Amphibian Research and Monitoring Initiative
2004 Report on Activities for the Upper Mississippi Region

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Overview

Populations of amphibians have been declining around the world. The Amphibian Research and Monitoring Initiative (ARMI) was created in 2000 to evaluate the statuses of populations of amphibians and to study causes of any declines. The US Geological Survey (USGS) was given primary responsibility for executing this initiative. We are conducting research in support of this program in the Upper Mississippi Region of ARMI (ARMI-UMR; Figure 1). Our offices and laboratories are at the Upper Midwest Environmental Sciences Center in La Crosse, Wisconsin.

Our work in the Upper Mississippi Region of ARMI is focused on establishing the infrastructure, studies, and partnerships necessary to address ARMI's core goals and objectives. Our general approaches are as follows.

- Address the goals of ARMI via methods described in the ARMI conceptual pyramid.
- Address the core elements of ARMI by surveying and monitoring populations of various species.
- Work with other organizations to obtain relevant data to allow a broader assessment of populations across the UMR of ARMI.
- Complement data from surveys and monitoring by assessing potential causes of previous and future declines.
- Conduct our work in locations and by methods that maximize our ability to gather high-quality data and provide direct benefits to resource managers and decision makers.

During 2004, we developed objectives to meet the goals of ARMI for the next 3 to 5 years, as listed below. The justification for these objectives is in the study plan for the Upper Mississippi Region of ARMI. Basically, these objectives address ARMI's needs to assess statuses of amphibian populations in our region via surveys and monitoring, to study populations of species that have declined (e.g., the Blanchard's cricket frog), to gather extant data to help evaluate the statuses of populations, and to study the primary factors that could cause populations to decline in our region (habitat loss and fragmentation, agricultural practices, and changes in climate).

Objectives

1. Assess the statuses of amphibian populations in at least three different areas managed by the Department of Interior via surveys and monitoring.

2. Assess the statuses of populations of Blanchard's cricket frogs (*Acris crepitans blanchardi*) in Wisconsin, Minnesota, Iowa, and Illinois via surveys.

3. Establish fine-scale study sites for Blanchard's cricket frogs in Wisconsin, Iowa, and Illinois to monitor statuses of populations and study causes of declines.

4. Assess the statuses of populations across our region by working with organizations outside of ARMI to obtain information regarding distributions and abundances.

5. Study a subset of our monitoring sites for the presence of atrazine and other pesticides in water and abnormal development of gonads in resident amphibians.

6. Conduct joint tests of survey methods of ARMI and the North American Amphibian Monitoring Program to determine comparability of the results.

7. Monitor resident populations at a set of apex sites for effects of climate change.

8. Work with Environment Canada to develop and implement monitoring of breeding sites of amphibians for any effects associated with climate change.

9. Continue to work with partners at The Earth Resources Observation Systems (EROS) Data Center and Iowa State University to analyze risks posed to populations by habitat loss and fragmentation.

10. Finalize and execute plans with partners at the University of Minnesota, Duluth, to collaborate on studies of amphibians and multiple stressors in the prairie pothole region.

11. Conduct surveys for amphibians and reptiles in support of the goals of the National Park Service's Inventory and Monitoring Program in parks of the Great Lakes Network.

I have described our efforts in 2004 in the following section according to the portion of the ARMI conceptual pyramid and the specific objectives to which they apply. For more details on the issues, challenges, and approaches for ARMI in the Upper Mississippi Region of ARMI (ARMI-UMR), please see http://www.umesc.usgs.gov/armi.html. For information on ARMI at the national level, please see http://edc2.usgs.gov/armi/.

Results

Surveys and monitoring

SURVEYS AND MID-LEVEL MONITORING

- Middle level of the ARMI conceptual pyramid [Figure 2]
- Objective 1

We began surveying populations of several species of amphibians in the Upper Mississippi River National Wildlife and Fish Refuge (UMRNWFR – located in Minnesota, Wisconsin, Illinois, and Iowa and managed by the U.S. Fish and Wildlife Service), the St. Croix National Scenic Riverway (SC – located in Minnesota and Wisconsin and managed by the National Park Service), and Voyageurs National Park (VNP – located in Minnesota and managed by the National Park Service) via standardized call and visual-encounter surveys (Heyer and others 1994) in 2002 (Figure 3). We surveyed those populations again in 2003 and continued monitoring them in 2004. The species confirmed as present are listed in Table 1 by management area and the proportion of sites in which we observed them in 2004.

We also surveyed the Neal Smith National Wildlife Refuge (NS – located in Iowa and managed by the U.S. Fish and Wildlife Service; Figure 3) for several species of amphibians (Table 1) for the first time in 2004. We expect to add these sites to our set of long-term mid-level monitoring sites.

In addition to the species listed in Table 1, we heard pickerel frogs (*Rana palustris*) at one location each in the NS and UMRNFWR that we did not survey during the day. We heard Cope's gray treefrogs (*Hyla chrysoscelis*), at 13, 3, and 12 locations in the NS, UMRNFWR, and SC, respectively, that we did not survey during the day. We also observed redback salamanders (*Plethodon cinereus*) at two locations near breeding sites in the SC.

The four management areas lie along a north-south gradient (Figure 3) that affords us an opportunity to study populations of several species across a changing landscape of land use, proximity to agricultural practices, and climate. In general, these are the three principal potential threats to the persistence of populations of amphibians in the Upper Mississippi Region of ARMI. These areas also contain a broad range of wetland types, from temporary to permanent and lentic to lotic.

In all of the above work, we complemented our measurements of the presence of species with measurements of weather and habitat variables to help us understand how the standard metric used by ARMI researchers, Percent Area Occupied (PAO), for each species might be related to environmental conditions. We currently are in the process of calculating PAO for all of our species in all of our areas using the new information we obtained at the PAO workshop in Altamonte Springs, Florida, in December 2004.

Malformations

- Middle level of the ARMI conceptual pyramid
- Objective 1

We surveyed our mid-level monitoring sites in four areas managed by the Department of Interior (plus one unit managed by the Wisconsin Department of Natural Resources) for the presence of metamorphs on 172 occasions and measured the frequencies and types of malformations visually in the field when metamorphs were present (Tables 2–4). We observed relatively few metamorphs across all sites; we expect that this is due partly to the timing of our surveys not being synchronized with metamorphosis of individual species at each site. This asynchrony is unavoidable when rotating multiple extensive surveys, of which surveys for metamorphs are but a part, at many mid-level sites in different management units with limited field crews.

The frequencies of malformations we observed per species and management unit ranged from 0 to 0.03. These frequencies are similar to those we observed in 2002 and 2003 and are typical of frequencies reported for many areas according to the database of the North American Reporting Center for Malformations (http://frogweb.nbii.gov/narcam/).

STUDIES OF EXPOSURES TO TRIAZINES AND ANY ASSOCIATED EFFECTS ON GONADAL DEVELOPMENT

- Apex level of the ARMI conceptual pyramid
- Objective 5

Atrazine is the herbicide used most heavily in the ARMI–UMR (Figure 4) and it is pervasive in the environment. Exposure to this compound has reportedly caused gonads of immature amphibians to develop abnormally, including at concentrations as low as 0.1 ppb. The U.S. Environmental Protection Agency (EPA) has reported uncertainty

regarding the range of concentrations of atrazine that cause such abnormalities based upon studies conducted in the laboratory. Given the quantities of atrazine used in the Midwest and the uncertainty regarding exposures and effects in the field, we began sampling our mid-level monitoring sites in 2003 to determine whether amphibians were exposed to atrazine and if frequencies of abnormal gonads in metamorphosed northern leopard frogs from these sites were associated with exposure.

We again sampled breeding sites in 2004, in collaboration with Perry Jones, our ARMI partner from the USGS's Water Resources Discipline, in NS, UMRNWFR, SC, and VNP. We collected water samples from 142 of 194 potential mid-level monitoring sites plus 26 sites nearby from April through mid-August (Table 5) and analyzed them for the presence of triazines (the class of compounds to which atrazine belongs and typically dominates in the environment).

We used Enzyme-linked Immunosorbent Assays (ELISAs – Abraxis Kits; Warminster, Pennsylvania) to analyze water samples for triazines. We also sent a subset of our samples to the USGS Organic Geochemistry Research Group in Lawrence, Kansas, for analyses of individual triazines and their degradates using Liquid Chromatography/Mass Spectrometry (LCMS).

The results of analyses for sites in the floodplain of the Mississippi River in the UMRNWFR showed that amphibians were exposed to concentrations of triazines that varied over space and with time (Figure 5). These concentrations also varied with the hydrograph for the Mississippi River, being highest at or near the time when water levels were highest. In general, samples with the highest concentrations were from the main channel of the river and from sampling sites located the farthest downstream.

Results across all sites we sampled in 2004 showed exposure was highest in areas closest to row crops, areas where triazines likely were applied (Figure 6). We observed a gradient of concentrations of triazines that followed a gradient of physical proximity and connectedness to areas of row crops.

We have not yet fully compared the results of our analyses using ELISAs with those using LCMS to better understand the comparability of the two methods and the ratios of individual triazines to their degradates and other triazines in our samples. However, based upon our preliminary comparisons and results in 2003, ELISAs appear to be a cost-effective method (ELISAs = approximately $300 for 100 samples; Liquid Chromatography/Mass Spectrophotometry via WRD = approximately $300 for one sample) for screening large numbers of sites for triazines in general and as an indicator of the potential presence of atrazine.

Our results demonstrate that amphibians at our monitoring sites were exposed to a range of concentrations of triazines. If atrazine and its degradates made up the vast majority of these concentrations as suspected, then concentrations of atrazine and its potentially toxic degradates were in the range of 0.1 ppb at several sites. This concentration and higher concentrations have been reported to cause gonads to develop abnormally in African clawed frogs (*Xenopus laevis*) and northern leopard frogs ((Hayes and others 2002a, 2002b, 2003).

As we did in 2003, we collected, fixed, and preserved metamorphosed northern leopard frogs from a subset of breeding sites during 2004. We collected metamorphs from two sites each in VNP and SC and three sites in UMRNWFR. Tyrone Hayes (University of California, Berkeley) is measuring the types and frequencies of abnormalities in the gonads of the metamorphosed northern leopard frogs we collected in 2003 and 2004. In 2004, we submitted 50 animals (of the 100 metamorphs we typically collected per site, except for 51 and 54 at two sites in 2004) from each site we sampled in 2003 and 2004 to Dr. Hayes for analyses. We coded all samples prior to submitting them, such that Dr. Hayes could not determine which animals came from which sites. We have not received the results of his analyses at this time.

SURVEYS FOR BLANCHARD'S CRICKET FROGS

- Middle level of the ARMI conceptual pyramid
- Objectives 2, 3, and 4

The Blanchard's cricket frog has declined considerably along the northern boundary of its range (Figure 7). We began conducting targeted searches for this species in Minnesota, Wisconsin, Illinois, and Iowa in 2002. We expanded those surveys in 2003 and again in 2004 (Figure 8), with the intent of identifying a set of apex sites where we can monitor breeding populations and study potential causes of declines.

We conducted call surveys for Blanchard's cricket frogs in 2004 at 89 locations we had not surveyed previously. (These sites were in addition to our mid-level monitoring sites within the range of this species that we surveyed again in 2004.) We heard Blanchard's cricket frogs at 29 of these new locations. We have not yet analyzed all of those results to determine how many of the 29 active sites were unique, that is, they could not have been heard from more than one listening location.

Biologists from the Wisconsin Department of Natural Resources (DNR) also surveyed parts of Wisconsin for Blanchard's cricket frogs in 2004 and shared their data with us. Between the active sites we located from 2002 to 2004 and those located by the DNR (Figure 8), we hope to establish up to 12 apex sites for this species in Wisconsin, Illinois, and Iowa in 2005.

Other related work with Blanchard's cricket frogs, but not surveying and monitoring – Collecting and mapping data on the distribution of Blanchard's cricket frogs

- Base level of the ARMI conceptual pyramid
- Objective 4

In late 2003, we joined a collaboration with Jason Irwin (Bucknell University), Gary Casper (Milwaukee Public Museum), Michael Lannoo (Ball State University), Alisa Gallant (USGS – EROS), and Robert Klaver (USGS – EROS) to map the historical and recent distributions of Blanchard's cricket frogs (ARMI–UMR) and analyze them (EROS) for any associations between climatic factors and declines of this species. We began the process of obtaining and mapping such data from Iowa, Minnesota, Wisconsin, Illinois, Indiana, Ohio, and Michigan during 2004. Whereas we have obtained and mapped data from several sources (Figure 9), we still are awaiting data from several others. We hope to obtain the remaining known records for this species and complete the initial mapping phase of this project during 2005.

SURVEYS IN THE MISSISSIPPI NATIONAL RIVER AND RECREATION AREA (MNRRA)

- Base and middle level of the ARMI conceptual pyramid
- Objectives 1 and 4

As part of a joint effort with the National Park Service (NPS) for one season, we conducted targeted searches via call and visual-encounter surveys to try to determine whether several species of amphibians were present in MNRRA. We observed bullfrogs (*Rana catesbeiana*), green frogs (*R. clamitans melanota*), northern leopard frogs (*R. pipiens*), chorus frogs (western or boreal: *Pseudacris triseriata* or *P. maculata*), eastern gray treefrogs (*Hyla versicolor*), eastern American toads (*Bufo americanus americanus*), and tiger salamanders (*Ambystoma tigrinum*). Aside from one possible visit in spring of 2005 to account for a time period we missed in 2004, we are not likely to survey or monitor populations in MNRRA as part of ARMI mid-level monitoring in the foreseeable future.

COLLABORATION WITH USGS, PATUXENT, AND THE UNIVERSITY OF MINNESOTA, DULUTH, TO FACILITATE COMPARABILITY AMONG RESULTS OF SURVEYS

- Base level of the ARMI conceptual pyramid
- Objective 10

Lucinda Johnson and Pat Schoff from the University of Minnesota, Duluth, and Glenn Guntenspergen from the U.S. Geological Survey, Patuxent, are funded by the U.S. Environmental Protection Agency (EPA) through 2005 to study the statuses of amphibian populations in the prairie pothole region in the United States (Figure 10) in relation to pesticides and other landscape-level factors. This group agreed to follow the protocols for visual-encounter and call surveys we use in the ARMI–UMR to the extent practical while conducting their work. We also plan to work with them to analyze their data using Percent Area Occupied as a descriptive metric. These common methods should enable direct comparability of the results of their surveys with those of ARMI–UMR. They also have agreed to submit their data to our regional database containing results of studies conducted by non-ARMI researchers (see below). This collaboration will add greatly to our ability to assess statuses of populations in the ARMI–UMR.

PLANNING COLLABORATIVE MONITORING OF WOOD FROGS IN RELATION TO CLIMATE WITH ENVIRONMENT CANADA IN CANADA AND THE U.S. FISH AND WILDLIFE SERVICE AND THE NATIONAL PARK SERVICE IN ALASKA

- Base level of the ARMI conceptual pyramid initially - ultimately base, middle, and apex levels
- Objectives 7 and 8

Changes in global climate have been accelerating in recent years. These changes portend potentially strong effects on populations of amphibians in terms of their fitness, distribution, and abundance. ARMI is uniquely positioned to monitor populations of amphibians in relation to climate and to develop distinctive partnerships to assess these issues across meaningful scales of space and time.

Anecdotal evidence shows that wood frogs have responded to warming in the Arctic by expanding their range northward into areas where they had been precluded by temperature. We do not know of any responses to changes in climate in more southerly populations. Nor do we know whether any climate-associated changes in hydroperiods have affected reproductive success. We have had several discussions with representatives from Environment Canada, and the US Fish and Wildlife Service and the National Park Service in Alaska, to develop a partnership to monitor the distribution, abundance, and reproductive success of populations of wood frogs in relation to climate across their range (Figure 11). I have submitted a proposal to USGS to obtain funding for the development of protocols. We are working to find other ways to leverage existing resources and obtain new resources to support this project.

<u>Obtaining and evaluating information on the statuses of populations from other researchers</u>

- base level of the ARMI conceptual pyramid
- Objective 4

Other organizations in the ARMI–UMR have collected data pertaining to the statuses of populations of amphibians via surveys, monitoring, and other specific research. Given our goal of evaluating and studying statuses of populations across our region and our limited resources to do so, one of our objectives is to assess data from others to determine their scope and usefulness to ARMI.

We began the process of developing a database that includes data from other organizations beginning in 2003 and continued through 2004. We have contacted representatives of several different kinds of organizations throughout the 13 states of our region via personal meetings, phone calls, and e-mails. We also included a questionnaire on our web page (http://www.umesc.usgs.gov/armi.html) that enables individuals to contact us and submit information. So far, we have obtained, or are in the process of obtaining, information and data from nine universities, eleven state departments of natural resources, the U.S. Fish and Wildlife Service (23 different locations), nine parks from the National Park Service, USGS, the Illinois Natural History Survey, the National Audubon Society, and two public museums. Data range from results of ongoing surveys to lists of species based upon literature reviews.

Interest in sharing information and data varies with the organization and their individual representatives. The quantity and quality of data from non-ARMI sources also varies considerably. Ultimately, we hope to have access to historical and current data from all willing collaborators contained in a database so that we can evaluate and refer to those data to help us assess statuses of populations throughout our region. This process will be ongoing.

<u>Collaborations with researchers at Iowa State University and EROS to study issues related to declines of amphibians</u>

- Base level of the ARMI conceptual pyramid
- Objective 9

In 2004, we met and talked with Jim Miller and Dave Otis (USGS) at Iowa State University to develop the foundations for collaborative studies of populations of

amphibians at risk in Iowa in relation to land use and other environmental stressors. We expect to meet again in 2005 to develop plans for a pilot project. We also collaborated with Eugenia Ferrar (Iowa State University) on her surveys of Blanchard's cricket frogs in Iowa.

We developed a proposal with Alisa Gallant at EROS (for the 2005 ARMI competition) to prioritize populations of amphibians that appear most at risk from a variety of environmental stressors across the 13 states of the ARMI–UMR. We will continue to pursue funding for this effort if we are not successful in this competition.

Meeting with personnel from the Wisconsin Department of Natural Resources and the national coordinator for the National American Amphibian Monitoring Program (NAAMP)

- Base level of the ARMI conceptual pyramid
- Objective 6

We met with representatives from the Wisconsin Frog and Toad Survey and Linda Weir of NAAMP in Madison, Wisconsin, to discuss personnel from ARMI–UMR conducting surveys side-by-side with volunteers from the Wisconsin Frog and Toad Survey to compare methods and results. We agreed that we would try to conduct such joint surveys in 2005 if resources are available.

Ongoing collaboration with David Green of the National Wildlife Health Center as part of the national ARMI effort to characterize the distribution of pathogens and malformations in amphibians

2004
In 2004, we submitted 15 embryos of blue-spotted salamanders (*Ambystoma laterale*) from one breeding site in the St. Croix National Scenic Riverway to Dr. Green as part of ARMI's program to evaluate diseases in amphibians and in response to Dr. Green's request for samples from species of the genus, *Ambystoma*. Some of these embryos were alive and some were dead when we collected them. Dr. Green reported that water mold was the only pathogen present on these embryos.

2002 – 2003
In 2004, we received the results of Dr. Green's analyses on samples we submitted to him from UMRNWFR, SC, and VNP in 2002 and 2003.

Upper Mississippi River National Wildlife and Fish Refuge
We submitted 30 animals in 2002 and 23 in 2003, all either bullfrogs, green frogs, or northern leopard frogs ranging in age from larvae to adults. These animals apparently did not have any significant diseases, but were infected with parasites such as lungworms and likely *Riberoia odantrae*.

St. Croix National Scenic Riverway
We submitted 35 animals in 2002 and 23 in 2003, all either green frogs, northern leopard frogs, wood frogs, or mink frogs [*Rana septentrionalis*] ranging in age from larvae to adults. These animals apparently did not have any significant diseases other than one

animal with ichthyophoniasis, but were infected with various parasites such as flukes, lungworms, and likely *Riberoia odantrae*.

Voyageurs National Park
We submitted 17 animals in 2002 and 23 in 2003, all either green frogs, northern leopard frogs, mink frogs, or blue-spotted salamanders ranging in age from larvae to adults. These animals were infected by at least 11 separate pathogens, ranging from Ranavirus to intestinal worms. The potentially most serious infectious diseases were presumptive ranaviral infections (three mink frogs from three different sites), chytridiomycosis (six mink frogs and two green frogs from three different breeding sites), and ichthyophoniasis (ten mink frogs from four different sites and two green frogs from one of the above sites). These infections were identified in recent metamorphs and all cases were slight to mild. We did not observe any die-offs associated with these infections, but such die-offs have been observed in other areas of the country and the world. If possible, we plan to submit more samples to Dr. Green in 2005 to try to characterize the geographical extent of these pathogens at breeding sites in VNP.

Plans for 2005 (subject to availability of operating funds)

- Calculate the Percent Area Occupied (PAO) for each species in each management unit we surveyed from 2002 to 2004 in the light of recent instructions provided at the ARMI-sponsored workshop on PAO in Altamonte Springs, Florida (December 2004).

- Survey historical and new mid-level monitoring sites in the ARMI–UMR.

- Survey and monitor new and known breeding sites for Blanchard's cricket frogs in collaboration with the Wisconsin DNR, Eugenia Farrar of Iowa State University, and possibly others to describe the current distribution of this species and to identify 9 – 12 apex-level sites for more detailed studies.

- Continue to collaborate with the EPA-funded researchers working in the prairie pothole region to maintain common methods and data analyses and to assess populations of amphibians in that region.

- Survey a subsample of extant and new mid-level monitoring sites for the presence of triazines and possibly other pesticides in collaboration with Perry Jones.

- Collect metamorphosed northern leopard frogs (and perhaps other species) to measure the frequency of abnormalities in gonads and possibly other effects in relation to exposure to pesticides.

- Continue to obtain and map data on historical breeding sites of Blanchard's cricket frogs and begin to analyze these data relative to climatic variables and other stressors.

- Continue to work with personnel from Environment Canada, as well as the National Park Service and U.S. Fish and Wildlife Service in Alaska, to develop and implement monitoring of wood frogs with respect to climate across their range.

- Continue to acquire and assess data collected by other organizations to help us understand the statuses of populations in the UMR.
- Continue to work with personnel from the National Park Service's Inventory and Monitoring Program to develop and implement protocols for monitoring herpetofauna in NPS management units.

- Disseminate information on our efforts through presentations, reports, and publications.

- Submit at least one manuscript on our work for publication in a scientific journal.

- Produce a USGS fact sheet on ARMI–UMR.

- Conduct coincidental surveys using ARMI methods and protocols with volunteers from the Wisconsin Frog and Toad Survey using NAAMP methods and protocols to compare results and assess the usefulness of NAAMP and other such data to ARMI.

Products

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2004 Staff

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2004 Collaborators and Partners

- Perry Jones, USGS – Water Resources Division
- David Green, DVM, USGS – National Wildlife Health Center
- Staff from the NPS's Inventory and Monitoring Program (Great Lakes Network)
- Staff from the USFWS's Upper Mississippi National Wildlife and Fish Refuge
- Staff from the USFWS's Neal Smith National Wildlife Refuge
- David Otis, Jim Miller, and Eugenia Ferrar – USGS and Iowa State University
- Jason Irwin (Bucknell University), Alisa Gallant (USGS), Michael Lannoo (Ball State University), and Gary Casper (Milwaukee Public Museum)
- Lucinda Johnson (University of Minnesota, Duluth), Pat Schoff (University of Minnesota, Duluth), and Glenn Guntenspergen (USGS – Patuxent)
- Bruce Pauli (Canadian Fish and Wildlife Service) and Elizabeth Kilvert (Environment Canada)

Suggestions

- As an integrated national science program focused on a high-profile issue in conservation biology, ARMI has enormous potential to leverage its resources within USGS and with other organizations to meet common goals more effectively. We have just begun to tap this potential. At the same time, ARMI faces immediate challenges to continue to meet its goals in the face of rising costs and flat budgets that pale in comparison to the challenge of remaining viable in the future given the federal budget deficit. Thus, vision, sound management, and clear thinking are required to ensure that ARMI realizes its potential and remains viable. One crucial way to meet these requirements is to make the national coordinator's position at least ¾ time or, preferably, full-time. Rick Kearney has done an excellent job for the very limited amount of time he has been able to devote to this program, but we need a coordinator who can work on ARMI in a focused and persistent manner and be prepared constantly to act upon opportunities to enhance the program. Many opportunities for such benefits have been, and will continue to be, lost until the national coordinator is able to play a stronger role.

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Table 1. The species we observed and the proportion of sites at which we observed them during daytime visual-encounter and call surveys. NS = Neal Smith National Wildlife Refuge; UMRNWFR = Upper Mississippi River National Wildlife and Fish Refuge; SC = St. Croix National Scenic Riverway; VNP = Voyageurs National Park. Seen indicates that we observed these species in locations other than at specific breeding sites.

Common Name	**Scientific Name**	**Proportion of Sites Surveyed Where Species X Was Observed**			
		NS	**UMRNFWR**	**SC**	**VNP**
bullfrog	*Rana catesbeiana*	5/16	7/54	0/63	0/54
green frog	*Rana clamitans melanota*	0/16	17/54	41/63	6/54
northern leopard frog	*Rana pipiens*	6/16	28/54	19/63	15/54
mink frog	*Rana septentrionalis*	0/16	0/54	6/63	12/54
wood frog	*Rana sylvatica*	0/16	1/54	28/63	44/54
chorus frog (western or boreal)	*Pseudacris triseriata* (*triseriata* or *maculata*)	11/16	2/54	16/63	4/54
northern spring peeper	*Pseudacris crucifer*	0/16	2/54	11/63	14/54
Blanchard's cricket frog	*Acris crepitans blanchardi*	9/16	6/54	0/63	0/54
eastern/Cope's gray treefrog	*Hyla versicolor* or *chrysoscelis*	5/16	9/54	9/63	11/54
eastern American toad	*Bufo americanus americanus*	1/16	5/54	18/63	5/54
blue-spotted salamander	*Ambystoma laterale*	0/16	3/54	11/63	11/54
spotted salamander	*Ambystoma maculatum*	0/16	0/54	3/63	0/54
eastern tiger salamander	*Ambystoma tigrinum*	3/16	0/54	0/63	0/54
central newt	*Notophthalmus viridescens louisianensis*	0/16	0/54	1/63	1/54

Table 2. The number of surveys we conducted, and the number of surveys in which metamorphs were present, at monitoring sites in 2004. NS = Neal Smith National Wildlife Refuge; UMRNWFR = Upper Mississippi River National Wildlife and Fish Refuge; SC = Saint Croix National Scenic Riverway; VNP = Voyageurs National Park; Sensiba = Sensiba Marsh (Wisconsin Department of Natural Resources)

Management Unit	**Number of Surveys**	**Number of Surveys Metamorphs Present**
NS	8	3
SC	65	28
UMRNWFR	34	14
VNP	64	30
Sensiba	1	1
Total	**172**	**76**

Table 3. The number of metamorphosed northern leopard frogs we examined for malformations at each management unit in 2004. NS = Neal Smith National Wildlife Refuge; UMRNWFR = Upper Mississippi River National Wildlife and Fish Refuge; SC = Saint Croix National Scenic Riverway; VNP = Voyageurs National Park ; Sensiba = Sensiba Marsh (Wisconsin Department of Natural Resources)

	Number of Metamorphs Examined/Management Unit				
Species	**NS**	**SC**	**UMRNWFR**	**VNP**	**Sensiba**
bullfrog	2				
eastern American toad		58		9	
eastern/Cope's gray treefrog		4		2	
green frog		148	27	53	
mink frog		8		122	
northern leopard frog	16	169	340	239	103
northern spring peeper		43		17	
western chorus frog		4			
wood frog		100		300	
Total	18	534	367	742	103

Table 4. The proportion of metamorphosed northern leopard frogs we sampled that were malformed by management unit in 2004. NS = Neal Smith National Wildlife Refuge; UMRNWFR = Upper Mississippi River National Wildlife and Fish Refuge; SC = Saint Croix National Scenic Riverway; VNP = Voyageurs National Park ; Sensiba = Sensiba Marsh (Wisconsin Department of Natural Resources)

	Proportion Malformed/Management Unit				
Species	**NS**	**SC**	**UMRNWFR**	**VNP**	**Sensiba**
bullfrog	0				
eastern American toad		0		0	
eastern/Cope's gray treefrog		0		0	
green frog		0.02	0	0.02	
mink frog		0		0.02	
northern leopard frog	0	0.01	0.02	0.01	0.03
northern spring peeper		0		0	
western chorus frog		0			
wood frog		0.03		0.03	

Table 5. The number of sites at which we collected water samples in 2004, the number of times we collected a sample from each site, and the mean concentraion of triazines per site per management unit. NS = Neal Smith National Wildlife Refuge; UMRNWFR = Upper Mississippi National Wildlife and Fish Refuge; SC = St. Croix National Scenic Riverway; VNP = Voyageurs National Park

Management Unit	**Number of sites sampled**	**Number of times sampled**	**Mean [triazines] (ppb) +/- s.e.**
NS	7	1	0.25+/- 0.082
UMRNWFR	60	up to 6	0.65 +/- 0.070
SC	54	2	0.13 +/- 0.018
VNP	46	2	0.03 +/- 0.004

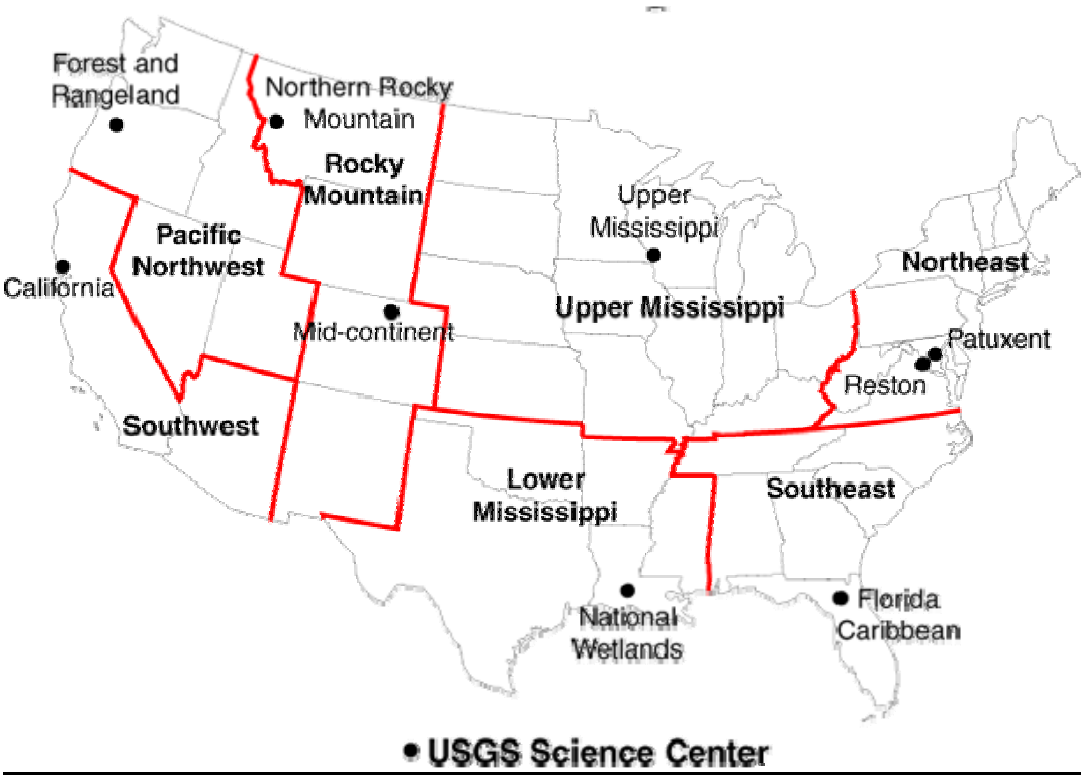

Figure 1. Regions of ARMI

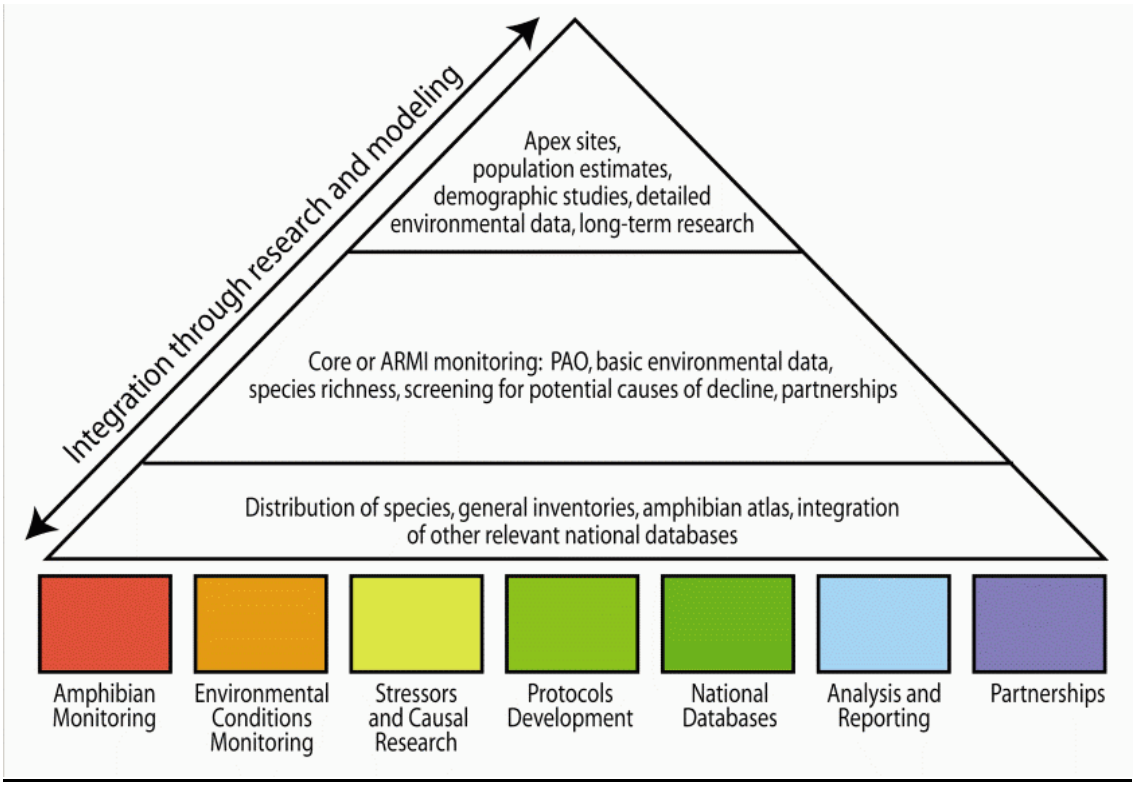

Figure 2. ARMI conceptual pyramid

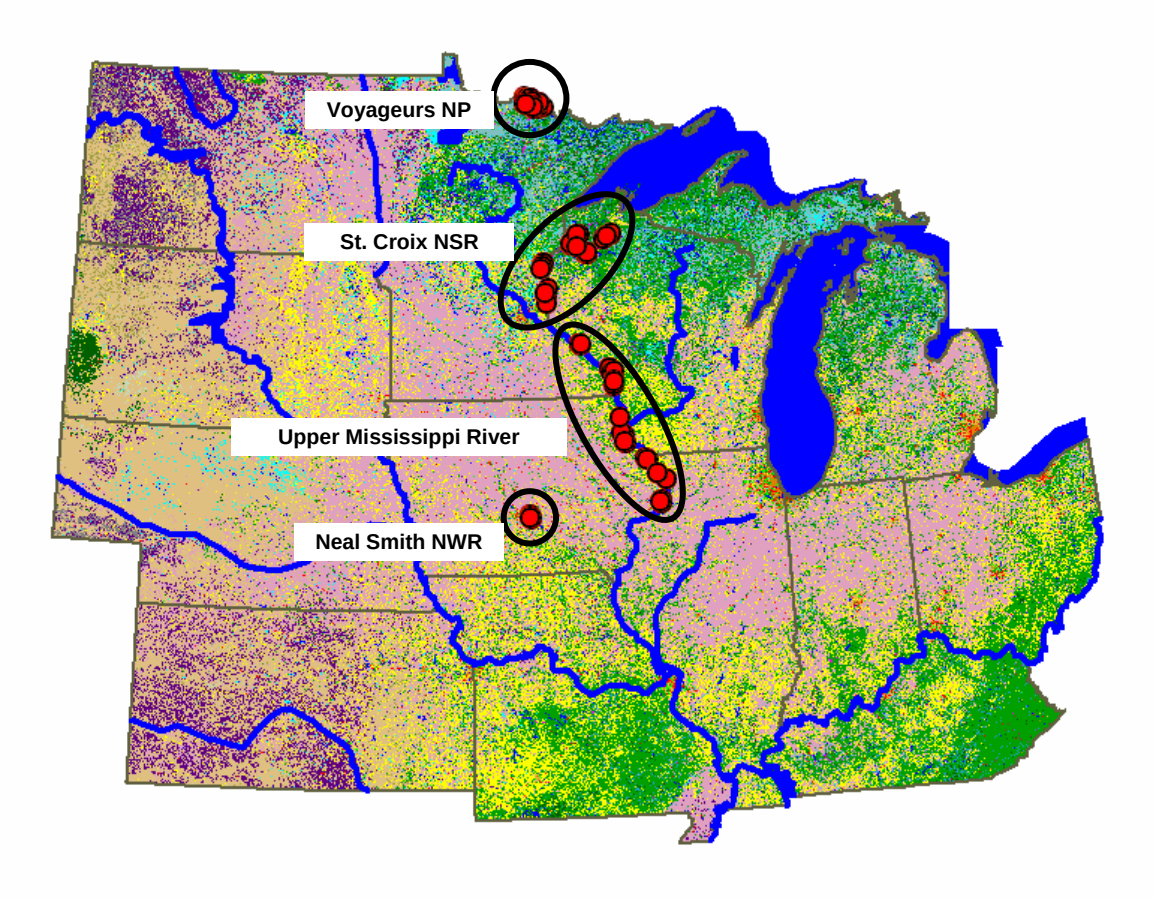

Figure 3. Management areas and land cover. Pink color = row crops

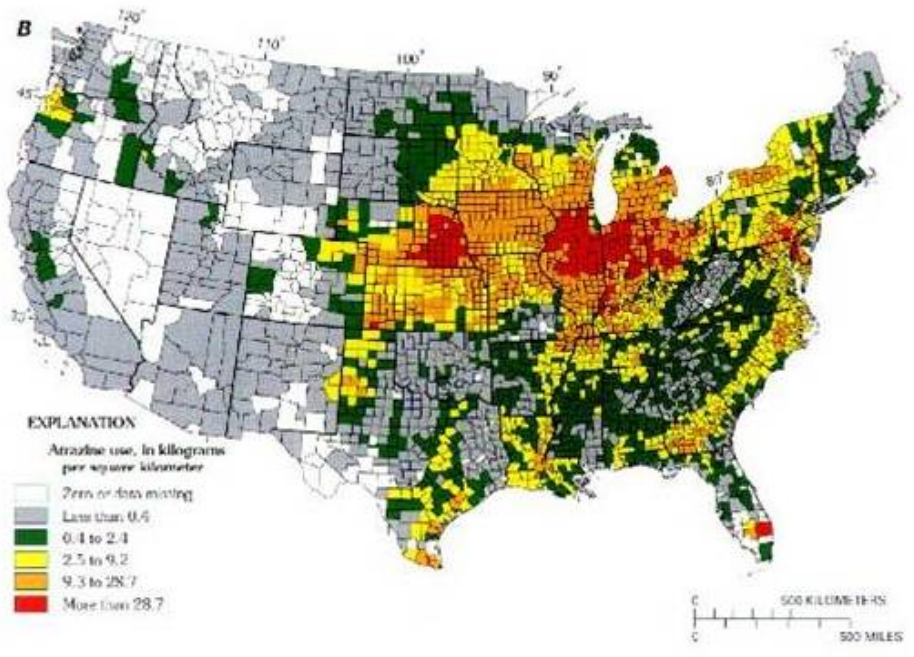

Figure 4. Atrazine use in the United States

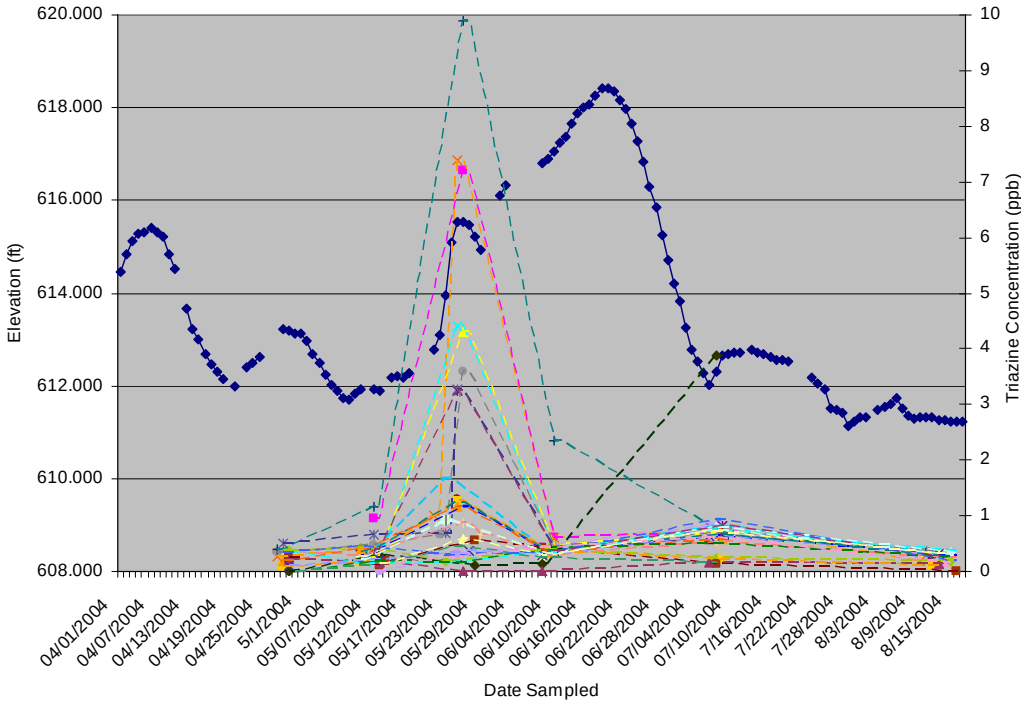

Figure 5. Mean concentrations of triazines at sampling locations in the floodplain of the Mississippi River (UMR) over several weeks in 2004. Dark blue dots at top = the average water levels (based upon data from the U.S. Army Corps of Engineers). Other lines represent individual sampling locations.

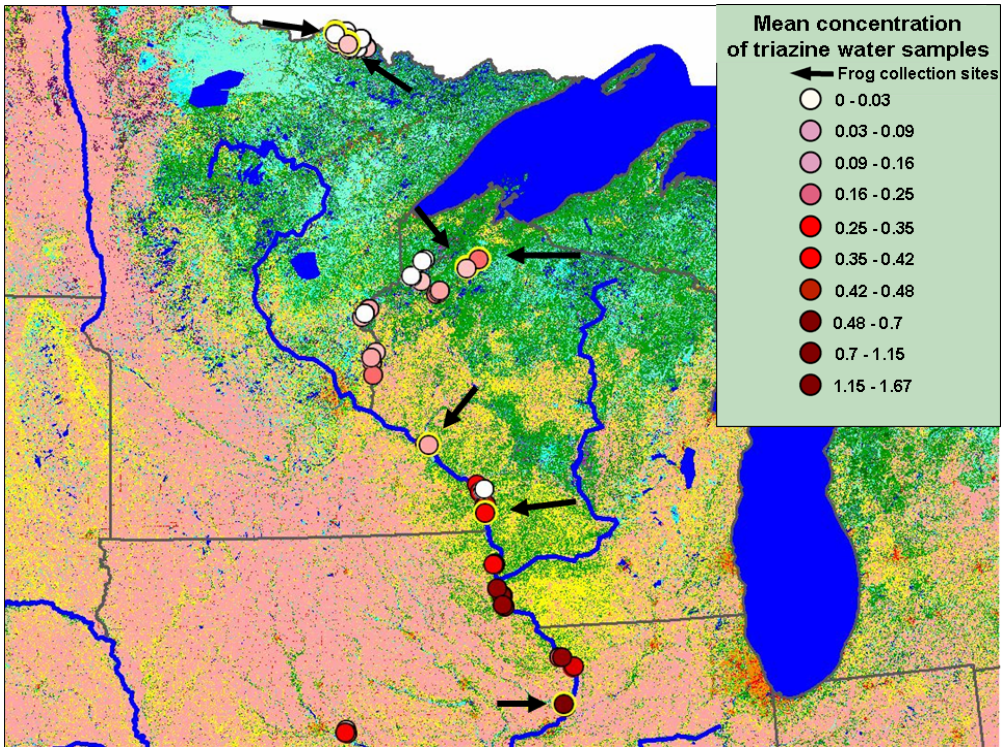

Figure 6. Results of sampling mid-level monitoring sites for triazines. Arrows point to sites where we collected metamorphs of northern leopard frogs for analyses of gonads. Pink color = row crops

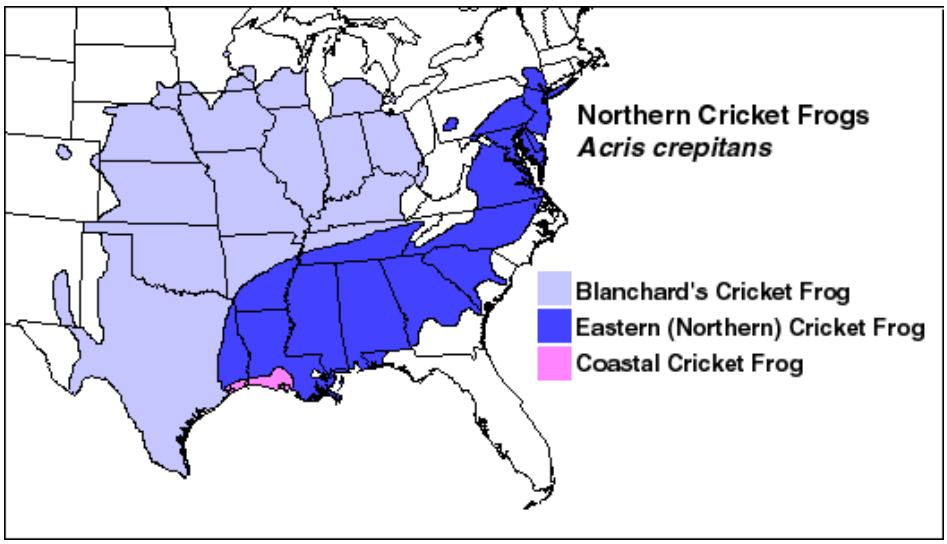

Figure 7. The range of the Blanchard's cricket frog.

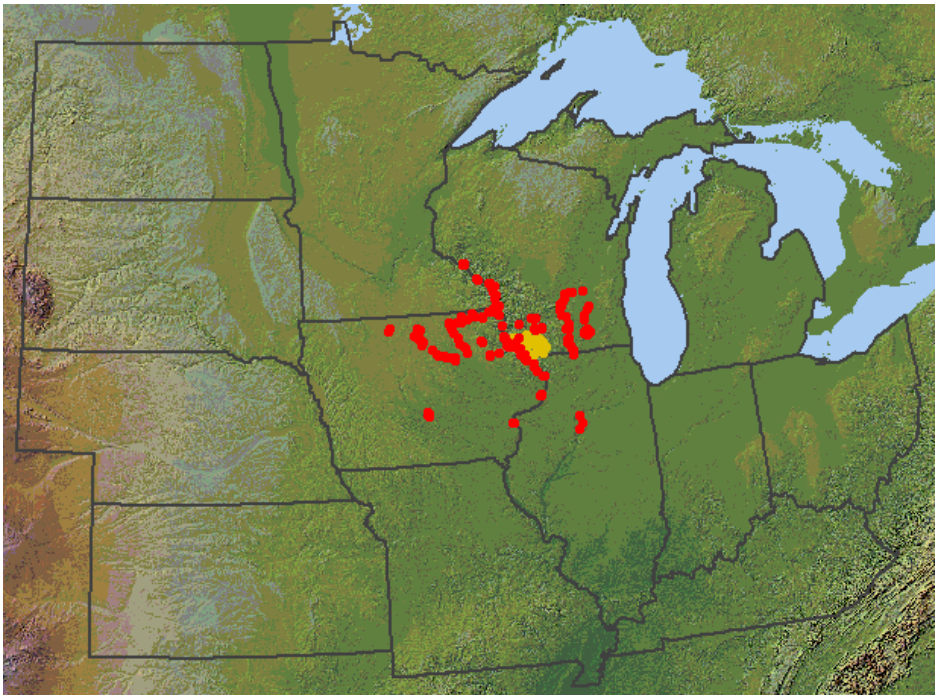

Figure 8. Red symbols = sites we surveyed for Blanchard's cricket frogs. Gold symbols = sites surveyed by the Wisconsin Department of Natural Resources

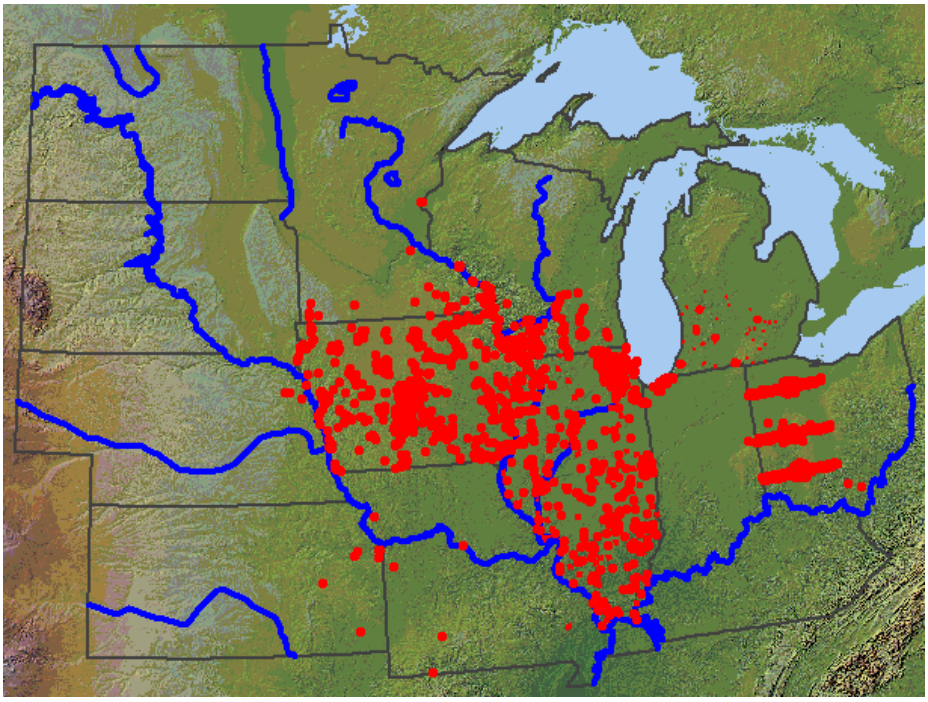

Figure 9. Historical locations of Blanchard's cricket frogs based upon information obtained to date.

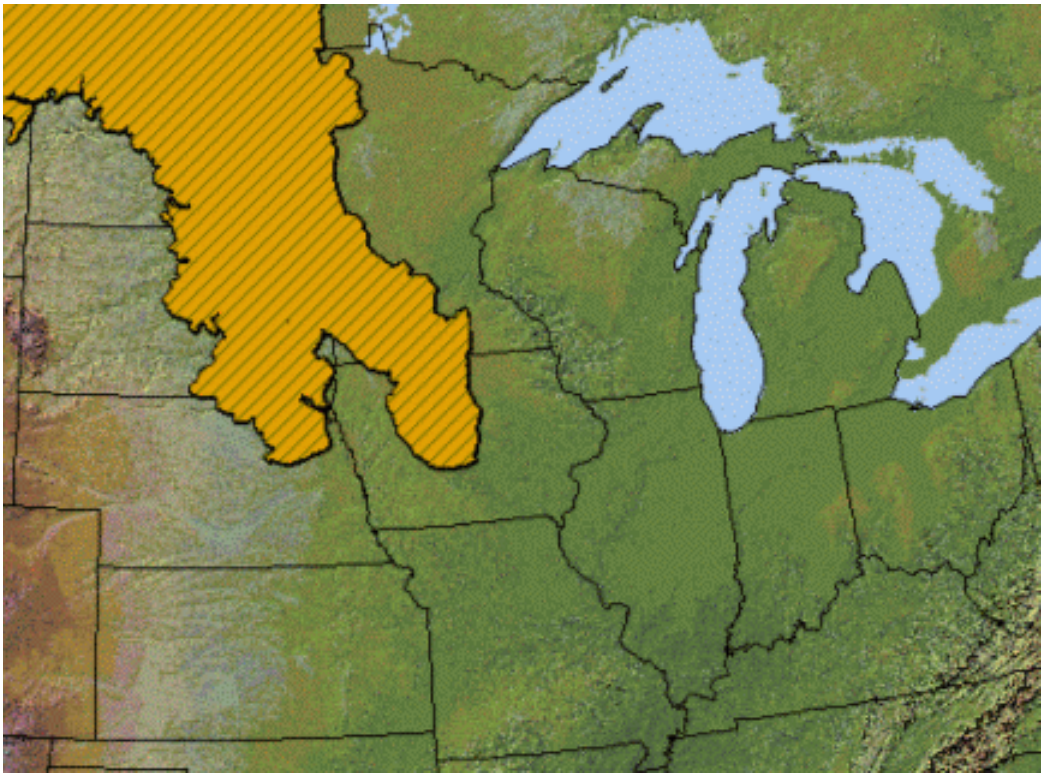

Figure 10. The prairie pothole region in the United States

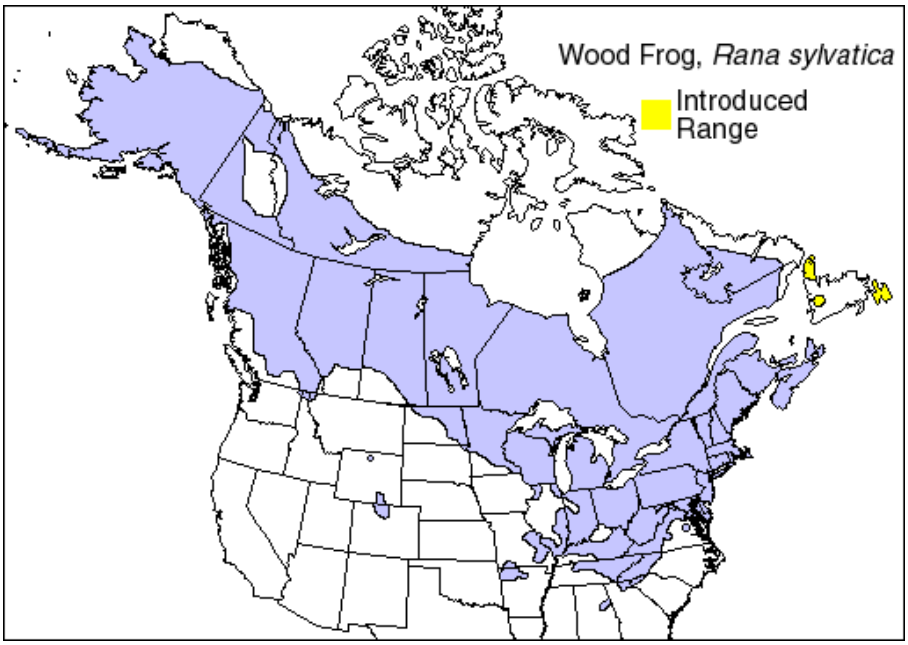

Figure 11. The range of wood frogs