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SOUTH DAKOTA DEPARTMENT OF GAME, FISH AND PARKS
STATE WILDLIFE GRANTS – FINAL REPORT

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Project Title: Prevalence of an Emerging Disease in South Dakota Amphibian Populations

Principal Investigator: Jacob Kerby, Department of Biology, University of South Dakota

Project Period:
June 2009-August 2011

Background information:

A recent paper by Wake and Vredenburg (2008) describes the severity and significance of the disappearance of amphibian populations across the world. Nearly one third of all amphibian species have been classified as threatened with extinction (Stuart et al. 2004), with several different factors listed as probable causes (Storfer and Collins 2006). While it is clearly difficult to untangle many of these factors (Blaustein and Kiesecker 2002), one has emerged as strikingly dangerous and incredibly fast moving, the chytrid fungus, *Batrachochytrium dendrobatidis (*Rachowicz et al. 2006). This pathogenic fungus has been found to extirpate populations across the world, with well documented cases on every continent examined. Due to the imminence of this emerging infectious disease, it is critically important that the presence of this fungus is identified immediately.

Even with this severe impact to amphibians nothing is know about the presence of chytrid in the state of South Dakota prior to this study. In addition, the status of amphibians is poorly understood in South Dakota. Obtaining baseline data around the state on species presence and abundance is a necessary starting point for assessing the status of these species. Some species are known to be in serious trouble in surrounding states, and obtaining data within South Dakota on these specific species is of utmost importance.

This study utilizes a collaborative effort of herpetologists across the state to determine the status of chytrid fungus infection prevalence on amphibians. This two year survey effort was also done in conjunction with both field and laboratory work to examine aspects of chytrid biology on South Dakota amphibians. This report represents a summary of the survey portion of the grant. Attached with this report is a Masters thesis that details the field and laboratory work.

Objectives of the grant:

I. Survey the prevalence of chytrid fungus in amphibian populations across South Dakota
II. Establish an Amphibian Disease Testing Center to provide timely and cost-efficient evaluations of amphibian disease outbreaks for researchers working in the state of South Dakota.
III. Disseminate information concerning the chytrid fungus to both wildlife biologists and the general public.

Outcome: All the above objectives of the grant were met and are discussed in detail below. In addition to these listed objectives, the grant also resulted in several additional beneficial outcomes:

1. This grant served as the primary support of a Masters student in Biology at USD, Jennifer Brown, who completed her degree in the summer of 2011. Her thesis is attached to this report as evidence of additional experimental work that was conducted regarding the impacts of chemical stressors on disease susceptibility. Each of these chapters will be submitted for publication this year and South Dakota Game, Fish, and Parks will be acknowledged as a funding source to support this student.
2. This project also served as an outreach opportunity to train several undergraduate and graduate students in both field survey methods and in pathogen quantification via the methodology of DNA extraction and quantitative PCR reactions.
3. Finally, this grant served as a large collaborative effort across the state which involved and integrated several herpetologists across South Dakota, particularly across the Dakota Amphibian Reptile Network (DARN). This work will likely play a strong role in furthering future collaborative relationships. This included a unique opportunity to work with Native American students on a South Dakota reservation.

Survey Project Methods:

This project was designed to conduct the most timely and cost-efficient way to examine the incidence and prevalence of the amphibian chytrid fungus at sites across the state of South Dakota. The proposal consisted of two separate components to successfully complete this project: several survey crews based across the state to collect samples, and a centralized Amphibian Disease Testing Center where infection data was analyzed.

Survey crews from across the state were provided with swabs and instructed on proper sterile swabbing techniques. At a minimum, each collaborator provided the date the sample was taken, the GPS coordinates of the sampling site, and designation of the swabbed species and life stage. In addition, a crew from my laboratory conducted more intensive sampling at different areas throughout the state. Along with the above data, measurements of water quality and habitat description were also recorded. Both air and water temperatures were recorded at each site, and standardized surveys were conducted

to better estimate abundance of amphibians at specific sites. Details of these surveys are outlined in the attached thesis.

During the summer of 2009, four regions across the state were sampled: the southeast Missouri River Valley, the Black Hills, the grasslands around Pierre, and the Northwest part of the state (Map 1). From these regions a total of 521 chytrid swabs were collected from 9 species of amphibians (Table 1). In addition to sampling from our own crew, we received assistance from several biologists across the state. Samples were received from Ruben Mars at Fort Pierre National Grasslands, Alyssa Kiesow from Northern State University, Shannan Miller with the US Forest Service, and Brian Smith at Black Hills State University in Spearfish, SD.

For the field season of 2010, we used methodology similar to above only with an earlier sampling time and an increase in number of sampled sites. This expanded coverage of the state included collected samples from six of the seven regions: the Black Hills, Northeastern, Pierre, Pine Ridge, and Custer (Map 1). In addition to the collaborators from 2009, new relationships were also established to get a better coverage in some regions and collection from a new part of the state. Our new collaborators include Mary Miller at the Nature Conservancy in Leola, SD; Patrice Lynch at the Mystic River Ranger District in the Black Hills National Forest (who additionally worked with other districts to collect swabs); and Alessandra Higa and her students at Oglala Lakota College in Kyle, SD. These collaborations were essential to obtaining data on chytrid in South Dakota and also have allowed us to connect and educate wildlife biologists working around the state on the chytrid fungus.

Also in 2010, we initiated a field study focused on 20 sites surrounding the Missouri River near Vermillion, SD to more closely monitor BD presence over time. Each site was visited during May, June, and July to study the spatial and seasonal distribution of BD within this region. Approximately 750 samples were collected for this portion of the study in addition to data on amphibian population estimates and habitat parameters. Water quality was tested at each site for pesticide presence. The results of this study are included in the attached thesis. In conjunction with another study on the delta region of the Niobrara, our crew also obtained swabs from amphibians captured in the stretch of Missouri river from the Niobrara river to Gavin's Point Dam.

Amphibian Disease Testing Center: We established a centralized testing facility that has not only successfully run samples on this project, but ran samples for several other researchers across the country on additional chytrid projects. A web page has been constructed (http://www.usd.edu/~Jacob.Kerby/chytrid.htm) containing detailed instructions on sampling, as well as a video demonstration. Our laboratory is now listed on the website: http://www.amphibianark.org/the-crisis/chytrid-fungus/ as one of the few in the country willing and able to provide this service. We are continuing operation of this disease testing center even after the completion of this grant, and have recently expanded to examining a second important amphibian disease- ranavirus.

Survey Results and Discussion:

The surveys conducted represent varied portions of the state, with sampling from nearly all species of amphibians found within the state of South Dakota. With our large collaborative effort, a total of 1,525 amphibians from ten species were swabbed for chytrid over the two years.

Due to the effort involved in sampling sites across the entire states, and our dependence on collaborators to provide swabs, sampling was unevenly distributed across both species and regions. To better estimate impacts, we focused our survey efforts across the state on one single species, the Northern Leopard Frog. Other species were sampled as available but are less well represented across the state. To compare prevalence among species, the survey crew focused on one area, the Missouri River Valley area near Vermillion, SD, with several intensive surveys throughout the summer.

Overall summary

Chytrid fungus was present in every region of the state sampled. Thus, chytrid is widespread in South Dakota although the prevalence was quite low overall (Table 1). This corroborates similar prevalence levels in surrounding regions (Ouellet 2005). In every species examined with sufficient sampling numbers (>100 individuals), we found chytrid infected amphibians. ***Thus, chytrid fungus was present in every area sampled, and also in every species sampled sufficiently***. Interestingly we found differences in prevalence data among both the particular species and the particular regions sampled.

Regional observations

Perhaps most interesting difference in chytrid prevalence was among geographic areas. While most of the regions were near or below the average for surrounding states, the Black Hills region had a much higher level of infection, with 19% of the swabbed individuals having chytrid in 2010 (Map 2). Of particular interest is that 50% of Northern Leopard Frog samples were infected in that same year with positives from three separate ponds in the area. Interestingly though, no infected animals were found in the Black Hills during the 2009 sampling.

To makes sense of this pattern, one must understand a little more regarding the established relationship of seasonal temperatures and chytrid infection (Berger et al. 2004; Piotrowski et al. 2004). Sampling in the Black Hills was done at a significantly higher elevation than all other sites. Higher elevation sites remain cooler for longer in the season and therefore are typically where chytrid die-offs tend to occur (Pounds et al. 2006). Therefore the high prevalence detected in the Black Hills can perhaps be simply associated to differences in temperature between regions. Our 2009 sampling was performed later in the summer when average temperatures were above the critical temperature for chytrid growth and therefore the reason for a lack of chytrid infection detected in those samples.

Differences in prevalence among lower elevation sites were not as apparent. Despite adequate sampling at several sites across the state, seemingly on average a very low number of individuals were typically carrying an infection. This is of some interest given that these pathogen do not seem to be causing immediate die-offs at these sites, and yet also are persisting at every site sampled. One site that we did not detect chytrid at was the Pine Ridge Reservation. Our sampling there was not high enough or done early enough in the season to conclude that chytrid is absent from this site. Further sampling will be done in future collaborations to verify its presence at the site.

Species observations

The Boreal Chorus Frogs had the highest overall prevalence (24%) of any other species. This finding though is heavily skewed because nearly all infected individuals were collected at one particular location in the Missouri River Valley in Vermillion, SD (Map 3). In 2010, 50 Boreal Chorus frogs were collected and swabbed from this site, out of those 47 were infected with chytrid. In 2009, 18 samples were collected from this site, and 17 of those were infected. Therefore 64/68 positive frogs in the region were from this single site. Removal of this single site from the dataset exhibits that this species is no more sensitive to chytrid infection than others.

Given that though, the high prevalence of chytrid infection at this site is of great interest. This particular site is an old golf course that is still managed for weed control by use of herbicides. Although American toads sampled in this area did not exhibit increased prevalence of chytrid, many individuals possessed what appeared to be chemical burn marks on their dorsal side. Further study of this particular site is underway as it may provide key insights into how chytrid can potentially have large effects even in warmer conditions if sites are heavily altered or impacted via chemical application.

The low prevalence of infection overall then makes it difficult to infer what the relative sensitivity of specific species are to chytrid infection. It may very well be that species such as cricket frogs are still highly sensitive to chytrid infection, and die too quickly in the field to be captured and noted. Experimental comparisons of species sensitivity to infection would provide key insights into which South Dakota species are most likely to be continually impacted by the ubiquitous presence of this fungus.

In our two year survey we found very little evidence that chytrid is actively causing massive die off events in South Dakota amphibian populations. In fact, given its widespread occurrence, it is highly likely that the fungus spread through the state some time ago. It is perhaps at that time when many species were hit hardest and caused precipitous reductions in abundance, although it is difficult to know. Recent methods have been developed to allow adequate DNA extraction from museum samples. It is only through the examination of these specimens will we have adequate knowledge of when chytrid might have arrived in the state. Anecdotal reports from farmers encountered while surveying claim that there were more amphibians around “when I was a kid.” Therefore, the fungus could be acting to impact current populations of amphibians still although without formal survey data, these claims are difficult to verify.

Despite the low prevalence of chytrid infection though, amphibian populations might still be significantly stressed by the presence of this pathogen. With other significant stressors such as habitat loss, climate change, introduced species, and particularly contaminant exposure, the presence of this pathogen might have a significant impact on reducing overall abundance in particular populations. As empirical evidence suggests, these stressors can have lethal outcomes when interacting, or can also influence several other important life history traits such as growth and reproduction. Further examination of these types of questions is clearly warranted and of relevance within South Dakota.

Management implications:

Massive die-offs due to chytrid are typically found at higher elevation sites across the world. This is likely due to the ideal growth temperatures for the fungus being cooler temperatures. Therefore, the largest concern for chytrid impacts is likely in the Black Hills area altough none of the sites exhibited massive die offs found in other areas of the world. Nonetheless, our data suggest that chytrid can be highly prevalent in certain populations in the spring season. We highly recommend intensive sampling in this area to better determine the potential impact of chytrid infection on these populations. Given the consideration of the Northern Leopard frog being considered for federal protection, and the high prevalence of infection that we found, this species in particular might be one to pay close attention.

Another key concern is the interaction of anthropogenic pollutants and chytrid infection on amphibian survival. Several studies suggest that contaminant exposure greatly increases risk for susceptibility to several pathogenic infections including trematodes and ranavirus (Rohr et al. 2008, Kerby and Storfer 2009, Kerby et al. 2010). Surprisingly little work has been done to investigate contaminant interactions with chytrid. The attached thesis highlights via empirical data that environmental concentrations of atrazine combined with chytrid exposure can significantly decrease survival. Given the clear overlap of agricultural lands with amphibian distributions within the state of South Dakota, this is a concern. That is, despite being at warmer temperatures, these lowland areas might still be significantly impacted by chytrid due to a synergism with contaminant exposure. The attached masters thesis details many of these concerns and provides empirical evidence of potential impacts.

Finally, we note that there is yet another amphibian pathogen classified as an emerging infectious disease that has received very little attention in South Dakota, ranavirus. This virus has been found in fish within the state as I was notified of this via email communication. This virus has been found to cause die offs in Tiger Salamanders in Canada, North Dakota, Colorado, and Arizona. It is highly likely that ranavirus is present in amphibian populations throughout the state, and might be causing additional issues in amphibian populations. Almost nothing is known regarding its potential for interaction with chytrid infection.

Dissemination of information

We have presented our findings at several regional meetings. In 2010, results were presented at the annual Dakota Amphibian and Reptiles Network (DARN) meeting in Pierre, SD and the annual Missouri River Institute (MRI) Symposium at USD in Vermillion, SD. Data were also presented at the Ecological Society of America in Pittsburgh, PA, and at the inaugural Emerging Amphibian Infectious Disease conference in Townsville, Australia. During the spring and summer of 2011 the information was presented at DARN, MRI Symposium, the Missouri River Natural Resources Committee (MRNRC) annual conference in Omaha, NE, and the Society of Ichthyologists and Herpetologists in Minneapolis, MN.

In addition, we have shared information at several public events held on the campus of University of South Dakota, and a public demonstration in Sioux City, IA. Public presentations were also given at the Henry Doorly Zoo in Omaha, NE and to students and community members at Oglala Lakota College.

The development of a brochure to present information on chytrid is nearly complete and will soon be printed. This informational brochure focuses on the impacts of both chytrid fungus and ranavirus, and highlights ways to prevent their spread (with a primary focus on the bait and pet risks of spread). I have discussed and developed content with Angela Picco of the US Fish and Wildlife Service and incorporated her insight in the final design. Once completed, we will forward a final draft to South Dakota Fish, Game, and Parks personnel for final input. Once complete, we plan to print and distribute copies across the state as well as post a downloadable web version on the PI's website and on the South Dakota Fish, Game, and Parks website if so desired.

A GIS-based database has been constructed based on the current site and infection status data. These layers have been sent to South Dakota Game, Fish, and Parks. An Access database has also been constructed that contains all the details of the surveys. These databases contain information on particular sites surveyed, as well as more detailed information on where pathogen detections are logged. The infection status data have been submitted to the Global Mapping Initiative at: http://www.spatialepidemiology.net/bd/.

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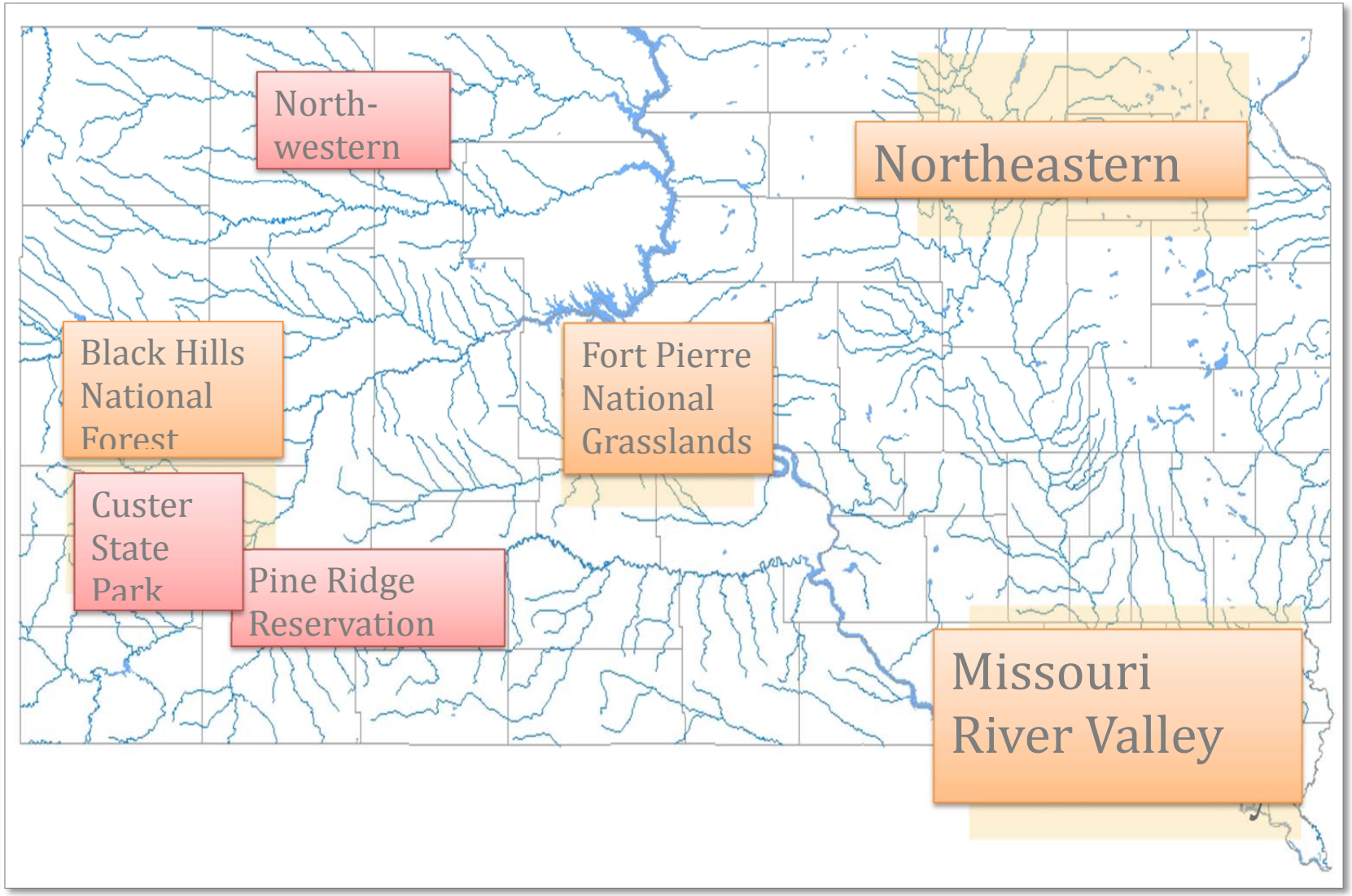
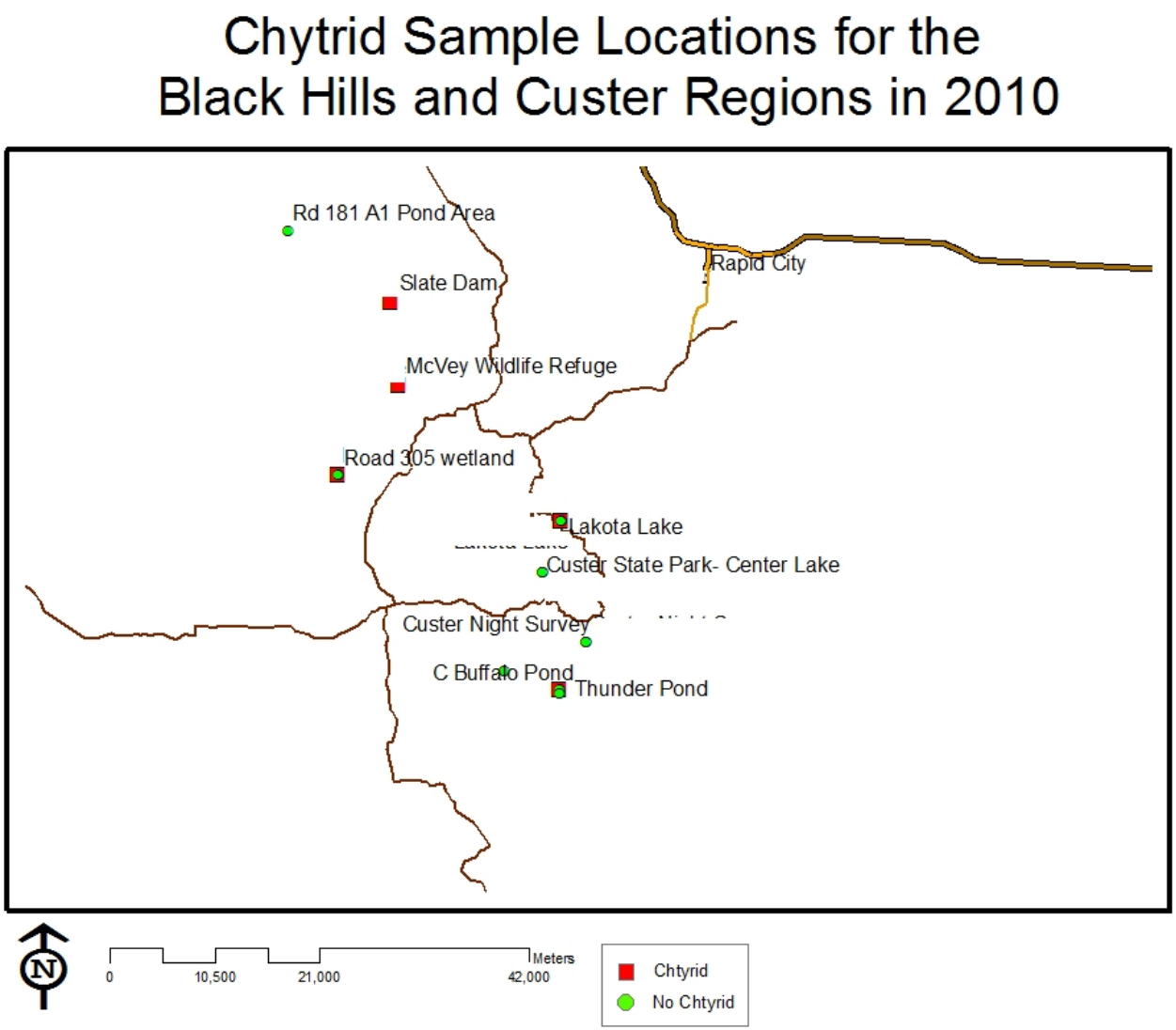
Table 1: **Chytrid Prevalence in South Dakota** Field results are shown from samples taking during the summers of 2009 and 2010. The table shows the total number of each species sampled by region with the number of infected individuals in parentheses. The total column indicates the total number of all species collected in each region with the total number of infected individuals and the percent prevalence for that region.

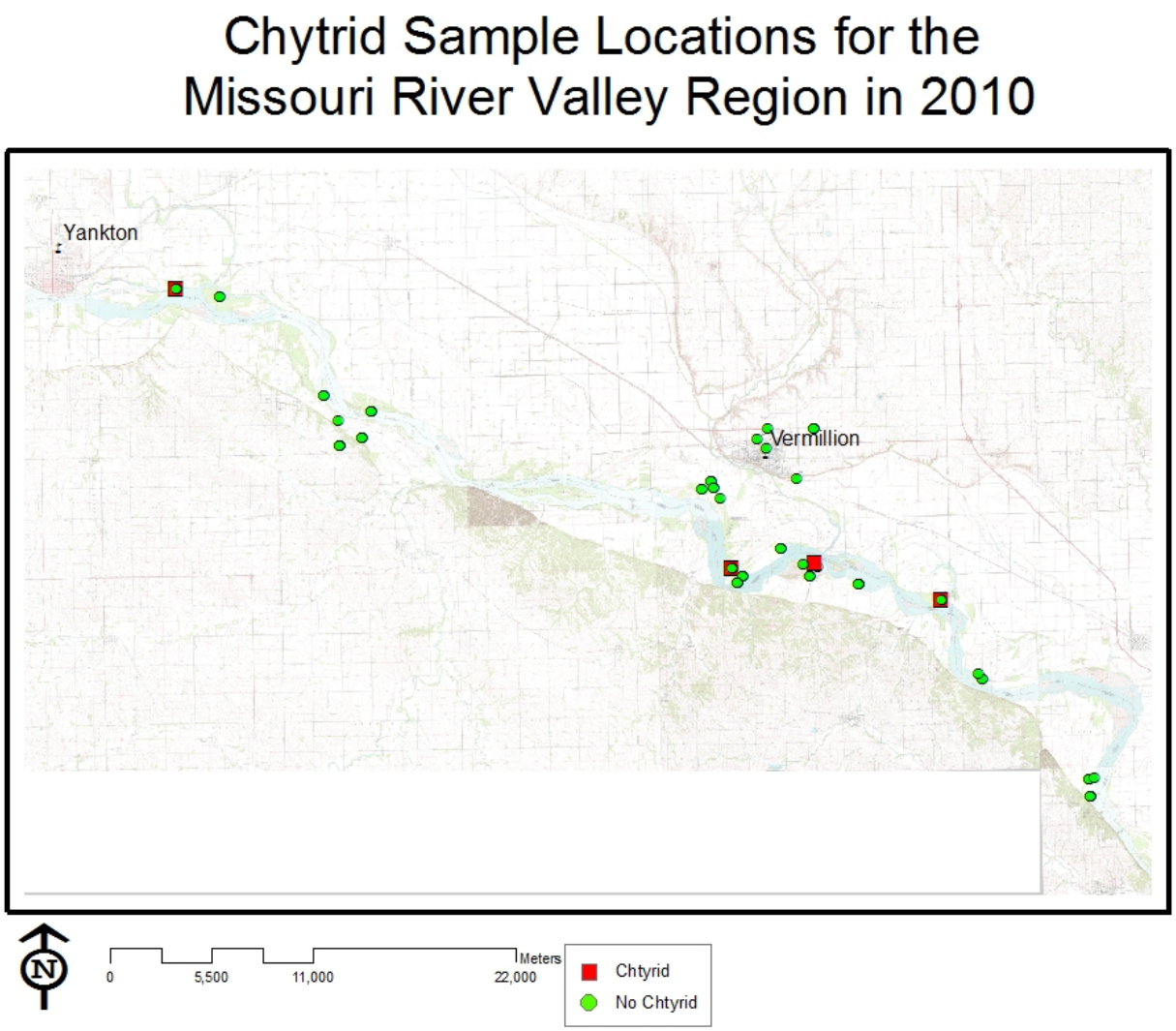
Region	Total	Northern Leopard Frog	Plains Leopard Frog	Wood house Toad	Northern Cricket Frog	Boreal Chorus Frog	Grey Tree Frog	Spade-foot Toad	Tiger Salaman -der	Great Plains Toad	Cana-dian Toad
2010	**1,024 (66) 6.4%**										
Missouri River Valley	887 (55) 6.2%	411 (1)	21 (0)	93 (2)	72 (2)	227 (50)	31 (0)		6 (0)	1 (4)	
Northwest	6 (0) 0%	3 (0)									2 (0)
Pierre	20 (1) 5%	8 (0)				12 (1)					
Black Hills	42 (8) 19%	14 (7)				28 (1)					
Pine Ridge Reservation	30 (0) 0%	9(0)						15 (0)	6 (0)		
Custer State Park	39 (2) 5%	37 (2)		1 (0)				1 (0)			
2009	**501 (20) 4.8%**										
Missouri River Valley	400 (19) 5%	17 (0)	34 (0)	250 (1)	30 (0)	22 (18)	22 (0)				
Northeast	28 (1) 4%	28 (1)									
Pierre	26 (0) 0%	26 (0)									
Black Hills	48 (0) 0%	46 (0)				2 (0)					
Total	1,525 (86) 5.6%	599 (11) 1.8%	55 (0) 0%	344 (3) 1%	102 (2) 2%	291 (70) 24%	53 (0) 0%	16 (0) 0%	12 (0) 0%	120 (4) 3%	2 (0) 0%



Map 1. Regions sampled in both 2009 and 2010 are shown in orange boxes. Regions sampled only in 2010 are shown in red boxes.



Map 2. Specific locations sampled in Custer State Park and the Black Hills of South Dakota.



Map 3. Detailed locations in the Missouri River Valley.