Climate change is predicted to increase the distribution of invasive threats to native amphibian populations in the northwestern US: *Batrachochytrium dendrobatidis* and the American bullfrog

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Abstract

Aim: Invasive species operate at multiple scales, from pathogen to predator, and may further expand their range as climate change eliminates limiting climatic factors. Amphibians are declining globally, with invasive species as a driving cause. We modeled habitat for two major invasive threats to amphibian populations, the newly-emerged pathogenic amphibian chytrid fungus (*Batrachochytrium dendrobatidis*; Bd) and the American bullfrog (*Lithobates catesbeianus*), to develop spatially-explicit predictions of their potential expansion under scenarios of climate change.

Location: North-central Idaho, USA, a topographically complex landscape that includes a wide range of climatic conditions.

Methods: We documented the presence of Bd and American bullfrogs through field surveys and quantitative PCR testing (for Bd), modeled the regional ecological niche for each using MaxEnt, and predicted their distribution under two emissions scenarios. Additionally, we tested for genetic bottlenecks associated with the presence of Bd.

Results: We found that Bd presence and infection intensity varied among species, was more prevalent at lower elevations, and was not associated with genetic bottlenecks in our sample. Models indicated that the presence of this pathogen was associated with areas having warmer winters and drier, cooler summers. Bullfrog distribution was associated with areas with warmer, wetter winters. Under climate warming scenarios, both of these invasive species are predicted to expand their range, dramatically in the case of the bullfrog, increasing threats to native amphibian species and populations in this area.

Main conclusions: Native species are being impacted by a host of invasive species functioning at multiple scales. We found that native amphibian populations in north-central Idaho will increasingly be challenged by two invasive species: the pathogen Bd and the predaceous American bullfrog. While the outcome for native amphibian species may vary by species and habitat, the increased exposure to both will present additional challenges for the persistence of these populations.

Keywords: American bullfrog, amphibian decline, *Batrachochytrium dendrobatidis*, climate change, Columbia spotted frog, Idaho giant salamander, invasive species, long-toed salamander, species distribution model, western toad

Introduction

The global ecological and economic impact of introduced species is extensive and occurs at multiple scales, from microbe to predator (Simberloff, 2000). Climate change will likely result in the range expansion of many invasive species, increasing these impacts (Simberloff, 2000; Harvell *et al.,* 2002). Understanding the potential spatial distribution of invasive threats to native communities is essential to designing effective mitigation and management efforts.

The decline of the world's amphibian populations has now been well documented, with one-third of all amphibian species threatened with extinction (Stuart *et al.,* 2004). While causes of these declines are multi-faceted and synergistic (Blaustein & Kiesecker, 2002), invasive species at multiple scales represent major threats to amphibian populations (Kats & Ferrer, 2003; Skerratt *et al.,* 2007). Two of the most important invasive threats to amphibian populations are the American bullfrog (*Lithobates catesbeianus*), which eats and outcompetes native amphibians as well as other species (Casper & Hendricks, 2005), and *Batrachochytrium dendrobatidis* (Bd), a pathogenic chytrid fungus which phylogenetic evidence indicates has been recently introduced around the world (Morehouse *et al.,* 2003).

Amphibians, especially those with complex life cycles (adult and larval stages), are particularly vulnerable to introduced predators (Kats & Ferrer, 2003). The American bullfrog is a voracious predator and an invasive species in the western United States and Europe, as well as other locations around the world (Casper & Hendricks, 2005). Bullfrogs have been implicated in the decline of many western native amphibian species (Kats & Ferrer, 2003; Casper & Hendricks, 2005), although whether they are a causative agent is unclear in some cases (Hayes & Jennings 1986; Pearl *et al.,* 2005). This species is highly fecund and extremely difficult to eradicate once established (Adams & Pearl, 2007). There is some evidence that the distribution

of bullfrogs is limited by cold temperatures; a global suitability model for bullfrogs found minimum temperature to be an important predictor, with probability of occurrence maximized between -20°C and 13°C (Ficetola *et al.*, 2007).

Many invasive species occur at a microbial scale that is much more difficult to observe. The most obvious of these invaders are those that cause disease. Bd is a newly emerged pathogen that has caused the decline or extinction of up to about 200 amphibian species (Skerratt *et al.*, 2007). According to the Global Amphibian Assessment (Stuart *et al.*, 2004), 92.5% of the amphibians listed as critically endangered are undergoing "enigmatic" declines, which are likely related to Bd (Skerratt et al. 2007). This invasive fungus reached the western hemisphere by 1961 (Ouellet *et al.*, 2005) but was not described until 1999 (Longcore *et al.*, 1999). This pathogen is now widespread globally, but with a patchy distribution that suggests limitations of survival and/or spread (Fisher *et al.*, 2009).

While most systems are predicted to experience more frequent or severe disease impacts under climate change, some pathogens may decline with warming (Harvell *et al.*, 2002). Bd is sensitive to high temperatures (Piotrowski *et al.*, 2004) and some amphibians have been shown to clear Bd infections by raising their body temperatures (Woodhams *et al.*, 2003, Chatfield & Richards-Zawacki, 2011). This limit has been identified climatically using species distribution modeling, with area suitable for Bd shrinking globally under predictions of climate change (Rödder *et al.*, 2010). However, there is evidence that amphibians at higher elevations and in cold headwater streams in the U.S. are less likely to be infected by Bd (Muths *et al.*, 2008, Hossack *et al.*, 2010). These areas may represent a lower temperature limitation for Bd and indicate where this pathogen may expand its range under global climate change.

The need to predict the changing spatial patterns of suitable habitat for species of concern as well as invasive species has recently lead to a plethora of papers modeling and mapping the predicted distribution of species under current and potential future conditions (Franklin, 2009). A popular tool for this task is maximum entropy modeling, implemented through the program MaxEnt (Phillips *et al.,* 2006), which has been demonstrated to be highly effective at predicting distributions from presence-only data (Elith *et al.,* 2006). While this approach has been criticized for ignoring interspecific interactions, land cover, species mobility, and for predicting to unprecedented conditions (Sinclair *et al.,* 2010), it can be highly informative and provide the basis for more dynamic modeling where more detailed data is available (Franklin, 2010). For datasets where only presence data is available (e.g., occurrence data and opportunistic disease sampling), this machine learning algorithm has outperformed alternative approaches (Elith *et al.,* 2006).

Managing native populations under climate change requires thorough spatial knowledge of invasive threats. To address this need, we documented the patterns of the two most prominent invasive threats to the native amphibian community, bullfrogs and Bd, in a topographically complex northern U.S. landscape that appears to currently encompass climatic limits for these species. We used these data to model the distribution of these invasive species as related to current climate conditions with MaxEnt and predicted changes to these distributions under scenarios of climate change to spatially explore the threat these species pose to the native amphibian community.

Methods

Study area. We collected data on the distribution of Bd at two scales. The fine-scale study was conducted in a 213 km^2 area in the vicinity of Moscow Mountain, Idaho. The land cover in this area is primarily dryland agriculture, with ponderosa pine forests at higher elevations. The broad-scale study was conducted over a 47,819 km^2 area covering north-central Idaho (Fig. 1). This area reaches from the shrub-steppe of Hells Canyon in the west through the forested area Bitterroot Mountains to the east. We collected both Bd and bullfrog information at this broad scale

Field collection. We collected Bd swab samples from 314 adult long-toed salamanders (*Ambystoma macrodactylum*) at 19 wetlands and 174 adult Columbia spotted frogs (*Rana luteiventris*) at 10 wetlands in north Idaho during the spring and summer of 2004 and 2005 (Fig. 2). Eight of these wetlands were the same for both species. As part of this study, we also collected DNA samples by mouthswab or toe-clip from these individuals and stored them in lysis buffer (50 mM Tris pH 8.0, 50 mM EDTA, 50 mM sucrose, 100 mM NaCl, 1% SDS; Goldberg *et al.*, 2003) or 95% ethanol, respectively. At the broad scale, we collected 261 samples from metamorphosed amphibians and 65 samples from paedomorphic and larval Idaho giant salamanders (*Dicamptodon aterrimus*) at 158 sites from the spring of 2008 through September 2010 (Table 1). Broad-scale sites were sampled opportunistically in the course of other surveys or specifically to gain information on areas that were unsampled. In the latter case, we chose sampling sites based on historical records and focused on Columbia spotted frogs to maximize the probability of detecting Bd. Because Bd grows only on keratinized skin, we focused on metamorphosed adults for this study. However, we also sampled larval and paedomorphic Idaho giant salamanders due to the dearth of knowledge on their skin composition and previous data

from this area that found one animal at this stage testing positive (Hossack *et al.,* 2010). We swabbed amphibians 30 times on the underside, including toe-tips. Swabs were stored in 95% ethanol until extraction.

We collected and mapped known bullfrog locations from the Idaho Conservation Data Center. We then conducted visual surveys at increasing distances to the east from known locations to determine the extent of bullfrog invasion on this front. Additionally, we conducted visual and dip-net surveys for bullfrogs at all sites sampled for Bd.

Laboratory analysis. We used established qPCR techniques to estimate the number of Bd zoospores on sampled amphibians. We extracted DNA from Bd swabs using the Prepman Extraction kit (Boyle *et al.,* 2004; Applied Biosystems) and used an Applied Biosystems 7500 Fast Real-Time quantitative PCR System (Applied Biosystems) to quantify Bd intensity following a protocol modified from Boyle *et al.* (2004). The following changes were made to the conditions in Boyle *et al.* (2004): total reaction volume was 10 µl, probe concentration was 125 nM, and 1 out of the 3 wells for each sample contained the internal control recommended by Hyatt *et al.* (2007) to ensure there was no inhibition of the reaction. The Australian Animal Health Laboratory (Geelong, Victoria, Australia) provided the quantification standards. Any sample yielding ambiguous results (testing positive for 1 or 2 replicates) was rerun and considered positive only if at least one positive detection occurred in a subsequent reaction (following Kriger *et al.,* 2006). We report intensity as the mean number of zoospores detected in all replicates for an individual swab. A negative control with all standard reagents was included in each extraction to confirm that there was no contamination.

We analyzed DNA samples using 8 microsatellite loci for each species. We extracted DNA using a DNeasy Tissue Kit (Qiagen, Inc). Microsatellite loci for Columbia spotted frogs

were: Rp3, Rp15, Rp17, Rp23, SFC128, SFC134, SFC139, and RP193 (Monsen & Blouin, 2003; Funk *et al.*, 2005). Microsatellite loci for long-toed salamanders were AjeD03, AmmF142, AmmH123, AmmH136, AcroB192, AcroD037, AcroD166, and AcroD300 for long-toed salamanders (Julian *et al.*, 2003; Savage, 2009). More information on the amplification of these microsatellites can be found in Goldberg & Waits (2010).

Bottlenecks. We tested for population bottlenecks for long-toed salamanders and Columbia spotted frogs in the fine scale study area to determine whether they were related to the presence of Bd in the population using the Wilcoxon test with the two-phase model in program BOTTLENECK (Piry *et al.*, 1999). We used the recommended variance of 12 and 95% single-step mutations.

Climate data. We obtained current and predicted future climate data from ClimateWNA (http://www.genetics.forestry.ubc.ca/cfcg/ClimateWNA/ClimateWNA.html). We used climate data from 1961-1990 to model current niches. Pixel size was 2.5 x 2.5 arcminutes, approximately 3755 m^2 at this latitude. For future climate predictions, we focused on the A2 and B1 scenarios. The A2 scenario is a “business as usual” scenario that represents the results of an increasingly divided and populated world, while the B1 scenario represents a more integrated and ecological friendly world, where emission rates hold steady and then decline starting at 2040. For each species, we narrowed down the number of climate variables modeled into a set of variables with low correlation. For logistic modeling of Bd, we focused on variables associated with physiological limits for this species and removed variables that were linearly correlated at $r > 0.7$. For maximum entropy modeling of both species, we did the same, but used qr decomposition to determine the multicollinearity of variables (Falkowski *et al.*, 2009).

Effects of climate on distribution. We conducted several tests to explore the patterns of Bd in this system. First, we tested whether the patterns of Bd presence and average intensity of infection at a site were correlated with elevation after accounting for the species tested and the sample size at a site using mixed effect models in program R (R Development Core Team, 2011), package lme4 (Bates *et al.*, 2011) for models of Bd presence and package nlme (Pinheiro *et al.*, 2011) for models of Bd intensity (number of zoospores). Because the distance to the nearest highway for these samples was highly correlated with elevation ($r = 0.69$), we also tested whether distance to highway had this pattern. We then used an information theoretic approach to test whether species sampled, climate variables, or both together were the most likely explanation for patterns of Bd distribution and average intensity (number of zoospores, log-transformed to better meet assumptions of normality) per site, again after accounting for sample size using mixed effect models. All of these tests were conducted for sites where we sampled metamorphosed amphibians; paedomorphic Idaho giant salamanders were excluded from these analyses.

For both Bd and bullfrogs, we used the maximum entropy approach applied in MaxEnt (Phillips *et al.*, 2006) to model the niche of each species under current climate conditions. For each species, we used 10-fold crossvalidation to test the predictive ability of the model, and removed variables with an average permutation importance of ≤1%. We then used these models to predict the distribution of each species under predicted climate change outcomes using an ensemble approach where we averaged predicted values across climate models for each scenario. Climate models consisted of the recommended runs provided in the ClimateWNA program, selected for their likelihood of predicting future climates. These consisted of selected runs of CGCM3, HadCM3, GFDLCM21, ECHAM5, NCARCCSM30, CSIROMK30, and

MIROC32(medres) (Spittlehouse & Murdock, 2010). For Bd models, we constrained the area in the model to the area sampled (Fig. 1)

Results

Bd distribution. In the fine-scale study area, 1.6% of long-toed salamanders tested positive, with 16% of the sites having at least one positive individual (Fig. 2A). For Columbia spotted frogs, 65% of individuals and all sites sampled tested positive (Fig. 2B). All detections of Bd for long-toed salamanders were quantified at <1 zoospore, while median zoospore count for Columbia spotted frogs testing positive was 1.5 (range: 1 – 1965 zoospores). In the broad-scale study area, Bd was found at 73% of wetland sites and 0% of stream sites (Fig. 1). Prevalence varied across species and ranged from 0% for Idaho giant salamanders and Rocky Mountain tailed frogs (*Ascaphus montanus*) to 82% for Columbia spotted frogs (Table 1). Median zoospore count for individuals testing positive was 5 (range: <1- 16,382) for Columbia spotted frogs, 9 (range <1-23,884) for western toads, and 8 for the one Sierran treefrog that tested positive.

Bottlenecks. We found no evidence of bottlenecks in populations testing positive for Bd ($P > 0.05$). For long-toed salamanders, we detected a bottleneck at one site where Bd was not detected ($P = 0.037$). For Columbia spotted frogs, we did not detect a bottleneck at any of the sampled sites.

Effects of climate on Bd distribution. Bd presence was negatively associated with elevation after accounting for the random effects of species and sample size at each site ($z = -2.60$, $P = 0.01$). There was some evidence that Bd intensity was also negatively correlated with elevation after accounting for these random effects ($t_{144} = -1.86$, $P = 0.06$). Distance to the

nearest highway did not show this pattern for either presence (z = -0.88, P = 0.38) or intensity (t_{144} = 0.44, P = 0.66). We used 3 uncorrelated climatic variables to test whether both species and climate played a role in explaining variation in Bd distribution in the study area: average minimum temperature in winter, temperature difference between the mean warmest and mean coldest month, and average maximum temperature in summer. Using these variables, we found that both species sampled and climate played a role in explaining the presence of Bd at a site, after accounting for the random effect of number of samples taken, but that the model with only species sampled was the most likely explanation for infection intensity at a site (Table 2).

We used 14 climate and landscape variables to model the current niche of Bd in north Idaho: mean temperature in the coldest month, precipitation in winter, spring, and summer, mean maximum temperature in winter and fall, mean minimum temperature in spring, summer, and fall, mean average temperature in spring and fall, degree days above 18°C, precipitation as snow (mm), and Hargreaves climatic moisture deficit. Bd was detected at 95 sites and these positives were used as the data for the model (multiple sites within a pixel were combined into a single data point for this analysis). Test AUC averaged over the 10 cross-validated models was 0.68. The variables contributing the most to the models (based on percent contribution) were: a positive relationship with average minimum temperature in the coldest month (39.9%), a negative relationship with precipitation in the summer (11.7%) and a negative relationship with average minimum summer temperature (8.4%). Future climate models under the B1 scenario predicted no overall landscape increase in suitability for Bd, but a shift to the south of the most suitable area. Under the A2 scenario, suitability is predicted to increase 14% between 2020 and 2080 (Fig. 3A).

Effects of climate on bullfrog distribution. We compiled 26 known locations of bullfrog populations and used 5 climate and landscape variables to model the current niche of this species in north Idaho: average temperature, average maximum temperature, and precipitation in winter, and average temperature and precipitation in summer. Test AUC averaged over the 10 cross-validated models was 0.90. The variables contributing the most to the models (based on percent contribution) were: a parabolic relationship with average temperature in winter, with a maximum at 0°C (52.1%), a positive relationship with average maximum temperature in winter (23.2%), and a positive relationship with precipitation in winter (16.6%). Future climate models predict a large increase in suitable area for bullfrogs under both B1 and A2 scenarios, with the average suitability across the entire broad-scale study area reaching 75% by 2080 under the A2 scenario (Fig. 3B).

Discussion

Native species are increasling being challenged by a host of invasive species functioning at multiple scales. Climate change is predicted to affect the potential distribution of both native and invasive species in a variety of ways. In this paper, we have shown that native amphibian populations in north-central Idaho will increasingly be challenged by two invasive species: the pathogen Bd and the predaceous American bullfrog. While the outcome for native amphibian species may vary by species and habitat, the increased exposure to both will present additional challenges for the persistence of these populations.

Bd distribution differs between syntopic species. We found that two co-occurring wetland-breeding species (long-toed salamanders and Columbia spotted frogs) differed greatly in their level of infection by Bd. Long-toed salamanders showed very low prevalence with low

zoospore counts, while the majority of Columbia spotted frogs tested were found to be infected and had higher zoospore counts. This could be due to innate differences among species with respect to skin peptides or immune response (Woodhams *et al.,* 2007) or could be due to temporal habitat partitioning. Long-toed salamanders overwinter terrestrially, arrive at wetlands for breeding while ice is still present, and remain at the breeding wetland for < 1 month, while Columbia spotted frogs overwinter aquatically and become active later than long-toed salamanders. Low water temperatures at the time when long-toed salamanders use wetlands may reduce their exposure and rates of infection, as Bd is limited to very slow growth near freezing (Piotrowski *et al.,* 2004).

Bd distribution is related to climatic conditions. Our data support the findings of two previous studies in the western U.S. showing that Bd prevalence decreases with elevation in this area, as had been found for western toads by Muths *et al.* (2008), and was very low in headwater streams, as had been found by Hossack *et al.* (2010). We confirmed that these patterns in our study were not due to proximity to highways, which may serve as corridors for the transportation of Bd (Morgan *et al.,* 2007), or due only to species sampled. These results are contrary to the predominant global pattern, where Bd has been shown to be primarily limited by high temperatures (Rödder *et al.,* 2010). However, species niches are multi-dimensional, and these data indicate that the extreme cold temperatures represented in the mountains of the northern U.S. may also be limiting for this pathogen.

Our species distribution model of Bd had far lower accuracy than the global model of Rödder *et al.* (2010) and slightly lower accuracy than the regional model of Puschendorf *et al.* (2009). This could be due to the coarse scale of the climate data, which may not represent the fine-scale climate conditions that influence the presence of Bd. Additionally, in our study area

factors in addition to climate, such as land cover and native species distributions, may be influencing these patterns.

Climatic suitability for Bd will increase with climate change. Predictions of Bd distribution under two scenarios of climate change showed little overall increase under the less extreme emissions scenario and a larger increase (14% average increase in suitability by 2080) under the business-as-usual scenario. In both cases, the low elevation area along the Clearwater River drainage has the highest suitability for Bd, with this area greatly expanded under the business-as-usual scenario. Additionally, by 2050 nearly the whole area is predicted to be at least moderately suitable for Bd under this scenario. Areas of lowest overall suitability for Bd under all scenarios include the Clearwater Mountains, St. Joe Mountains, and Coeur d'Alene Mountains.

Impacts of Bd in this area are unknown. We found that while the probability of being infected with Bd was best explained by local climate and species sampled, the intensity of infection was best explained only by species sampled. However, the impact of Bd on native amphibian species in this area is largely unknown. Generally, some species (e.g., bullfrogs) can carry this pathogen without showing detrimental effects, while other species have high mortality rates after exposure (Daszak *et al.*, 2004; Blaustein *et al*., 2005; Kilpatrick *et al.*, 2010). We did not find any evidence of population bottlenecks associated with infection by Bd for Columbia spotted frogs or long-toed salamanders. No die-offs associated with Bd have been recorded in this area, and no quantitative evidence of declines is available (although anecdotal evidence suggests a reduction of western toad populations; Goldberg & Waits, 2009). Other studies have found reduced body condition of Oregon spotted frog juveniles (*Rana pretiosa*, sister species to the Columbia spotted frog) infected with Bd (Pearl *et al.*, 2009) and high susceptibility of

western toads to this pathogen (Carey *et al.*, 2006). For some anuran species, a threshold of 10,000 zoospores must be reached before population die-offs are seen (Vredenburg *et al.*, 2010; Kinney *et al.*, 2011); we analyzed only three samples that reached this level of infection (two Columbia spotted frogs and one western toad).

Climatic suitability for the American bullfrog will increase dramatically. Our models predict a dramatic increase in suitability for American bullfrogs in this landscape under scenarios of climate change, even if greenhouse gases are reduced. This model was highly accurate in cross-validation tests, demonstrating a strong relationship of current bullfrog distribution to local climatic conditions. There are a number of isolated but well-established bullfrog populations in this area, and the window of time when eradication is an option may be closing in this area. Eradication of bullfrog populations is notoriously difficult, leading some authors to suggest focusing on alternative approaches (Adams & Pearl, 2007). The establishment of bullfrogs is assisted by the presence of non-native fish (especially centrarchids) that reduce predators that would otherwise eat bullfrog tadpoles and the conversion of temporary wetlands into year-round water that allows for the full life cycle of bullfrogs (Adams, 2000; Adams *et al.*, 2003). Removing these non-native fish from vulnerable wetlands may inhibit the spread of bullfrogs, especially in concert with the establishment of a hydroperiod regime involving a dry season in the late summer. Additionally, regulations preventing the movement of bullfrogs between wetlands may reduce their rate of spread across this landscape. Finally, focusing research efforts on situations where native amphibian species currently co-exist with bullfrogs may yield insight into managing for the persistence of these native species in the presence of bullfrogs (Adams & Pearl, 2007).

Conclusion

Climate change will affect native and invasive species through numerous interacting processes. We have highlighted here what to the best of current knowledge are the two most important invasive threats to native amphibian populations in the inland northwestern U.S.: Bd and the American bullfrog. Suitability for the pathogenic chytrid fungus Bd is predicted to increase in this area under the business-as-usual emissions scenario, especially at lower elevations. The potential impacts of this expansion are unknown. Naïve stream amphibians and wetland amphibian populations at higher elevations may be vulnerable to this pathogen in ways that have not yet been seen for wetland amphibians at lower elevations. Additionally, sublethal impacts may be occurring in wetland amphibian populations testing positive for Bd that have not yet been detected, and western toad populations may have already been impacted by this invasive pathogen.

Suitability for the invasive American bullfrog is predicted to increase dramatically under all scenarios, placing additional competition and predation pressure on these native populations. Bullfrogs are not yet widespread in this area, and there is currently a window of opportunity to attempt to limit their spread into higher elevation areas. The most likely invasion front into the higher elevation areas of the study area is along the middle fork of the Clearwater River, followed by the Coeur d'Alene River and the north fork of the Clearwater above Dworshak Reservior, and we recommend efforts at limiting further invasion focus in those areas.

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Literature Cited

Adams, M.J. (2000) Pond permanence and the effects of exotic vertebrates on anurans. *Ecological Applications*, **10**, 559-568.

Adams, M.J., Pearl, C.A. & Bury, R.B. (2003) Indirect facilitation of an anuran invasion by non-native fishes. *Ecology Letters*, **6**, 343–351.

Bates, D., Maechler, M. & Bolker, B. (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-39.

Blaustein, A.R. & Kiesecker, J.M. (2002) Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters* **5**, 597-608.

Blaustein, A.R., Romansic, J.M., Scheessele, E.A., Han, B.A., Pessier, A.P. & Longcore J.E. (2005) Interspecific variation in susceptibility of frog tadpoles to the pathogenic fungus *Batrachochytrium dendrobatidis*. *Conservation Biology*, **19**, 1460-1468.

Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T. & Hyatt, A.D. (2004) Rapid quantitative detection of chytridiomycosis *(Batrachochytrium dendrobatidis)* in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, **60**, 141–148.

Carey, C., Bruzgul, J.E., Livo, L.J., Walling, M.L., Kuehl, K.A., Dixon, B.F., Pressier, A.P., Alford, R.A. & Rogers, K.B. (2006) Experimental exposures of boreal toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). *EcoHealth*, **3**, 5-21.

Casper, G.S. & Hendricks, R. (2005) Rana catesbeiana Shaw 1802: American bullfrog. *Amphibian declines: the conservation status of United States species* (ed. by M.J. Lannoo), pp. 540–546. University of California Press, Berkeley.

Chatfield, M.W.H & Richards-Zawacki, C.L. (2011) Elevated temperature as a treatment for *Batrachochytrium dendrobatidis* infection in captive frogs. *Diseases of Aquatic Organisms*, **94**, 235-238.

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

Elith, J., Graham, C.H., Anderson, R.P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J., Lohmann, L.G., Loiselle, B.A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J. McC., Peterson, A.T., Phillips, S.J., Richardson, K.S., Scachetti-Pereira, R., Schapire, R.E., Soberón, J., Williams, S., Wisz, M.S. & Zimmermann, N.E. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129-151.

Falkowski, M.J., Evans, J.S., Martinuzzi, S., Gessler, P.E.& Hudak AT (2009) Characterizing forest succession with lidar data: An evaluation for the Inland Northwest, USA. *Remote Sensing of Environment*, **113**, 946-956.

Ficetola, G.F., Thuiller, W. & Miaud, C. (2007) Prediction and validation of the potential global distribution of a problematic alien invasive species – the American bullfrog. *Diversity and Distributions* **13**, 476-485.

Fisher, M.C., Garner, T.W.J. & Walker, S.F. (2009) Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology* **63**, 291-310.

Franklin, J. (2010) Moving beyond static species distribution models in support of conservation biogeography. *Diversity and Distributions,* **16**, 321-330.

Franklin, J. (2009) *Mapping species distributions: spatial inference and prediction*. Cambridge University Press, Cambridge.

Funk, W.C., Blouin, M.S., Corn, P.S., Maxell, B.A., Pilliod, D.S., Amish, S. & Allendorf F.W. (2005) Population structure of Columbia spotted frogs (Rana luteiventris) is strongly affected by the landscape. *Molecular Ecology*, **14**, 483–496.

Goldberg, C.S., Kaplan, M.E. & Schwalbe, C.R. (2003) From the frog's mouth: buccal swabs for collection of DNA from amphibians. *Herpetological Review*, **34**, 220-221.

Goldberg, C.S. & Waits LP (2009) Using habitat models to identify conservation priorities for pond-breeding amphibians in a privately owned landscape. *Biological Conservation*, **142**, 1096-1104.

Goldberg, C.S. & Waits, L.P. (2010) Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. *Molecular Ecology* **19**, 3650-3663.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D. (2002) Climate warming and disease risks for terrestrial and marine biota. *Science*, **296**, 2158-2162.

Hayes, M.P. & Jennings, M.R. (1986) Decline of Ranid frog species in western North America: are bullfrogs (*Rana catesbeiana*) responsible? *Journal of Herpetology*, **20**, 490–509.

Hossack, B.R., Adams, M.J., Campbell Grant, E.H., Pearl, C.A., Bettaso, J.B., Barichivich, W.J., Lowe, W.H., True, K., Ware, J.L. & Corn, P.S. (2010) Low prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*) in amphibians of U.S. headwater streams. *Journal of Herpetology*, **44**, 253-260.

Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F. & Colling, A. (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, **73**, 175–192.

Julian, S.E., King, T.L. & Savage, W.K. (2003) Novel Jefferson salamander, *Ambystoma jeffersonianum*, microsatellite DNA markers detect population structure and hybrid complexes. *Molecular Ecology Notes*, **3**, 95–97.

Kats, L.B. & Ferrer, R.P. (2003) Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, **9**, 99-110.

Kilpatrick, A.M., Briggs, C.J & Daszak, P. (2010) The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends in Ecology and Evolution, **25**, 109-118.

Kinney, V.C., Heemeyer, J.L., Pessier, A.P. & Lannoo, M.J. (2011) Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality and *Lithobates areolatus*: affirmation of Vredenburg's "10,000 zoospore rule." *PLoS One*, **6**, e16708.

Kriger, K.M., Hero, J.M. & Ashton, K.J. (2006) Cost efficiency in the detection of chytridiomycosis using PCR assay. Diseases of Aquatic Organisms, **71**, 149–154.

Longcore, J.E., Pessier, A.P. & Nichols, D.K. (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia*, **91**, 219-227.

Monsen, K.J. & Blouin, M.S. (2003) Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. *Molecular Ecology*, **12**, 3275–3286.

Morehouse, E.A., James, T.Y., Ganley, A.R.D., Vilgalys, R., Berger, L., Murphy, P.J. & Longcore, J.E. (2003) Multilocus sequence typing suggest the chytrid pathogen of amphibians is a recently emerged clone. *Molecular Ecology*, **12**, 395-403.

Morgan, J.A.T., Vredenburg, V.T., Rachowicz, L.J., Knapp, R.A., Stice, M.J., Tunstall, T., Bingham, R.E., Parker, J.M., Longcore, J.E., Moritz, C., Briggs, C.J. & Taylor, J.W. (2007) Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*. *Proceedings of the National Academy of Sciences*, **104**, 13845-13850.

Muths, E., Pilliod, D.S. & Livo, L.J. (2008) Distribution and environmental limitations of an amphibian pathogen in the Rocky Mountains, USA. *Biological Conservation*, **141**, 1484-1492.

Ouellet, M., Mikaelian, I., Pauli, B.D., Rodrigue, J. & Green, D.M. (2005) Historical evidence of widespread chytrid infection in North American amphibian populations. *Conservation Biology*, **19**, 1431–40.

Pearl, C.A., Adams, M.J., Leuthold, N. & Bury, R.B. (2005) Amphibian occurrence and aquatic invaders in a changing landscape: implications for wetland mitigation

in the Willamette Valley, Oregon, USA. *Wetlands*, **25**, 76–88.

Pearl, C.A., Bowerman, J., Adams, M.J. & Chelgren, N.D. (2009) Widespread occurrence of the chytrid fungus *Batrachochytrium dendrobatidis* on Oregon spotted frogs (*Rana pretiosa*). *EcoHealth*, **6**, 209-218.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling* **190**, 231-259.

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

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & the R Development Core Team (2011) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-100.

Piry, S., Luikart, G. & Cornuet, J.M. (1999) BOTTLENECK: a computer program for detecting recent reductions in effective population size using allele frequency data. *Journal of Heredity*, **90**, 502-503.

Puschendorf, R., Carnaval, A.C., VanDerWal, J., Zumbado-Ulate, H., Chaves, G., Bolaños, F. & Alford, R.A. (2009) Distribution models for the amphibian chytrid *Batrachochytrium dendrobatidis* in Costa Rica: proposing climatic refuges as a conservation tool. *Diversity and Distributions* **15**, 401-408.

R Development Core Team (2011). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna.

Rödder, D., Kielgast, J. & Lötters, S. (2010) Future potential distribution of the emerging amphibian chytrid fungus under anthropogenic climate change. *Diseases of Aquatic Organisms*, **92**, 201-207.

Savage, W.K. (2009) Microsatellite loci for the critically endangered Santa Cruz long-toed salamander (*Ambystoma macrodactylum croceum*) and other Ambystoma taxa. *Conservation Genetics*, **10**, 619–622.

Simberloff, D. (2000) Global climate change and introduced species in United States forests. *The Science of the Total Environment*, **262**, 253-261.

Sinclair, S., White, M.D. & Newell, G.R. (2010) How useful are species distribution models for managing biodiversity under future climates? *Ecology and Society*, **15**, 8.

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

Spittlehouse, D. & Murdock, T. (2010) Introduction to selecting and using climate scenarios for British Columbia. Available at: *http://www.for.gov.bc.ca/hfd/library/FIA/2010/FSP_F100215b.pdf* Accessed 3 July 2011.

Stuart, S.N., Chanson, J.S, Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., & Waller, R.W. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science*, **306**, 1783-1786.

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

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

Woodhams, D.C., Ardipradja, K., Alford, R.A., Marantelli, G., Reinert, L.K. & Rollins-Smith, L.A. (2007) Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. *Animal Conservation*, **10**, 409-417.

Table 1. *Batrachochytrium dendrobatidis* samples taken in the broad-scale study area, north-central Idaho, USA. Idaho giant salamanders are classified as either metamorphosed adults, larvae, or paedomorphic adults (paed.). Prevalence is by individual and presented with 95% confidence interval.

Species	Samples	Sites	Habitat	Prevalence
Columbia spotted frog (*Rana luteiventris*)	227	128	Wetland	73% (67-79%)
Idaho giant salamander (*Dicamptodon aterrimus*)	5 metamorphosed 65 larvae or paed.	13	Stream	0%
Rocky Mountain tailed frog (*Ascaphus montanus*)	13	7	Stream	0%
Sierran treefrog (*Pseudacris sierra*)	5	4	Wetland	20% (0.5-72%)
Western toad (*Anaxyrus boreas*)	11	6	Wetland	82% (48-98%)

Table 2. Ranking of mixed-effect models of Bd presence and average intensity at 150 sites sampled in north-central Idaho, USA. The random effect is number of individuals sampled at a site. Models consist of climate data only, species sampled only, and both together (full model).

Response	Model	AIC	ΔAIC
Presence	Full	154.66	0
	Species only	166.34	11.68
	Climate only	185.00	30.34
# zoospores	Species only	601.54	0
	Full	608.42	6.88
	Climate only	633.96	32.42

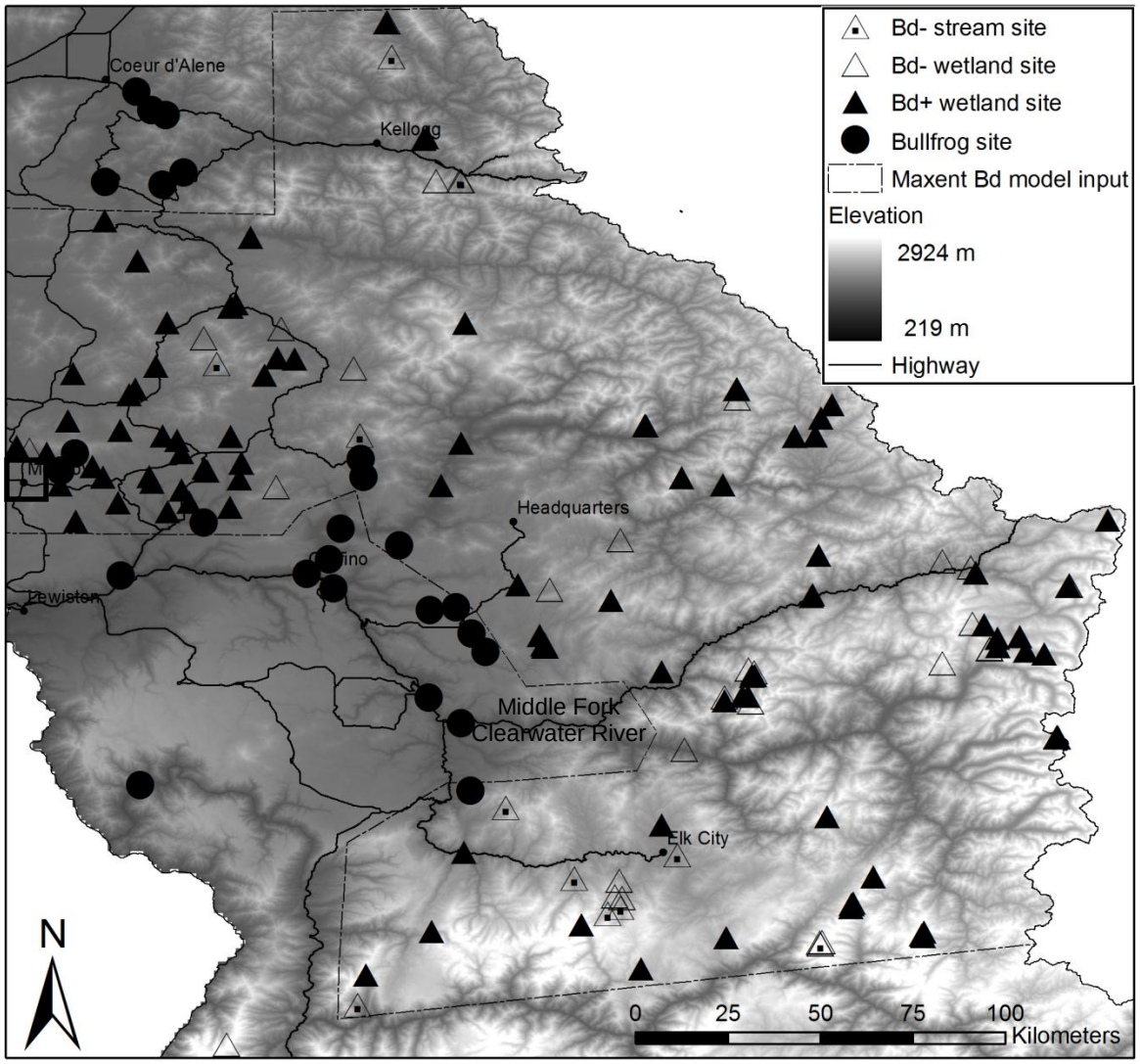

Figure 1. Distribution of Bd samples and American bullfrog known locations in north-central Idaho used in broad-scale analyses, and the spatial extent of background data used to model Bd climatic niche. Open square indicates fine-scale study area.

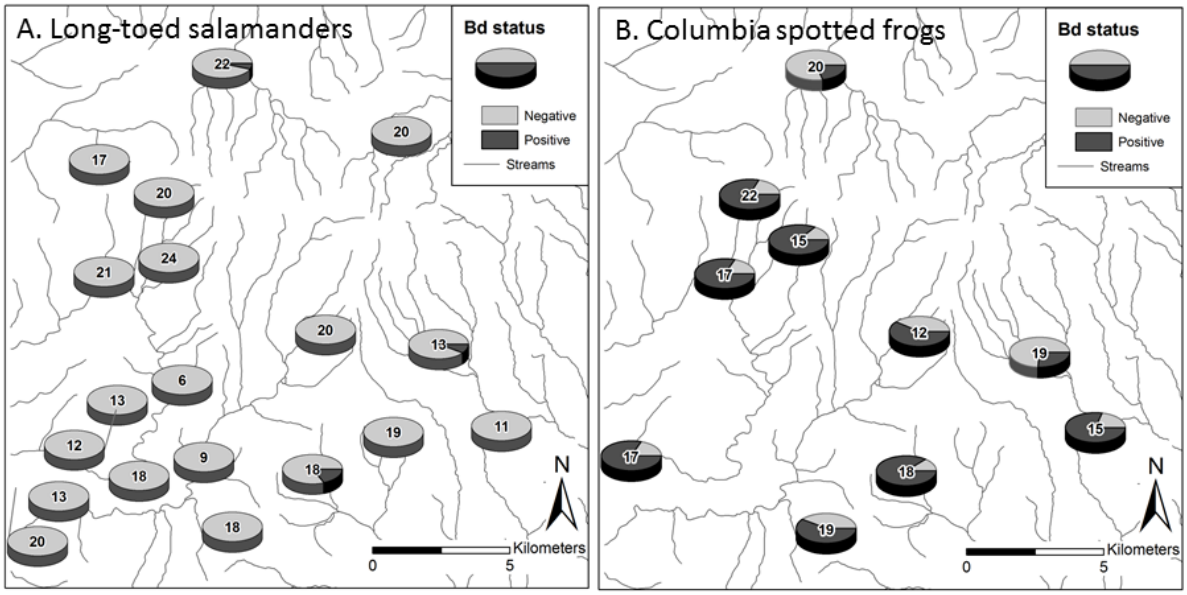

Figure 2. Distribution of long-toed salamanders (A) and Columbia spotted frogs (B) that tested positive for Bd in the fine-scale study area, in the vicinity of Moscow Mountain, ID. Numbers represent sample sizes for each site.

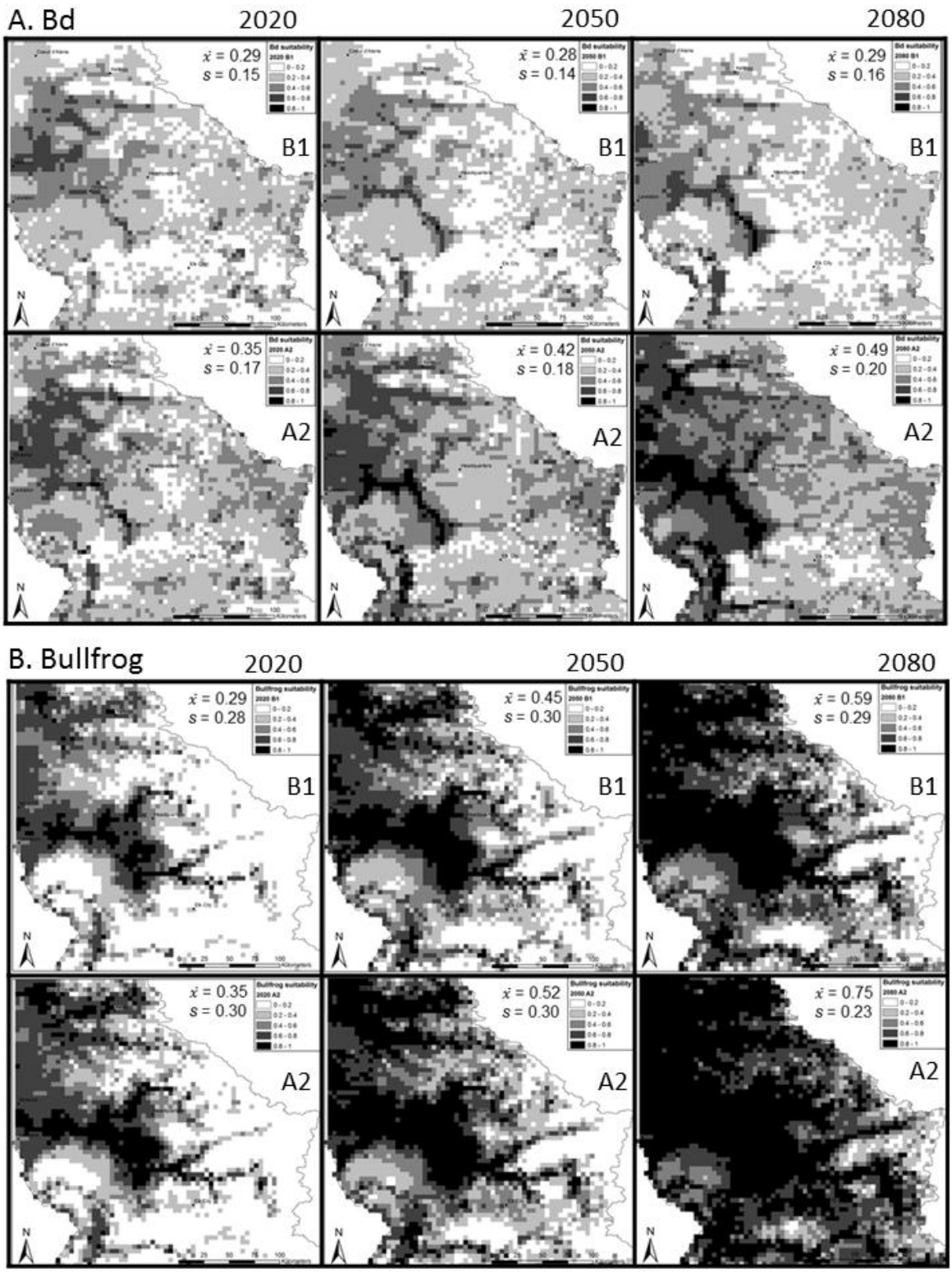

Figure 3. Predicted suitability for Bd (A) and American bullfrogs (B) in north-central Idaho under predictions of climate change, ranging from 0 to 1, under B1 and A2 emission scenarios.