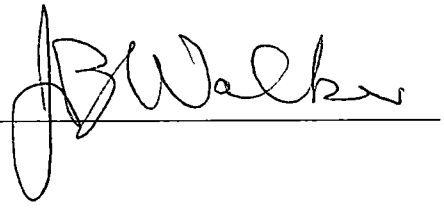
Comparison of prevalence and intensity of the fungal pathogen *Batrachochytrium dendrobatidis* in Turnbull National Wildlife Refuge and northern Idaho

Philip Campos

Dr. Jenifer Walke

Faculty advisor signature 

Statement of Purpose

The fungal pathogen *Batrachochytrium dendrobatidis* (Bd) poses a great threat against the biodiversity of amphibian species, which play an important role in healthy ecosystems. My goal is to compare the prevalence and intensity of Bd on frogs at Turnbull National Wildlife Refuge (TNWR) with those sampled from populations in northern Idaho to assess how geographic location and amphibian biology influences disease patterns.

Background and Significance

Emerging infectious diseases threaten global biodiversity by contributing to population declines and extinctions, having been associated with declines in a large range of organisms, including amphibians, birds, lions, invertebrates, and plants (1, 2). Chytridiomycosis, the disease caused by Bd, has resulted in the decline or extinction of nearly 500 frog species globally (3-7). The disease spreads in epidermal cells, which is problematic because amphibian skin is crucial in regulating the exchange of respiratory gases, electrolytes, and water (8). Bd prevalence, defined as the proportion of sampled individuals that had Bd detected, and Bd infection intensity, defined as the estimated number of Bd zoospores found on an infected individual, were measured in Columbia spotted frogs (*Rana luteiventris*) sampled from northern Idaho in a large scale study in 2013. Bd prevalence was found to be high, with 65% (N = 261) testing as Bd positive out of 399 frogs, and when summarized by number of sites, Bd was detected in 80% (N =123) of 153 sites (Figure 1) (9). However, infection intensity was found to be low, ranging from 0 to 98, far below numbers seen in populations facing decline, e.g. 10,000 zoospores (10). It is unknown how the high Bd prevalence affects other amphibian species in northern Idaho that coexist in the surveyed area but were not screened for Bd. The same study area was sampled again in 2018, with

analysis currently underway to quantify Bd prevalence and infection intensity. These results will provide an opportunity to compare disease status in northern Idaho after approximately 5 years.

Eastern Washington is another potential area of concern for Bd, as Bd has been detected on Columbia spotted frogs and a second species, the Pacific chorus frog (*Pseudacris regilla*), from TNWR based on our lab's preliminary results. Pacific chorus frogs in Sierra Nevada of California have been found to act as a reservoir species, being highly tolerant of the disease despite carrying higher loads of Bd than low tolerance sympatric species facing population declines (11). In this study by Reeder et al., Pacific chorus frog persisted at 100% of sites where sympatric species where extirpated from 72% of sites in a wave of disease. Similar patterns of high tolerance and high infection intensity are seen in known Bd carriers such as the American bullfrog (*Rana catesbeiana*) and African clawed frog (*Xenopus laevis*) (12, 13). This pattern differs to that observed for Columbia spotted frogs in northern Idaho, where frogs are also suspected to act as carriers because of the high Bd prevalence observed, but instead carry low loads of zoospores.

By measuring Bd prevalence and infection intensity in frogs sampled in TNWR, I will be able to compare these factors in two species that have shown different patterns when Bd is present. Together with our sample set from northern Idaho, I will be able to use the TNWR sample set to compare Bd prevalence and infection intensity in populations of Columbia spotted frogs from two study areas. Results from these comparisons could help inform a future study involving characterization of the skin microbiomes of TNWR frogs, which would supplement my current work involving characterization of skin microbiomes on frogs from the 2013 northern Idaho sample set. Comparisons between the bacterial communities of 1) the two species in TNWR and 2) Columbia spotted frogs in the two locations could demonstrate how skin bacteria

are involved in tolerance of Bd, which could contribute to conservation efforts of amphibian species.

Objectives

First, I will measure Bd prevalence and infection intensity in sampled frogs from TNWR. Results will be used for two types of comparisons: 1) compare Bd prevalence and infection intensity between two species in TNWR and 2) compare Bd prevalence and infection intensity between Columbia spotted frogs from northern Idaho and TNWR. I hypothesize that 1) Pacific chorus frogs will exhibit higher infection intensity than Columbia spotted frogs as seen in the Reeder et al. study (11) and 2) Columbia spotted frogs in TNWR will have low infection intensity similar to numbers seen in northern Idaho Columbia spotted frogs.

Procedures and Rationale

The Idaho Department of Fish and Game sampled *Rana luteiventris* during the periods of May-September 2013 (N=396) and 2018 (N=163) (Table 1). The study area consisted of most of the Idaho Panhandle, the northernmost region of Idaho encompassing 10 counties (see Figure 1). Skin swab samples were collected using sterile buccal swabs to swab the underside of the frog back and forth 15 times (30 total swipes) as described in the Multi-species Baseline Initiative project report (9). DNA isolation and screening for Bd prevalence and infection intensity have previously been completed for Idaho and are currently being processed for 2018 samples. I am requesting funds to assess Bd loads for the 2019 TNWR samples.

***Rana luteiventris* Skin Swab Samples: # Samples (# Sites)**		
Idaho 2013	Idaho 2018	Sites Sampled In Both Years
396 (153)	163 (68)	53 Sites

Table 1. Number of skin swab samples and sites sampled from the years 2013 and 2018. Sampling effort was lower in 2018. Data from Idaho Department of Fish and Game.

Skin swab samples were collected as above from 29 Columbia spotted frogs and 52 Pacific chorus frogs at TNWR in May and June of 2019. I isolated DNA from these samples using the Qiagen DNeasy Blood & Tissue Kit for DNA purification. I will run a preliminary screening to detect presence of Bd using conventional PCR with primers Bd1a and Bd2a (14), which is a cost-effective way to assess presence/absence of Bd, but is limited in that it does not provide critical infection intensity data. Samples will then be sent for qPCR to measure infection intensity (i.e. Bd zoospore loads per individual). DNA will be sent to Dr. Jake Kerby's lab at University of South Dakota for duplex qPCR reactions, which allow for simultaneous screening of prevalence and intensity of two amphibian pathogens: Bd and the newly emerging chytrid fungus, *Batrachochytrium salamandrivorans* (Bsal), which is not yet widespread in North America, but its surveillance is critical for a rapid response if necessary (15, 16).

Bd infection intensity data, measured in zoospore equivalents, or genomic equivalents based on the quantity of Bd DNA found through qPCR, will be log-transformed to normalize the data. Separate student's t-tests will be used to test for statistically significant differences in the two comparisons: 1) Bd prevalence and infection intensity between two species in TNWR and 2) Bd prevalence and infection intensity between Columbia spotted frogs from northern Idaho and TNWR.

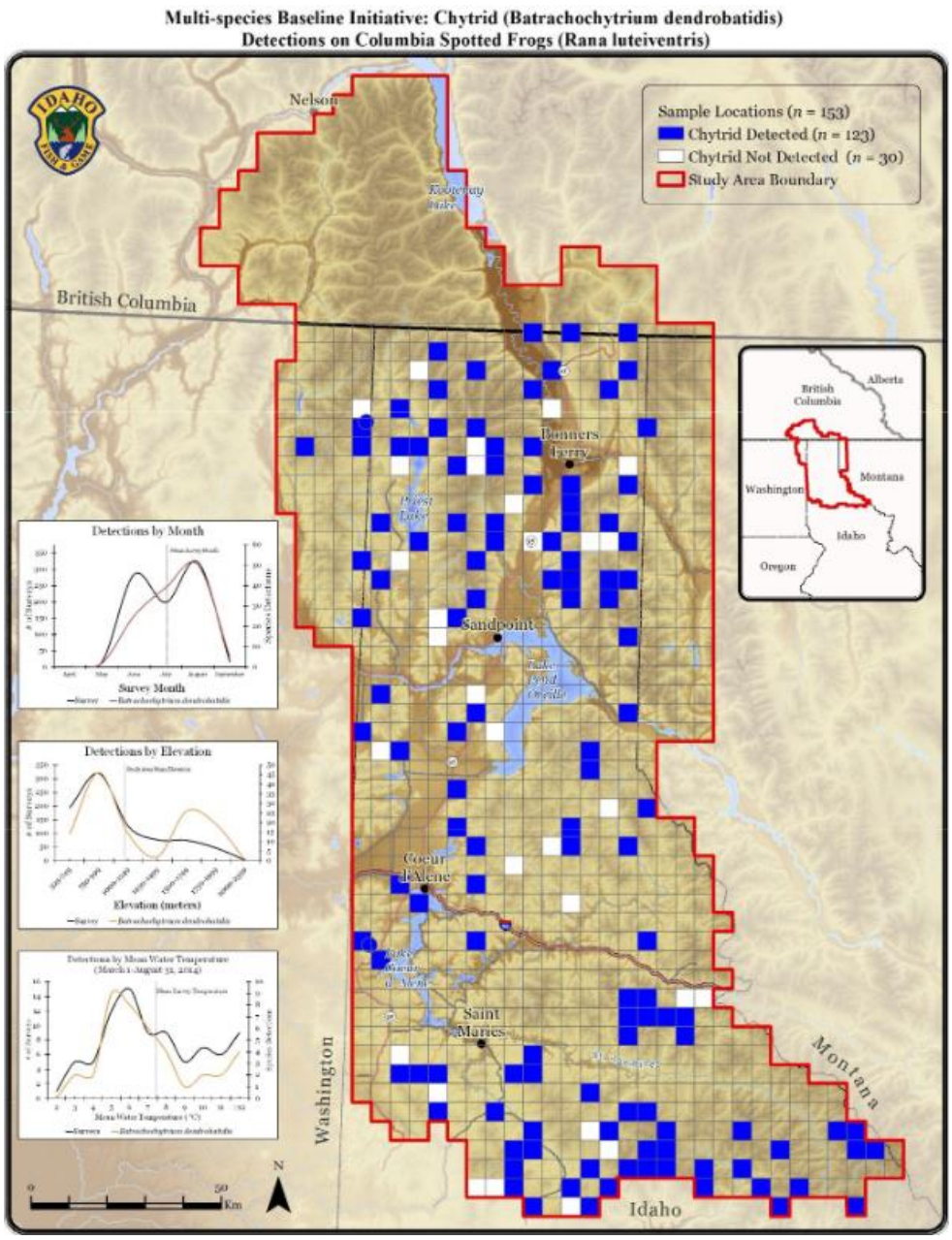


Figure 1. Map of northern Idaho study area by Idaho Fish and Game. Bd was detected on Columbia spotted frogs in 80% of sampled locations (N = 123 out of total N = 153).

Budget Justification

Item	**Cost**	**Quantity**	**Total Cost (+ shipping)**
qPCR	*$10 per sample*	*81*	*$810.00**
TOTAL BUDGET REQUESTED			***$750.00***

*Remaining $60 to be supplemented with Dr. Walke's discretionary research funds.

Timeline

Quarter	Winter 2020	Spring 2020
Tasks	• Receive Bd prevalence and infection intensity results for 2018 Idaho samples • Run Bd presence/absence PCR on 2019 Turnbull samples • Send 2019 Turnbull samples for qPCR to measure Bd intensity (depending on funding) • Data analysis to characterize skin microbiomes in 2013 samples and compare bacterial communities in infected and uninfected frogs*	• Receive qPCR results and conduct statistical analyses • Thesis defense • Submit paper for publication

*Described in Fall 2018 mini grant

References

1. Harvell C, Mitchell C, Ward J, Altizer S, Dobson A, Ostfeld R, et al. Ecology - Climate warming and disease risks for terrestrial and marine biota. *Science* (2002) **296**:2158-62 doi: 10.1126/science.1063699.

2. Daszak P, Cunningham A, Hyatt A. Wildlife ecology - Emerging infectious diseases of wildlife - Threats to biodiversity and human health. *Science* (2000) **287**:443-9 doi: 10.1126/science.287.5452.443.

3. Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, et al. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* (2019) **363**:1459-63.

4. Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, et al. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *Ecohealth* (2007) **4**:125-34 doi: 10.1007/s10393-007-0093-5.

5. Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, et al. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc Natl Acad Sci U S A* (1998) **95**:9031-6.

6. Garner T, Walker S, Bosch J, Hyatt A, Cunningham A, Fisher M. Chytrid fungus in Europe. *Emerg Infect Dis* (2005) **11**:1639-41.

7. Ouellet M, Mikaelian I, Pauli B, Rodrigue J, Green D. Historical evidence of widespread chytrid infection in North American amphibian populations. *Conserv Biol* (2005) **19**:1431-40 doi: 10.1111/j.1523-1739.2005.00108.x.

8. Rosenblum EB, Voyles J, Poorten TJ, Stajich JE. The deadly chytrid fungus: a story of an emerging pathogen. *PLoS Pathogens* (2010) **6**:e1000550.

9. Lucid M, Robinson L, Ehlers S. Multi-species Baseline Initiative Project Report: 2010-2014. *Idaho Department of Fish and Game, Coeur d'Alene* (2016).

10. Clare F, Daniel O, Garner T, Fisher M. Assessing the ability of swab data to determine the true burden of infection for the amphibian pathogen Batrachochytrium dendrobatidis. *EcoHealth* (2016) **13**:360-7.

11. Reeder NMM, Pessier AP, Vredenburg VT. A Reservoir Species for the Emerging Amphibian Pathogen Batrachochytrium dendrobatidis Thrives in a Landscape Decimated by Disease. *PLoS One* (2012) **7**:e33567 doi: 10.1371/journal.pone.0033567.

12. Daszak P, Strieby A, Cunningham A, Longcore J, Brown C, Porter D. Experimental evidence that the bullfrog (Rana catesbeiana) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. *Herpetolog J* (2004) **14**:201-7.

13. Solís R, Lobos G, Walker SF, Fisher M, Bosch J. Presence of Batrachochytrium dendrobatidis in feral populations of Xenopus laevis in Chile. *Biol Invasions* (2010) **12**:1641-6.

14. Annis SL, Dastoor FP, Ziel H, Daszak P, Longcore JE. A DNA-based assay identifies Batrachochytrium dendrobatidis in amphibians. *J Wildl Dis* (2004) **40**:420-8.

15. Standish I, Leis E, Schmitz N, Credico J, Erickson S, Bailey J, et al. Optimizing, validating, and field testing a multiplex qPCR for the detection of amphibian pathogens. *Dis Aquat Org* (2018) **129**:1-13.

16. Gray MJ, Lewis JP, Nanjappa P, Klocke B, Pasmans F, Martel A, et al. Batrachochytrium salamandrivorans: The North American Response and a Call for Action. *PLoS Pathog* (2015) **11**:e1005251 doi: 10.1371/journal.ppat.1005251 [doi].