Office of Resource Conservation

State of Illinois

Grant Proposal

PROJECT NUMBER: T-56 R-1

PROJECT TITLE: Survey for the pathogen, *Batrachochytrium dendrobatidis,* in Illinois.

PURPOSE:
The purpose of our project is to describe the distribution of *Bd* within the state of Illinois. This project will directly contribute to action items in the Illinois Wildlife Conservation Plan that cover Wetlands Campaign, Invasive Species, Monitoring and Research of Wildlife Disease, and Monitoring and Research on Amphibian Species of Conservation Priority (http://dnr.state.il.us/ORC/WildlifeResources/theplan/final/). As outlined in the IWCP (pg. 31), "Fourteen of Illinois' 41 amphibian (34%)" species are considered "Species in Greatest Need of Conservation, eight are threatened or endangered, and 1 has a Global Conservation Rank of G3."

The goal of this project is to assess the current and historic status of the invasive fungus *Batrachochytrium dendrobatidis* (*Bd*) among amphibian species at selected sites throughout the state of Illinois. The presence of *Bd* in Illinois increases the vulnerability of all Illinois species to population decline, and species that are rare endangered, or which have special habitat needs will be particularly affected. It will be especially important to determine the infection status of amphibians inhabiting important reserves and habitats, and the species listed as either threatened or endangered in Illinois to better manage their habitats and populations, and to evaluate the feasibility of conservation activities such as captive breeding, translocations, and reintroduction programs (Mendelson et al. 2006). This information can also guide future management practices to minimize the spread of the fungus into new areas or new populations. This project involves collaborative efforts among SIUC, IDNR, and USFWS to produce recommendations related to actions to address research, monitoring, management, and recovery of rare and declining amphibian species in Illinois and threats from an invasive wildlife disease.

I. NEED:

Our primary objectives are 1) to determine the current distribution of *Bd* in Illinois and 2) to determine the time when *Bd* was first found infecting Illinois amphibians and the pattern in which it spread across the state. We need to survey for *Bd* in Illinois because it is likely to be infecting amphibians today, and to have caused declines in Illinois amphibians in the past; yet there is no database of infected sites, habitats, or species. A statewide survey would produce distributional information on *Bd* needed for individual managers to determine whether they have a healthy or infected site, and how to either prevent introduction, or prevent future spread of this disease. This study would determine infection status of sites allowing the development and dispersal of guidelines on disease containment, prevention of spread, disease surveillance, and/or amphibian population monitoring. We also need to conduct historic surveys to determine when it arrived in the state to determine whether it might have impacted amphibian populations. A historic survey might also indicate the location of original introductions which could suggest potential routes of transmission.

General background: The case of amphibian population declines began very recently in the Midwest, with the few documented losses reported within the last 30 years, and many from within protected reserves. *Bd* is emerging in new areas and species, causing epidemics in many naïve populations of wild species and resulting in die-offs, population declines and even extinctions. *Bd* has infected over 200 species around the globe, and can potentially cause mortality of all amphibian species. The World Organization for Animal Health (the OIE) has begun the process to list *Bd* as a disease of global significance and is advising all nations to conduct surveys to determine the geographic and taxonomic distribution of this disease. This pathogen is of particular concern because it is an invasive species, it can infect and kill many species, it does not respect geopolitical boundaries, and it persists in the environment, thus limiting the effectiveness of traditional conservation tools such as reintroductions, translocations, and establishment of protected areas.

Amphibian species vary in their responses to the disease; some show high prevalence, high mortality and extinctions while others have limited infections and little evidence of population declines. These patterns likely result from differences in host sensitivity to infection which may be influenced by habitat use (aquatic, terrestrial, temperate, tropical), behaviors (basking, diurnal, hibernation, aggregations), but also as a result of natural defenses in the skin such as antimicrobial peptides and symbiotic bacteria. For example, in the southeastern US, *Desmognathus auriculatus* has disappeared form headwater springs, while *Eurycea cirrigera* and *Pseudotriton ruber* both persist (Means and Travis 2007), suggesting either differences in habitat use or in species biology or behavior.

Multiple strains of *Bd* are present in the US (Morgan et al. 2007), and certain strains are more virulent than others, suggesting that even if *Bd* is present, continued management to minimize the spread may help prevent invasion by more lethal strains. Results from this project will identify infected and uninfected sites and will inform management actions that are intended to slow or prevent the spread of this fungus into healthy populations. *Bd* is naturally transmitted between amphibians, or between infected environments (damp substrates, water) and amphibians. Sites that have not yet been affected may become infected in the future unless major management plans are developed. Amphibians at sites infected with a less virulent strain might show greater losses if more virulent strains were introduced. *Bd* can spread among sites through many means; once it invades, it remains to infect surviving amphibians, new colonists, or reintroduced animals; therefore, knowledge of infected sites should direct future reintroduction decisions. *Bd* is likely to be more widespread than currently known because of limited surveys in remote regions, high cost of analyses, and few historic surveys of museum specimens. Illinois is no exception as no large-scale surveys have been completed in the state to date, thus the need to conduct histological surveys as described in this proposal. This fungus as an emerging infectious disease first was discovered in 1998 and much remains to be learned about its biology, how it is transmitted, its impacts on native amphibians, and its distribution in Illinois. This project will provide information that may enable us to conserve amphibians in Illinois via specific management techniques despite the spread of this fungus infection.

Bd has been detected in all regions of the US where field surveys have been conducted, often with very little effort (e.g., a few days, a few ponds, <100 animals), suggesting *Bd* is widespread in the US. Many Illinois amphibians species have been found infected with *Bd* elsewhere, including *Bufo americanus, Ambystoma maculatum, Hyla versicolor, Notophthalmus viridescens, Pseudacris triseriata, Rana pipiens, R. sylvatica, R. palustris, R. clamitans, R. catesbeiana* (Green et al. 2002). Terrestrial (Highton 2005) and stream (Banks et al. 2006) salamander populations have also declined throughout the eastern half of the US, including populations of *Plethodon cinereus* and *P. dorsalis* in Illinois, Indiana, and Missouri (Highton 2005). These populations

have not been tested for *Bd*, but the timing of the declines was in the mid-1980's when *Bd* was known to be causing population declines in amphibians elsewhere in the US. *Bd* has been involved in many die-offs and declines in amphibian populations throughout the United States (e.g., California, Arizona, and Colorado).

Based on these reports, *Bd* is likely to be widespread in certain regions of Illinois presently. *Bd* has been reported from 8 Illinois individuals of *Acris crepitans* collected in 1996 (Pessier et al. 1999) and in larval *Rana clamitans* collected in 1998 (Lips unpubl. data), although there have been no large-scale surveys to determine its presence or prevalence among species or habitats. *Bd* has been present in the states that surround Illinois since at least the 1980's (Indiana, Wisconsin) or 1990's (Missouri; Ouellet et al. 2005), and may be causing population declines in Missouri hellbenders (Jeff Briggler pers. comm.). No retrospective surveys have been done to determine when the disease first appeared and its impact on the Illinois fauna. The status of Illinois amphibian populations has never been fully quantified, although there is anecdotal evidence that certain species are in decline in certain parts of the state. There are very few long term studies of amphibian populations in Illinois, which prevents any comparative studies of population trends. However, extensive collections of amphibians have been made over the decades by state herpetologists (e.g., Smith, Brandon, Phillips), which have produced an excellent understanding of the historic and current distribution of Illinois species.

II. OBJECTIVES:

Objective 1:
Survey protected areas of the State of Illinois for the presence of *Bd*, focusing on protected areas located within Natural Divisions (Northeastern Morrainal, Rock River Hill Country, Grand Prairie, Southern Till, Upper Mississippi / Illinois River Bottomlands, Wabash Border, Shawnee Hills, Coastal Plain, Middle Mississippi Border). (Spring and Summer 2008 & 2009, 31% of budget).

1.1) Determine sites to be surveyed, choosing 2 in the north, 2 in the center, and 2 in the south in year 1 and 6 additional sites in year two.
1.2) Over 8 sample dates for each site, capture visually located amphibians. Swab captured amphibians to collect samples for *Bd* testing. Record individual data. Release all amphibians once swabbing completed.
1.3) Measure microenvironmental and habitat variables and obtain GPS location at each capture.

Objective 2:
Use histology to survey for *Bd* in museum specimens collected prior to 1990 from the 9 Natural Divisions of Illinois (Summer 2008-Fall 2010; <3% budget).

2.1) Obtain list of all Illinois specimens (including species, collection date and locality data) deposited at SIUC, INHS/UIUC, FMNH from curators. Select appropriate species and individuals to sample based on division and date of collection.
2.2) Remove a 4x4 mm snip of pelvic patch skin from at least 366 historical specimens collected prior to 1990 (the earliest record of *Bd* in the state) in each of the 9 natural divisions in the study area, choosing approximately 90 individuals from each decade (1950, 1960, 1970, 1980). Place collected tissue samples in alcohol to be transported to the SIUC Histology lab.
2.3) Prepare histological slides of skin, examine under microscope for the presence of *Bd*. Record dates and locations of infection.
2.4) Enter date and GPS locality of each positive and negative record of *Bd* into database. Quantify degree and prevalence of infection by *Bd* among decades in each Natural Division.

Objective 3:
Organize and analyze field survey data. (Summer 2008 – Spring 2009; 65% of budget)

3.1) Analyze amphibian swabs to determine the presence of *Bd* using PCR analysis.
3.2) Enter site data and GPS location into database.
3.3) Quantify degree and prevalence of infection by *Bd* among amphibian species, sites, and habitat types. Determine current *Bd* distribution: ubiquitous, patchily distributed (by region, habitat, or species), or not present.

Objective 4:
Produce Reports and Maps (Fall 2010-Spring 2011; <1% of budget)

4.1) Produce maps of current and historic dates and locations of *Bd* distribution.
4.1) Develop management plans to assist land managers based on presence/absence of *Bd* across state, in particular habitats, and in particular species.
4.1) Produce progress reports for IDNR and FWS.

III. EXPECTED RESULTS OR BENEFITS

This project will directly contribute to action items in the Illinois Wildlife Conservation Plan that cover Wetlands Campaign, Invasive Species, Monitoring and Research of Wildlife Disease, and Monitoring and Research on Amphibian Species of Conservation Priority (http://dnr.state.il.us/ORC/WildlifeResources/theplan/final/).

1) We will combine data produced from these objectives. We can produce a broad scale picture of the current and historic distribution of *Bd* in the state, among Natural Divisions, and among species over the past 50 years.
We will determine the current and historic distribution of *Bd* throughout the state of Illinois and provide information on the infection status of many species found in natural habitats of Illinois. We will import GPS localities of sampled individuals into ArcView to produce a map of *Bd* distribution in the state. This map can then be used by site managers and other scientists to manage specific species and habitats for *Bd* occurrence. This Expected Result will address part of the "Required Elements of a Comprehensive Wildlife Conservation Plan/Strategy" of the IWCP (pg. 4, part iii) by describing "problems that could adversely affect species" and habitats, assisting in "restoration and improved conservation" of the aforementioned.

2) We will relate current and historic patterns of infection to microhabitat features to prioritize future, in-depth and long-term studies of amphibian populations and the disease.
We will create a survey tool designed for use by IDNR, USFWS and other groups to administer to other Illinois amphibian populations to continue and elaborate upon this study. This project will provide managers with a better idea of how amphibian populations are currently being affected so that more fine-scaled management can focus on species and habitats of highest *Bd* susceptibility. This Expected Result addresses "Element 3, part b" in the IWCP (pg. xi) by describing "threats/problems" "in sufficient detail to develop focused conservation actions." This is further supported by Table 6 "Stresses considered as potentially having adverse effects on Illinois' SGCN" (pg. 266) in studying "Parasites-Diseases and Invasives/Exotics" as "community stressors."

3) We will use results to recommend particular management activities to minimize the spread of *Bd* in the state, to protect amphibian populations and species threatened by *Bd*, and to inform management and conservation activities necessary to stabilize and restore native Illinois amphibian species.
Using the data collected during this study, we can recommend management actions to better manage the threat of *Bd* to amphibians, and allocate resources to effective conservation activities, such as preventing future amphibian population declines, inadvertently introducing pathogenic disease to healthy sites, or losing captive bred or translocated individuals by exposing them to infected habitats. These results will contribute to proactive management and recovery of T&E species and their habitats in Illinois. Any appropriate conservation responses must be formulated on geographic and taxonomic distributions of the pathogen. Proactive conservation measures will depend on statewide surveys such as this for the presence and prevalence of the disease among habitats and among species coupled with population monitoring of key Illinois amphibian populations. This information will also be of concern to industries such as fisheries, agriculture, timber and trade to ensure that their activities do not facilitate the spread of the disease. This Expected Result addresses "Element 3, part b" in the IWCP (pg. xi) by describing "threats/problems" "in sufficient detail to develop focused conservation actions." This is further supported by Table 6 "Stresses considered as potentially having adverse effects on Illinois' SGCN" (pg. 266) in studying "Parasites-Diseases and Invasives/Exotics" as "community stressors."

4) We will use results to prioritize geographic areas for future conservation efforts related to amphibian diversity.
Understanding the effects of microenvironmental and habitat variables and *Bd* on amphibian communities will better enable researchers and land managers to facilitate the conservation and possibly the recovery of affected species. This Expected Result follows IWCP's "Element 3, part c" (pg. xii) by "considering threats/problems, regardless of their origins (local, state, national, and international), where relevant to the State's species and habitats." It further addresses conservation needs of "Illinois Species in Greatest Need of Conservation" as when the Illinois population of a species represents a significant proportion of the species' global population" (e.g., crayfish frog, Illinois chorus frog; pg. 262).

5) We will communicate results to conservation partners and develop a final report.
Information will be communicated though presentations at professional meetings or conferences and public talks. We will distribute annual reports and a final report to conservation partners to provide a review of our activities and survey results. This Expected Result is in alignment with IWCP's recommendation for "Research, Monitoring, and Evaluation" (pg. 98) by providing information on *Bd's* "threat" to Illinois species, particularly those "Illinois Species in Greatest Need of Conservation and the ecosystems upon which they depend." Furthermore, this information will be shared with "resource managers, decision-makers, and the public at large." This will also follow "Element 3, part e" (pg. xii) in providing research results to "allow for the development of research and survey projects."

IV. APPROACH:

We will determine the distribution of this disease among amphibian populations in wetland sites located within 9 of the Natural Divisions of Illinois. We will target habitat areas most important to species of conservation priority, such as Cache River, LaRue Swamp, Wabash River, and the Fox River.

Objective 1:

Survey protected areas of the State of Illinois for the presence of *Bd*, focusing on protected areas located within Natural Divisions (Northeastern Morain, Rock River Hill Country, Grand Prairie, Southern Till, Illinois River Bottomlands, Wabash Border, Shawnee Hills). (Spring 2008 & 2009, 31% of budget).

1.1) Determine sites to be surveyed, choosing 2 in the north, 2 in the center, and 2 in the south in year 1 and 6 additional sites in year two.

We will compile species lists of potential study sites by consulting with IDNR, FWS and INHS staff and site managers and by conducting library and internet research. We will arrange site visits to speak with managers and determine whether appropriate habitat and sufficient abundance of focal species exists at each site and to assess feasibility of each site.

1.2) Capture visually located amphibians. Swab captured amphibians to collect samples for Bd testing. Record individual data. Release all amphibians once swabbing completed.

A field crew of 2-3 SIUC students will conduct surveys to establish the distribution of *Bd* among wetlands in 9 Natural Divisions (Coastal Plain, Middle Mississippi Border, Northeastern Morain, Rock River Hill Country, Grand Prairie, Southern Till, Upper Mississippi / Illinois River Bottomlands, Wabash Border, Shawnee Hills). Multiple protected areas, and most species, including those of conservation priority (e.g., Bird-voiced treefrog, Crayfish frogs, Illinois chorus frogs) will be included. We will sample during spring and early summer when amphibians are most abundant and when environmental conditions are within the optimal range for growth and survival of *Bd* and it is likely to be widely distributed among species and habitats.

We will sample 35 individuals in each of 10 species per site to ensure collection of 366 swabs per site. This is considered a sufficient sample size according to research by Ross Alford, Forrest Brem, and other researchers studying *Bd* infection in the wild (e.g., Brem et al. MS submitted to Herp. Rev.). We will maximize our chances of finding *Bd* if it is present, by sampling species that have been shown to be infected elsewhere (e.g., most species of *Rana* and *Bufo*). We will target two types of amphibians: widespread species and those of special interest. At each site, we will sample 5 species that are widespread in the state and which have been shown to be infected elsewhere (e.g., *Bufo americanus, Acris crepitans, Ambystoma maculatum, Hyla versicolor, Notophthalmus viridescens, Pseudacris triseriata, Rana pipiens, R. sphenocephala, R. sylvatica, R. palustris, R. clamitans, R. catesbeiana)* to determine how local environments influence infection. At each site we will also sample species that have shown declines in the state (e.g., *A. crepitans*, *R. areolata*), are restricted in range (*Desmognathus fuscus, Eurycea lucifuga*) or are Threatened or Endangered (e.g., *P. triseriata*, *H. avivoca*).

We will use standard field techniques to survey adult amphibians (Heyer *et al.* 1994) with distance-constrained visual encounter surveys (VES). Teams of 2-3 researches will walk along transects and visually search for frogs. Individuals will be captured by hand, swabbed for *Bd* (Hyatt et al. 2006), toe-clipped for individual identification, measured (SVL, mass) and then released. Each cotton tip used for swabbing will be stored in a DNA storage buffer (Lips *et al.* 2006). Additional information such as age, sex, perch substrate, reproductive behavior will be recorded when possible. We will determine GPS coordinates at the midpoint of each transect with a Garmin™ GPS 12 personal navigator.

1.3) Measure microenvironmental and habitat variables, and obtain GPS location at each capture.

At each sampling location we will obtain a GPS reading and record relevant environmental variables including: air temperature, substrate temperature, water temperature, wind speed, humidity, cloud cover, and precipitation. We will also record habitat variables including: canopy cover, depth and width of water bodies, wetland area, and vegetation type. We will plot locations of all sampled amphibians with ArcView to plot current and historical distributions of *Bd* in the state, and if possible, to search for environmental cofactors (e.g. Witte 2005) of infection. Fieldwork will include collection of environmental cofactors at each capture location, to be included in a separate database.

Objective 2:
Use histology to survey for *Bd* in museum specimens collected prior to 1990 from each of the Natural Divisions of Illinois (Summer 2008-Fall 2010; <3% budget).

2.1) Obtain list of all Illinois specimens (including species, collection date and locality data) deposited at SIUC INHS, UIUC, FMNH from curators.

We will request information from curators of museums with major holdings of Illinois material, and obtain listings of all Illinois specimens collected between 1950-1990. We will summarize those lists to determine how many individuals of which species are available from each Natural Division in each decade from 1950-1990. We will determine which species to sample by identifying those with $\geq$40 individuals collected from each Division per decade.

2.2) Remove a 4x4 mm snip of pelvic patch skin from at least 366 historical specimens collected prior to 1990 (the earliest record of Bd in the state) in each of the 9 natural divisions in the study area, choosing approximately 90 individuals from each decade (1950, 1960, 1970, 1980). Place collected tissue samples in alcohol to be transported to the SIUC Histology lab.

We will visit each museum with specimens that fit our profile (above) and remove a small (~4x 4 mm) piece of skin from the ventral side of the animal in the area of the pelvic patch. Based on the literature, this region is most likely to show an infection (Puschendorf and Bolanos, 2006).

2.3) Prepare histological slides of skin, examine under microscope for the presence of Bd. Record dates and locations of infection.

We will work with Maureen Dornan in the SIUC Histology lab to prepare slides for standard H&E histology (Puschendorf and Bolanos, 2006). This approach involves fixing the tissue in formalin, dehydrating the tissue in ethanol baths, embedding the tissue into paraffin, sectioning the skin to make very thin slices that will be placed on a glass slide for staining. Hematoxylin and eosin (H&E) are the most commonly used stains in histology and histopathology. Hematoxylin colors nuclei blue; eosin colors the cytoplasm pink. To see the tissue under a microscope, the sections are stained with one or more pigments.

2.4) Enter date and GPS locality of each positive and negative record of Bd into database. Quantify degree and prevalence of infection by Bd among decades in each Natural Division.

We will use results of the histology surveys to calculate *Bd* infection prevalence and 95% Clopper-Pearson binomial confidence intervals for each Natural Division and for each decade. We will plot

locations of all sampled amphibians with ArcView to plot historic distributions of *Bd* in the state, and if possible, to search for environmental cofactors (e.g. Witte 2005; Lips et al. 2003; Brem 2006) of infection.

Objective 3.
Organize and analyze survey data. (Summer 2008 – Spring 2009; 65% of budget)

3.1) Analyze amphibian swabs to determine the presence of Bd using PCR analysis.

Each swabbed sample will be tested for *Bd* in the lab using PCR primers (Annis *et al.* 2004; Boyle et al. 2004; Hyatt et al. 2006). Genetic screening for *Bd* will be conducted in the lab of Dr. Ed Heist at SIUC.

3.2 Enter site data and GPS location into database

All microenvironmental and habitat variables will be entered into a database. GPS locations of sampled sites and historical occurrence will be plotted into ArcView. We will plot locations of all sampled amphibians with ArcView to plot current distributions of *Bd* in the state,

3.3) Quantify degree and prevalence of infection by Bd among amphibian species, sites, and habitat types. Determine current Bd distribution: ubiquitous, patchily distributed (by region, habitat, or species), or not present.

We will use results of the PCR assay to calculate *Bd* infection prevalence and 95% Clopper-Pearson binomial confidence intervals for each species at each site. Using data entered in Obj. 3.2, we will attempt to search for environmental cofactors (e.g. Witte 2005) of infection. We will relate infection status to differences in species ecology and microhabitat characteristics (Lips et al. 2003; Brem 2006).

We will summarize and analyze distribution patterns of current and historical *Bd* presence in the state using spatial analysis software. We will determine whether infection is associated with particular landscape features, seasons, or species. We will assess whether amphibian populations are likely to have declined as a result of historic introductions of *Bd* to the state.

Objective 4.
Produce Reports and Maps (Fall 2010-Spring 2011; <1% budget)

4.1) Produce maps of current and historic dates and locations of Bd distribution.

We will use spatial analysis and mapping software to create maps of the current and historic distribution in the state.

4.2) Develop management plans to assist land managers based on presence/absence of Bd across state, in particular habitats, and in particular species.

We will use results from the statistical analysis and spatial mapping to recommend potential management options to minimize or control the spread of *Bd*, to evaluate feasibility of reintroduction programs, and to conduct disease surveillance in particular regions or species. We will identify specific

management needs depending on whether a region, a site, a habitat, or a particular species is infected or not, and to what degree. These would include ideas for active prevention of spread (e.g., management of human activities, placing bleaching stations at key sites, designing informational signs and brochures, etc), monitoring of wild amphibian populations, management of captive and reintroduced populations, and assessing possibility of future spread (e.g., routes, direction, rates, vectors).

Some examples of management recommendations resulting from information gathered through this study are as follows:

Ubiquitous: (A) If we determine that *Bd* is ubiquitous throughout Illinois, managers could use this information to take actions to minimize the spread of *Bd* (e.g., educating site users, providing bleaching stations). There are multiple strains of *Bd* known to date, some more lethal than others, so even though a site is infected we recommend minimizing additional introductions. We would also recommend molecular analyses to identify the distribution of different strains throughout the state to better manage amphibian populations. (B) In addition, we would not recommend reintroduction of threatened and endangered species anywhere in Illinois until methods are developed to eradicate *Bd* from the wild. (C) Furthermore, we would recommend determination of the population status and changes thereof for all wild populations of threatened and endangered species, as well as those species with restricted ranges. We would use results of the historic survey to determine the likely date and location of the earliest introductions of *Bd* into Illinois and also to evaluate when each region or population was likely to have first been exposed to *Bd* and whether the timing was associated with any information regarding change in populations status.

Patchily distributed: (A) If we determine that *Bd* is patchily distributed (either by region, habitat, or species), then managers could implement activities to minimize the spread of *Bd* (see above). (B) Since *Bd* is easily transported through many types of moist substrates (e.g., water, soil, plants), managers could contain or minimize the spread of *Bd* into healthy amphibian populations by promoting the cleaning of recreational items, footwear and vehicle tires. For example, anglers fishing in infected water bodies could be encouraged to clean bait buckets and fishing gear before leaving the site. (C) We will compare the current distribution to the distribution determined by histological surveys of museum specimens to determine whether currently uninfected areas have always been uninfected and when they are likely to be invaded, and from which direction. We will use GIS maps of current and historic infection to identify potential routes, vectors, and rates of spread of *Bd* to help managers block routes, slow rates or manage vectors. (D) If *Bd* is present in specific species or regions, management strategies could be developed to monitor the population status of both healthy and infected populations, and to determine whether additional protection is needed (e.g., captive breeding, translocations, introductions) under the Endangered Species Act.

Not present: In the unlikely event that *Bd* is currently absent from IL and the historic survey also produces no infections, then state agencies should focus on preventing any future introductions by communicating with members of the pet industry, the horticultural industry and the fisheries industry about the threat of *Bd* introduction, and steps that could be taken to prevent its accidental introduction. Managers could evaluate particular areas that are likely to be infected in the future by either monitoring the population status of focal amphibian species or the disease prevalence of amphibians in regions or habitats of interests. Since surrounding states have documented cases of *Bd*, management might focus on water bodies flowing between two states, or industries and activities that facilitate spread of *Bd* into

Illinois. For example, the bait industry, pet industry, anglers and reserve managers may need to be advised of potential contamination of healthy areas from contaminated sources.

4.3) Produce progress reports for IDNR and FWS.

We will provide yearly written progress reports and a final written report from our work to the IDNR, the FWS, and all managers of sites where we conducted the study. We will give presentations at scientific meetings and to the general public.

Project schedule:

Objective	**Sp 08**	**Su 08**	**F 08**	**W 08-09**	**Sp 09**	**Su 09**	**F 09**	**W 09-10**	**Sp 10**	**Su 10**	**F 10**	**W 10-11**
Objective 1: Survey protected areas of Illinois for the presence of *Bd*	**X**	**X**			**X**	**X**						
Objective 2: Analyze (PCR) field samples			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Objective 3: Survey for *Bd* in museum specimens (histology)		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	
Objective 4: Produce Reports and Maps, give presentations at meetings				**X**		**X**		**X**		**X**	**X**	**X**

Any work performed before the effective start date of the grant will be paid for through funds provided by Dr. Lips at no cost to the SWG '07 project.

Personnel: SIUC will contribute 2.25 months per year salary time for Dr. Karen Lips. Dr. Lips will serve as project coordinator and will supervise the graduate (Brooke Talley) and undergraduate student(s). SIUC will also contribute 1 month per year salary time for Dr. Ed Heist. Dr. Heist will serve as lab coordinator and will supervise the graduate student in the lab. We have recruited many undergraduate volunteers who will be assisting us in the field in the capture and swabbing of animals

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Appendix

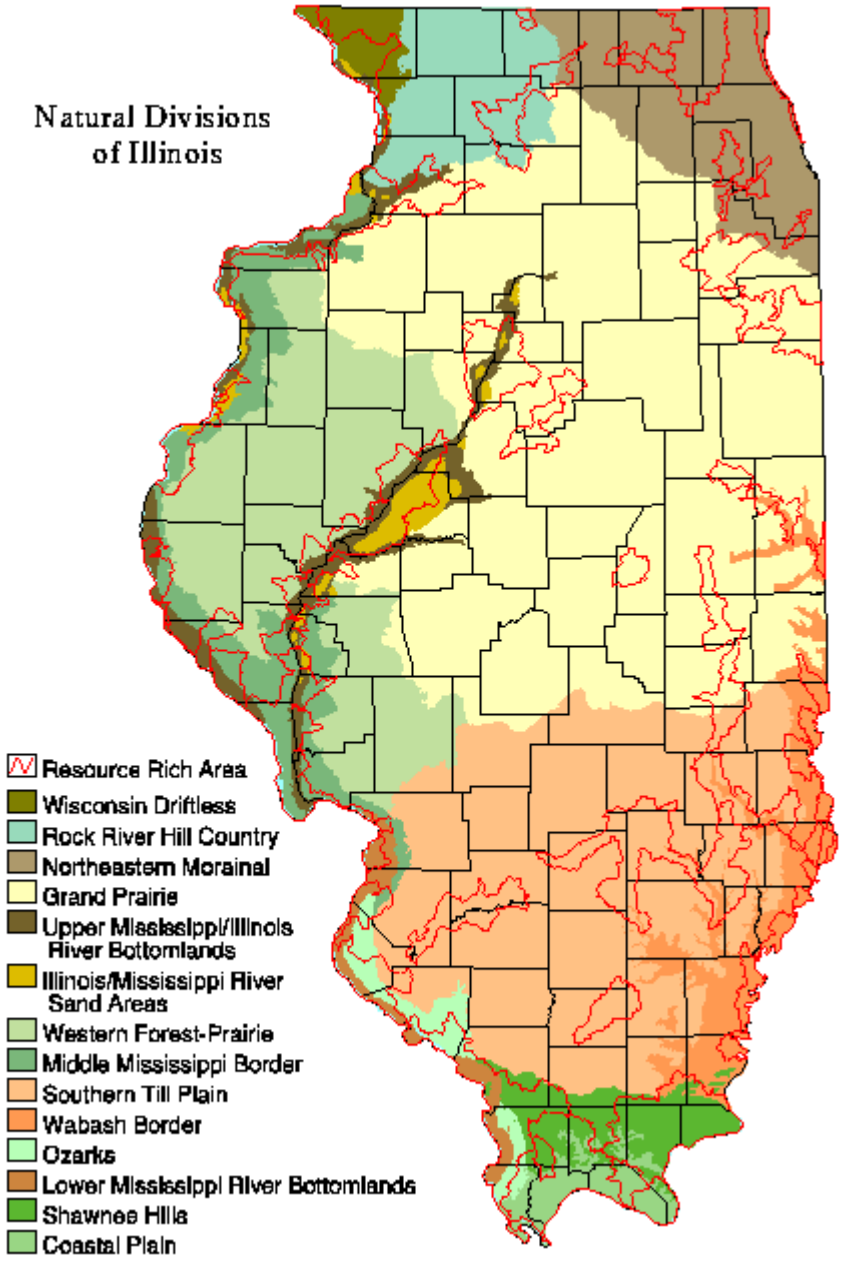

Figure 1: Natural Divisions of Illinois (http://www.inhs.uiuc.edu/cwe/rra/figure13.html). The 9 Natural Divisions to be sampled are (1) Northeastern Morrainal, (2) Rock River Hill Country, (3) Grand Prairie, (4) Southern Till, (5) Upper Mississippi / Illinois River Bottomlands, (6) Wabash Border, (7) Shawnee Hills, (8) Coastal Plain, and (9) Middle Mississippi Border.

Table 1: Species to be handled during surveys.

Species
Frogs / Toads
Acris crepitans
Bufo americanus
Bufo fowleri
Gastrophryne carolinensis
Hyla avivoca
Hyla cinerea
Hyla chrysoscelis / versicolor
Pseudacris crucifer
Pseudacris feriarum
Pseudacris illinoensis
Pseudacris streckeri
Pseudacris triseriata
Rana areolata
Rana blairi
Rana catesbeiana
Rana clamitans
Rana palustris
Rana pipiens
Rana sphenocephala
Rana sylvatica
Scaphiopus holbrookii
Salamanders
Ambystoma jeffersonianum
Ambystoma laterale
Ambystoma maculatum
Ambystoma opacum
Ambystoma platineum
Ambystoma talpoideum
Ambystoma texanum
Ambystoma tigrinum
Cryptobranchus alleganiensis
Desmognathus conanti
Eurycea cirrigera
Eurycea longicauda /guttolineata
Hemidactylium scutatum
Necturus maculosus
Notophthalmus viridescens
Plethodon cinereus
Plethodon dorsalis
Plethodon glutinosus
Siren intermedia