana*) *chiricahuensis*), the Northern Leopard Frog (*Lithobates* (*Rana*) *pipians*), the Lowland Leopard Frog (*Lithobates* (*Rana*) *yavapaiensis*) and the Boreal Toad (*Anayraxus* (*Bufo*) *boreas*; Bradley et al. 2002; Muths et al. 2003; Scherer et al. 2005, Lannoo et al. 2011). In New Mexico we have an incomplete understanding of the impacts of Bd on other anuran species due to lack of sampling effort. Recent surveys have detected Bd on 53% (9/17) of species of New Mexican anurans including the endemic, terrestrial Jemez Mountain Salamander (*Plethodon neomexicanus*; Cummer et al. 2005) and on the Arizona Toad (*Anaxyrus microscaphus*; Ryan et al. 2014). Additionally, Bd has been detected in several counties of New Mexico, but the distribution and prevalence of Bd throughout the state is the subject of on-going investigation (Painter et al-unpublished). Understanding the potential impact of Bd on amphibian communities and the broad distribution of Bd is critical for management and conservation around the state.

To date there has been no thorough evaluation of the population trends or presence/absence of the Boreal Chorus Frog (*Pseudacris maculata*) within New Mexico. The Boreal Chorus frog was previously considered a subspecies in the *P. triseriata* species complex, but was elevated to a unique species based off of a combination of mitochondrial DNA, morphology and behavior (Lemmon et al. 2007a; Lemmon et al. 2007b). Declines of the Boreal Chorus Frog have been significant reported from Canada and the loss of wetlands, native vegetation, contaminants and Bd have been suggested to be responsible for these declines (Seburn et al. 2014).

The Boreal Chorus Frog was previously ubiquitous throughout central and northern New Mexico and historically occurred in many different habitat types. Breeding populations were found along the Rio Grande and lakes and ponds in every county of northern New Mexico, in the Rio Grande Valley, in northern Socorro County, with disjunct populations in the Gila National Forest (Degenhardt et al. 1996).

Several factors make the Boreal Chorus Frog an ideal focal species for investigating the community-wide effects of Bd on New Mexican amphibians. First, the distribution of the Boreal Chorus Frog overlaps with several other species that are known to be susceptible to chytridiomycosis (e.g., Boreal Toad and Northern Leopard Frog). Second, the distribution of Boreal Chorus Frogs spans an altitudinal and latitudinal gradient and therefore, populations occurring from low to high elevations may exhibit differences in their thermal ecology that could help explain chytridiomycosis dynamics in multiple species in New Mexico. Lastly, declines in related Chorus Frog populations have been reported in parts of the US and Canada and Bd has been detected at a relatively low prevalence (37.8%) in previous surveys (Oullet et al 2005). Further investigation is needed to determine if Bd is a causative factor in reported declines from New Mexico and in other parts of North America.

Methods

Establishing survey sites — We obtained historical data on Boreal Chorus Frog locales was from HerpNet online catalogue and the Museum of Southwest Biology at the University of New Mexico (HerpNet.org), which includes data collected from multiple natural history museums dating back over 50 years, and from conversations with New Mexico-based herpetologists.

Surveys for amphibians— We conducted standardized surveys in 61 historic and new Boreal Chorus Frog sites within three different geographic regions: The Southern Rocky Mountains of New Mexico (Rio Arriba, Taos, Colfax, and Mora County), the Rio Grande Valley (Bernalillo, Valencia and Socorro County) and Jemez Mountains (Sandoval County; Fig 1). We used a combination of visual encounter and acoustic surveys during the months of March to June 2014, when Boreal Chorus Frogs are most likely to be active (Degenhardt et al. 1996).

We made 145 total visits to sites, with 1-13 visits per site (mean visits per site 2.4, S.D. 2.1) and 1-6 researchers assisted with sampling. At each site, we arrived by sunset, collected ecological data (including air and water temperature, humidity, wind speed, barometric pressure) and conducted standardized visual and acoustic surveys. For acoustics surveys, we sat quietly for a minimum of 10 minutes, listening for frog calling. For visual encounter surveys, we slowly walked the perimeter of the water bodies, carefully searching for amphibians. We recorded frog presence/absence and estimated relative abundance for all anuran species that we encountered. Based on these surveys, anuran abundance was quantified for every sampling event at each site as the number of anurans detected divided by the Search Effort. We calculated Search Effort as search time in hours x number of searchers.

Sample collection for Bd — When we encountered frogs, we collected adult frogs with clean dip nets. We handled each individual using new latex gloves and with strict adherence to field hygiene protocols to reduce pathogen transmission (Phillot et al. 2010). Once an amphibian was captured, we recorded each animal's mass (measured to the nearest 0.01 g), snout-to-vent length (SVL), sex (male, female), life-stage and/or reproductive status (e.g., gravid females). We collected skin swab samples using a non-invasive standardized protocol that involves rubbing a sterile cotton-tipped swab across the ventral epidermis on the abdominal and inguinal surfaces, and on the digits of the hands and feet (Hyatt et al. 2007). We transferred the swab sample to a sterile tube for transport to the laboratory at New Mexico Tech (NMT) for sample processing (described below). We placed the sample into a labeled screw cap tube and kept all samples cool for transport to NMT. The samples were placed in a -20C freezer until analysis. One swab sample was collected per individual and we aimed to capture 20-40 individuals at each site. A minimum of 20 samples per site should provide a reasonable estimate of Bd prevalence with 95% confidence intervals (Skerratt et al. 2007).

Environmental variables — Because amphibian susceptibility to chytridiomycosis is highly dependent on environmental conditions, we measured relevant environmental variables (e.g., site elevation, habitat type, water temperature, total dissolved solids, conductivity and pH) in historic and new Boreal Chorus Frog sites. Where frogs were found, we measured body and microhabitat temperatures. Specifically, once an anuran was sighted, and before the anuran was captured, we used a non-contact infrared thermometer (Raytek ST80 Pro-Plus Non-contact thermometer, RAYST80 IR) to determine the body and microhabitat temperatures. We held the device approximately 5 cm away from the frog and aimed it at the lower dorsal area (Rowley & Alford 2007).

Additionally, we collected daily temperature fluctuation data using temperature recording devices implanted into agar frog models. In brief, we constructed agar frog models of a similar body size to Boreal Chorus Frogs (to mimic the Boreal Chorus Frog's body size) with temperature recording devices (i.e., "ibuttons", IButtonLink Thermochron DS1921G-F5) inside the agar models. We set the loggers to collect temperature every ten minutes for two weeks. We distributed the agar models around the sites in the same microhabitat locations where frogs were found. After two weeks we retrieved the agar models to collect the temperature data. The advantage of using temperature recording devices in agar models is that we could get more accurate thermal ecology data with minimal disturbance to the frog populations. Similar methods have been used to study amphibian thermal ecology (Rowley & Alford 2007) and chytridiomycosis dynamics (Richards-Zawacki 2010) in amphibian habitats (e.g., in the tropical rainforests).

Processing of swab samples — We processed the skin swab samples to determine the presence, prevalence and intensity of infection of Bd using quantitative real-time Polymerase Chain Reaction (qPCR). For this analysis, Bd DNA was extracted using Qiagen Blood and Tissue DNA Extraction Kit (Qiagen catalog #69504). During PCR analysis, samples were run in triplicate. For the qPCR assay, we analyzed all samples in triplicate with an internal positive control (Hyatt et al., 2007) and used a dilution set of plasmid standards (obtained from Pisces Molecular, Boulder, Colorado) to quantify pathogen load. We converted plasmid copy numbers to zoospore copy numbers using the

line of best fit ($r^2 > 0.999$) from a linear regression of log (plasmids) vs. log (zoospores) ($t4 = 210.6$, $P < 0.0001$) that we obtained by running the plasmid standard set alongside a series of standards containing known quantities of zoospores (obtained from Alex Hyatt, Australian Animal Health Laboratory). If 1 of 3 replicate wells turned up positive, we checked Cycle Threshold (Ct) value to determine whether non-amplification in 2 of 3 wells could have been caused by a low-level infection (near the detection threshold) and verified that the qPCR was not inhibited (IPC amplified normally). In cases of inhibition or Ct values far from the detection threshold, we re-ran and considered them positive if Bd was detected in any of the three re-run wells.

Results

Sampling Areas — We conducted field surveys for amphibians in three regions in New Mexico (Fig 1); the Southern Rocky Mountain Region, the Rio Grande Valley and the Jemez Mountain Region. In the Southern Rocky Mountain region sampling took place in Rio Arriba, Taos, Colfax, and Mora counties. In the Rio Grande Valley, we sampled in Bernalillo, Valencia and Socorro counties. In the Jemez Mountain region, we sampled in Sandoval County. These locations include a diverse array of riparian areas such as perennial streams, perennial marsh, ephemeral catchments, ephemeral marsh, ephemeral ponds, mountain lakes, wet meadows, flooded fields and lakes within all eight counties.

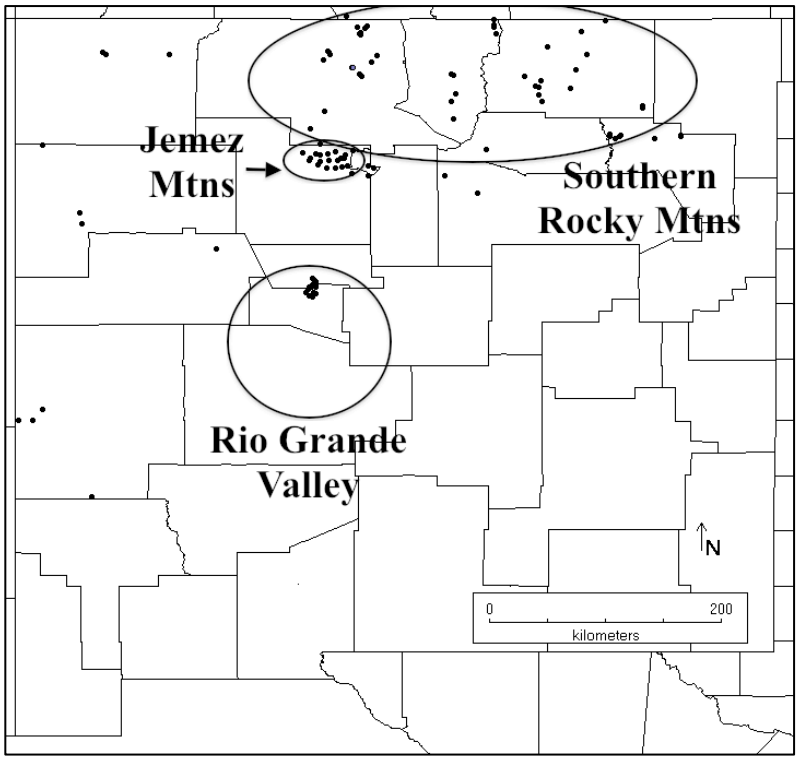

Figure 1| Sampling occurred in 61 sites within three regions where populations of Boreal Chorus Frogs occurred: the Southern Rocky Mountain region, the Rio Grande Valley and the Jemez Mountain region. Points indicate the historical populations (HerpNet Database).

Boreal Chorus Frog Surveys — We conducted visual and acoustic surveys at 61 sites throughout these regions (Table 1). We captured a total of 156 Boreal Chorus Frogs in 16 of 61 historical sites sampled (Table 1).

At all sites where frogs were detected, we observed that Boreal Chorus Frogs were more likely to be calling at night after sunset when the daylight was almost completely gone (variable, but approximately 1 hr after sunset) during the field season, from May 2014 to June 2014 (late spring). Calling occurred intermittently during the day at many sites but was much more frequent after sunset (1 or 2 individuals vs. 20-30 individuals). We also report that calling began during or right after the last snowfalls of the season in locations where the frogs were detected. In many habitats, calling persisted in the snow and during light snowing events. We observed that this behavior in Carson National Forest (May 23, 2014, 8:51 pm 9,816 ft). This calling behavior has been reported in Arizona for the same species (Bezy et al. 2004) but to our knowledge has not been reported in New Mexico. We also observed males chorusing were often grouped close together in a particular areas of the breeding pond. These males would sit at the top of the water with their heads above water level to call. Often the males were not exposed and could only be found calling under grasses or reeds in the same type of position, making them cryptic for researchers.

Figure 2. Boreal Chorus Frogs (*Pseudacris maculata*) found in Mora, NM (A). Two Boreal Chorus Frogs (upper is male; lower is female) in an ephemeral pond in Carson National Forest, Rio Arriba Co. (B).

We found Boral Chorus Frogs in a wide variety of habitat types. Frogs were detected in irrigation ditches, along cow pastures, pristine high mountain ponds, marshy meadows, and slow moving, sinuous riparian rivers. In many cases, calling males were cryptic such that it took researchers several hours to locate the calling males. In other cases, when the aquatic habitat was smaller and more exposed, the capture rates were higher.

Figure 3. The slow-moving East Fork of the Jemez River in the Jemez Mountains in Sandoval Co. creates several wet meadows and ponds, a rich natural aquatic habitat for Boreal Chorus Frogs.

Below we detail our presence/absence results for Boreal Chorus Frogs in each region.

The Southern Rocky Mountain Region: This area includes the some of the northernmost counties of New Mexico including, Rio Arriba, Taos, Colfax and Mora County. Within Rio Arriba County, 2 of the 8 sites surveyed Boreal Chorus frogs were present (25% of sites). Within Mora County, 5 of the 6 sites surveyed Boreal Chorus frogs were present (83% of sites). Within Colfax County, 1 out of 6 sites Boreal Chorus frogs were detected (17% of sites). Within Taos County, 0 out of the 8 surveyed sites had Boreal Chorus frogs present. For this region, 29% of the sites (8/28) were detected for Boreal Chorus Frog. There were a total of 48 visits to 24 sites with an average of 1.5 visits per site and a Search Effort of 4.3.

The Rio Grande Valley: This area includes the counties of Socorro, Valencia, and Bernalillo. We surveyed nine historical Boreal Chorus Frog sites and no Boreal Chorus frogs were detected in any site (0/9, 0% of sites). We visited these sites a total of 56 times with an average of 3.5 visits per site and a Search Effort of 1.2.

The Jemez Mountain Region: This area includes Sandoval County. We surveyed at 13 historical locations and detected eight sites where Boreal Chorus Frogs were present (62% of sites). We visited a total of 17 sites, 50 times with a Search Effort of 4.3.

Overall, we found that Boreal Chorus frogs could be detected in 30% of historical sites with relatively minimal effort (Table 1).

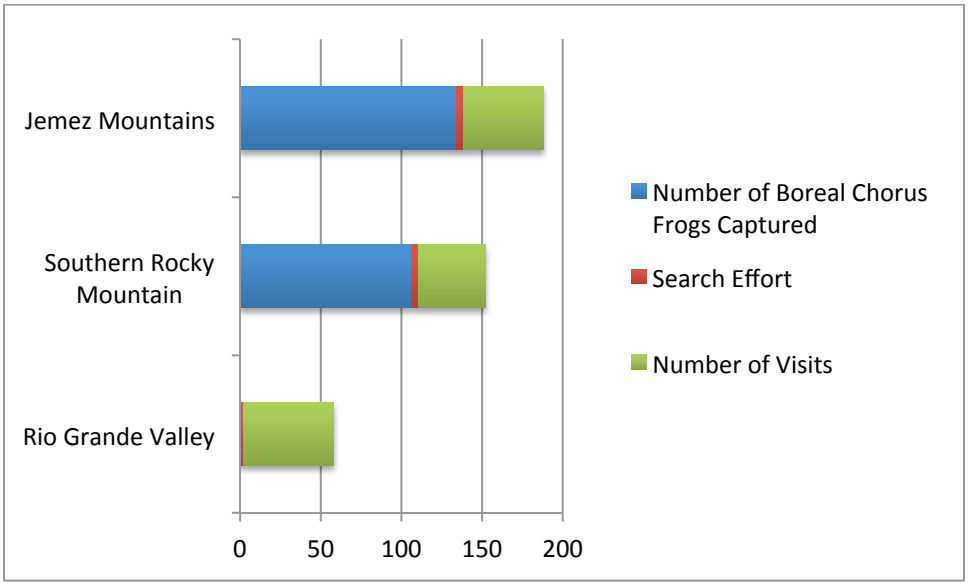

Table 1. The sampling effort and capture success per region by comparing the amount of visits to site regions vs. number of anurans captured [Search Efforts are similar across sites (1.2-4.3)]

For each region we intensively surveyed for Boreal Chorus Frogs but we also found other anuran species in the sites where Boreal Chorus Frogs were historically present (Table 2).

Region	County	# of Sites	Chorus Frogs detected	Species Detected
Jemez Mountains	Sandoval Co.	15	Y	*Ambystoma mavortium, Pseudacris maculata, Lithobates pipiens*
Rio Grande Valley	Bernalillo Co	9	N	
Southern Rocky Mountains	Colfax Co.	6	N	*Anaxyrus woodhouseii*
	Mora Co	5	Y	*Pseudacris maculata, Lithobates pipiens, Lithobates catesbeiana, Ambystoma mavortium*
	Rio Arriba Co.	8	Y	*Pseudacris maculata, Ambystoma mavortium*
	Taos Co.	8	N	

Table 2. Boreal Chorus Frog survey results in three regions of northern and central New Mexico

Figure 4. Other sympatric amphibian species captured and sampled were the New Mexico Species of Concern, Northern Leopard Frog (*Rana* (*Lithobates*) *pipiens*).

Pathogen Surveys — We intensively surveyed for Bd in 61 locations, within eight New Mexican counties, within three separate eco-regions where Boreal Chorus Frogs were historically found. From these populations we collected a total of 139 diagnostic samples to evaluate the presence/absence and prevalence of Bd pathogen in anuran communities.

We tested samples for Bd presence/absence, prevalence and intensity of infection. The total prevalence for Bd in northern and central New Mexico was 25.9 % (36/139); with prevalence by county at 22% in Sandoval Co. (11/50), 33.3% in Colfax Co. (1/3), 28.% in Mora Co. (15/53), and 27.3% in Rio Arriba Co. (9/33) (Table 3). We also collected samples from five different species, including the Northern Leopard Frog (*Lithobates* (*Rana*) *pipiens)*, Woodhouse's Toad (*Anaxyrus* (*Bufo*) *woodhousii*), Boreal Chorus Frog (*Pseudacris maculata*), American Bullfrog (*Lithobates* (*Rana*) *catesbeiana*) and Barred Salamander (*Ambystoma mavortium*) (Table 4).

Region	**County**	**# of Sites**	**# Bd Positive**	**Total Swabbed**	**Prevalence by County**
Jemez Mountains	Sandoval Co.	17	11	50	22%
Rio Grande Valley	Socorro Co.	2	0	0	N/A
Rio Grande Valley	Bernalillo Co	11	0	0	N/A
Southern Rocky Mountains	Colfax Co.	6	1	3	33.3%
Southern Rocky Mountains	Mora Co	6	15	53	28.3%
Southern Rocky Mountains	Rio Arriba Co.	8	9	33	27.3%
Southern Rocky Mountains	Taos Co.	8	0	0	N/A
TOTAL		**58**	**36**	**139**	**25.9%**

Table 3. Bd survey results in three regions of New Mexico

Site	Species	Bd prevalence
Mora County, Coyote Creek State Park	*Rana pipiens*	100% (6/6)
Mora County, Coyote Creek State Park	*Pseudacris maculata (2)*	
Mora County, Coyote Creek State Park	*Lithobates catesbeiana (1)*	
Carson NF, Hopewell Lake	*Ambystoma mavortium (1)*	
Carson NF, Hopewell Lake	*Pseudacris maculata*	21% (6/28)
Mora, NM (1A)	*Pseudacris maculata*	8% (2/24)
Mora, NM (1A)	*Rana pipiens (1)*	
Mora, NM (1B)	*Rana pipiens (1)*	
Mora, NM (1B)	*Pseudacris maculata (4)*	
Mora, NM, Horse Field	*Rana pipiens*	100% (2/2)
Mora, NM, Horse Field	*Pseudacris maculata (4)*	
Mora, NM, Site 2	*Pseudacris maculata*	20% (1/5)
Mora, NM, Site 2	*Rana pipiens*	100% (1)
Mora, NM, Morphy Lake State Park	*Lithobates catesbeiana (1)*	
Mora, NM, Morphy Lake State Park	*Rana pipiens*	100% (3/3)
Jemez Mountains, Santa Fe NF, Thompson Ridge	*Pseudacris maculata*	20% (3/15)
Jemez Mountains, Santa Fe NF, Thompson Ridge	*Ambystoma mavortium (1)*	
Carson NF, Trout Lakes	*Rana pipiens*	67% (2/3)
Carson NF, Trout Lakes	*Pseudacris maculata*	100% (1/1)
Valles Caldera NP, Valle Seco	*Pseudacris maculata*	22% (4/18)
Valles Caldera NP, Valle Seco	*Rana pipiens*	100% (2/2)
Valles Caldera NP, Valle Seco	*Ambystoma mavortium (1)*	
Valles Caldera NP, Pond (8)	*Ambystoma mavortium*	100% (1/1)
Valles Caldera NP, Pond (8)	*Pseudacris maculata (2)*	
Valles Caldera NP, Pond (4)	*Pseudacris maculata*	10% (1/10)
Vermejo Park Ranch	*Anaxyrus woodhousii*	33% (1/3)

Table 4. Bd Results for every species by site

Environmental factors — We collected temperature data (microhabitat and body temperature) from all 61 sites visited. We found that body temperatures ranged from 5-22.6 °C and averaged 11.8°C on Boreal Chorus frogs. Microhabitats of Boreal Chorus frogs temperature fluctuations ranged from 3.1-21.5 °C and averaged 12 °C, while air temperatures at habitats ranged from -0.5-21.6 °C and averaged at 14 °C. We found that chorus frog body temperatures are closely linked to their immediate microhabitat and the air temperature (Fig 5). Data collected from agar frog temperature loggers showed differences among microhabitat temperatures across elevations and that body temperatures generally match microhabitat temperatures (Fig 5).

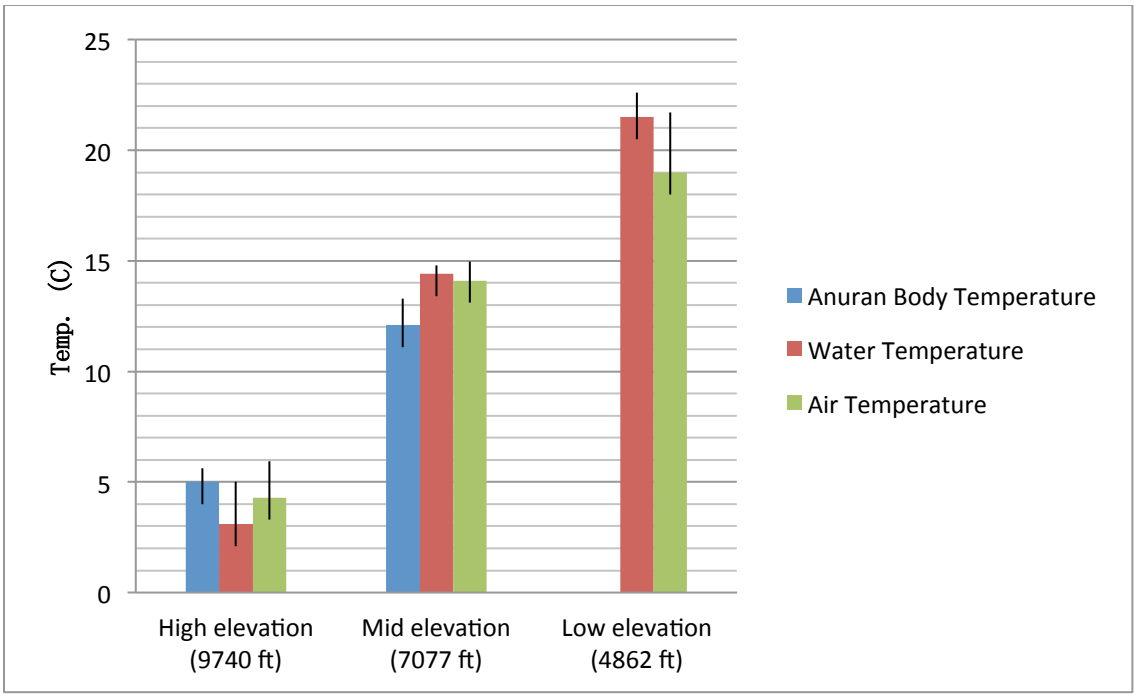

Figure 5. Average Boreal Chorus Frog body temperatures, microhabitat temperatures and air temperatures for three sites with varying elevational gradients.

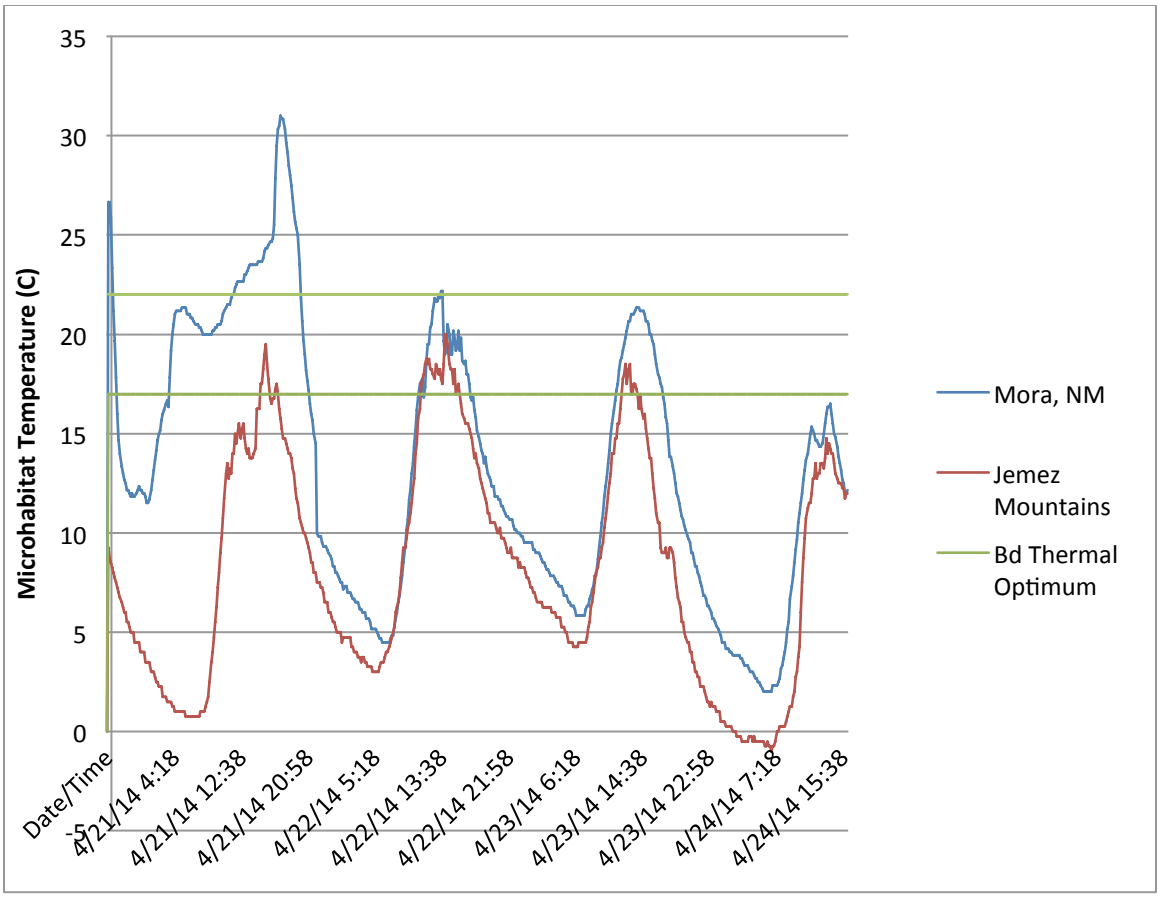

Figure 6. Microhabitat temperatures collected by agar models with temperature sensors taken between April 20, 2014 and April 24, 2014. During this period we sampled for Bd at: Mora, NM (28% prevalence) and the Jemez Mountains (22% prevalence).

Discussion

After a considerable search effort using standardized surveying methods, we found that Boreal Chorus frogs could be detected in only 30% of their historic sites. More specifically, we captured a total of 156 Boreal Chorus frogs in only 16 of 61 sites from three regions: the Southern Rocky Mountains (8 of sites present/ 28 of sites absent), the Jemez Mountains (8 of sites present/ 13 of sites absent) and the Rio Grande Valley (0 of sites present/ 9 of sites absent). To make an additional survey effort in the Rio Grande Valley (i.e., in addition to surveys in the historic sites), we visited six new sites, but unfortunately, we did not detect any Boreal Chorus frogs, or any other amphibian species, anywhere in the Rio Grande Valley.

In regions where we could find Boreal Chorus frogs, the frogs occupied a diverse array of riparian habitats such as perennial streams and marshes, ephemeral catchments and marshes, ephemeral ponds, mountain lakes, wet meadows, flooded fields and playa lakes. We also found that detectability (by hearing Boreal Chorus frog calling) was improved when we conducted surveys under optimal conditions, including sampling during the late spring months (from May to June), 1 hour after sunset and with the following weather

conditions: relatively high humidity (~35-70% relative humidity), low temperatures (~ -1.0-15.0 °C), low wind speed (~0-5) and no precipitation (i.e., rain). (However, we observed that Boreal Chorus frog calling during light snow events.) Using infrared thermometers, we found that Boreal Chorus frog body temperatures are closely linked to microhabitat and air temperatures. Using agar models, we found that daily temperatures will fluctuate from -1-20°C in high elevation sites and 2-32°C in low elevation sites. These temperature ranges fall within the optimal temperature range for Bd growth.

In this study we also tested extensively for Bd in New Mexican amphibians, and we especially focused on the Boreal Chorus Frog. Although we detected Bd in all of the sites where we found Boreal Chorus Frogs, and in other sympatric species, we suggest that chytridiomycosis may not be the only cause of population losses in Boreal Chorus Frogs in New Mexico. More specifically, the overall pattern of infection in our study suggests that Boreal Chorus Frogs, at least those in northern and central New Mexico, may not be highly susceptible to lethal chytridiomycosis. Three lines of evidence support this idea.

First, Boreal Chorus Frog populations are persisting with Bd infection, in some cases at a relatively high prevalence (e.g., 75% in Carson National Forest, Trout Lakes). We collected diagnostic samples (139 samples from 14/61 sites) and found that Bd prevalence in Boreal Chorus frog populations is generally low (ranging from Mora, NM, Robert Ortega's Cow fields site: 8% - 20% to the Jemez Mountains, Thompson Ridge site: 10% and Carson National Forest, Hopewell Lake: 21%). The prevalence for Bd in all species in northern and central New Mexico was 25.9% (36/139); with prevalence by county at 22% in Sandoval Co. (11/50), 33% in Colfax Co. (1/3), 28% in Mora Co. (15/53), and 27% in Rio Arriba Co. (9/33).

Second, the intensities of infection in this species were relatively low (~1,000-2,000 genome equivalents), especially when compared to other susceptible species (e.g., Northern Leopard frog, *Lithobates pipiens* at ~20,000-50,000 genome equivalents). We analyzed the relative intensity of Bd infection on all individual amphibians, which is measured in genome equivalents. In most cases, Boreal Chorus Frogs carried relatively low infection intensity compared to other more susceptible species.

Third, we found no evidence of disease-related mortality Boreal Chorus Frogs even with a considerable search effort (13 visits, >5 hours search effort) in a site where this species tested positive for Bd. We did, however, find two moribund Leopard frogs (*Lithobates pipiens*) at Coyote Creek, in the Southern Rocky Mountain Region. These individuals tested positive for Bd and this site had relatively high prevalence of Bd (67%). Although we attempted to isolate Bd and performed a post-mortem necropsy, we were unable to definitively determine the proximate cause of disease in these individuals.

Our results do not rule out the possibility that Bd played a role in past declines of Boreal Chorus Frog populations. Nor do our results suggest that Bd is not an on-going threat to Boreal Chorus Frogs and other New Mexico amphibian species. Rather, our findings suggest that the role of disease, as well as other causes, in on-going declines warrant further investigation.

We suggest that conservation management efforts consider the following outstanding concerns. First, we need a better understanding of disease dynamics in multi-species communities, particularly those amphibian assemblages in high elevation sites. For example, we found that Boreal Chorus frogs are persisting with infection in Trout Lakes (a high elevation site) where they share water bodies with the highly susceptible Boreal Toad (*Anaxyrus boreas*). It is unknown if the Boreal Chorus frogs may act as a pathogen reservoir for the Boreal Toad or if the specific environmental conditions in these high elevation habitats may exacerbate disease for either or both species. Second, we suggest that in some regions it may be appropriate to consider additional causes of Boreal Chorus Frog declines. For example, we did not detect any Boreal Chorus frogs (or any species of amphibians) in the Rio Grande valley. However, we noted that historic sites appeared to be heavily impacted by drought, habitat destruction and human encroachment (e.g., commercial development). There may also be additional causes of amphibian losses in this area that were not immediately apparent during our surveys.

Acknowledgements

The following individuals were closely involved with this project and provided abundant advising for New Mexico field sites, input on the amphibian species and assistance with field collections and sample processing in the laboratory. We would like to thank Gabriela Rios-Sotelo (New Mexico Tech), Carley Barron (NMT), Roberto Ortega (New Mexico Highlands University), Mason Ryan (University of New Mexico), Michelle Christman (US Fish and Wildlife), Bruce Christman (USFW), Dr. Sarah Corey-Rivas (NMHU), Leland Pierce (New Mexico Game and Fish), Steve Jerome (UNM), Cliff Richardson (NMT) and Tom Giermakowski (UNM - Museum of Southwest Biology).

Data were obtained from records held in the following institutions and accessed through the HerpNet data portal (http://www.herpnet.org) on 13 August 2013:

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Permits

Permits were granted by New Mexico State Parks and the Valles Caldera National Preserve. Dr. Voyles currently has IACUC approval for multiple amphibian species from New Mexico Tech. Updated IACUC amendments to include specific amphibians to New Mexico have approval.