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Title: Effects of *Batrachochytrium dendrobatidis* on amphibian communities in New Mexico
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Project Findings Summary

- Boreal Chorus Frogs were present in 32% (7/22) of the historical sites visited. No populations of Boreal Chorus Frogs could be found in the Gila Wilderness or the Apache Sitgraves National Forest.

- *Batrachochytrium dendrobatidis* **(**Bd) prevalence was of 56% across all sites where amphibians were detected. Mean infection intensity was 13,219 (range of 231– 251,868) genomic equivalents.

- Bd prevalence at Trout Lakes, where we sampled at multiple time points, was 75% and mean infection intensity (among three amphibian species) was 18,290 (range of 481-251,868) genome equivalents. Bd could not be detected in these populations until late May, when Boreal Toads were just emerging.

Management Implications

We suggest management continue to and intensify efforts to monitor Boreal Chorus Frogs and sympatric species throughout New Mexico, especially in the Gila Wilderness where no chorus frogs were detected. Additionally, our study indicates that Bd prevalence and infection intensity fluctuate seasonally in the Trout Lakes system of Carson National Forest. Efforts for disease eradication and reintroduction efforts for the Boreal Toad should be concentrated at a specific time point. Spefically, timing of infection peak should be considered when conducting species reintroductions and disease eradication should be implemented when Bd prevalence and infection intensity are at their peak, which is late spring (towards the end of May in 2015) at Trout Lakes.

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

Project Abstract

The fungal disease chytridiomycosis, which is 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, so disease dynamics can vary across biogeographic zones. In New Mexico, the threat of chytridiomycosis to New Mexico's amphibian communities has not yet been fully resolved. The Boreal Chorus Frog (*Pseudacris maculata*) is an excellent species for Bd investigation due to its distribution in multiple aquatic eco-regions and its co-occurrence with a high number of other native amphibian species. To determine Bd disease dynamics within the amphibian communities of New Mexico, we conducted field surveys to determine the presence/absence of Boreal Chorus Frogs and collected diagnostic samples to determine Bd prevalence and intensity of infection. We surveyed in three regions where Boreal Chorus Frogs have historically been found in counties in Northern New Mexico, the Jemez Mountains, and in Western New Mexico's Gila Wilderness. We also intensively focused our efforts in a site in Northern New Mexico, the Trout Lakes system. This site provides habitat for three species of special concern, including the Boreal Chorus Frog (*Pseudacris maculata),* the Northern Leopard Frog (*Rana* (*Lithobates*) *pipiens*), and the endangered Boreal Toad (*Anaxyrus boreas*). From surveys in 2015, we detected Boreal Chorus Frogs in 32% of all sites visited, a higher percentage than 2014 surveys (26%). However, Boreal Chorus Frogs were not detected in the Gila Wilderness or other sites in Western New Mexico. We also found a relatively high prevalence of Bd among all species sampled at all sites at 56% (44/79 individuals), which was higher than the prevalence in our 2014 surveys (26% in 2014). Bd was detected in Northern New Mexico at most sites from April until June. However, Trout Lakes remained Bd free until the last week of May when infection spiked and stayed relatively constant until end of sampling in June. From our results, we suggest that management focus on determining the causes of the loss of Boreal Chorus Frog populations from the Gila Wilderness where no chorus frogs were detected. We also suggest conservation managers consider seasonal disease fluctuations when implementing eradication and/or reintroduction strategies. In the case of sites such as Trout Lakes, where repatriation efforts are being implemented for the Boreal Toad, it may be a better strategy to time management efforts with knowledge of peak pathogen prevalence.

Introduction

Chytridiomycosis has devastated amphibian populations worldwide and is implicated in the extirpation, and in some cases 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 (*Anyraxus boreas*), Chiricahua Leopard Frog (*Rana* (*Lithobates*) *chiricahuensis*), Northern Leopard Frog (*Rana* (*Lithobates) pipiens*), and Lowland Leopard Frog (*Rana (Lithobates) yavapaiensis*). Understanding several key features of the disease, such as differential susceptibility and environmental factors that exacerbate disease infection, is essential for assessing the impact and potential threat of chytridiomycosis for the amphibians of New Mexico.

Wide variation in species responses to Bd-infection presents a central challenge for conservation programs and wildlife management because it is difficult to identify which species are most vulnerable and therefore require conservation intervention. An additional concern is that species that are inherently tolerant (or become tolerant) of Bd infection can act as reservoirs for more susceptible species. Thus, understanding differences in current infection patterns among species is critical for implementing effective management of threatened species.

This project focused on understanding infection patterns in the Boreal Chorus Frog (*Pseudacris maculata*). This species has been traditionally underrepresented in statewide surveys. It was previously thought to be ubiquitous in New Mexico; it historically occurred in many different habitat types and populations were found in every county of northern New Mexico, in the Rio Grande Valley, and populations in the Gila National Forest (Degenhardt et al. 1996).

We focused on the Boreal Chorus Frog for several reasons. First, declines in the group of Western 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). Second, the distribution of the Boreal Chorus Frog overlaps with several other species that are known to be susceptible to chytridiomycosis (e.g., Boreal Toad, Jemez Mountain Salamander and Northern Leopard Frog). Third, the distribution of Boreal Chorus Frogs spans a large portion of the state and a wide range of elevations. Therefore, we hope to determine if Bd is a proximate causative factor of reported declines in the Boreal Chorus Frog and other sympatric species in New Mexico and other parts of North America.

Another important consideration is that Bd is an environmentally sensitive pathogen so can change according to seasonal fluctuations among and within populations (Berger et al. 2004; Longo et al. 2010; Lenker et al. 2014). In temperate zones, Bd infection intensity and prevalence has been documented to increase at higher elevations and during the cooler seasons, presumably due to cooler temperatures (Sapsford et al 2013, Phillot et al 2013). Additionally, host individuals that can maintain higher body temperatures can reduce infection and infection risk (Rowley and Alford 2003). Thus, it is critical that environmental factors are investigated to determine disease risk to amphibians.

Methods

Surveys for Boreal Chorus Frog— One of our primary objectives was to add to the results from our previous study. In 2014, we conducted surveys for Boreal Chorus Frogs and collected diagnostic samples for Bd. We found Boreal Chorus Frogs in 26% (16/61) of the historical sites that we surveyed. However, we were unable to survey in all 35 historical sites in Northern New Mexico or in the 10 historical sites in the Gila Wilderness. For the present study,

we conducted rigorous, multi-day visual and acoustic surveys at 15 additional sites in the following counties: San Miguel, Mora, Catron, Sandoval and Rio Arriba counties (Fig 1).

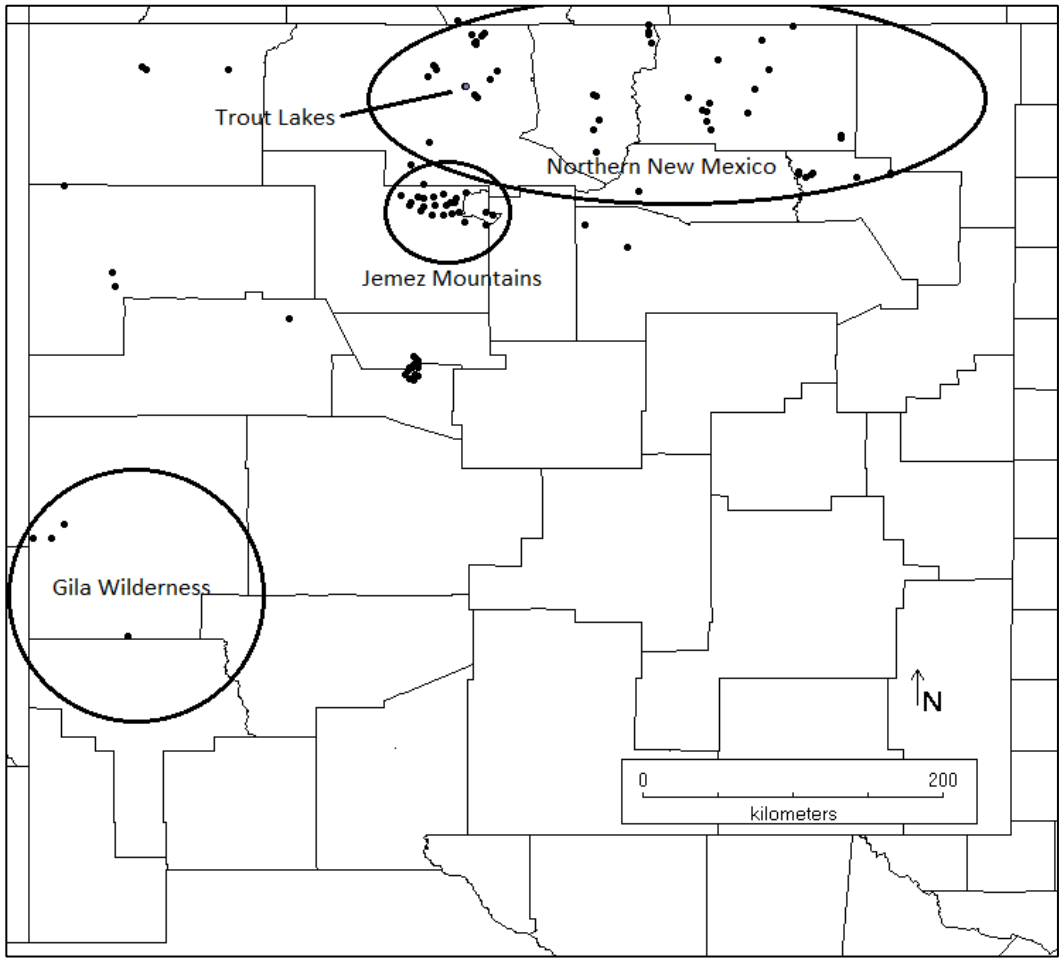

Fig 1| Sampling took place within three regions where populations of Boreal Chorus Frogs were thought to occur (from historic surveys): the Rocky Mountains region of Northern New Mexico including the Trout Lakes of Carson National Forest, the Jemez Mountains, and the Gila Wilderness. Points indicate the historical distribution of Boreal Chorus Frogs (*Pseudacris maculata*) in New Mexico (data collected from HerpNet.org).

Infection Patterns— We previously collected Bd diagnostic samples and found that Boreal Chorus Frogs are infected with Bd at a relatively low prevalence (18%- 22%) and that theses frogs are co-occurring with other threatened and endangered species. However, due to the timing of our surveys last year (focused on Boreal Chorus Frogs), we were not able to survey for the emergence of other species. To address this challenge, we conducted intensive surveys at Trout Lakes in Carson National Forest, Rio Arriba County by visiting the site at multiple time points. We sampled for Bd among all species that we encountered. We conducted diurnal and nighttime surveys after dusk to 1 am (MST zone) starting in April, when we believe is the best time to find Boreal Toads and Boreal Chorus Frogs. From our 2014 fieldwork, we know that Bd

is in this area. However, the timing of our sampling was late in the season when we believe the Boreal Toad had already dispersed. Therefore, we increased our survey efforts and made multiple trips to the same site in order to provide a more complete view of the infection patterns in this amphibian community.

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 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).

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 (IPC, 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

Boreal Chorus Frogs Presence/Absence

We visited a total of 22 historical and new sites in three separate regions where chorus frogs breed in New Mexico (The Jemez Mountain range, Northern New Mexico and Western New Mexico Fig1), but only seven of the total 22 sites contained Boreal Chorus Frogs (32% of sites; Table 1). Additionally, we found only three (3/12) populations of Boreal Chorus Frogs out of 12 historic sites. These sites were found in Northern New Mexico and the Jemez Mountains regions. The new sites had not been noted in historical records. These were located in Abiquiu, NM, and isolated populations surrounding Ledoux, NM. During our surveys in Western New Mexico, in the Gila Wilderness and Apache Sitgraves National Forest, we did not detect any Boreal Chorus Frogs (0/4 of historic sites surveyed). However, it is important to note that some these historic sites, especially in the Gila Wilderness, were severely burned from previous forest fires (Fig 2).

Fig 2. Gila Wilderness historical Boreal Chorus Frog site, Adam Hoague Pond, post 2012.

Table 1: All sites surveyed for Boreal Chorus Frogs (*Pseudacris maculata*) and all chorus frog detections in the three regions.
See supplemental file

Infection patterns across New Mexico

All sites combined- In all of the sites where we found Boreal Chorus Frogs, we collected samples for diagnostic analyses (n = 79). Additionally, we collected samples for diagnostic analysis from all other amphibian species found, including several other endemic New Mexican amphibians (Table 2). When this data is compiled, we found that Bd prevalence was 56% (44/79 individuals) across all sites. The mean infection intensity was 13,219 genome equivalents (range 231 – 251,868 genome equivalents).

Northern New Mexico- We assessed presence and absence of Bd at 4 sites in Northern New Mexico. We found a Bd prevalence of 58% (40/69 individuals) and a mean infection intensity of 21,151 (range from 231-251,868) genome equivalents (Table 2). Species sampled included Woodhouse's Toad, Boreal Chorus Frog, Boreal Toad, and Northern Leopard Frog.

Jemez Mountains- We detected Bd prevalence of 25% (1/4) and infection intensity of 51,738 genome equivalents (Table 2). Species sampled were Boreal Chorus Frogs.

Western New Mexico- Although we did not find Boreal Chorus Frogs in the Gila Wilderness, we did encounter other amphibian species, which were tested for Bd infection. These amphibians included Woodhouse's Toad (*Bufo woodhouseii*), Canyon Tree Frog (*Hyla arenicolor*) and Arizona Toad (*Bufo microscaphus*). Only Woodhouse's Toad and Arizona Toad tested positive for Bd (50%, 3/6). Taken together, we detected a Bd prevalence of 50% (3/6) and a mean infection intensity of 2,914 genome equivalents (5,099-6,983 genome equivalents) across all the sites and species within the Gila Wilderness.

Disease among species- Bd was detected on six endemic and disease susceptible species of New Mexico, three of these being species of special conservation status: Boreal Toad, Arizona Toad and Northern Leopard Frog. Boreal Toads, all found at Trout Lakes, had a very high Bd prevalence of 75% (9/12). Arizona Toads, only found in the Gila Wilderness, also had a high prevalence of 50% (2/4). Northern Leopard Frogs, found throughout northern New Mexico, also had a high prevalence of 75% (6/8). Boreal Chorus Frogs overlapped in the habitats of several of these species (i.e., Northern Leopard Frogs and Boreal Toads), and also exhibited a high prevalence of 50% and high infection intensities (mean 18,260 genome equivalents).

Table 2. Infection intensities for all sites and individuals.
See supplemental file.

Table 3. Bd prevalence for all sites. P = positive; N = negative.
See supplemental file.

Amphibian emergence and infection patterns across one season

We conducted intensive survey efforts at Trout Lakes in order to understand patterns of disease dynamics over time among three New Mexico amphibian species (Boreal Chorus Frogs, Boreal Toads and Northern Leopard Frogs). Specifically, we completed a total of eight visits to the Trout Lakes system in the spring and summer (from April until June) to sample Bd from

every amphibian encountered. This period of sampling was chosen because it includes the emergence from overwintering and breeding season of each of the amphibian species.

We found Boreal Chorus Frogs occupying numerous types of water bodies found in Trout Lakes, such as the small lakes making up what is designated Trout Lakes, as well as ephemeral pools and small beaver made ponds which connect the lakes. Boreal Toads have been extirpated from New Mexico since presumably the 1970's but have been recently repatriated into Trout Lakes. Historically, they have been found throughout the Trout Lakes system, including in small beaver ponds and the larger lakes at Trout Lakes. Northern Leopard Frogs have been consistently occupying the Trout Lakes system but are found mostly in the deeper lakes and not the surrounding ponds and ephemeral pools that the toads and chorus frogs occupy.

We detected adult Boreal Chorus Frogs and Northern Leopard Frogs emerging from overwintering (Fig 3). However, we only found young-of-the-year Boreal Toads, not adults (Fig 4). Boreal Chorus Frogs were the first amphibians detected and were most abundant earlier in the season, from April until May (Fig 5). Northern Leopard Frogs also emerged in April and peaked in abundance around late May. However, Boreal Toads were not detected until late May and peaked in abundance in mid June (Fig 5).

Fig 3. Adult Boreal Chorus Frog.

Fig 4. All Boreal Toads encountered were 1-2 year old juveniles.

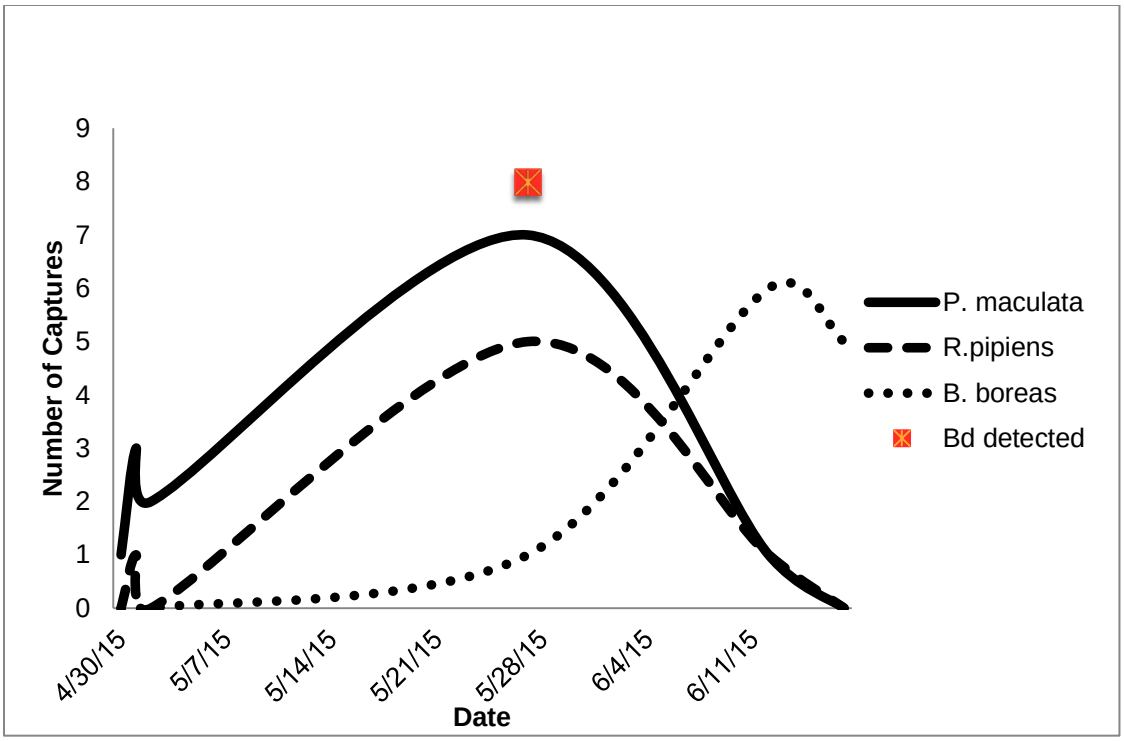

Fig 5. Species abundances based on successful captures and sightings at Trout Lakes, Carson National Forest and the date of Bd detection at the site.

Although we sampled for Bd early in the season in Boreal Chorus Frogs and Northern Leopard Frogs, Bd was not detected until the last week of May, when Boreal Toads were just beginning to emerge and Northern Leopard Frogs and Boreal Chorus Frogs were at their peak of abundance (Fig 5). Following this initial detection, all species tested positive for Bd infection.

Prevalence and infection intensity also varied over the season at Trout Lakes (Figs 6 and 7). For Northern Leopard Frogs and Boreal Toads, prevalence and infection intensities were highest at the end of May and decreased in June. Boreal Chorus Frogs and Northern Leopard Frogs had no detectable infection until the last week of May. Infection intensities peaked for Boreal Chorus Frogs in the beginning of June. Northern Leopard Frogs infection peaked at the end of May but was undetected after that. Lastly, Boreal Toads maintained their infection as they emerged and became more abundant. They also peaked their infection intensities at the end of May, but were reduced in early June and increased slightly in mid June (Fig 6).

To compile the results across the whole season at Trout Lakes, we collected a total of 33 samples for diagnostic analysis of disease and found a Bd prevalence of 51% among all species (17/33). At Trout Lakes, the mean infection intensity for all three species was 18,290 (ranged from 487 to 251,867) genome equivalents (Fig 7). Twelve samples that were collected from the repatriated, endangered Boreal Toads at Trout Lakes indicated 75% prevalence (9/12 toads tested positive for Bd) and a mean infection intensity of 39,085 (ranged from 487-251,867) genome equivalents. In contrast, Bd prevalence in the Northern Leopard Frogs and Boreal Chorus Frogs was 71% (5/7) and 21% (3/14) respectively. Northern Leopard Frogs had a mean infection intensity of 13,710 genomic equivalents (ranged from 3,298-46,635 genomic equivalents). Boreal Chorus Frogs had a lower mean infection intensity of 2,757 genomic equivalents (4,950-28,451 genomic equivalents).

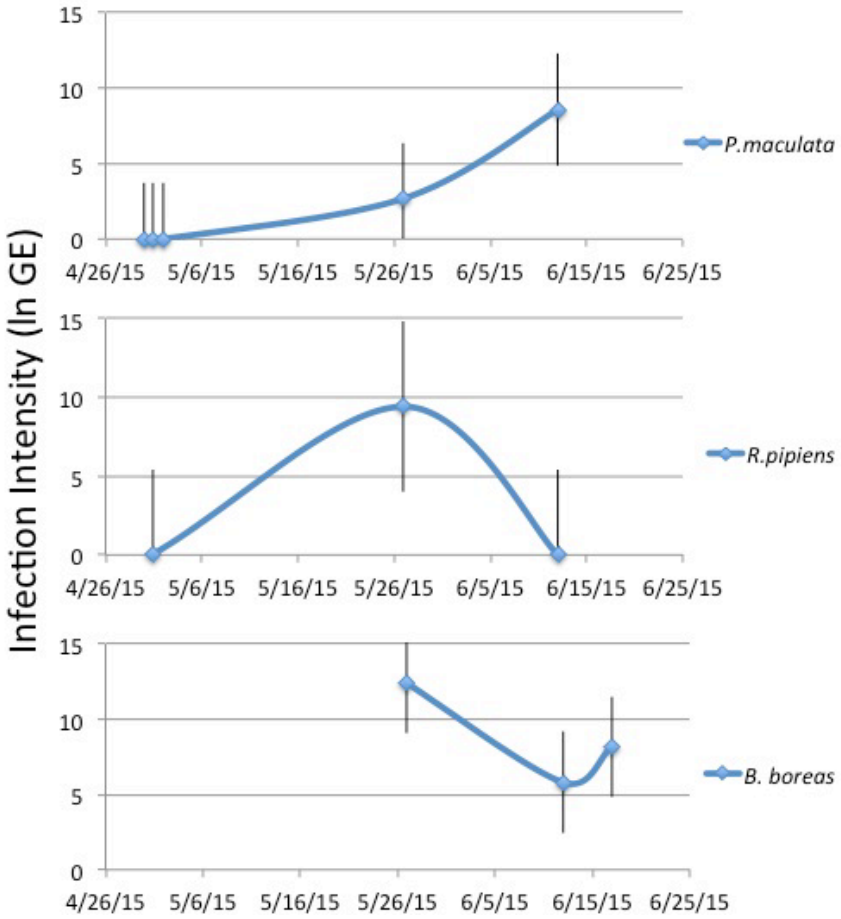

Fig 6. Infection intensities (i.e., genomic equivalents) at Trout Lakes for each species encountered over one season in spring and early summer of 2015

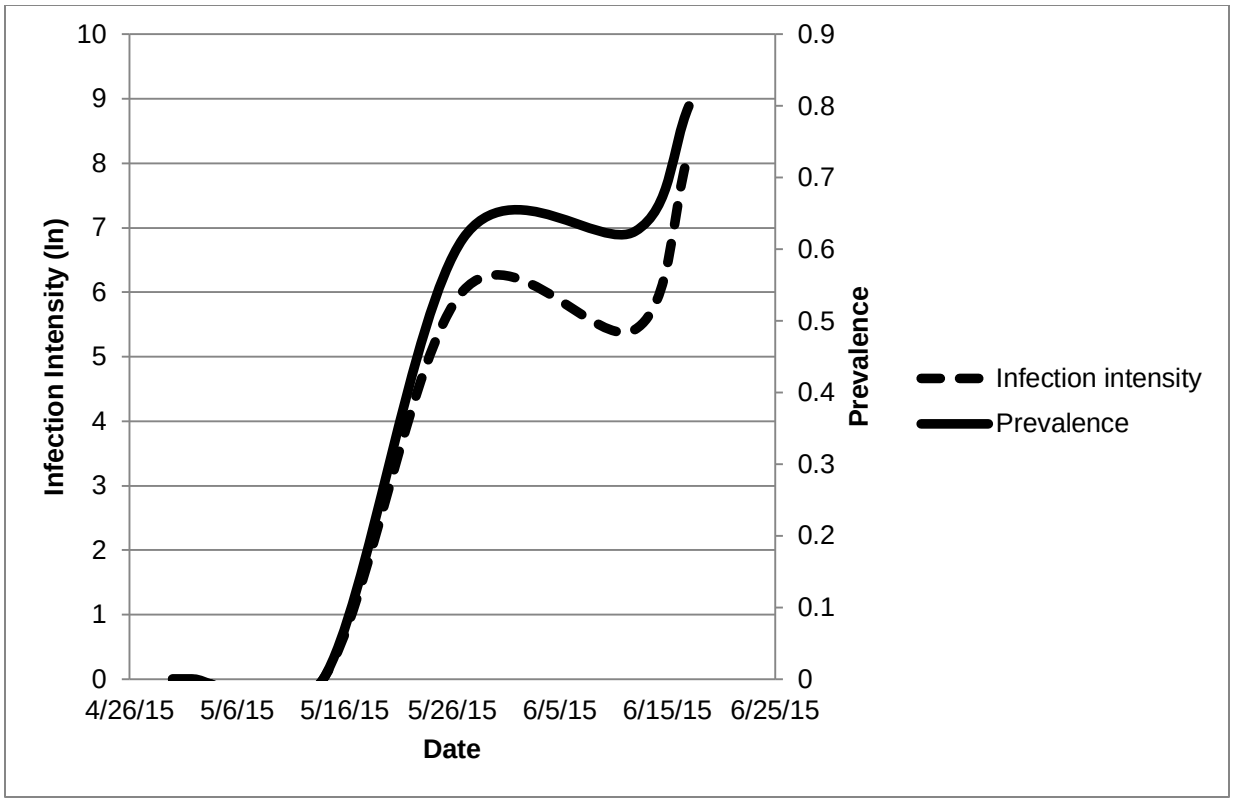

Fig 7. Prevalence of Bd infection (infected sampled individuals/total sampled individuals) and infection intensities (genomic equivalents from PCR diagnostic analysis) over one season at Trout Lakes.

Discussion

Over the past two seasons (2014 and 2015), we have been able to intensively survey for Boreal Chorus Frog (*Pseudacris maculata*) populations in Northern New Mexico, the Jemez Mountains, and Western New Mexico, where these frogs were historically found from the spring into the summer. Additionally, we collected diagnostic samples to evaluate the prevalence of Bd pathogen within amphibian communities. At many of our targeted sites, Boreal Chorus Frogs co-occur with other species of special concern (e.g., the Boreal Toad) so we were able to collect diagnostic samples from multiple species. These samples allowed us to provide a better understanding of the infection dynamics in entire communities throughout New Mexico.

We expanded our survey effort from last year and surveyed in areas we were previously unable to visit and new sites in Northern New Mexico and the Gila Wilderness. Although we conducted rigorous acoustic and visual encounter surveys, only a very small percentage (32%) of these historical sites still contain Boreal Chorus Frogs. Additionally, no Boreal Chorus Frogs could be detected in surveys within Western New Mexico, specifically within Gila Wilderness and Apache Sitgraves National Forest.

With the absence of Boreal Chorus Frogs from the Gila Wilderness, it will be challenging to determine the role of Bd in these disappearances. Due to other evidence in this species (described below), we suggest that Boreal Chorus Frogs (at least those in Northern New Mexico) are not currently particularly susceptible to chytridiomycosis and may be acting as a reservoir for Bd in sites where they are still found. Nevertheless, we cannot rule out the possibility that Boreal Chorus Frogs were negatively impacted by Bd in the past or that chytridiomycosis has

exacerbated declines due to other causes such as wildfire or drought. We suggest that additional research should focus on amphibians in the Western New Mexico region, as this appears to be an area of marked declines. We do know Bd is present in the historical sites in the Gila Wilderness [positive Bd from other amphibian species, including the Arizona Toad (*Bufo microscaphus*)]. Therefore, we suggest it may be useful to monitor other amphibian species for chytridiomycosis and fully investigate the losses of Boreal Chorus Frogs, potentially from other causes, in this region.

From our surveys in Northern New Mexico, it appears that frog populations are more heavily infected with Bd in this region and should be monitored for chytridiomycosis. Similar to surveys from last year, Bd prevalence and infection intensities were the highest in sites in the Southern Rocky Mountain region of northern New Mexico; specifically Ledoux, NM and at Trout Lakes in Carson National Forest. In Ledoux NM, the Bd prevalence was the highest at 85% (23/27). At Trout Lakes, Bd prevalence was also high at 53% and included sampling of Boreal Toads, Boreal Chorus Frogs and Northern Leopard Frogs. In both the Gila Wilderness and Valles Caldera National Preserve in the Jemez Mountains our capture rates were low (4-6 individuals) so our sample size is not large enough to determine a strong pattern of disease. These results suggest there are considerable differences in infection patterns among these different regions of New Mexico and conservation plans may benefit from adaptive management in each of these regions.

Boreal Chorus Frogs may be acting as a reservoir for Bd in Northern New Mexico sites. During our surveys, we observed that Boreal Chorus Frogs are persisting with high levels of infection in many areas. For example, in Ledoux, NM chorus Boreal Chorus Frogs show high infection intensities and prevalence, but no clinical signs of disease or mortality. At Trout Lakes, Boreal Chorus Frogs do not have a high Bd prevalence, but they do have high infection intensities beginning in late spring. While we see no evidence of declines in Boreal Chorus Frogs at these sites, declines of other Bd-sensitive species, such as Boreal Toads and Northern Leopard Frogs, have been a concern for management. Additional research, including laboratory infection experiments, could determine if Boreal Chorus Frogs are indeed tolerant of Bd infection and if they are able to transmit Bd to other sensitive species, resulting in lethal disease.

Our results for Bd surveys in Trout Lakes suggest that the disease dynamics follow a seasonal pattern. Specifically, we see increase in Bd prevalence and infection intensities in late spring. This period corresponds to the emergence from overwintering, and the beginning of breeding season, for the Northern Leopard Frog and the Boreal Toad. There are a variety of factors that may be contributing to this pattern. In Trout Lakes seasonal temperature changes may be facilitating an increase in Bd prevalence in late spring. Additionally, amphibian species that are known to be Bd infected (e.g., Boreal Chorus Frogs) may be surviving long enough (1-2 years) to re-infect their own populations and/or other species the following breeding season. This possibility is concerning for more susceptible species, such as the Boreal Toad. We only found juvenile Boreal Toads at Trout Lakes and no sign of older adults. While this does not necessarily mean they are absent (it is possible we were unable to detect them), it does suggest that these individuals are not surviving through metamorphosis and may be succumbing to disease (Carey et al 2006). Additional research focused on survival of Boreal Toads (e.g., using mark-recapture techniques) at Trout Lakes may provide further insight on this question.

Overall, based on our survey findings in 2015, we suggest that the factors contributing to amphibian declines in New Mexico are complex. Compared to historic records, Boreal Chorus Frog populations appear to be far less abundant today. Most concerning is that we could not find any populations in the Gila Wilderness or Apache Sitgraves National Forest. The role of disease in these losses is equivocal. Bd is present in this region, and so may play a role, but disease-induced declines cannot be definitively determined without the ability to sample some frogs. In other regions, Boreal Chorus Frogs may be contributing to seasonal patterns of diseases and may be acting as pathogen reservoirs for more susceptible species. With these insights, we suggest several key areas for additional research and management. To begin with, we suggest that further investigations in Western New Mexico are needed to determine if drought or wildfire could be contributing to the losses of Boreal Chorus Frogs in this region. In addition, we suggest that additional research on the role of Boreal Chorus Frogs as pathogen reservoirs is warranted for management of other sensitive species in Northern New Mexico. Management efforts that focus on reintroduction (and disease eradication), such as the effort at Trout Lakes, should be aware of the seasonal dynamics to improve the chances of success. Lastly, we also suggest Boreal Chorus Frogs may be a key species in monitoring disease in areas where sympatric frogs are rare or declining and chorus frogs are persisting, such as Mora County or the Jemez Mountains.

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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: Historical herpetological data used in this study was obtained from the Sam Noble Oklahoma Museum, University of Oklahoma; Museum of Southwest Biology, University of New Mexico; Carnegie Museum of Natural History, Pittsburgh, PA; University of Colorado Museum; Utah Museum of Natural History; The Centennial Museum, University of Texas El Paso; Amphibian and Reptile Collection, University of Arizona; American Museum of Natural History, NY; University of Kansas Natural History Museum and Biodiversity Research Center; Cornell University Museum of Vertebrates; Museum of Vertebrate Zoology, University of California Berkeley; Smithsonian National Museum of Natural History, Washington DC; and the Natural History Museum of Los Angeles County (Accessed through the HerpNet2 Portal, www.HerpNet2.org, 2014-07-28 which is now called VertNet.org).

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 and from New Mexico Department of Game and Fish (NMDGF License Number 1767099).

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