Farm Ponds as Critical Habitats for Native Amphibians: Final Report

Submitted to

Legislative Commission on Minnesota Resources
100 Constitution Avenue, Room 65
St. Paul, Minnesota 55155-1201

June 2002

U.S. Geological Survey
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

USGS graphic by Joel Jahimiak

1999 Project Abstract
For the Period Ending June 30, 2002

TITLE: Farm Ponds as Critical Habitats for Native Amphibians
PROJECT MANAGER: Melinda G. Knutson
ORGANIZATION: USGS Upper Midwest Environmental Sciences Center
ADDRESS: 2630 Fanta Reed Road, La Crosse, Wisconsin 54603
WEB SITE ADDRESS:
http://www.umesc.usgs.gov/terrestrial/amphibians/mknutson_5003869.html
FUND: Minnesota Environment and Natural Resources Trust Fund
LEGAL CITATION: ML 1999, Ch. 231, Sec. 16, Subd. 12 Benchmarks and Indicators, (i) Farm Ponds as Critical Habitats for Native Amphibians

APPROPRIATION AMOUNT: $250K

Overall Project Outcome and Results

We studied constructed farm ponds and natural wetlands in southeastern Minnesota during the spring and summer of 2000 and 2001. The objectives were to identify land management practices that sustain healthy populations of amphibians in southeastern Minnesota farm ponds and to recommend monitoring methods suitable for assessing amphibian habitat quality. We collected amphibian and habitat data from 40 randomly selected ponds, ten ponds in each of four surrounding land-use classes: row crop agriculture, grazed grassland, ungrazed grassland, and natural wetlands. We identified 10 species of amphibians at the ponds. Surveys indicated that at least five fish, six snake, two turtle, 18 mammal, and 100 bird species were associated with the study ponds. We found no differences in amphibian species richness among the pond types, and very few frogs had malformations. In a mesocosm study, there were no differences in amphibian larval survival between agricultural and natural wetlands. The highest amphibian reproductive success was found in ponds with no fish, low amounts of vegetation, and low concentrations of nitrogen. Ponds used for watering cattle had elevated concentrations of nitrogen and higher turbidity, indicating lower quality habitat for amphibians. Constructed farm ponds designed to serve the needs of farmers can be managed to provide valuable aquatic breeding habitat for amphibians in this region. Important management actions include fencing cattle away from the pond, maintaining a wide grass buffer strip around the pond to trap sediment and nutrients, and avoiding fish introductions.

Project Results Use and Dissemination

We are distributing 2,500 amphibian larvae and egg field guides to wildlife biologists. State and federal agriculture and natural resources agencies are receiving 10,000 USGS Fact Sheets and 2,000 posters containing practical advice on how to manage farm ponds to benefit wildlife. Details of research documenting the above results are found in the attached report.

Executive Summary

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EXECUTIVE SUMMARY

Result 1: Objective: *Identify land management practices that sustain healthy populations of amphibians in southeastern Minnesota farm ponds.*

We studied constructed farm ponds and natural wetlands in southeastern Minnesota during the spring and summer of 2000 and 2001. We collected amphibian and habitat data from 40 randomly selected ponds, 10 ponds in each of four surrounding land use classes: row crop agriculture, grazed grassland, ungrazed grassland, and natural wetlands. We identified 10 species of amphibians at the ponds, including the Tiger Salamander *(Ambystoma triginum)*, American Toad *(Bufo americanus)*, Gray Treefrog *(Hyla versicolor)*, Western Chorus Frog *(Pseudacris triseriata)*, Spring Peeper *(Pseudacris crucifer)*, Green Frog *(Rana clamitans)*, Wood Frog *(Rana sylvatica)*, Northern Leopard Frog *(Rana pipiens)*, Pickerel Frog *(Rana palustris)*, and the Blue-spotted Salamander *(Ambystoma laterale)*. The Blue-spotted Salamander was a new record for Houston County, Minnesota. Amphibian species richness among the pond types was similar and deformity rates were low (< 5% deformed individuals) at all ponds. The parasite, *Ribeiroia* (linked to amphibian malformations elsewhere), was identified at 3 of 16 ponds examined for parasites in 2000 and 6 of 13 ponds examined in 2001. Of the 260 amphibians necropsied for parasites only 11 were considered to be malformed and five of these harbored *Ribeiroia*. *Ribeiroia* was found only in Northern Leopard Frogs and Green Frogs. Six species of snakes and two turtle species were observed at the ponds over the two years of the study. The common garter snake (*Thamnophis sirtalis*) was the most frequently encountered reptile, followed by painted turtles (*Chrysemys picta*). One hundred species of birds were observed at the ponds. The song sparrow (*Melospiza melodia*) was the most frequently observed bird species, followed by the red-winged blackbird (*Agelaius phoeniceus*), common yellowthroat (*Geothlypis trichas*), and the American robin (*Turdus migratorius*). Eighteen species of mammals were recorded, based on tracks at scent stations. The raccoon (*Procyon lotor*) was found at the most ponds, followed closely by the white-tailed deer (*Odocoileus virginianus*). Five species of fish were identified from the ponds, with brook stickleback (*Culaea inconstans*) the most frequently observed. A wide variety of invertebrate taxa were observed in the ponds. Midge larvae (Chironomidae), crawling water beetles (Haliplidae), and water boatmen (Corixidae) were the most common invertebrate taxa observed.

We found the highest amphibian reproductive success in ponds lacking fish, and in those containing sparse vegetation, and low concentrations of nitrogen. Ponds used for watering cattle had elevated concentrations of nitrogen and higher turbidity, indicating lower quality habitat for amphibians. In a mesocosm study, there were no differences in amphibian larval survival between agricultural and natural wetlands. In a study of post-breeding habitat use for the Northern Leopard Frog, we found that frogs selected wetland, grassland, and forest/shrub habitats post-breeding. Hayfields were frequently used during the summer; mowing resulted in frog mortality.

Constructed farm ponds, designed to serve the needs of farmers, can be managed to provide valuable aquatic breeding habitat for amphibians in this region. Important management actions include restricting cattle access to the pond, not introducing fish, and maintaining a wide grass buffer strip around the pond to trap sediment and nutrients.

Result 2: Objective: ***Recommend monitoring methods suitable for assessing amphibian habitat quality.***

We describe our recommendations regarding amphibian monitoring methods in Chapter 6, *Resources for Monitoring Pond-breeding Amphibians in the Northcentral USA* and the *Field Guide to Amphibian Larvae and Eggs for Minnesota, Wisconsin, and Iowa*. Correct identification of eggs and larvae is critical to the success of amphibian monitoring programs and no suitable field guide existed. We found that the most efficient time frame for surveying amphibian larvae using dip nets was a six-week sampling frame, centered on June (last week of May through the first week of July). This time frame sampled six species in southeastern Minnesota (American Toad, Western Chorus Frog, Spring Peeper, Green Frog, and Northern Leopard and Pickerel Frogs). Two species (Gray Treefrog and Tiger Salamander) were most efficiently sampled during July.

We are distributing 2,500 amphibian larvae and egg keys, as well as 10,000 USGS Fact Sheets and 2,000 posters containing practical advice on managing farm ponds to benefit wildlife. The field guides are being distributed to wildlife biologists, herpetologists, and students. The USGS Fact Sheets and posters are being distributed to USDA Service Centers, US Fish and Wildlife Service offices, and state departments of natural resources in Minnesota, Wisconsin, and Iowa.

Contents of Final Report

7. Role of *Ribeiroia ondatrae* (Platyhelminthes: Trematoda) Metacercariae in the Development of Malformed Frogs in Minnesota and Wisconsin

Separately bound:

A. A Field Guide to Amphibian Larvae and Eggs of Minnesota, Wisconsin, and Iowa (in press).

B. Kapfer, J. M., and J. R. Parmelee. 2001. *Ambystoma laterale* (Blue-spotted salamander). Herpetological Review 32:267.

C. Farm Ponds Work for Wildlife (Fact Sheet/brochure).

D. Farm Ponds Work for Wildlife (Poster, in press).

E. Malformed frogs in Minnesota: an update. USGS Fact Sheet.

Acknowledgments

This was a collaborative project. We are most indebted to the private landowners and Jon Cole, Minnesota Department of Natural Resources, for granting us access to the study ponds. Major funding was provided by the Minnesota Environment and Natural Resources Trust Fund, as recommended by the Legislative Commission on Minnesota Resources, the USGS Upper Midwest Environmental Sciences Center, and the USGS Amphibian Research and Monitoring Initiative. Cooperators include the University of Wisconsin - La Crosse, Gundersen-Lutheran Medical Center, Simpson College, the USDA Natural Resources Conservation Service, the Minnesota Department of Natural Resources, and the U.S. Fish and Wildlife Service. We thank Shawn Weick, Josh Kapfer, Brian Pember, James Lyon, Sam Bourassa, Joel Jahimiak, Ben Campbell, Bart Bly, Dean Jobe, Meredith Kline, Shane Jones, Andy Kimball, Kara Vick, German Musch, Richard Fox, Nick Strasser, Tom Kelly, Fred Kollmann, Irene Nissalke, Gene Amsrud, Jerry Cox, Georginia Ardinger, Arthur (Tex) Hawkins, John Moriarty, Mark Kunz, Mel Bower, Thomas Custer, Kevin Kenow, and Tim Fox for their assistance.

Accomplishments and Collaborations

Publications

1. Kapfer, J. M., and J. R. Parmelee. 2001. *Ambystoma laterale* (Blue-spotted salamander). Herpetological Review 32:267.
2. Parmelee, J. R., M. G. Knutson, and J. E. Lyon. 2002. A field guide to amphibian larvae and eggs of Minnesota, Wisconsin, and Iowa. Information and Technology

Report USGS/BRD/ITR-2002-0004. U.S. Geological Survey, Biological Resources Division, Washington, D.C. 38 pp. In press.

3. Knutson, M. G. 2002. Farm Ponds Work for Wildlife. USGS Fact Sheet. FS-043-02. U.S. Geological Survey, Biological Resources Division, Washington, D.C.
4. Knutson, M. G. 2002. Farm Ponds Work for Wildlife-poster. USGS Poster. U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. In press.
5. Knutson, M. G., and M. Wise. 2002. Farm Ponds Work for Wildlife. People, Land, and Water. U.S. Department of the Interior. In press.

Scientific Presentations

1. Bly, B. L., D. A. Jobe, M. B. Sandheinrich, M. G. Knutson, B. R. Gray, and S. Weick. 2002. Flow cytometry as a tool for detecting geonotoxic effects in amphibians breeding in southeastern Minnesota farm ponds (poster). Proceedings of the Mississippi River Research Consortium 34:55. 25 April 2002.
2. Bourassa, S. J., J. E. Lyon, and M. G. Knutson. 2002. Amphibian Research and Monitoring Initiative (ARMI) in the Midwest (poster). Proceedings of the Mississippi River Research Consortium 34:56. 25 April 2002.
3. Kapfer, J. M., M. B. Sandheinrich, and M. G. Knutson. 2001. Effects of agricultural pond water on the survival of anurans in the Upper Midwest (poster) *in* National Society of Environmental Toxicology and Chemistry 22nd Annual Meeting. 15 November 2001. National Society of Environmental Toxicology and Chemistry, Baltimore, Maryland.
4. Kapfer, J. M., M. B. Sandheinrich, and M. G. Knutson. 2002. Effects of agricultural pond water on the survival of anurans in the Upper Midwest (seminar, won Best Student Paper Award) *in* Mississippi River Research Consortium 2002 Annual Meeting. 25 April 2002. La Crosse, Wisconsin.
5. Kapfer, J. M., M. B. Sandheinrich, M. G. Knutson, and D. R. Sutherland. 2001. Effects of agricultural pond water on the development and metamorphosis of anurans native to the Upper Midwest (poster) *in* Midwest Chapter of the Society of Environmental Toxicology and Chemistry. 26 April 2001. Midwest Chapter of the Society of Environmental Toxicology and Chemistry, Racine, Wisconsin.
6. Knutson, M. G. 1999. Riparian zones as breeding habitat for birds, reptiles, and amphibians, Riparian Management Symposium (seminar) *in* 61st Midwest Fish and Wildlife Conference. 7 December 1999. Chicago, Illinois.
7. Knutson, M. G. 2000. Declining amphibians: what's the big picture? (seminar) *in* Partnership Coordination Meeting. 15 March 2000. USGS Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin.
8. Knutson, M. G. 2000. Farm ponds, radios, and ARMI (seminar) *in* USGS ARMI, First Annual Workshop. 6 December 2000. USGS Amphibian Research and Monitoring Initiative (ARMI), Reston, Virginia.
9. Knutson, M. G. 2001. Managing farm ponds as amphibian breeding sites in the Driftless Area Ecoregion (seminar) *in* Wisconsin Chapter of the Wildlife Society, 2001 Winter Meeting. 29 February 2001. Eau Claire, Wisconsin.

10. Knutson, M. G. 2001. Managing farm ponds as amphibian breeding sites in the Driftless Area Ecoregion (seminar). 21 March 2001. Coulee Region Audubon Society, La Crosse, Wisconsin.
11. Knutson, M. G. 2001. Managing farm ponds as amphibian breeding sites in the Driftless Area Ecoregion (seminar). 25 April 2001. Black Hammer Lutheran Church, Ladies' Aide Society, Spring Grove, MN.
12. Knutson, M. G. 2001. New Direction: Amphibians (seminar). 12 April 2001. Upper Midwest Environmental Sciences Center Science Review, La Crosse, Wisconsin.
13. Knutson, M. G. 2001. USGS Amphibian Research and Monitoring Initiative and farm pond research (seminar) *in* Minnesota Frog Malformation Meeting. 17 January 2001. Mounds View, Minnesota.
14. Knutson, M. G. 2002. From frog ponds to forests and flyways: natural connections across the Driftless landscape (seminar) *in* Questions of Scale: integration of efforts within the Greater Blufflands Region. Holmen, Wisconsin.
15. Knutson, M. G., R. W. B., B. Knights, and S. Weick. 2002. Farm ponds are working wetlands: agriculture and biodiversity in the heartland (seminar) *in* People and Environment Lecture Series. 1 March 2002. University of Wisconsin - La Crosse,
16. Knutson, M. G., and W. B. Richardson. 2002. Farm ponds are working wetlands: conservation practice benefits amphibians (seminar) *in* Women in Science Lecture Series. 10 April 2002. Iowa State University, Ames, Iowa.
17. Pember, B., B. Knights, M. G. Knutson, S. Weick, and D. Sutherland. 2001. Effects of wetland type and land use practices on movement and habitat selection by northern leopard frogs (*Rana pipiens*) (poster) *in* USGS Upper Midwest Environmental Sciences Center Science Review. 10 April 2001. La Crosse, WI.
18. Pember, B., M. G. Knutson, B. Knights, and S. Weick. 2002. Effects of agricultural and urban land uses on movement and habitat selection by northern leopard frogs (*Rana pipiens*) (poster). Proceedings of the Mississippi River Research Consortium 34:68. 25 April 2002.
19. Sutherland, D. R., J. M. Kapfer, M. Lannoo, and M. Knutson. 2002. The role of *Ribeiroia ondatrae* (Platyhelminthes: Trematoda) metacercariae in the development of malformed frogs in Minnesota and Wisconsin. Working together in a climate of change to manage Minnesota's water resources (seminar). Minnesota Water 2002 and Minnesota Lakes and Rivers Conference, St. Cloud, Minnesota.
20. Weick, S., M. G. Knutson, W. B. Richardson, M. B. Sandheinrich, D. Sutherland, and J. Parmelee. 2002. Farm ponds as critical habitats for amphibians (poster). Proceedings of the Mississippi River Research Consortium 34:71. 25 April 2002.

Outreach/Education

1. Knutson, M. 2000. USGS Biological Resources Division national staff orientation to Upper Mississippi Science Center science projects (tour of farm ponds and Upper Mississippi River sites). 20 June 2000. USGS Upper Mississippi Science Center, Houston, Crawford, Allamakee, and La Crosse counties, MN, IA, and WI.

2. Knutson, M. G. 2000. Amphibian display, outdoor classroom. 12 May 2000. Dakota Elementary School, Dakota, Minnesota.
3. Knutson, M. G. 2000. USDA Natural Resources Conservation Service (NRCS) Technical Note uses USGS published research. 2 October 2000. (D. Stratman, Ed.). USDA Natural Resources Conservation Service, Indianapolis, Indiana.
4. Knutson, M. G. 2000-2001. Represented UMESC at a national stakeholder meeting to launch the USGS ARMI initiative. USGS, Shepherdstown, West Virginia.
5. Knutson, M. G. 2001. Led farm pond tour and described the importance of amphibian research in small farm ponds to USGS Headquarters staff, including USGS Director, Dr. Chip Groat. 8 June 2001. USGS Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin.
6. Jahimiak, J. 2002. Award: 2 Honorable Mentions, National Wetland Photo Contest: Gray Treefrog and American Toads, Houston County, Minnesota. May 2002. US Environmental Protection Agency, Washington, D.C.
7. Knutson, M. G., D. R. Sutherland, and W. B. Richardson. 2000. Discussion session on amphibian mesocosm research from field season 2000. 27 October 2000. Minnesota Pollution Control Agency, USDA Agricultural Research Service, USGS Upper Midwest Environmental Sciences Center, La Crosse Wisconsin.
8. Knutson, M. G., D. R. Sutherland, and W. B. Richardson. 2000. Planning session to discuss Minnesota amphibian deformities and results of field season 2000. 16 October 2000. Minnesota Pollution Control Agency, USGS Water Resources, USDA Natural Resources Conservation Service, USGS Upper Midwest Environmental Sciences Center, La Crosse Wisconsin.

Media Inquiries

1. Dankert, J. 2001. Newpaper article. *Leaping leopard frogs in* Winona Daily News. July 1, 2001. Winona, Minnesota.
2. Knutson, M. G. 2000. Newspaper interview prompted by USGS News Release on amphibian research, farm pond research. 29 March 2000. Reporter Tim Krohn, Mankato Free Press, Mankato, Minnesota.
3. Pember, B. 2001. Radio interview. *Leopard frogs as environmental bioindicators in southeastern Minnesota.* 29 November 2001. Morning Show with Bob Seebo, reporter, KWNO Radio, AM 1230, Winona, Minnesota.

Related Publications

1. Johnson, P. T. J., K. B. Lunde, E. M. Thurman, E. G. Ritchie, S. N. Wray, D. R. Sutherland, J. M. Kapfer, T. J. Friest, J. Bowerman, and A. R. Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs 72:151-168.
2. Rosenberry, D. O. 2001. Malformed frogs in Minnesota: an update. USGS Fact Sheet. FS-043-01. U.S. Geological Survey, Water Resources Division, Mounds View, Minnesota, USA. (http://water.usgs.gov/pubs/FS/fs-043-01/)

Collaborations, Matching Contributions

Match dollars included salaries of Upper Midwest Environmental Sciences Center science staff ($130K federal), funding for a University of Wisconsin-La Crosse graduate student ($45K federal), a USGS Amphibian Research and Monitoring Initiative grant ($98K federal), pesticide analysis of farm pond water (John Elder, USGS Water Resources; $10K federal), in-kind use of laboratory equipment (flow cytometer) by Gundersen-Lutheran Medical Center ($25K private, non-profit capital assets), and in-kind staff time (Fred Kollmann, USDA Natural Resources Conservation Service; $15K federal), additional contributors were the University of Wisconsin-La Crosse, the US Fish and Wildlife Service, the Milwaukee Zoological Society, Milwaukee, Wisconsin, and John Moriarty.

Curriculum Vitae of Principal Investigators and Collaborators

VITA

Melinda G. Knutson

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Education and Training

BSN	1977	University of Minnesota (Nursing)
MPH	1984	University of Minnesota (Public Health)
MS	1991	State University of New York (Environmental Science and Forestry)
PhD	1995	Iowa State University (Ecology and Evolutionary Biology)

Areas of Specialization & Research Interests

Conduct research on migratory and resident bird populations with an emphasis on passerines. Develop research initiatives incorporating theory and concepts of ecology, conservation biology, and landscape ecology. Current research includes estimating nesting success for forest-dwelling songbirds in the Driftless Area and assessing the amphibian habitat values of farm ponds in the Driftless Area Ecoregion. Consult with DOI agencies, states, and citizens concerned with migratory birds, biodiversity, and factors affecting wildlife populations and communities in the Upper Midwest.

Professional Experience	Dates	Location
Graduate research/teaching assistant	1988-1991	State University of New York, Syracuse, NY
Graduate research/teaching assistant	1991-1995	Iowa State University, Ames, IA
Research Wildlife Biologist	1995-present	USGS Upper Midwest Environmental Sciences Center, La Crosse, WI
Graduate Faculty (Affiliate)	1996-present	University of Wisconsin, La Crosse

Recent publications

Knutson, M. G., G. Butcher, J. Fitzgerald, and J. Shieldcastle. 2001. Partners in Flight Bird Conservation Plan for The Upper Great Lakes Plain (Physiographic Area 16). USGS Upper Midwest Environmental Sciences Center in cooperation with Partners in Flight, La Crosse, Wisconsin. (http://www.blm.gov/wildlife/pifplans.htm). 1 November 2001.

Gustafson, E. J., M. G. Knutson, G. J. Niemi, and M. Friberg. 2002. Evaluation of spatial models to predict vulnerability of forest birds to brood parasitism by cowbirds. Ecological Applications 12:412-426.

Fox, T. J., M. G. Knutson, and R. K. Hines. 2000. Mapping forest canopy gaps using air photo interpretation and ground surveys. Wildlife Society Bulletin 28:882-889.

Knutson, M. G., S. J. Gutreuter, and E. E. Klaas. 2000. Patterns of artificial nest depredation in a large floodplain forest. Journal of Wildlife Management 64: 576-583.

Knutson, M. G., J. R. Sauer, D. O. Olsen, M. J. Mossman, L. H. Hemesath, and M. J. Lannoo. 2000. Landscape associations of frog and toad species in Iowa and Wisconsin, U.S.A. Journal of the Iowa Academy of Sciences 107: 134-145.

Hochachka, W. M., T. E. Martin, V. Artman, C. R. Smith, S. J. Hejl, D. E. Andersen, D. Curson, L. Petit, N. Mathews, T. Donovan, E. E. Klaas, P. B. Wood, J. C. Manolis, K. P. McFarland, J. V. Nichols, J. C. Bednarz, D. M. Evans, J. P. Duguay, S. Garner, J. Tewksbury, K. L. Purcell, J. Faaborg, C. B. Goguen, C. Rimmer, R. Dettmers, M. Knutson, J. A. Collazo, L. Garner, D. Whitehead, and G. Geupel. 1999. Scale dependence in the effects of forest coverage on parasitization by brown-headed cowbirds. Studies in Avian Biology 18: 80-88.

Knutson, M. G., R. K. Hines, C. M. Sveum, T. J. Fox, and C. E. Korschgen. 1999. Floodplain forest songbirds of the Upper Mississippi River. Passenger Pigeon 61: 307-310.

Knutson, M. G., J. R. Sauer, D. A. Olsen, M. J. Mossman, L. M. Hemesath, and M. J. Lannoo. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. Conservation Biology 13: 1437-1446.

Knutson, M. G., and E. E. Klaas. 1998. Floodplain forest loss and changes in forest community composition and structure in the Upper Mississippi River: a wildlife habitat at risk. Natural Areas Journal 18: 138-150.

Knutson, M. G., and E. E. Klaas. 1997. Declines in abundance and species richness of birds following a major flood on the Upper Mississippi River. Auk 114: 367-380.

Knutson, M. G., J. P. Hoover, and E. E. Klaas. 1996. The importance of floodplain forests in the conservation and management of neotropical migratory birds in the Midwest. Pages 168-188 in F. R. Thompson, ed. Management of Midwestern landscapes for the conservation of neotropical migratory birds. General Technical Report NC-187. USDA Forest Service, North Central Forest Experiment Station, St. Paul, MN.

VITA

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EDUCATION:

B.Sc., Michigan State University, Department of Fisheries and Wildlife, 1975-1979.
M.Sc., Central Michigan University, Department of Biology, 1980-1983.
Ph.D., University of Oklahoma, Department of Zoology, 1983-1989.

POSITIONS HELD:

Post-doctoral Research Associate, University of Georgia, Savannah River Ecology, Aiken, SC., 1989-1991.
Aquatic Ecologist, U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, WI, 1991-present.
Adjunct Professor, Department of Biology and Microbiology, University of Wisconsin, La Crosse, WI, 1992-present.

SELECTED PUBLICATIONS:

Richardson, W.B., E.A. Strauss, E.A. Monroe, L.A. Bartsch, D.A. Soballe, L. Rabuck. (In review at Freshwater Biology). Spatial and temporal patterns of denitrification in the Upper Mississippi River.

Strauss, E.A., **W.B. Richardson**, L.A. Bartsch, J. Heinz, and D.A. Soballe. (In review at Freshwater Biology). Spatial and temporal patterns of nitrification and the potential coupling with denitrification in the Upper Mississippi River.

Richardson, W. B., S. J. Zigler, and M. R. Dewey. 1998. Bioenergetic relations in submerged aquatic vegetation: an experimental test of prey use by juvenile bluegills. Ecology of Freshwater Fish 7: 1-12.

Bartsch, L. A., **W. B. Richardson** and T. J. Naimo. 1998. Sampling benthic macroinvertebrates in a large flood-plain river: considerations of sample design, sample size, and cost. Environmental Monitoring and Assessment 52: 425-439.

Richardson, W. B. and L. A. Bartsch. 1997. Effects of zebra mussels on food webs: interactions with hydraulic retention time and juvenile bluegill. Hydrobiologia 354: 141-150.

Dewey, M. R., **W. B. Richardson**, and S. J. Zigler. 1997. Patterns of foraging and distribution of bluegill sunfish in a Mississippi River backwater: influence of macrophytes and predation. Ecology of Freshwater Fish 6: 8-15.

Johnson, B.L., **W.B. Richardson,** and T.J. Naimo. 1995. Past, present, and future concepts in large river ecology. BioScience 45(3): 134-141.

Richardson, W.B. and S.T. Threlkeld. 1993. Complex interactions between multiple aquatic consumers: an experimental mesocosm manipulation. Canadian Journal of Fisheries and Aquatic Sciences 50: 29-42.

Richardson, W.B. 1992. Microcrustacean zooplankton in flowing water: experimental analysis of washout times and a field test. Freshwater Biology 28: 217-230.

Richardson, W.B. 1991. Seasonal dynamics, benthic habitat use, and drift of zooplankton in a small stream in southern Oklahoma, U.S.A. Canadian Journal of Zoology 69: 748-756.

Richardson, W.B. 1990. A comparison of detritus processing between permanent and intermittent headwater streams. Journal of Freshwater Ecology 5: 341-357.

Richardson, W.B., S.A. Wickham and S.T. Threlkeld. 1990. Foodweb response to the experimental manipulation of a benthivore (*Cyprinus carpio*), zooplanktivore (*Menidia beryllina)* and benthic insects. Archiv für Hydrobiologie 119: 143-165.

Fairchild, G.W., R.L. Lowe, and **W.B. Richardson**. 1985. Algal periphyton growth on nutrient diffusing substrates: an in-situ bioassay. Ecology 66: 467-472.

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EDUCATION

Ph.D., Applied Statistics, Air Force Institute of Technology, Wright Patterson AFB, OH, 1999
M.S., Applied Statistics, Wright State University, Dayton, OH, 1994
B.S., Secondary Mathematics Education, Wright State University, Dayton, OH, 1991

EXPERIENCE

- Assistant Professor of Statistics, University of Wisconsin - La Crosse, 1999-present
- Associate Graduate Faculty Member, University of Wisconsin - La Crosse, 1999-present
- Instructor of Mathematics & Statistics, Wright State University, 1994-1999
- ASEE Student Researcher, Air Force Institute of Technology, June-Sept. 1997,1998, 1999
- Part-time Consultant, Statistical Consulting Center, WSU, June-Sept. 1996
- Adjunct Instructor of Mathematics, Edison State Community College, 1993 - 1996
- Graduate Teaching Assistant, Department of Mathematics & Statistics, WSU 1993-1994

PROFESSIONAL AFFILIATION

American Statistical Association: Member (1993-1994, 1997-2002).

PUBLICATIONS

A Bayesian Look at Classical Estimation: The Exponential Distribution, *Journal of Statistics Education*, vol. 9, no. 1 or 2, 2001, (with Abdulazziz M. Elfessi).
Censored data reliability analysis, *2000 Annual Reliability and Maintainability Symposium Tutorial Notes*, (with W. Paul Murdock).

Maintenance policy cost analysis for a series system with highly censored data, *IEEE Transactions on Reliability*, vol. 48, no. 4, 1999, pp. 413-419, (with Edward A. Pohl and W. Paul Murdock).

Improving availability and cost performance for complex systems with preventive maintenance, *Proceedings of the Annual Reliability and Maintainability Symposium* (*1999*), 383-388, (with W. Paul Murdock, Edward A. Pohl and Ian Rehmert).

Survival analysis and maintenance policies for a series system with highly censored data, *Proceedings of the Annual Reliability and Maintainability Symposium* (*1998*), 182-188, (with Edward A. Pohl and W. Paul Murdock).

TECHNICAL REPORTS

Farm Ponds As Critical Habitats for Native Amphibians: Field Season 2000 Report, with a team of members from UMESC and UW-L.

TALKS PRESENTED

Modeling Lifetime Data for a Split Population: A Censored Data Approach, UW-Milwaukee seminar, March 30, 2001.

Modeling Lifetime Data for a Split Population: A Censored Data Approach, UW-Eau Claire seminar, April 28, 2000.

Monte Carlo Simulation, *Math Club*, University of Wisconsin – La Crosse, 1999.

Maintenance policy cost analysis for a series system with highly censored data, *INFORMS*,Cincinnati, OH, 1999.

Improving availability and cost performance for complex systems with preventive maintenance, *Annual Reliability and Maintainability Symposium*, Washington, D.C., 1999.

Research Interests

My research interests include goodness-of-fit, randomly censored data, lifetime data analysis, nonparametric density estimation, preventive maintenance and reliability. Also, developing statistical models for applications in ecology. Furthermore, I would like to participate in joint research with other departments in the university community.

VITA

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DATE AND PLACE OF BIRTH

March 23, 1952, Vermillion, South Dakota

EDUCATION AND TRAINING

1994-present: Full Professor, Department of Biology, University of Wisconsin-La Crosse
1987-94: Associate Professor, Department of Biology, Wartburg College, Waverly, Iowa
1983-87: Research Associate (post doc), Department of Veterinary Science, University of Wisconsin-Madison
1981-82: Adjunct Assistant Professor, Department of Biology, University of Wisconsin-Eau Claire
1977-81: Ph.D. in Zoology (Parasitology), Iowa State University, Ames
1974-76: M.S. in Zoology (Parasitology), University of North Dakota, Grand Forks
1970-74: B.S. in Biology, Wayne State College, Wayne, Nebraska (Summa cum laude)

TEACHING EXPERIENCE

1994-present: Parasitology (BIO 406/506), Aquatic AnimalHealth (BIO 463/563), Introductory Microbiology (MIC 230), Human Anatomy and Physiology labs (BIO 312, BIO 313)
1987-94: Parasitology (BIO 403), Microbiology (BIO 305), Immunology (BIO 405), Research and Methods (BIO 460), Field Biology/Great Lakes (BIO 295), Field Biology/Guyana (BIO 295)
1981-82: General Zoology (BIO 200)
1977-81: Comparative Chordate Anatomy, Parasitology, Histology, Invertebrate Zoology, Vertebrate Zoology

SCIENTIFIC ASSOCIATIONS AND AWARDS

American Society of Parasitologists, 1976-present
Helminthological Society of Washington, 1976-present
American Microscopical Society, 1976-2001
Annual Midwestern Conference of Parasitologists, 1978-present
Fish Health Section, American Fisheries Society, 1986-96
Wartburg College Professor of the Year, 1991-92

GRANTS FUNDED AS PI or co-PI

University of Wisconsin Sea Grant Institute, NOAA, "Assessing the risk of whirling disease becoming established in the Great Lakes: Field and laboratory evaluation of a novel polymerase chain reaction diagnostic assay," $139,161, 1998-2000.

Whirling Disease Initiative, National Partnership on the Management of Wild and Native Coldwater Fisheries, "Assessing the risk of whirling disease becoming established in the Great Lakes: Field and laboratory evaluation of a novel polymerase chain reaction diagnostic assay," $25,000, 1997-98.

Minnesota Department of Natural Resources and Wisconsin Department of Natural Resources, "*Heterosporis* sp. (Microsporida, Pleistophoridae): A new parasitic pathogen from yellow perch and walleye in Minnesota and Wisconsin," $8,230, 2000-01.

U.S. Geological Survey, "Role of trematode metacercariae in the development of malformed frogs in Minnesota and Wisconsin," $10,000, 2001.

U.S. Fish and Wildlife Service, "Role of trematode metacercariae in the development of malformed frogs in eastern national wildlife refuges,"$6,900, 2000-01.

National Science Foundation, CCLI, "Improving undergraduate instruction through an integrated program in microscopic techniques," $54,078 plus match, 1994.

GRANTS FUNDED AS COLLABORATOR

U.S. Geological Survey, Amphibian Research and Monitoring Initiative (ARMI), "Use of radio-transmitters in monitoring movements of frogs," $98,000, 2000-02. [M. Knutson, P.I.]

National Science Foundation, CCLI, "Incorporating structural and image analysis investigations across the biology curriculum," $74,126 plus match, 2002 [D. Howard and J. Miskowski, P.I.'s]

AQUATIC ANIMAL PARASITE RESEARCH PUBLISHED IN PEER REVIEW JOURNALS

1. Johnson, P.T.J., K.B. Lunde, E.M. Thurman, E.G. Ritchie, S.N. Wray, D.R. Sutherland, J.M. Kapfer, T.J. Frest, J. Bowerman and A.R. Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs 72:151-168.
2. Courtney, C.C., D.R. Sutherland and B.M. Christensen. 1993. Ecology of metazoan parasites infecting *Catostomus* spp (Catostomidae) from southwestern Lake Superior. Canadian Journal of Zoology 71:1646-1652.
3. Lasee, B.A. and D.R. Sutherland. 1993. Bacterial colonization of tegumental surfaces of *Culaeatrema inconstans* Lasee et al., 1988 (Digenea) from the brook stickleback, *Culaea inconstans* (Kirtland). Journal of Fish Diseases16:83-85.
4. Sutherland, D.R. 1989. Seasonal distribution and ecology of three helminth species infecting carp in NW Iowa. Canadian Journal of Zoology 67:692-698.
5. Lasee, B.A., D.R. Sutherland and M.E. Moubry. 1988. Host-parasite relationships between burbot (*Lota lota*), and adult *Salmincola lotae* (Copepoda). Canadian Journal of Zoology 66:2459-2463.
6. Lasee, B.A., W.F. Font and D.R. Sutherland. 1988. *Culaeatrema inconstans* sp. n. (Digenea: Allocreadiidae) from brook stickleback (*Culaea inconstans*). Canadian Journal of Zoology 66:1328-1335.
7. Sutherland, D.R. and D.D. Wittrock. 1986. Surface topography of the branchiuran *Argulus appendiculosus* Wilson, 1907 as revealed by scanning electron microscopy. Zeitschrift fur Parasitenkunde 72:405-415.
8. Sutherland, D.R. and D.D. Wittrock. 1985. The effects of *Salmincola californiensis* (Copepoda: Lernaeopodidae) on the gills of farm-raised rainbow trout, *Salmo gairdneri*. Canadian Journal of Zoology 63:2893-2901.
9. Williams, D.D. and D.R. Sutherland. 1981. *Khawia sinensis* (Caryophyllidea: Lytocestidae) in *Cyprinus carpio* in North America. Proceedings of the Helminthological Society of Washington 48:253-255.
10. Sutherland, D.R. and H.L. Holloway, Jr. 1979. Parasites of fish from the Missouri, James, Sheyenne and Wild Rice rivers in North Dakota. Proceedings of the Helminthological Society of Washington 46:128-134.

AQUATIC ANIMAL PARASITE RESEARCH PUBLISHED IN NON-PEER REVIEW ARTICLES

1. Lannoo, M.J., D.R. Sutherland, P. Jones, D. Rosenberry, R.W. Klaver, D.M. Hoppe, P.T.J. Johnson, K.B. Lunde, C. Facemire and J.M. Kapfer. 2003. Multiple causes for the malformed frog phenomenon. In Symposium on Multiple Stressor Effects in Relation to Declining Amphibian Populations. ASTM STP 1443, G. Linder, E. Little, S. Krest and D. Sparling, Eds. American Society for Testing and Materials, West Conshoshocken, PA.
2. Sutherland, D.R. 2002. Parasites of frogs. In Status and Conservation of US Amphibians. M.J. Lannoo, Ed. University of California Press, Berkeley, CA.

VITA

Jeffrey R. Parmelee

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EDUCATION

Ph.D., Systematics and Ecology, The University of Kansas, Lawrence, Kansas (1998).
Dissertation: Trophic ecology of a tropical anuran assemblage. 196 pp.
Organization for Tropical Studies Tropical Biology: An ecological approach course in Costa Rica (1991).
M.S., Biological Sciences, Illinois State University, Normal, Illinois (1990).
Thesis: Microhabitat segregation and spatial relationships among four species of mole salamanders (Genus *Ambystoma*). 108 pp.
B.A., Biological Sciences, Illinois Wesleyan University, Bloomington, Illinois (1987).

EXPERIENCE

Assistant Professor of Biology, Simpson College, 1999 - present
Co-Investigator, Amphibian and reptile surveys of Wapsipinicon river public lands in Clinton and Scott counties, Iowa. (with J. Christiansen, Spring-Summer 2002)
Herpetological Consultant, USGS project: Farm ponds as critical habitats for native amphibians. 2000-2002
Adjunct Faculty, Johnson County (Kansas) Community College, 1998-1999
Graduate Teaching assistant at Illinois State University and the University of Kansas, 1987–1998
Curatorial Assistant in the Herpetology Department, Natural History Museum, The University of Kansas (Fall 1990–Summer 1991)
Research Assistant, Feeding ecology of a lizard community from Cuzco Amazónico, Peru (Fall, 1997)
Research Assistant, Herpetofaunal Survey and Natural Community Analysis of the Fort Riley Military Reservation (Spring, Summer, and Fall 1993) and Identification and Delineation of Loggerhead Shrike Habitat on the Fort Riley Military Reservation (Summer, 1995)

SELECTED PUBLICATIONS

Parmelee, J. R. and S. Ron. Mass changes in fluid-preserved anuran specimens. *In Press* Herpetol. Rev.
Parmelee, J. R.**, M. G. Knutson, and J. E. Lyon. 2002. A field guide to amphibian larvae and eggs of Minnesota, Wisconsin, and Iowa . USGS Upper Midwest Environmental Sciences Center in cooperation with Simpson College. La Crosse, Wisconsin, USA. *In Press.***
Knutson, M. G., J. E. Lyon, and **J. R. Parmelee**. 2002. Resources for monitoring pond-breeding amphibians in the Midwest. USGS publication, *In Press.*
Emerman, S. H. and **J. R. Parmelee**. 2002. The control of infiltration as a mechanism for the self-regulation of prairie ecosystems: Preliminary studies at Rolling Thunder Prairie Preserve, Warren County, Iowa. Proceedings of the 22nd annual American Geophysical Union Hydrology Days.
Kapfer, J. M. and **J. R. Parmelee**. 2001. Geographic Distribution: *Ambystoma laterale*. Herp. Rev. 32:267.
C. R. Bursey, S. R. Goldberg, and **J. R. Parmelee**. 2001. Helminths of fifty one species of anurans from Reserva Cuzco Amazónico, Peru. Comp. Parisitol. 68(1):21-35.
Parmelee, J. R. 1999. Trophic ecology of a tropical anuran assemblage. Sci. Pap. Nat. Hist. Mus. Univ.Kansas 11:1–59.
Meinhardt, D. J. and **J. R. Parmelee**. 1996. A new species of *Colostethus* (Anura: Dendrobatidae) from Venezuela. Herpetologica 52(1):70–77.
Busby, W. and **J. R. Parmelee**. 1996. Historical changes in a herpetofaunal assemblage in the Flint Hills of

Kansas. Amer. Midl. Nat. 135:81–91.
Busby, W., J. T. Collins, and **J. R. Parmelee**. 1996. The Reptiles and Amphibians of Fort Riley and Vicinity. Kansas Biological Survey. 72 pp., 64 color photographs.
Parmelee, J. R. and H. S. Fitch. 1995. An experiment with artificial shelters for snakes: effects of material, age, and surface preparation. Herpetol. Nat. Hist. 3(2):187–191.
Parmelee, J. R. and C. Guyer. 1995. Sexual differences in foraging behavior of an anoline lizard, *Norops humilis*. J. Herpetol. 29(4):619–621.

PROFESSIONAL MEMBERSHIPS
Iowa Academy of Science (vice chair of the Zoology section), Iowa Natural History Association, Iowa Herpetological Society, Society for the Study of Amphibians and Reptiles, The Herpetologists' League, Herpetological Natural History, Organization for Tropical Studies Associate

RECENT GRANTS
Iowa Science Foundation Grant (with Paul Frese), Ensuring the survival of a species: the ecology of juvenile timber rattlesnakes (*Crotalus horridus*) in Iowa. 2002 ($2080).
Maytag Grant for student/faculty research, The ecology and conservation of the timber rattlesnake (*Crotalus horridus*) in an agricultural landscape. 2001 ($2000).
Iowa Science Foundation Grant (with Paul Frese) ($4701), Iowa Department of Natural Resources Wildlife Diversity Grant (with Paul Frese) ($1425), The ecology and conservation of the timber rattlesnake (*Crotalus horridus*) in an agricultural landscape. 2001.
Maytag Grant for student/faculty research (with Steven Emerman), Fluorescein Dye as a tracer for the study of soil-plant-animal water relations. 2000 ($2000).
Maytag Grant for student/faculty research (with Ron Warnet), Geographic and ontogenetic variation in the skin toxins of the American toad, *Bufo americanus*. 2000 ($1733).

SERVICE
Associate Editor, Zoology Section, Journal of the Iowa Academy of Science 2002
Society for the Study of Reptiles and Amphibians Grant's in Herpetology Committee 2002
Copy Editor, Herpetological Review 1998–2000
Reviewer for: Biological Conservation (2002), Herpetologica (2001), Journal of Herpetology (1997, 1998, 1999, 2001, 2002), Copeia (1998, 2001), Alytes (1999), Journal of the Iowa Academy of Science (2000)
Editorial board member, Contemporary Herpetology (an on-line herpetological journal) 1997–present
Local organizing committee for the 1996 Annual meeting of the Society for the Study of Amphibians and Reptiles, University of Kansas, 1995–1996

VITA

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Biological Statistician
USGS Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, WI 54603
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Education and Training

PhD	2001	University of South Carolina	Biostatistics
MS	1993	University of Kentucky	Biology
Diploma	1982	Lincoln University	Natural Resources
BS	1981	University of Auckland	Botany

Areas of Specialization and Research Interests
Statistical applications within ecology and environmental biology, generalized mixed models, multilevel models, spatial statistics

Recent Professional Experience

Biological Statistician	2001–present	UMESC, USGS
Biostatistician (part time)	1997–2001	Baruch Institute for Marine Biology and Coastal Research, and Schools of Medicine and Public Health, University of Southern California
Sediment Toxicologist		1993-1997 AScI Corporation, Vicksburg, MS

Professional Activities and Memberships
American Statistical Association
International Environmetrics Society
Society of Environmental Chemistry and Toxicology
- Member, Editorial Board, *Environmental Toxicology and Chemistry* (2002-2004)
- Reviewer, *Environmental Toxicology and Chemistry* (2000-2001)

Honors and Awards

Travel Award	2000	Society for Risk Analysis
Performance Awards	1994, 1995	AScI Corporation
Fellowship Award	1992-1993	Oak Ridge Associated Universities
Grants-in-Aid of Research Award	1992	Sigma Xi Scientific Research Society
'A' Bursary Award (full tuition waiver)	1977-1981	New Zealand government

Publications

Gray, BR, WR Hill and AJ Stewart. 2001. Effects of development time, biomass and ferromanganese oxides on nickel sorption by stream periphyton. *Environmental Pollution* 112: 61-71.
Gray, BR, S McDermott and S Butkus. 2000. Effect of job coaches on employment likelihood for individuals with mental retardation in South Carolina. *Journal of Vocational Research* 14: 5-11.

Gray, BR, VL Emery, DL Brandon and others. 1998. Selection of optimal measures of growth and reproduction for the sublethal *Leptocheirus plumulosus* sediment bioassay. *Environmental Toxicology and Chemistry* 17: 2288-2297.

Emery, VL, DW Moore, BR Gray and others. 1997. Development of a chronic sublethal sediment bioassay using the estuarine amphipod *Leptocheirus plumulosus* (Shoemaker). *Environmental Toxicology and Chemistry* 16: 1912-1920.

Moore, DW, TS Bridges, BR Gray and BM Duke. 1997. Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus*. *Environmental Toxicology and Chemistry* 16:1020-1027.

Bridges, TS, RB Wright, BR Gray, AB Gibson and TM Dillon. 1996. Chronic toxicity of Great Lakes sediments to *Daphnia magna*: elutriate effects on survival, reproduction, and population growth. *Ecotoxicology* 5:83-102.

Gray, BR and WR Hill. 1995. Nickel sorption by periphyton exposed to different light intensities. *Journal of the North American Benthological Society* 14:299-305.

VITA

Joshua M. Kapfer

River Studies Center, Biology Department,
University of Wisconsin-La Crosse
La Crosse, WI 54601
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Education

Master of Science, Biology (Aquatic Science Concentration)- January, 2000 to August, 2002 (expected graduation)
Bachelor of Science, Biology - Diploma granted December 1999
Undergraduate GPA: 3.83 (within Biology Dept.), 3.06 (cumulative). Credits earned (undergad.): 167
Dean's List: spring of '99, fall of '98, spring of '97 and fall of '96

Research Interests

- Endangered snakes and turtles native to the Upper Midwest
- Amphibian toxicology
- Role of larval tiger salamanders as keystone predators
- Wetland ecology and restoration
- Prairie ecology and restoration

Technical training and experience

Herpetology

Several years of experience in field herpetology, including; identifying "herp" habitats and life histories/ecology; comfortable identifying any herp native to the Upper Midwest by sight or sound (frog calls); also skilled in herptile external and internal anatomy.

Seven years experience with care, maintenance, and handling of amphibians and reptiles (native and exotic). Through my research and as a hobby I have cared for "herptiles" native to arid, temperate, and tropical environments including potentially dangerous members of the lizard genus *Varanus*. In addition, I have field experience handling venomous snakes in the genus *Crotalus*.

Ecology

Extensive experience in performing field work as part of my Master's research; have spent time in the field taking pictures to be used for poster and seminar presentations, as well as for my website, and am comfortable outdoors; experience identifying birds (especially wetland birds) and their calls within the Upper Midwest; likewise, a fair amount of experience identifying fish species of the Upper Midwest; limited experience identifying aquatic vascular plants.

Technical skills

-Experience using radio telemetry equipment
-Have performed surgeries to implant transmitters into anurans
-Experience with GPS units, and PDA databases
-Experience using microscopes (compound and dissecting)
-Administering solutions via syringe and stomach pipette to amphibians and birds

Research and publications

- Johnson, P. T. J., K. B. Lunde, E. M. Thurman, E. G. Ritchie, S. N. Wray, D. R. Sutherland, **J. M. Kapfer**, T. J. Frest, J. Bowerman, and A. R. Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs 72:151-168.
- **Kapfer, J. M.**, and S. N. Jones. A method for rearing and keeping the eastern tiger salamander (*Ambystoma tigrinum tigrinum*). 2002. Bulletin of the Chicago Herpetological Society. 37:25-28.
- **Kapfer, J. M.**, and J. R. Parmelee. 2001. Geographic distribution: *Ambystoma laterale*. Herpetological Review. 32:267.
- **Kapfer, J. M.**, and D. R. Sutherland. 2000. Larval trematodes in the Upper Midwest and the Pacific Northwest which are known to induce skeletal malformations in anurans. University of Wisconsin, La Crosse Journal of Undergraduate Research. 3:115-124.
- **Kapfer, J.M.** 2002. Effects of agricultural pond water on the survival of anurans in the Upper Midwest (M. S. research).

Poster presentations

- Kapfer, J. M., M. B. Sandheinrich, M. G. Knutson. "The Effects of Agricultural Pond Water on the Development of Anurans in the Upper Midwest" (poster presentation, National Society of Environmental Toxicology and Chemistry annual meeting, Baltimore, MD, Nov., 2001).
- Kapfer, J. M., M. B. Sandheinrich, M. G. Knutson, D. R. Sutherland "The Effects of Agricultural Pond Water on the Development and Metamorphosis of Anurans Native to the Upper Midwest" (poster presentation, Midwest Chapter of the Society of Environmental Toxicology and Chemistry annual meeting in Racine, WI. March, 2001).
- Kapfer, J. M., J. Mulchay, and D. R. Sutherland "Larval Trematodes in the Upper Midwest and the Pacific Northwest, Which Are Known to Induce Skeletal Malformations in Anurans" (poster presentation, UW-La Crosse Undergraduate Research Consortium, 2000).

Platform presentations and seminars

- Kapfer, J.M., M.B. Sandheinrich, M.G. Knutson. "The Effects of Agricultural Pond Water on the Survival of Anurans in the Upper Midwest" (Thesis defense seminar. May 29th 2002).
- Kapfer, J.M., M.B. Sandheinrich, M.G. Knutson. "The Effects of Agricultural Pond Water on the Survival of Anurans in the Upper Midwest" (platform presentations, Midwest Chapter of the Society of Environmental Toxicology and Chemistry annual meeting in Duluth, MN. April, 2002. Mississippi River Research Consortium annual meeting in La Crosse, WI. April, 2002)
- Kapfer, J. M. "Living With Snakes: An Overview of Snakes in the La Crosse Area" (Public Presentation at the Hixon Forest Nature Center in LaCrosse, WI, October, 2001).
- Sutherland, D. R. and J. M. Kapfer. "Larval Trematodes (Digenea) Infecting Anurans in Minnesota and Wisconsin" (Declining Amphibian Workshop held by the USGS in Mounds View, MN 2001).
- Kapfer, J. M. "Amphibian Water Conservation Tactics With Respect to Certain Water Characteristics" (graduate seminar presentation, UW-La Crosse, March, 2001).
- Kapfer, J. M. "Habitat Selection and Landscape Fragmentation Effects on Amphibians" (graduate seminar presentation, UW-La Crosse, December, 2001).
- Kapfer, J. M. "An Overview of Amphibian Malformations and Population Declines" (graduate seminar presentation, UW-La Crosse, December, 2000).

VITA

Brian C. Pember

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Education

B. A. (Environmental Biology), Saint Mary's University of Minnesota, Winona. 1991
M.S. (Biology) Currently enrolled at University of Wisconsin – La Crosse

Professional Experience

1991-present: Biological Science Technician (Wildlife), U. S. Fish & Wildlife Service, Upper Mississippi River National Wildlife & Fish Refuge, Winona, Minnesota.

1989-1991: Saint Mary's University of Minnesota. Student intern assigned to a bathometry project with the U. S. Geological Survey, La Crosse, Wisconsin.

1982-1986: Operations Specialist. United States Navy

Research Papers

Pember, B. C. and G. J. Mastey. 1991. Gilmore creek macroinvertebrate recovery following a shock dose of calcium hypochlorite. Undergraduate thesis presented to the faculty of Saint Mary's University of Minnesota, May 1991

VITA

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Education and Training

Masters of science	2001	Saint Mary's University of Minnesota	Resource Analysis (GIS)
Bachelor of arts	1995	Saint Mary's University of Minnesota	Environmental Biology

Areas of Specialization & Research Interests

Conduct field studies and surveys to collect wildlife and vegetation data and/or specimens. Conduct a variety of data manipulation and analyses such as compiling and tabulating data and other resource management information, including research and long-term monitoring projects. Create, manage, and manipulate data files. Run computer programs to enter and verify data input into automated database and /or geographic information systems (GIS). Provide GIS support and analysis relating to the landscape and radio telemetry. Prepare data summaries. Support work that applies to mammology, ornithology, biology, ecology, entomology, invertebrate zoology, zoology, and geography. Coordinate and supervise field crews. Provide the full range of coordination, logistical support, and area orientation for contract and cooperating scientists, students, and technicians. Oversee and train new technicians and field crews in standard field data collection and analytical techniques.

Professional Experience	Dates	Location
Biological Science Technician	1993-1996	USGS, Environmental Management Technical Center 575 Lester Ave. Onalaska, WI 54650-8552
Biologist	1996-present	USGS, Upper Midwest Environmental Sciences Center 575 Lester Ave. Onalaska, WI 54650-8552

Honors and Awards	Date	Organization
Performance Awards	1998(2), 2000	Upper Midwest Environmental Sciences Center
Certificate of Appreciation	2001, 2002	Upper Midwest Environmental Sciences Center

Significant recent publications

Johnson, B. L., D. M. Soballe, R. F. Gaugush, B. C. Knights, T. J. Newton, E, M. Monroe, S. J. Rogers, J. S. Sauer, S. E. Weick, W. F. James, and A. Stevens. 2000. Evaluation of hydrologic modification for habitat improvement: the Finger Lakes habitat rehabilitation and enhancement project biological response study. Final Report submitted to the U.S. Army Corps of Engineers, St. Paul, Minnesota, Chapters 1-6.

Recent Presentations

"Farm ponds as critical habitats for amphibians," Poster presented by S. Weick, M. G. Knutson, W. B. Richardson, M. B. Sandheinrich, D. Sutherland, and J. Parmelee Mississippi River Research Consortium, La Crosse, Wisconsin (April 2002).

"Effects of wetland type and land use practices on movement and habitat selection by northern leopard frogs (Rana pipiens)," Poster presented by B. Pember, B. Knights, M. G. Knutson, S. Weick. Mississippi River Research Consortium, La Crosse, Wisconsin (April 2002).

Ecological Communities and Water Quality Associated with Agricultural Farm Ponds in Southeastern Minnesota

by

Melinda G. Knutson, William B. Richardson, Shawn E. Weick, David M. Reineke, Jeffrey R. Parmelee, and Dan R. Sutherland

Farm Ponds as Critical Habitats for Native Amphibians: Final Report

Submitted to

Legislative Commission on Minnesota Resources
100 Constitution Avenue, Room 65
St. Paul, Minnesota 55155-1201

June 2002

U.S. Geological Survey
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
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Suggested citation:

Knutson, M. G., W. B. Richardson, S. E. Weick, D. M. Reineke, J. R. Parmelee, and D. R. Sutherland. 2002. Ecological communities and water quality associated with farm ponds in southeastern Minnesota *in* Farm ponds as critical habitats for native amphibians: Final report. Submitted to the Legislative Commission on Minnesota Resources, St. Paul, Minnesota. U.S. Geological Survey Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, USA, June 2002. 47 pp. (http://www.umesc.usgs.gov/terrestrial/amphibians/mknutson_5003869.html)

Contents

Tables

Table

4. Probability of observing the presence of tadpoles or metamorphs for each species by number of visits and monitoring time frame in farm ponds in Houston and Winona Counties, Minnesota, 2000. Estimates are for visits conducted during the full season April – July (27 March – 7 August), or during a shortened season of June (24 May and 6 July) or July (29 June to 27 July). Estimates in bold show a higher probability of detecting a species with the fewest visits in either the June or July shortened season.

5. Deformity and *Ribeiroia* infection rate of amphibians based on deformity assessments at farm ponds in Houston and Winona Counties, Minnesota, 2000-2001.

6. The area (ha) of different types of land uses within a 2,500-m radius circle surrounding each farm pond, by the four types of ponds used in our study of Houston and Winona Counties, Minnesota. Pond types were determined by the land use immediately surrounding the pond and the width of the grassed buffer strip (see *Methods*).

Figures

Figure

1. Farm pond study sites in southeastern Minnesota, Houston and Winona counties.

2. Amphibian species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

3. Comparison of detections of amphibians based on different survey methods, all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC). Species include (A) tiger salamanders, (B)American toad, (C) eastern gray tree frog, (D) chorus frogs, (E) spring peeper, (F) green frog, (G) wood frogs, (H) leopard frog, and (I) pickerel frog.

4. Mean species richness as determined by each survey method for the four land use classes measured in 2000 (A) and 2001 (B). Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

5. Reptile species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

6. Bird species present within four types of surrounding land uses, based on detection by point counts, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use

categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

7. Mammal species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001 (scent stations excluded grazed ponds, but incidental observations included all ponds. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

8. Fish species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

9. Invertebrate species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

10. Mean (1 SD of mean) of (A) nitrate, (B) total ammonia nitrogen, (C) total phosphorus, (D) total nitrogen, (E) conductivity, and (F) turbidity for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

Ecological Communities and Water Quality Associated with Agricultural Farm Ponds in Southeastern Minnesota

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Abstract

We studied constructed farm ponds and natural wetlands in southeastern Minnesota during spring and summer 2000 and 2001. We collected amphibian and habitat data from 40 randomly selected ponds, 10 ponds in each of four surrounding land use classes: row crop agriculture, grazed grassland, nongrazed grassland, and natural wetlands. In this paper we describe the terrestrial and aquatic ecological communities we observed at farm ponds and describe the water quality habitat characteristics. We identified 10 species of amphibians at the ponds, including the tiger salamander *(Ambystoma triginum)*, American toad *(Bufo americanus)*, eastern gray treefrog *(Hyla versicolor)*, chorus frog *(Pseudacris triseriata)*, spring peeper *(Pseudacris crucifer)*, green frog *(Rana clamitans)*, wood frog *(Rana sylvatica)*, leopard frog *(Rana pipiens)*, pickerel frog *(Rana palustris),* and the blue-spotted salamander *(Ambystoma* *laterale).* The blue-spotted salamander was identified from two larval specimens at one natural wetland. The American toad, eastern gray tree frog, and green frog were the most commonly observed species. There were no significant differences in amphibian species richness among the four classes of surrounding land use. To most efficiently sample amphibian larvae using dip nets, we found that a six-week sampling frame, centered on June (last week of May through the first week of July) sampled six species in the Driftless Area Ecoregion (American toad, chorus frog, spring peeper, green frog, leopard and pickerel frog). Two species (eastern gray treefrog and tiger salamander) were most efficiently sampled during July. Deformity rates were low (< 5% deformed individuals) at all ponds. The trematode parasite, *Ribeiroia* (linked to amphibian malformations elsewhere), was identified at 3 of 16 ponds examined for parasites in 2000 and 6 of 13 ponds examined in 2001. Of the 260 amphibians necropsied for parasites only 11 were considered to be malformed and five of these eleven harbored *Ribeiroia*. *Ribeiroia* was found in only northern leopard frogs and green frogs. Six species of snakes and two turtle species were observed at the ponds over the two years of the study. The common garter snake (*Thamnophis sirtalis*) was the most frequently encountered reptile (18 ponds), followed by painted turtles (*Chrysemys picta*) (11 ponds). One hundred species of birds were observed at the ponds. The song sparrow (*Melospiza melodia*) was the most frequently observed bird species (40 ponds), followed by the red-winged blackbird (*Agelaius phoeniceus*) (34 ponds), common yellowthroat (*Geothlypis trichas*) (30 ponds), and the American robin (*Turdus migratorius*) (25 ponds). Eighteen species of mammals were recorded, based on tracks at scent stations. The raccoon (*Procyon lotor*) was found at the most ponds (34 ponds), followed closely by the white-tailed deer (*Odocoileus virginianus*) (33 ponds). Five species of fish were identified from the ponds, with brook stickleback (*Culaea inconstans*) the most frequently observed (6 ponds). A wide variety of invertebrate taxa were observed in the ponds. Midge larvae (Chironomidae), crawling water beetles (Haliplidae), and water boatmen (Corixidae) were the most common invertebrate taxa observed. Total nitrogen and turbidity tended to be higher at grazed and agricultural ponds vs. non-grazed and natural ponds. The majority of the land use surrounding the ponds is row crop agriculture and forests. Constructed agricultural farm ponds are providing breeding habitat for amphibians in the Driftless Area ecoregion and support a species assemblage comparable with natural wetlands. In addition, a wide range of invertebrates, reptiles, birds, and mammals are associated with constructed farm ponds.

Key words: Farm pond, amphibian, reptile, bird, mammal, land use, agriculture, habitat

Introduction

Conservation practices on 'working' agricultural land will be receiving substantial financial support ($17 billion) from the Farm Security and Rural Investment Act of 2002 (http://agriculture.house.gov/farmbill.htm). These conservation incentive payments go beyond traditional price support and conservation reserve payments to farmers. The Act will provide a financial incentive to farmers to conserve soil, produce clean water, and provide wildlife habitat on working agricultural land. Last year, the National Governor's Association published a white paper describing how 'working lands conservation' can produce numerous public benefits, such as cleaner air, cleaner water, and more abundant wildlife

(NGA/CBP 2001). The report called for government programs to demonstrate that they produce valuable and measurable 'environmental goods' or 'conservation commodities'.

In the Driftless Area Ecoregion of southeastern Minnesota, a landscape where natural wetlands are scarce (Eckblad and Coon 1984), constructed farm ponds represent potentially significant breeding, rearing, and over-wintering habitat for amphibians. This region contains thousands of farm ponds constructed with cost-sharing dollars from the US Department of Agriculture and state lands. The purpose of these farm ponds is to prevent soil erosion; no studies have been conducted to determine how the ponds benefit wildlife.

Farm pond construction has been subsidized in the past by state and federal agencies, primarily because the ponds prevent gully development and control the movement of sediment and nutrients into rivers and streams (Pavelis et al. 1995; Helms et al. 1996). Farm ponds are one component of a suite of conservation practices that may be subsidized in the future, if it is demonstrated that they produce multiple conservation benefits. In particular, small farm ponds may provide important breeding habitat for amphibians, an at-risk group of vertebrates. Identifying high quality breeding habitats located on agricultural land may be the key to sustaining populations of many amphibian species in the agricultural Midwest, a region where over 95% of the land is privately owned and the majority is in agricultural use (Knutson et al. 1999; Knutson et al. 2000).

We were interested in the ecological communities associated with small farm ponds in southeastern Minnesota and the water quality and landscape characteristics of these ponds. We focused on the following questions: 1) Which species of amphibians and other wildlife are associated with small farm ponds? 2) What are the optimal sampling methods and times for monitoring amphibians in farm ponds? 3) What rates of deformities are experienced by amphibians breeding in small farm ponds? 4) What are the water quality characteristics of small farm ponds? 5) What is the composition of the landscape surrounding these ponds?

Study Area

Our study ponds were located in Houston and Winona counties, Minnesota, USA. The study area is part of the Driftless Area Ecoregion of southeastern Minnesota, western Wisconsin, and northeastern Iowa (McNab and Avers 1994; Fig. 1). This ecoregion was not covered by ice during the last (Wisconsin) glaciation, a feature that distinguishes it from other ecoregions in the agricultural Midwest (Mickelson et al. 1982). The landforms are characterized by maturely dissected, upland plateaus with steep bedrock ridges descending to river drainages that flow to the Mississippi River (McNab and Avers 1994). Prior to European settlement, the ecoregion was covered by an oak savanna complex (*Quercus* spp.) of mixed grasslands with forests in areas protected from fire (Curtis 1959). Forests today are mixed oak and maple hardwoods and are interspersed with pastures, hay fields, small towns, and cities. Natural wetlands are found in the floodplains of rivers and streams; most natural fen wetlands were drained and tiled to convert the land to agriculture. Complex topography and erosive soils support less intensive agriculture than in many parts of the Midwest, with agriculture occupying only 30–40% of the landscape.

Small constructed farm ponds represent nearly all the available lentic wetlands in this well-drained landscape dominated by small streams and rivers; they are potentially significant habitats for amphibians (Hall 1997). The region contains thousands of small farm ponds designed to prevent soil erosion. Most farm ponds are privately owned and adjacent land uses are row crops, livestock grazing, and forestry. Some ponds are surrounded by fallow grasslands enrolled in the U.S. Department of Agriculture's Conservation Reserve Program (CRP).

Methods

We randomly selected 40 ponds representing four contrasting amphibian breeding habitats, based on adjacent land uses and wetland type: constructed farm ponds adjacent to (1) row crop agriculture, (2) grazed grassland, and (3) nongrazed grassland, and (4) natural wetland in Houston and Winona counties, Minnesota (Fig. 1). The study ponds and wetlands (hereafter referred to as ponds) were identified using USFWS National Wetland Inventory (NWI) maps (1979-1988, 1:24,000) overlaid on USGS Digital Orthophoto Quarter Quad (DOQQ) maps (1991) (http://deli.dnr.state.mn.us/metadata/index_th.html). The ponds were selected from ponds classified as palustrine, unconsolidated bottom, intermittently flooded wetlands (Cowardin et al. 1979); in addition, the 30 constructed farm ponds were classified as diked or impounded. Ponds identified on the DOQQ maps but not on the NWI maps (constructed after 1988) were added to the set of ponds from which the study ponds were selected.

The constructed farm ponds were generally located high on the watershed, were < 5 ha in size, and utilized a minimal engineering design referred to as a 'push-up' dike. A bulldozer was used to redistribute soil to construct a small, diked impoundment. The land uses immediately surrounding the pond determined the treatment class of the pond. The width of the grass buffer surrounding the pond differentiated our row crop and nongrazed pond types. If the grass buffer was < 30 m wide and adjacent to row crop agriculture (corn or soybeans) the pond was considered agricultural. If the buffer strip was ≥ 30 m wide and had no cattle grazing, the pond was considered nongrazed. If domestic livestock (cattle or horses) had direct access to the pond, it was considered grazed. Our natural wetlands represented wetland habitats available in the Driftless Area Ecoregion in the absence of constructed farm ponds. We were unable to control for land uses surrounding the natural wetlands because natural wetlands were scarce. Ephemeral wetlands and ponds within 80 m of barnyards or livestock confinement areas were excluded. Most ponds were privately owned and written permits for access were obtained from all landowners and public land managers. An amphibian collection permit was obtained from the Minnesota Department of Natural Resources (Special Permit No. 9516).

Amphibian Community

We surveyed amphibians at each pond in 2000 and 2001 using chorus surveys, egg mass surveys, larval surveys, and visual encounters. We surveyed calling anurans beginning late March and ending in late July. We used standard chorus survey methods developed by the Wisconsin DNR (Mossman et al. 1998) and conducted 2 surveys within each of the 3 survey time periods established by the Minnesota Frog Watch Program (15 to 30 April; 20 May to 5 June; and 1 to 15 July). Names of all species follow the Integrated Taxonomic

Information System (Appendix A, ITIS 2002). The leopard frog and pickerel frog larvae could not be reliably differentiated in the field, so these species were considered together as larvae.

Reproductive effort was assessed through egg mass counts. The littoral zone of each pond was searched for egg masses ~ every 2 weeks. The densities of eggs per mass were estimated as 1-100, 100-1000, >1000 (Thoms et al. 1997). The visibility of egg masses is species-specific; some species have large, visible egg masses (e.g., *Rana pipiens*), whereas others lay clusters of 2-3 eggs on vegetation (e.g., *Pseudacris crucifer*). Larval surveys detect anurans with weak or infrequent calls and salamanders, which do not vocalize. We conducted larval dip net surveys at each pond every 2-3 weeks (Scott and Woodward 1994; Thoms et al. 1997). We identified and scored each larval species for the following categories of abundance: 0, 1 (1-10), 2 (11-99), 3 (≥100). Larval forms that could not be identified to species, were identified to genus or species complex. We also conducted visual encounter surveys for adult amphibians along with the egg mass and larval surveys (Crump and Scott 1994). All adult amphibians captured were identified, weighed, and snout-vent length measured. We informally tested the feasibility of using funnel traps (Adams et al. 1997) for capturing larval amphibians, and drift fences and pitfall traps for capturing salamanders (Corn 1994) in this study. Amphibian voucher specimens were collected to aid in accurate identification of specimens and as a permanent public record. Specimens for a permanent voucher record were deposited with the Bell Museum of Natural History, Minneapolis, Minnesota.

Deformity Assessment

We conducted several deformity assessments of amphibian metamorphs during June to August (NARCAM 2002). Up to 100 individual metamorphs per assessment were counted, identified to species, and examined for deformities in the field. A sample of 10 metamorphs per assessment were collected for laboratory examination. If deformed individuals were found, up to 5 were included in the collection for laboratory assessment; the balance included apparently healthy individuals. The 10 animals were examined in the laboratory for parasites.

Amphibian Statistical Analysis

Separate analysis of variance procedures (Littell et al. 1991) were used to determine if differences existed in counts of species across treatments for larvae, adult visual surveys, and all survey methods combined. Nonparametric Kruskal-Wallis tests were employed to detect differences in counts of species across treatments for egg masses and choral surveys due to the non-normality of the data. Differences were deemed significant at $\alpha<0.05$.

We approached the problem of optimal allocation of field effort for standardized field surveys of amphibians through a probability-based statistical analysis of larval amphibian sampling, based on our dip net data from 2000. Larval amphibians included tadpoles, salamander larvae, and metamorphs. For a given species of amphibian, let p represent the true probability of observing at least one larva for a single visit when dip netting is the means of detection. We used $\hat{p} = \frac{\#\ \text{successes}}{\#\ \text{visits}}$ as an estimator of p, where a success is defined as observing at least one larva of a given species during the visit.

We modeled the number of visits needed for detection of larvae of a given species as a geometric probability distribution. The model contains the assumption that each visit is independent of the others and that the probability of observing a given species remains constant from one visit to the next and over time. In the model a "success" was defined as a visit in which the presence of tadpoles or metamorphs for a given species is observed.

The estimated mean for a geometric probability distribution is given by the reciprocal of $\hat{p}$. Using the same assumptions needed for the geometric probability distributions discussed above, binomial distributions with the estimated success probabilities ($\hat{p}$) were used to estimate the probabilities of observing the presence of larval amphibians within a certain number of visits for each species. For a binomial probability distribution, the probability of *at least one* success in n visits is given by the expression $P(x \geq 1) = 1 - P(x = 0) = 1 - (1 - p)^n$, where x represents the number of successes. Monitoring options such as varying the sampling frame and the number of visits can be evaluated with this information.

Reptiles, Birds, Mammals, Fish, Invertebrates

Mammal presence was monitored once in August at each pond each year (excluding grazed ponds). We placed 3 scent stations equidistant around the riparian zone perimeter of each pond using protocols modified from the Minnesota DNR Predator - Furbearer Scent Post Station Survey (Bill Berg, Minnesota DNR, pers. comm., Sargeant et al. 1998). We excluded grazed ponds because of the likelihood that the cattle would disturb the scent stations. We created the scent station in a 1-m diameter circle of sifted and smoothed mud at the edge of the pond. Stations were checked 1-2 days after placement and all animal tracks were identified to species.

We used cover objects (0.6 m X 2 m strips of corrugated sheet metal) to estimate reptile and small mammal presence (Parmelee and Fitch 1995). Cover objects were initially placed in the grass buffers, equidistant around the riparian zone perimeter in March 2000; they were left in place until the end of the study. The cover objects were checked at each pond visit (every 2-3 weeks). We also recorded all incidental observations of mammals and reptiles, including turtles. All small mammals and reptiles captured were identified to species, weighed, and snout-vent length recorded. We collected relative abundance information on birds using a 10-minute point count of birds within 100 m of the pond once each year (Ralph et al. 1995). Incidental observations of birds (ducks, herons, swallows, shorebirds, nocturnal birds) at each visit were also recorded.

We surveyed farm ponds for the presence of fish using dipnets at each pond visit, in conjunction with the larval amphibian surveys. Fish were also surveyed using funnel traps. Our invertebrate sampling concentrated on potential amphibian larval predators and snails and was not a comprehensive inventory of all invertebrates in the ponds. Snail species were identified because of their role as definitive hosts for the *Riberoria* parasite, which is linked to amphibian deformities. Potential macroinvertebrate predators on amphibian larvae (particularly odonates, hemipterans, and crayfish) were sampled at 2 locations in the littoral zone of each pond with 3 sweeps of a long-handled benthos net. We collected the 2 samples in contrasting vegetation types, if vegetation varied around the perimeter of the pond. We targeted riparian vegetation and shallow open sediments for sampling, habitats known to harbor most predatory macroinvertebrate species. We sampled each pond 3 times each year, twice in June and once in July.

Water Chemistry

We collected water for chemical analysis once every two weeks (seven times) from 24 April through 24 July 2000 at 26 selected ponds. The remaining 14 ponds were sampled once for chemical analysis during the week of 22 May 2000 (193 total pond samples). In 2001, water samples were collected once a month (four times) at all ponds (except pond 15 which dried) for chemical analysis from 23 April through 25 July (156 total pond samples). Each sample was a composite of separate water samples collected from four equidistant locations along the pond perimeter. Water samples were collected approximately 1 m from the shoreline at mid-depth. Water samples were acidified (pH < 2 with H_2SO_4), labeled, immediately placed in coolers on ice, then kept at 4 degrees C in the laboratory until analysis. Sample numbers and codes were assigned to each sample to ensure blind testing of each sample by laboratory staff.

Nutrient analyses were conducted at the USGS Upper Midwest Environmental Sciences Center Water Quality Laboratory in La Crosse, Wisconsin within 30 days of collection Unfiltered water samples from both 2000 and 2001 were analyzed for total nitrogen and total phosphorus following standard methods (APHA 1998). In 2001 water was also filtered (Whatman CA 0.45 μm) and analyzed for nitrate and ammonium concentrations. Nutrient analyses were completed on a Bran+Luebbe TrAAcs 800 Continuous Flow Analysis System. Quality assurance for all nutrient analyses included sample splits, spike recovery, and routine evaluation of external standards. We measured dissolved oxygen concentration, pH, conductivity, and turbidity in the field with calibrated water quality probes (e.g., YSI Model 57 multiparameter probe, Hach Model 2100P Turbidimeter) according to standard methods (APHA 1998) and UMESC standard operating procedures.

Simple two-way analysis of variance (General Linear Models, SAS, Littell et al. 1991) was used to determine difference in means of nutrient concentrations and water quality variables across treatments and years. Models were accepted as significant at $\alpha<0.05$. Where necessary, natural log transformations were used to stabilize variance and induce homogeneity of variance. Nutrient concentrations of several ponds were repeatedly abnormally high; these data were excluded from analyses. These "outliers" were defined as values falling between the 3rd quartile (75th percentile) + 1.5 x the interquartile (range between 25th and 75th percentile) range. Extreme values were values greater than the range defined for outliers. Models were accepted as significant at $\alpha<0.05$.

Landscape

We used International Coalition Land Use Land Cover maps (1990, 1:24,000 scale, http://deli.dnr.state.mn.us/metadata/index_th .html) to measure the proportion and number of patches of land in different cover classes, the densities of roads, area of urban development, and nearest neighbor distances to wetlands, forests, and row crops (corn, soybeans) within 500, 1000, and 2500 m of the breeding pond. We used NWI maps to measure the area of wetlands surrounding the breeding ponds. This range of distances corresponds to home range sizes for many amphibian species (Stebbins and Cohen 1995). Other landscape studies of amphibian habitat have used this range of distances (Vos and Stumpel 1995; Knutson et al. 1999; Lehtinen et al. 1999; Knutson et al. 2000).

Results

Amphibian Community

We identified 10 species of amphibians at the ponds (Fig. 2, Appendix A), including the tiger salamander (*Ambystoma tigrinum)*, American toad (*Bufo americanus)*, eastern gray treefrog (*Hyla versicolor)*, chorus frog (*Pseudacris triseriata)*, spring peeper (*Pseudacris crucifer)*, green frog (*Rana clamitans)*, wood frog (*Rana sylvatica)*, leopard frog (*Rana pipiens)*, pickerel frog (*Rana palustris)* and the blue-spotted salamander (*Ambystoma laterale)*. The blue-spotted salamander was identified from two larval specimens at a single natural wetland (Kapfer and Parmelee 2001). We made a total of 1644 visits to ponds in 2000 (842 visits) and 2001 (802 visits); visit frequency was every 2-3 weeks at each pond.

Calling surveys detected the most species at each pond, followed by dip net surveys, and adult visual searches (Fig. 3A-I). Some species were detected by egg mass surveys, but not all. Calling surveys had the highest correlation with the total number of ponds where each species was identified, followed by dip net surveys and visual searches for adults (Table 1). Calling surveys were not as useful for leopard frogs and pickerel frogs, missing about half of the ponds where these species were ultimately found. For leopard frogs and pickerel frogs, adult visual searches were the most successful survey method. Egg mass surveys were least successful in identifying species presence. However, egg mass surveys were useful for wood frogs and American toads because their egg masses are easily observed and identified. Tiger salamanders were detected primarily with dip net surveys. We did not detect any additional species using either baited or unbaited funnel traps vs. dip net surveys. Pitfall traps were not feasible for use at a relatively large number of sites, so we abandoned them for this study.

Amphibian Species Richness

Species richness did not differ among land use classes when presence was determined by a combination of all survey methods, or when based only on larvae, or adults (from visual surveys), or egg mass methods in 2000 or 2001. When we used only choral survey data, natural ponds had significantly higher species richness than grazed or non-grazed ponds ($P = 0.006$) in 2000, but not in 2001 (Fig. 4). Square-root transformation of the species counts did not change the above results nor did it result in normally distributed data, so the untransformed results are report here.

Amphibian Detection

We conducted dip net surveys for amphibian larvae at the 40 farm ponds from 27 March to 7 August 2000, for a total of 202 visits. The dates for first and last observation vary by species (Table 2). The estimated probability of observing a larval amphibian species for a single visit on a randomly selected day between 27 March and 7 August 2000 varied from 0.23 (chorus frog) to 0.37 (American toad) (Table 3).

During the time frame 24 May through 6 July 2000, five of the observed species were present as larvae (American toad, chorus frog, green frog, leopard/pickerel frog, and spring peeper) (Table 3). A total of 112 visits were made at the 40 ponds during this time window. The estimated probability of observing the presence of tadpoles or metamorphs for a single visit on a randomly selected day between 24 May and 6 July 2000 varied from 0.18 (chorus frog) to 0.67 (American toad) (Table 3). Eastern gray tree frog larvae were concentrated from 29 June to 27 July 2000. A total of 32 visits were made at the 40 ponds during this time window. The estimated probability of observing at least one larvae of the eastern gray tree frog for a single visit on a randomly selected day between 29 June and 27 July 2000 was 0.78 (3). Significance testing for differences in proportions is not appropriate here because the samples are not independent, but some practical information can be gleaned. Using the same assumptions needed for the geometric probability distributions discussed above, binomial distributions with the estimated success probabilities ($\hat{p}$) (Table 4) can be used to estimate the probabilities of observing the presence of tadpoles or metamorphs for species in these two shortened sampling frames.

Deformities and Ribeiroia

Thirty-three deformity assessments among 8 species of amphibians (metamorphs) were conducted at 20 ponds (Table 5). Deformity rates for all ponds were < 5% of individuals examined. All deformities found were minor, i.e. missing digits, limb truncations, and an eye deformity. Some deformities were determined to be the result of trauma. The trematode parasite, *Ribeiroia ondatrae,* was identified at 3 of 16 ponds examined for parasites in 2000 and 6 of 13 ponds examined in 2001. Of the 260 amphibians necropsied for parasites only 11 were considered to be malformed and five of these eleven harbored *Ribeiroia. Ribeiroia* was found in only northern leopard frogs and green frogs.

The mean infestation rate for individuals with *Ribeiroia* was 17.8 metacercariae per individual. Except for one site (Hou-ag) in 2001 where the ten normal leopard frogs examined harbored many *Ribeiroia* (mean intensity of 33.6 worms per infected host), all other sites were characterized by either single infections (a normal green frog from site Bro-ag with 68 *Ribeiroia* and a severely deformed leopard frog from site Lew-ag with 5 *Ribeiroia*) or mean intensities of *Ribeiroia* infection of less than five. Several frogs with missing limbs were determined to have suffered failed predation attempts or traumatic injuries at the time of capture; these injuries were easy to diagnose because they were hemorrhagic and often had bone projecting beyond the end of the limb stub.

Reptiles, Birds, Mammals, Fish, Invertebrates

Six species of snakes and two turtle species were observed at the ponds over the two years of the study (Fig. 5, Appendix A). The common garter snake (*Thamnophis sirtalis*) was the most frequently encountered reptile (18 ponds), followed by painted turtles (*Chrysemys picta*) (11 ponds). One hundred species of birds were observed at the ponds (Fig. 6A-D). The song sparrow (*Melospiza melodia*) was the most frequently observed bird species (40 ponds), followed by the red-winged blackbird (*Agelaius phoeniceus*) (34 ponds), common yellowthroat (*Geothlypis trichas*) (30 ponds), and the American robin (*Turdus migratorius*) (25 ponds). In addition, we observed the whip-poor-will (*Caprimulgus vociferous*), American woodcock (*Scolopax minor*), and common nighthawk (*Chordeiles minor*) during night and evening hours. Eighteen species of mammals were recorded. The raccoon (*Procyon lotor*) (34 ponds) and the white-tailed deer (*Odocoileus virginianus*) (33 ponds) were the most commonly recorded mammals (Fig. 7). Five species of fish were identified from the ponds, with brook stickleback (*Culaea inconstans*) the most frequently observed (6 ponds) (Fig. 8). No fish were observed at ponds surrounded by row crops (agricultural). A wide variety of invertebrate taxa were observed in the ponds (Fig. 9). Midge larvae (Chironomidae), crawling water beetles (Haliplidae), and water boatmen (Corixidae) were the most common invertebrate taxa observed.

Water quality

Concentrations of water column nutrients were generally higher in the agricultural and grazed ponds than in the natural or nongrazed ponds (Fig. 10). Nitrate concentrations (measured only in 2001) were low in all ponds, ranging from 0.22 mg/l in the grazed ponds to 0.52 mg/l in the natural ponds (Fig. 10a.). There were no statistical differences among pond types. Ammonium concentrations were significantly higher in the agriculture (2.86 mg/l) and grazed (1.2 mg/l) pond than the nongrazed (0.05 mg/l) and natural (0.11 mg/l) ponds (Fig. 10b.). Average total phosphorus concentrations were higher in the grazed (mean=3.1 mg/l) and agricultural (mean=3.2 mg/l) ponds (P=0.0007) compared to nongrazed (mean=0.8 mg/l) and natural (mean=0.9 mg/l) ponds and concentrations were higher during 2000 than 2001(P=0.0001, Fig. 10c.) When averaged over both years, total nitrogen concentrations were highest in the grazed ponds (mean=3.65 mg/l), followed by those in agricultural ponds (mean=2.7 mg/l), natural (mean=1.03 mg/l) and nongrazed (mean=1.0 mg/l). However, total nitrogen (TN) concentrations varied between years (Fig. 10d.) so that during 2000 there were no significant differences between grazed (mean=1.5 mg/l) and agriculture (mean=1.76 mg/l) ponds (although both were significantly higher than natural (mean=0.25 mg/l) or nongrazed (mean=0.3 mg/l) ponds). During 2001 TN in grazed ponds (mean=5.7 mg/l) was significantly higher than all other pond types. Natural ponds showed significantly higher (P=0.0003) conductivity relative to the other pond types (mean=427 vs. 148, 359, and 311 μmhos/cm^2, natural, nongrazed, grazed, and agricultural, respectively), and this pattern did not vary between years (Fig. 10e). Finally, turbidity was significantly different among pond types (P=0.0001). Typically, turbidity was highest in grazed ponds (mean=39.7 nephelometric turbidity units, NTU), followed by row crop and nongrazed ponds (mean=22.7 and 18.7 NTU); the lowest turbidity was consistently found in the natural ponds (mean=11.7 NTU, Fig. 10f). Turbidity did not differ among ponds by years.

Land use

The analysis of the landscape data shows that the dominant land use surrounding the ponds is row crop agriculture (43%), followed by forest (38%) and grassland (15%) (Table 6). Only about 2% of the landscape is in wetlands. This composition reflects a less intensively tilled landscape, typical of the Driftless Area Ecoregion.

Discussion

Amphibian Community

Constructed agricultural farm ponds are providing breeding habitat for most species of pond-breeding amphibians expected in this ecoregion (Oldfield and Moriarty 1994) and they support a species assemblage comparable with natural wetlands. Even wood frogs were found calling at natural, agricultural and grazed ponds. We expected that ponds surrounded by row crops (agricultural ponds) and ponds surrounded by grazed grassland would provide less suitable breeding habitat than nongrazed grassland or natural ponds because of high nutrient and agricultural chemical loading and disturbances from livestock. However, we did not find support for that assumption using presence/absence data. Based on our observations of calling, egg masses, and larvae for multiple species, it appears that farm ponds are important amphibian breeding habitats in the Driftless Area Ecoregion. Given the large numbers of farm ponds (as many as 1,000 in a single county, M. Kunz, Natural Resources Conservation Service, pers. comm.) and the scarcity of natural wetlands in the Driftless Area, constructed agricultural farm ponds are likely significant breeding habitats for amphibian populations in this region. More information on habitat associations based on indices of amphibian reproductive success is presented in another paper in this report (Knutson et al. 2002).

Amphibian Detection

Detection by any single survey method is dependent on the life history of the individual species. A combination of methods, including calling surveys, dip net and egg mass surveys will detect most species. In addition, adult visual searches should be used where leopard or pickerel frogs are suspected, as they have weak calls and differentiating their eggs and larvae in the field is difficult. Calling surveys do not adequately survey leopard and pickerel frogs because their calls are infrequent and often low or muffled. For tiger salamanders, dip net surveys or funnel traps are the best methods of capture. We found drift fences with pitfall traps to be too time-intensive for our purposes. Traps must be set and checked on a strict timetable to avoid mortality. Drift fences are most useful when the number of sites is small and travel distances to check them short.

Most anurans (except leopard, pickerel, and wood frogs) were observed calling at ponds where their larvae were not found, indicating either a larval detection problem or that some ponds were unsuitable for the growth and survival of larvae. In the case of one pond with particularly poor water quality, three species of amphibians attempted to breed there and none were successful. Assessing habitat quality only on the basis of calling data will overestimate habitat quality if some sites are unsuitable for supporting eggs and larvae.

Calling surveys are useful for identifying potential anuran habitat for some species at a large number of sites with minimal training of observers. Calling surveys are routinely conducted by volunteers in many Midwestern states (Hemesath 1998; Mossman et al. 1998; NAAMP 2002). We encourage public and private conservation land managers to consider larval dip net surveys for monitoring amphibian management units because larvae (especially late-stage larvae and metamorphs) provide direct evidence of breeding habitat quality, i.e. that reproduction was successful. Dip net surveys are also successful for salamanders and for anurans with weak or infrequent calls. They have an added advantage from a safety and logistical standpoint; dip net surveys can be conducted during the day.

We found that a six-week sampling frame, centered on June (last week of May through the first week of July) will most efficiently sample six species in the Driftless Area Ecoregion (American toad, chorus frog, spring peeper, green frog, leopard and pickerel frog). Two species (eastern gray treefrog and tiger salamander) were most efficiently sampled during July. However, if very early breeders (wood frog) or very late breeders (cricket frog) are of interest, then an April-July season may still be required. Our data indicate that at least 5 visits will be needed to bring detection probabilities in the range of 0.48 - 0.90 for most species. Concentrating efforts in June will improve detection probabilities for the most species.

Deformities and Ribeiroia

Deformity rates in our study were lower (all < 5%) than those reported from many other sites in Minnesota (Rosenberry 2001). The numbers of *Ribeiroia* metacercariae per individual in our study are comparable with other published literature on anurans (Johnson et al. 2002).

The two species of anurans infected with *Riberioa* during the current study were northern leopard frogs and green frogs. Both of these frogs produce larger metamorphs than do toads and treefrogs, and therefore their tadpoles spend more time in the pond in order to reach the larger size. We suspect that anurans producing larger metamorphs will require exposure to more *Ribeiroia* than anurans producing smaller metamorphs in order to elicit formation of malformations. For example, at various malformation 'hotspots' in Minnesota, >50% of mink frogs in 1999, >80% of leopard frogs in 2000, and >25% of American toads in 2001 were malformed, with mean intensities of *Ribeiroia* ranging from 18-155 per individual (D. Sutherland and J. Kapfer, University of Wisconsin, La Crosse, unpublished data).

Deformity surveys are easy to incorporate into an amphibian monitoring program. The timing of these surveys is late in the season (June to early August) or whenever late-stage metamorphs are present. Because severely deformed individuals are more vulnerable to predation and unlikely to survive long, it is important that deformity surveys be conducted as closed to peak emergence as possible. Otherwise, there will be a tendency to underestimate deformity rates. For species that disperse into surrounding habitats immediately after metamorphosis, deformed individuals will be less able to move away from the natal ponds as readily as normal individuals and there may be a tendency to overestimate deformity rates.

Reptiles, Birds, Mammals, Fish, Invertebrates

A wide range of wildlife species were detected in or around the farm ponds. While some of these species are generally found in upland habitats (several of the snake, bird, and mammal species), many others are unlikely to be found away from wetland habitat (turtles, bitterns, herons, ducks, shorebirds). Also, many upland species, especially mammals, require water sometime during the day. Small farm ponds provide drinking water for some of these species. While our survey methods were not intensive enough to comprehensively survey these other taxa, we provide evidence that these small ponds are visited by a wide range of species.

The few fish species we observed were primarily small, native species tolerant of low oxygen. While we attempted to identify species from each pond, our survey methods were designed to detect only presence or absence of fish as a taxa. We suggest that amphibians are successfully reproducing in all types of farm ponds partially because fish populations are low or non-existent, and because the fish species are primarily small, inefficient predators on amphibian larvae. The literature is clear that amphibian populations are generally depressed in the presence of fish predators (Kats et al. 1988; Hecnar and M'Closkey 1997). Large, complex wetlands may have enough habitat (plant) diversity to provide some refuges for amphibians from fish predation. However, small farm ponds generally have low plant diversity and predatory fish populations could easily wipe out any amphibian larvae.

These ponds do not appear to be refuges from invertebrate predators because we found many invertebrate predators were present in most ponds.

Water Quality

Dry weather conditions prevailed in May 2000, followed by heavy rainfall in late May and June and continued wet weather through July (NOAA 2000). Weather conditions during 2001 contrasted with 2000 in that steady April and May rains led to very wet conditions early in the season, followed by a summer drought (NOAA 2001). We were fortunate to study the same 40 ponds in both seasons; however, one of the ponds dried up in July 2001.

We found that ponds situated in agricultural landscapes with disturbed soils (row crop or grazed) typically had turbid waters and higher concentrations of dissolved nutrients. Water in natural ponds had higher conductivity. While these findings were not unexpected they do have implications for biota residing in or frequenting the impacted ponds. For example, increased nutrient concentrations will often result in elevated phytoplankton populations (if the water clarity is high enough), which could provide food for filter-feeding and grazing amphibian larvae (Dickman 1968, Osborne and McLachlan 1985). Overenrichment, particularly by nitrogen, however, can have detrimental effects (Seale 1980) through enhancement of microbial biological oxygen demand and benthic anoxia, development of sediment ammonia, and noxious algae (particularly associated with overenrichment of phosphorus). Benefits accrued through enhanced food resources may be offset through such detrimental effects, resulting in a net reduction of amphibian production in highly enriched ponds.

Outright lethality occurs at concentrations of nitrogen much higher than those we observed. Rouse et al. (1999) showed that lethal effects of nitrate for a variety of anurans ranged from 14 to 385 mg/l, while sub-lethal developmental effects on larvae ranged from 2.5-10 mg/l nitrate. These responses were species and life-stage specific, with early life stages always being more sensitive than adults, and bufonid adults tending to be the least sensitive species and life stage. In a study combining field surveys and laboratory exposures, Bishop et al. (1999) observed reduced amphibian diversity and density in an Ontario, Canada wetland-agricultural complex in proximity to nutrient-laden agricultural runoff. The proximate causal factor appeared to be reduced reproductive success and abnormalities during early life stage development. Water from agriculturally-impacted zones contained relatively high phosphorus (reactive phosphorus: 0.8 mg/l), nitrogen (total Kjeldahl nitrogen: 4.2 mg/l), and ammonia (total ammonia nitrogen: 0.2 mg/l). It is impossible to determine which, if any, of these constituents were causally responsible for the effect seen by Bishop et al. (1999); it is likely that the agricultural runoff mixture acted synergistically and any one element by itself was less harmful than the combined mixture. Despite the uncertainty of causal mechanisms in the field, it is clear from many other studies that nitrogenous compounds have potent negative effects on amphibian development, growth, and survival (Huey and Beitinger 1980b, a; Baker and Waights 1993; Baker and Waights 1994; Marco et al. 1999).

Contrary to this finding, Hecnar and M'Closkey (1996) surveyed 180 ponds in the agricultural region of southwest Ontario for anuran diversity and water chemistry. They found little relationship between diversity and soluble nutrients. Anuran diversity was related to water conductivity; this effect, though, was confounded by geographic latitude. In our study, natural ponds had significantly higher conductivity than the agricultural ponds. Clearly, the position of natural ponds on the landscape was such that they tended to be located in areas receiving higher inputs of water with elevated dissolved minerals or were situated in sites tending to be exposed to highly soluble lithology (e.g., exposed limestone outcrops). Because most natural ponds were in flood plains of streams and rivers, it is likely that they were receiving substantial inputs of high solute water during floods.

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Table 1. Correlation among methods for the number of ponds within each land use type where a species was present for farm ponds in Houston and Winona Counties, Minnesota, 2000.

	All methods	Calling surveys	Dipnet or larval trap	Egg surveys	Visual search, adults
All methods	1.00	0.91	0.83	0.39	0.59
Calling surveys		1.00	0.70	0.39	0.43
Dipnet or larval trap			1.00	0.41	0.66
Egg surveys				1.00	0.37
Visual search, adults					1.00

Table 2. Summary of number of observations and first and last dates of observation for amphibian larvae between 27 March and 7 August 2000 for farm ponds in Houston and Winona Counties, Minnesota.

Common name	N*	Date of first observance	Date of last observance
American toad	75	May 10	August 7
Chorus frog	25	May 11	July 6
Eastern gray tree frog	44	June 15	August 7
Green frog	43	April 4	August 2
Leopard frog/pickerel	43	May 10	August 7
Spring peeper	31	May 24	July 12
Tiger salamander	9	May 2	July 26

*The sum of the number of observations is greater than the total number of visits (202), because more than one species may be observed in a single visit.

Table 3. Estimated probability of observing the presence of tadpoles or metamorphs in a single visit to farm ponds, comparisons among a full sampling season (27 March – 7 August), or two shortened seasons (24 May to 6 July, 29 June to 27 July) in Houston and Winona Counties, Minnesota, 2000.

Common Name	27 March – 7 August $\hat{p}$ (95% CI)	27 March – 7 August Est. mean visits to 1st obs.	24 May and 6 July $\hat{p}$ (95% CI)	24 May and 6 July Est. mean visits to 1st obs.	29 June to 27 July $\hat{p}$ (95% CI)	29 June to 27 July Est. mean visits to 1st obs.
American toad	0.37 (0.30, 0.44)	2.7	0.67 (0.58, 0.76)	1.5	-	-
Chorus frog	0.12 (0.08, 0.17)	8.1	0.18 (0.11, 0.25)	5.6	-	-
Eastern gray tree frog	0.22 (0.16, 0.27)	4.6	-	-	0.78 (0.64, 0.92)	1.3
Green frog	0.26 (0.20, 0.32)	3.9	0.30 (0.22, 0.39)	3.3	-	-
Leopard/pickerel frog	0.21 (0.16, 0.27)	4.7	0.21 (0.13, 0.28)	4.9	-	-
Spring peeper	0.15 (0.10, 0.20)	6.5	0.26 (0.18, 0.34)	3.9	-	-
Tiger salamander	0.04 (0.02, 0.07)	22.4	-	-	0.13 (0.01, 0.24)	8.0

Table 4. Probability of observing the presence of tadpoles or metamorphs for each species by number of visits and monitoring time frame in farm ponds in Houston and Winona Counties, Minnesota, 2000. Estimates are for visits conducted during the full season April – July (27 March – 7 August), or during a shortened season of June (24 May and 6 July) or July (29 June to 27 July). Estimates in bold show a higher probability of detecting a species with the fewest visits in either the June or July shortened season.

# Visits	April –July	June	April –July	June	April –July	July	April –July	June	April –July	June	April –July	June	April – July	July
	American toad		Chorus frog		E. gray treefrog		Green frog		Leopard frog		Spring peeper		Tiger salamander	
1	0.37	**0.67**	0.12	**0.18**	0.22	**0.78**	0.26	**0.30**	0.21	**0.21**	0.15	**0.26**	0.04	**0.13**
2	0.60	**0.89**	0.23	**0.33**	0.39	**0.95**	0.45	**0.52**	0.38	**0.37**	0.28	**0.45**	0.09	**0.23**
3	0.75	**0.96**	0.33	**0.45**	0.52	**0.99**	0.59	**0.66**	0.51	**0.50**	0.39	**0.59**	0.13	**0.33**
4	0.84	**0.99**	0.41	**0.54**	0.63	**1.00**	0.70	**0.76**	0.62	**0.60**	0.49	**0.70**	0.17	**0.41**
5	0.90	**1.00**	0.48	**0.63**	0.71	**1.00**	0.77	**0.84**	0.70	**0.68**	0.57	**0.78**	0.20	**0.40**
6	0.94	**1.00**	0.55	**0.69**	0.77	**1.00**	0.83	**0.89**	0.76	**0.75**	0.63	**0.83**	0.24	**0.55**
7	0.96	**1.00**	0.60	**0.75**	0.82	**1.00**	0.88	**0.92**	0.81	**0.80**	0.69	**0.88**	0.27	**0.61**
8	0.98	**1.00**	0.65	**0.79**	0.86	**1.00**	0.91	**0.94**	0.85	**0.84**	0.74	**0.91**	0.31	**0.66**
9	0.98	**1.00**	0.70	**0.83**	0.89	**1.00**	0.93	**0.96**	0.88	**0.87**	0.78	**0.93**	0.34	**0.70**
10	0.99	**1.00**	0.73	**0.86**	0.91	**1.00**	0.95	**0.97**	0.91	**0.90**	0.81	**0.95**	0.37	**0.74**

Table 5. Deformity and *Ribeiroia* infection rate of amphibians based on deformity assessments at farm ponds in Houston and Winona Counties, Minnesota, 2000-2001.

		2000					2001				
Common Name	Pond Name	Number Deformed	Number Examined	Deformity Rate[a]	Number Collected	*Ribeiroria* Infection[b]	Number Deformed	Number Examined	Deformity Rate[a]	Number Collected	*Ribeiroria* Infection[b]
American Toad	Mou-Graze	0	43	0.00%	8	0					
American Toad	Stc-Agric	0	114	0.00%	10	0					
Chorus Frog	She-Natur	2	103	1.94%	2	0					
Chorus Frog	Stc-Agric	0	146	0.00%	6	0					
Gray Tree Frog	Cal-Ngraz	2	66	3.03%	9	0	5	135	3.70%		
Gray Tree Frog	Mou-Agric	1	156	0.64%	11	0					
Green Frog	Alt-Graze	0	25	0.00%	10	5					
Green Frog	Bro-Agric						0	50	0.00%	10	1
Green Frog	Bro-Graze	0	30	0.00%	17	5	1	60	1.67%		
Green Frog	Lew-Natur	0	42	0.00%	9	0	3	100	3.00%	10	1
Green Frog	Uti-Agric	0	276	0.00%	8	0					
Green Frog	Uti-Graze	0	51	0.00%							
Green Frog	Uti-Ngraz	2	102	1.96%	10	3	1	114	0.88%	10	0
Leopard Frog	Cal-Graze	4	91	4.40%	4	0					
Leopard Frog	Hou-Agric						1	186	0.54%	10	10
Leopard Frog	Hou-Ngraz	0	36	0.00%	7	0					
Leopard Frog	She-Agric	0	107	0.00%	7	0	1	254	0.39%	10	2
Pickerel Frog	Eit-Natur	0	18	0.00%							
Rana (Leopard/Pickerel)	Eit-Ngraz	0	37	0.00%	10	0	0	101	0.00%	10	0
Rana (Leopard/Pickerel)	Stc-Natur	0	52	0.00%	8	0					
Rana (Leopard/Pickerel)	Uti-Graze	0	70	0.00%			1	110	0.91%	10	0
Spring Peeper	Bro-Agric	1	31	3.23%							
Spring Peeper	Bro-Ngraz						0	80	0.00%	9	0
Spring Peeper	Hou-Ngraz						0	87	0.00%	9	0
Spring Peeper	She-Agric	0	41	0.00%							
Tiger Salamander	Cal-Graze						2	216	0.93%	3	0

Table 6. The area (ha) of different types of land uses within a 2,500-m radius circle surrounding each farm pond, by the four types of ponds used in our study of Houston and Winona Counties, Minnesota. Pond types were determined by the land use immediately surrounding the pond and the width of the grassed buffer strip (see *Methods*).

Land use type	Agriculture (ha)	Grazed (ha)	Nongrazed (ha)	Natural Wetland (ha)	Total (ha)	Percent of total
Agriculture	9744	9715	8131	5931	33521	43
Farmstead	333	333	284	227	1177	2
Forest	6372	6446	7946	9008	29773	38
Grassland	2937	2875	3031	2931	11774	15
Other	25	6	8	20	59	< 1
Shrub	17	6	14	31	68	< 1
Urban	7	16	0.3	52	76	< 1
Wetland - Permanent	49	48	45	422	564	< 1
Wetland - Temporary	45	84	70	909	1108	1
Wetland -Total	94	132	116	1331	1673	2
Total	19529	19529	19529	19529	78116	100

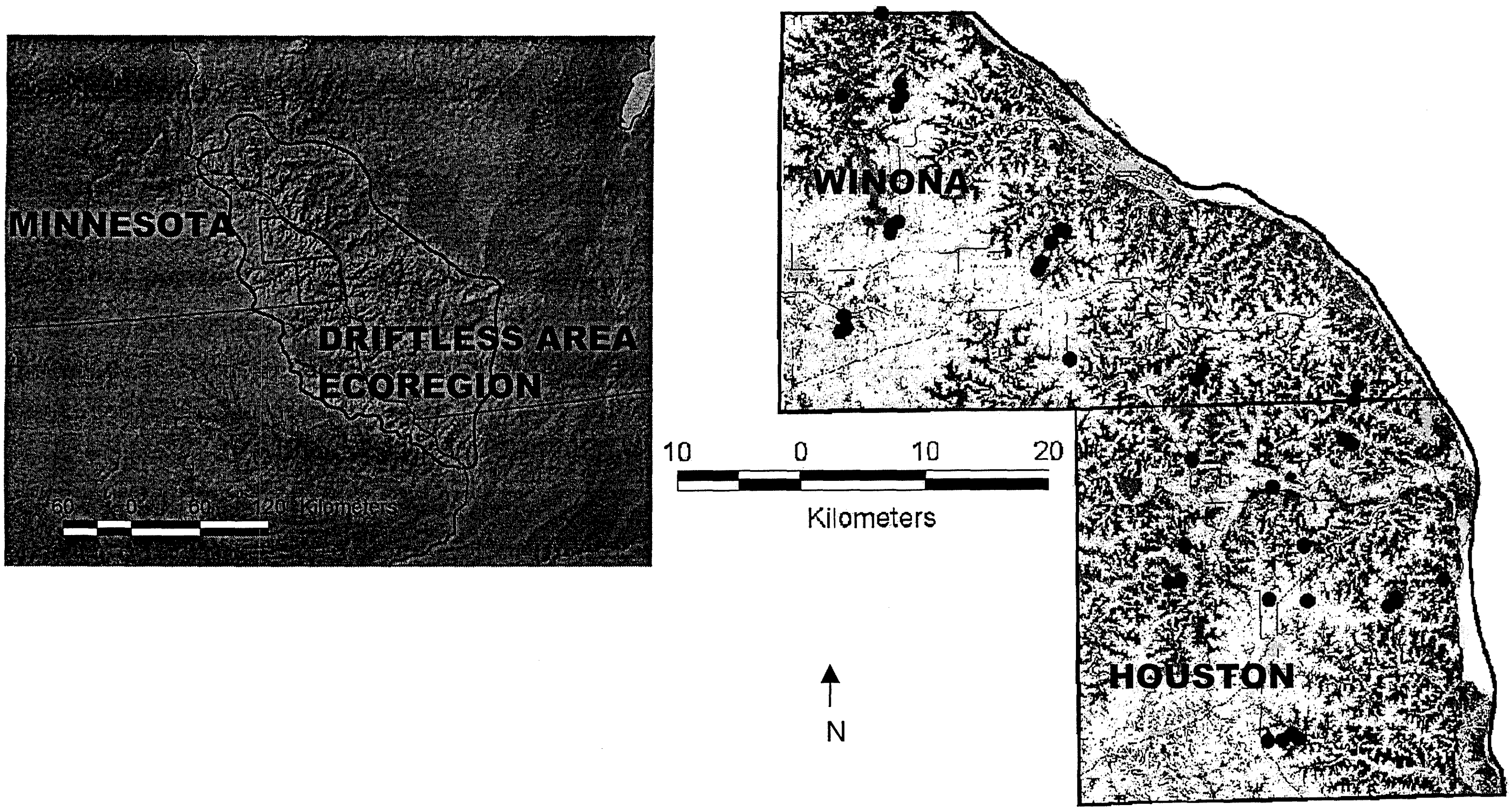


Figure 1. Farm pond study sites in southeastern Minnesota, Houston and Winona counties.

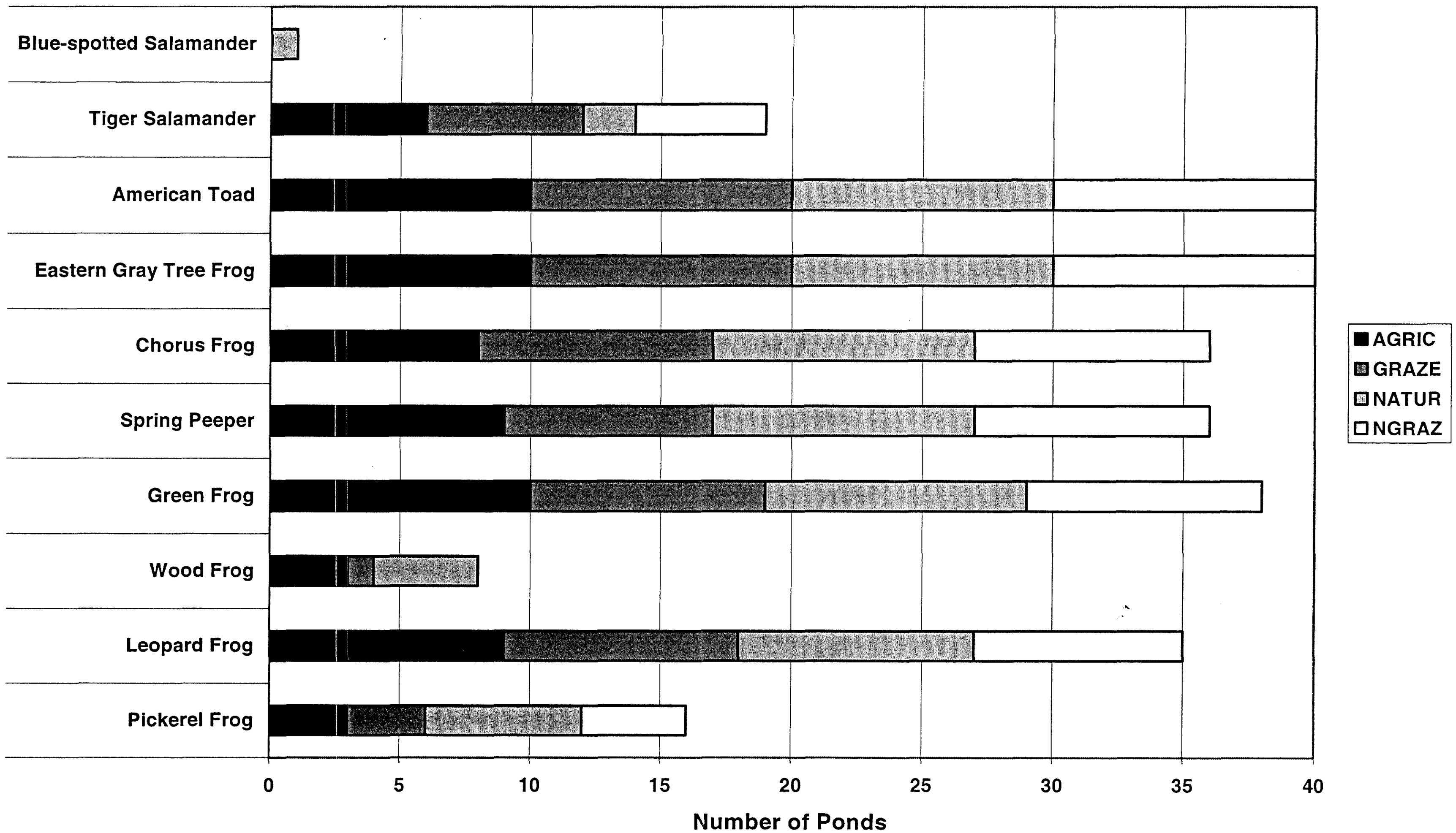


Figure 2. Amphibian species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

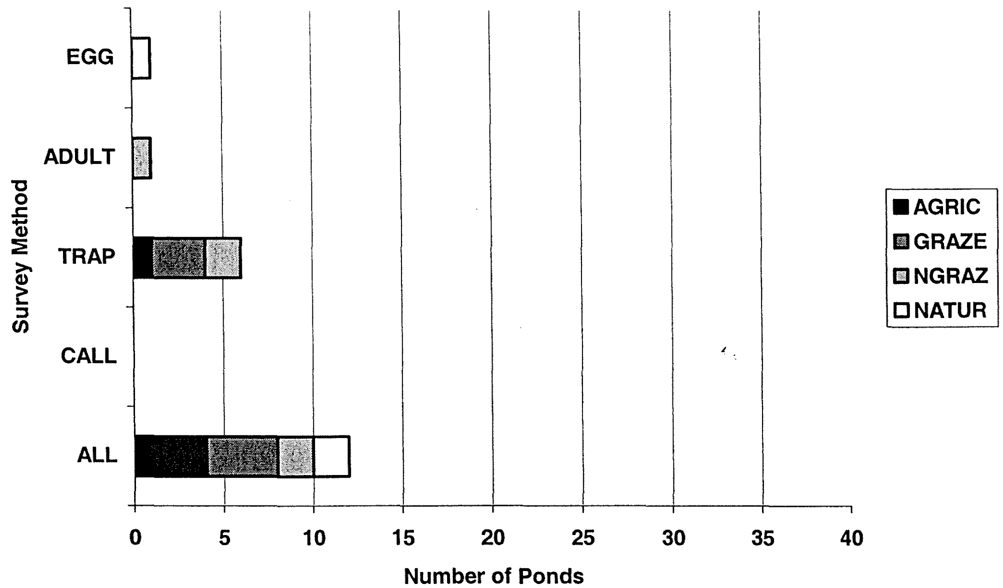


Figure 3A. Comparison of detections of tiger salamanders based on different survey methods, all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

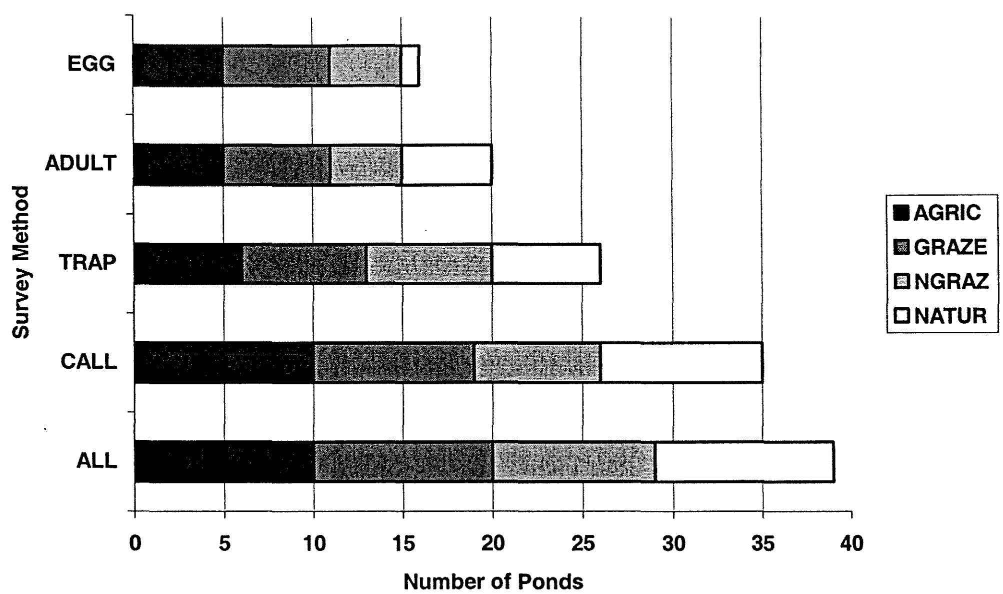


Figure 3B. Comparison of detections of American toads based on different survey methods, all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

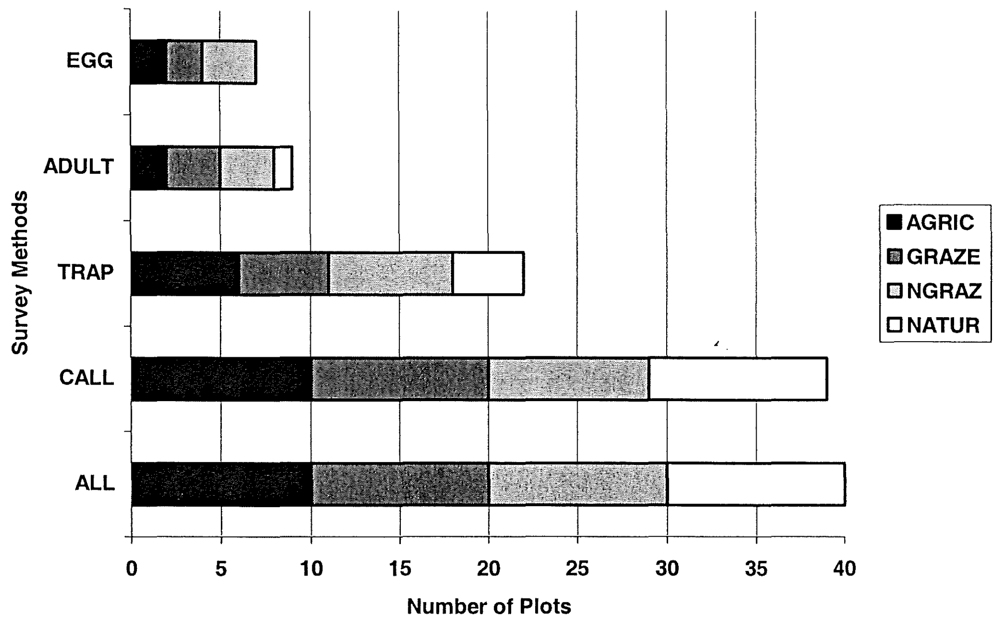


Figure 3C. Comparison of detections of eastern gray tree frogs based on different survey methods, all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

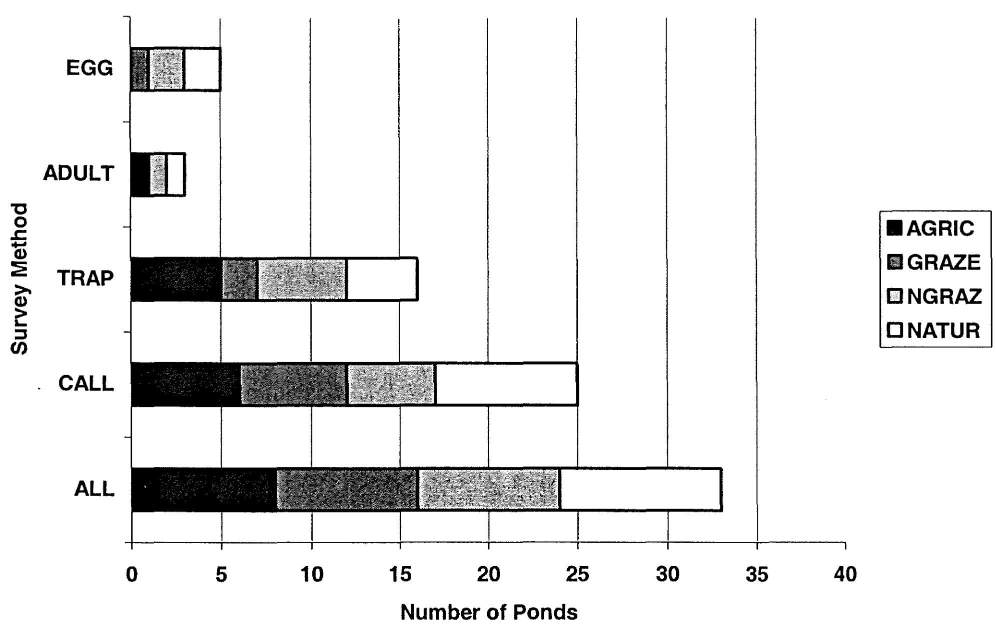


Figure 3D. Comparison of detections of chorus frogs in 4 types of surrounding land uses, based on different survey methods: all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

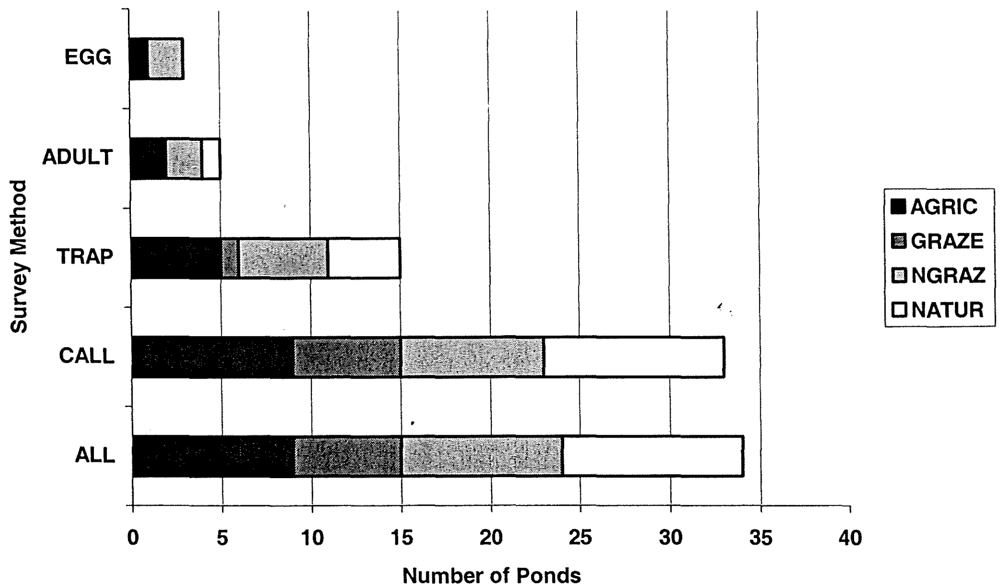


Figure 3E. Comparison of detections of spring peepers in 4 types of surrounding land uses, based on different survey methods: all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

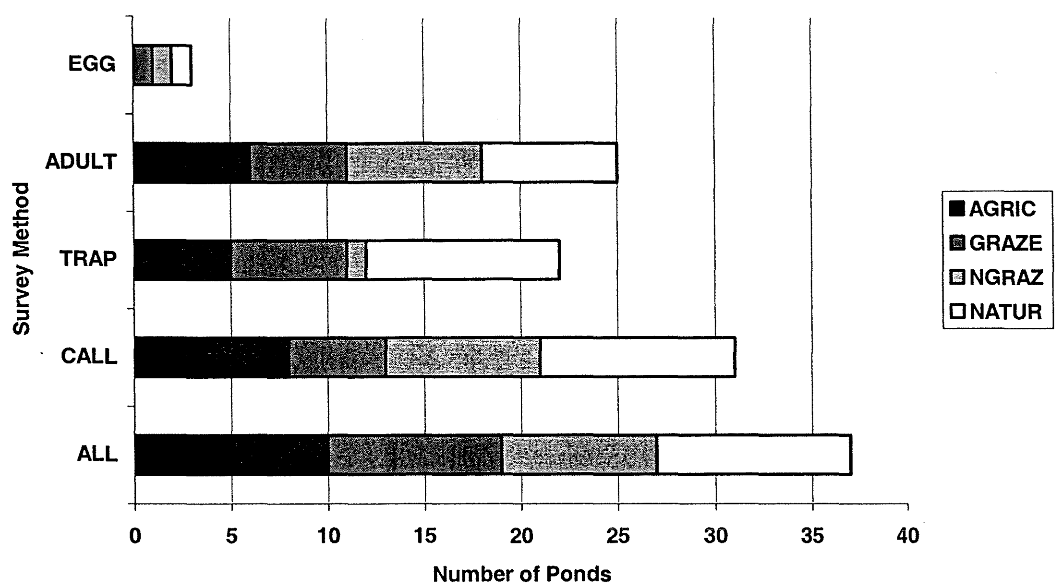


Figure 3F. Comparison of detections of green frogs in 4 types of surrounding land uses, based on different survey methods: all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

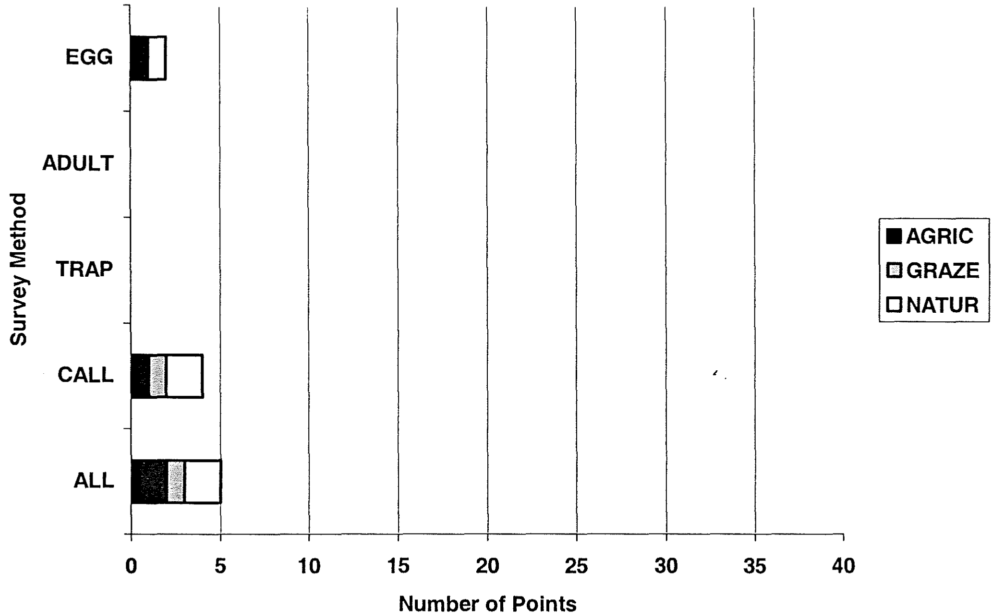


Figure 3G. Comparison of detections of wood frogs in 4 types of surrounding land uses, based on different survey methods: all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

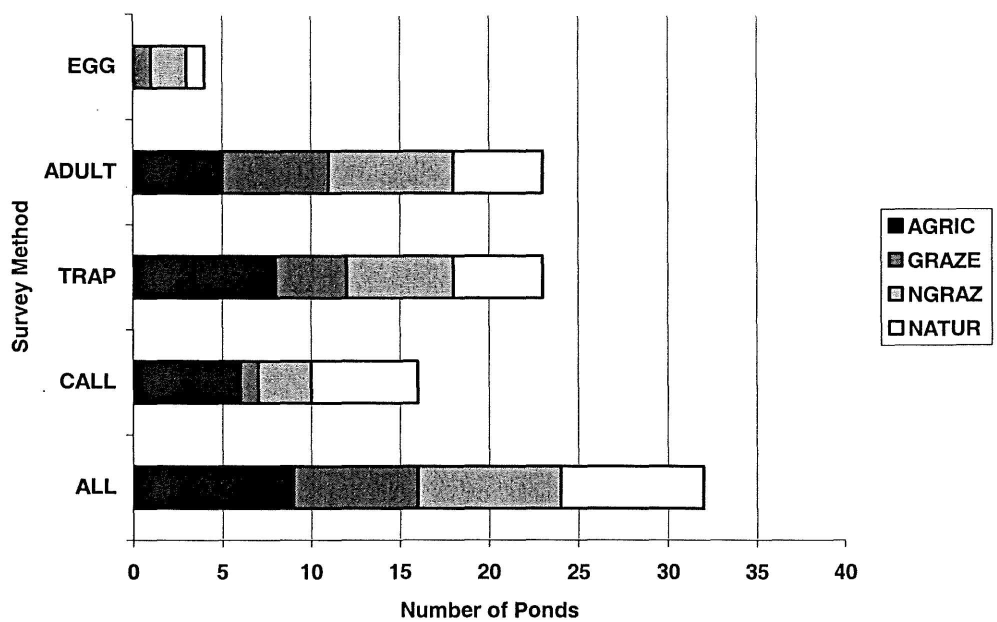


Figure 3H. Comparison of detections of leopard frogs in 4 types of surrounding land uses, based on different survey methods: all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

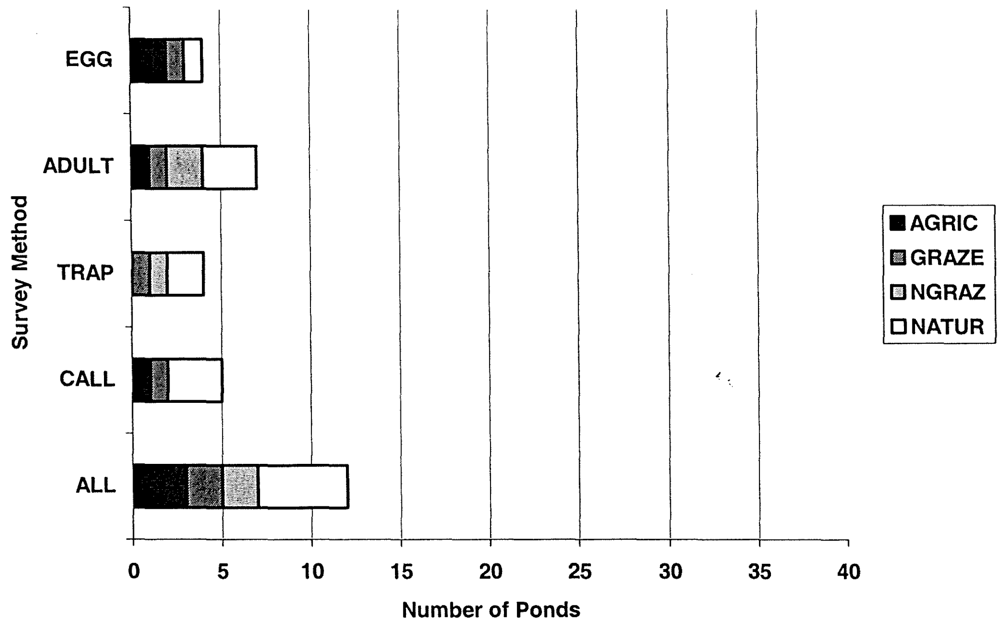
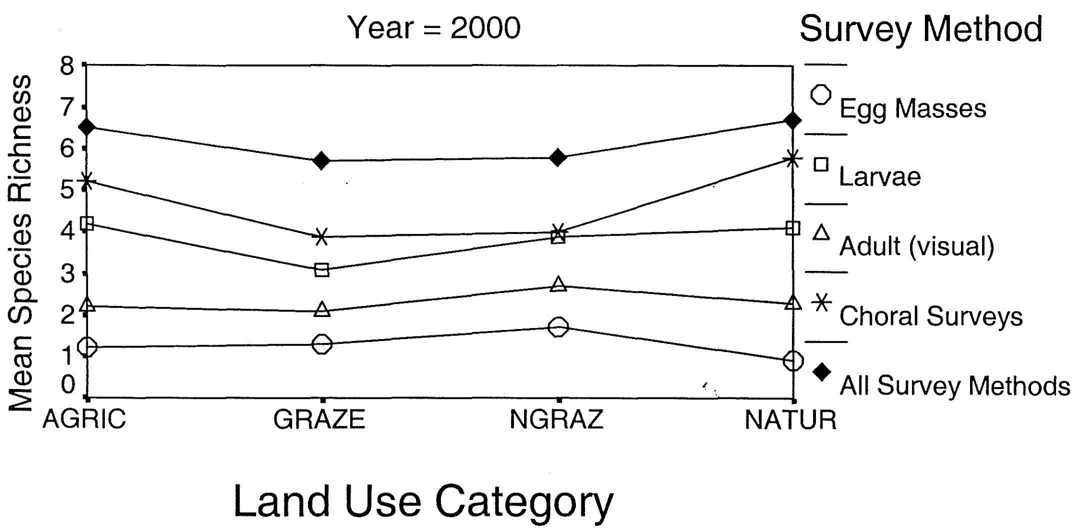


Figure 3I. Comparison of detections of pickerel frogs in 4 types of surrounding land uses, based on different survey methods: all methods (ALL), calling surveys (CALL), dipnet or larval traps (TRAP), egg mass surveys (EGG) and visual surveys for adults (ADULT), for farm ponds in Houston and Winona Counties, Minnesota, 2000. Note TRAP data is not reliable because larval pickerel frogs could not be distinguished from larval leopard frogs in the field, so these larvae were coded as leopard frogs. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

A



* NATUR is significantly higher than NGRAZ and GRAZE (P=0.006)

B

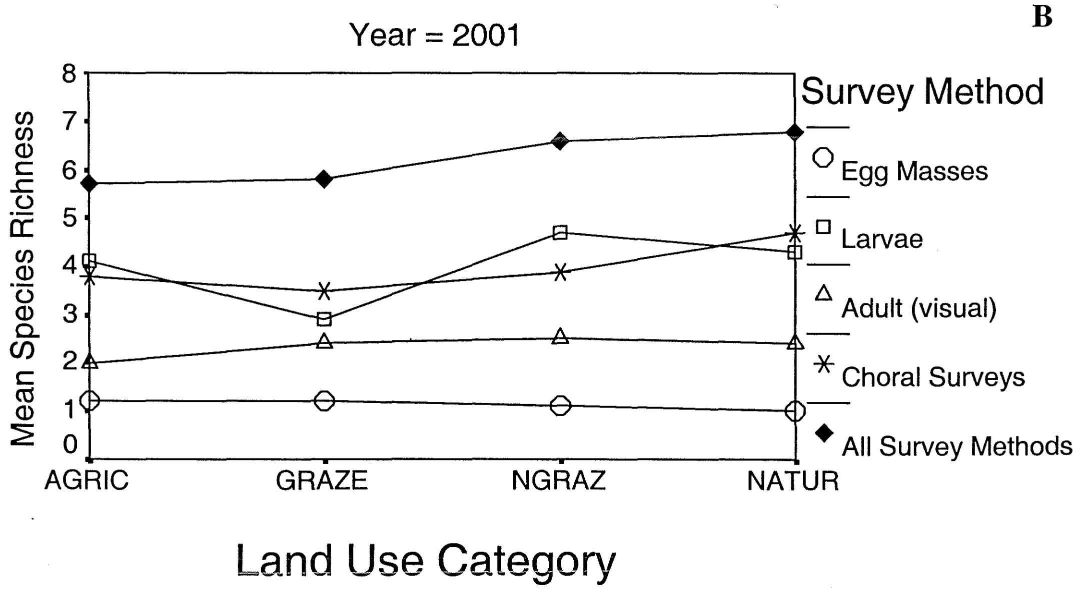


Figure 4. Mean species richness as determined by each survey method for the four land use classes measured in 2000 (A) and 2001 (B). Land use categories include agricultural (AGRIC), grazed (GRAZE), nongrazed (NGRAZ), and natural (NATUR).

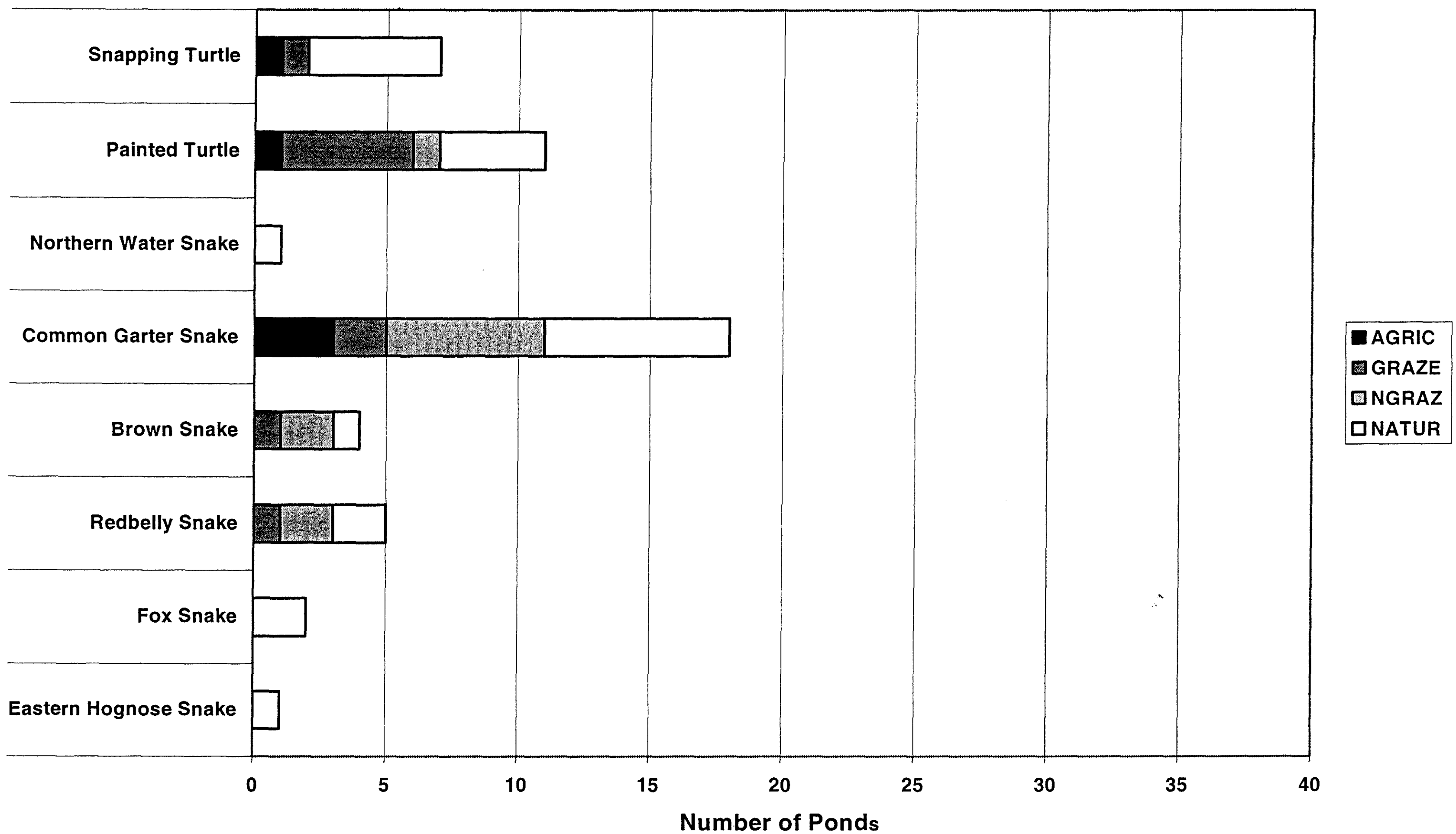


Figure 5. Reptile species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

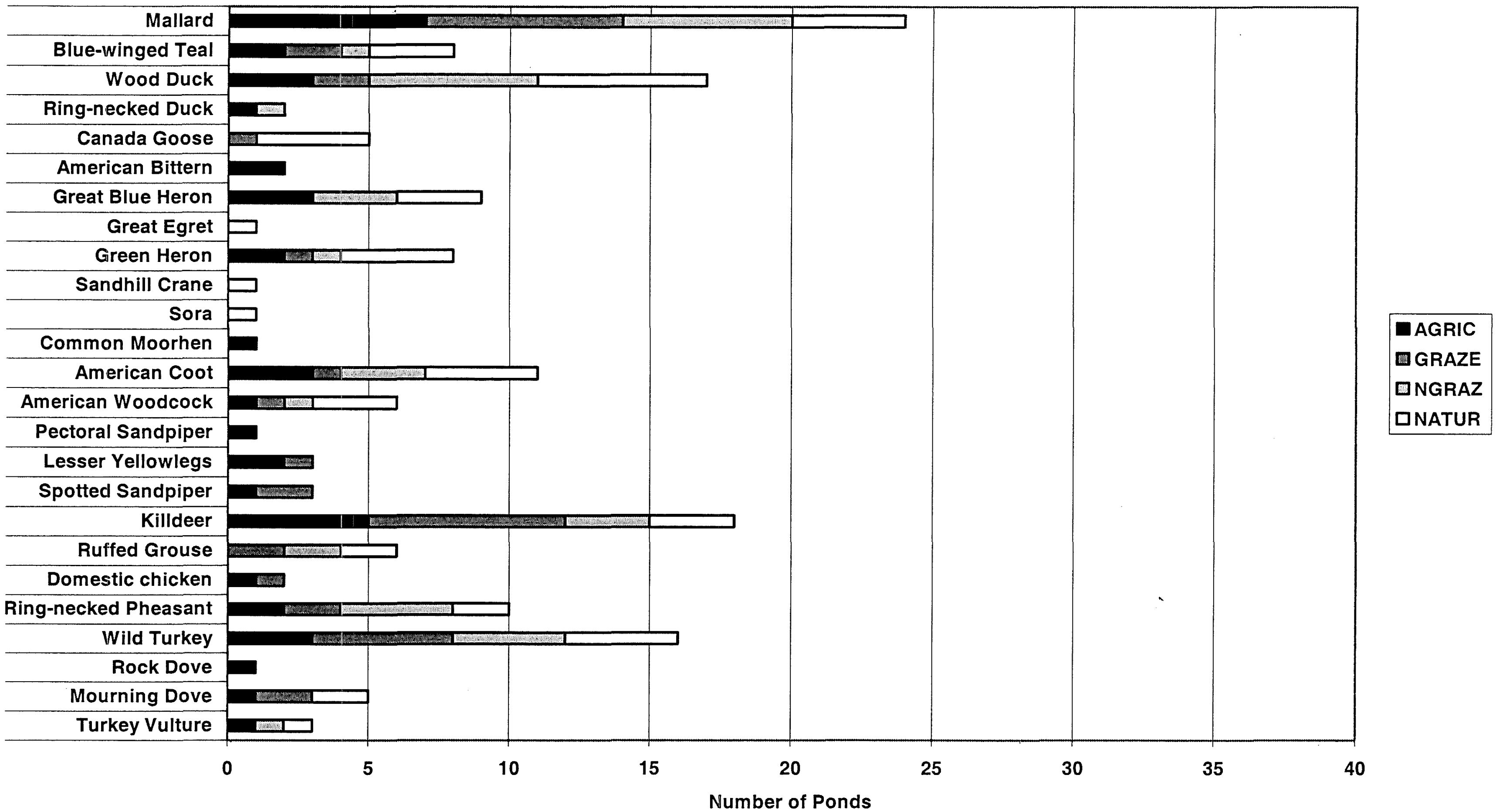


Figure 6A. Bird species present within four types of surrounding land uses, based on detection by point counts, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

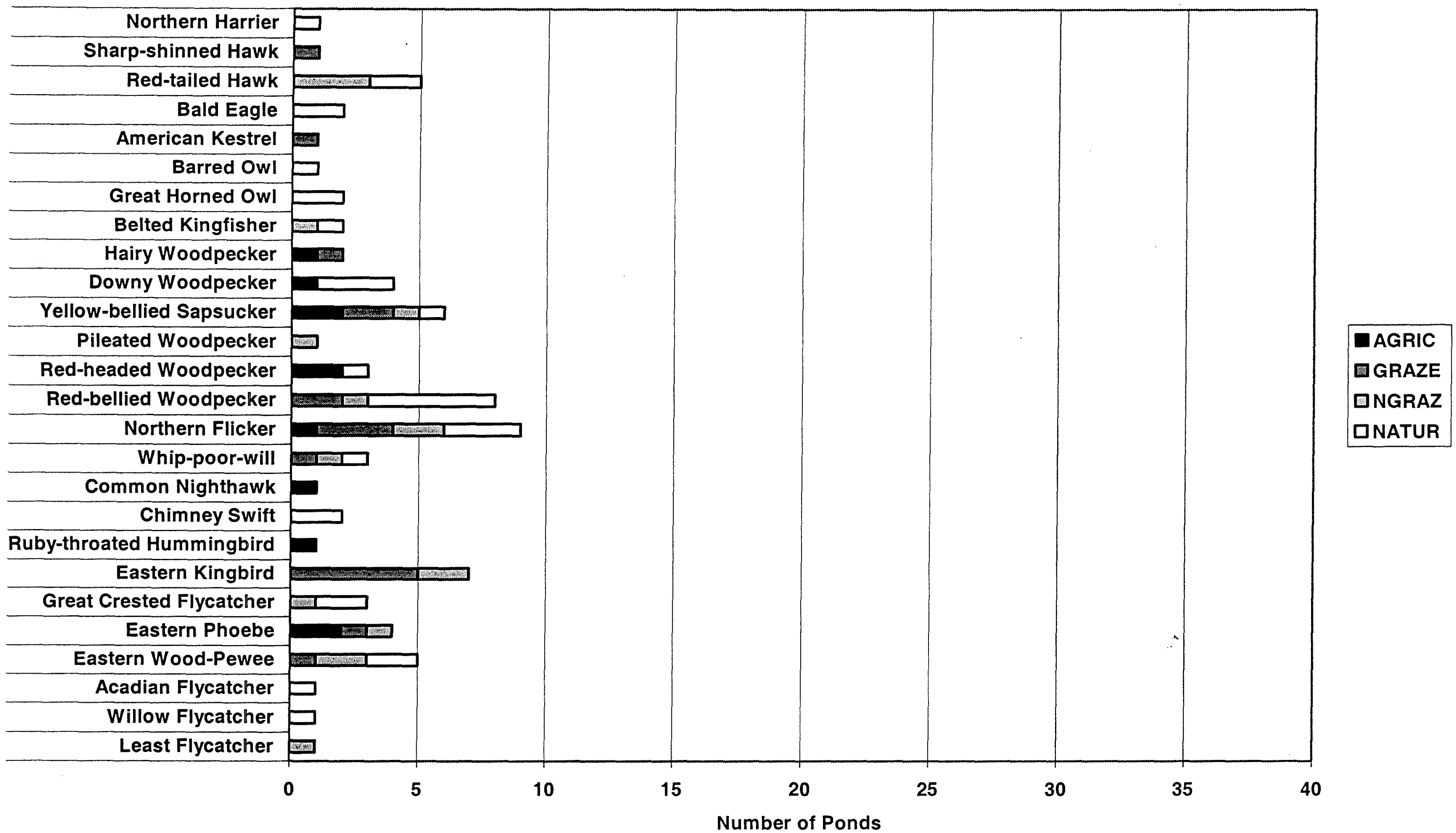


Figure 6B. Bird species present within four types of surrounding land uses, based on detection by point counts, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

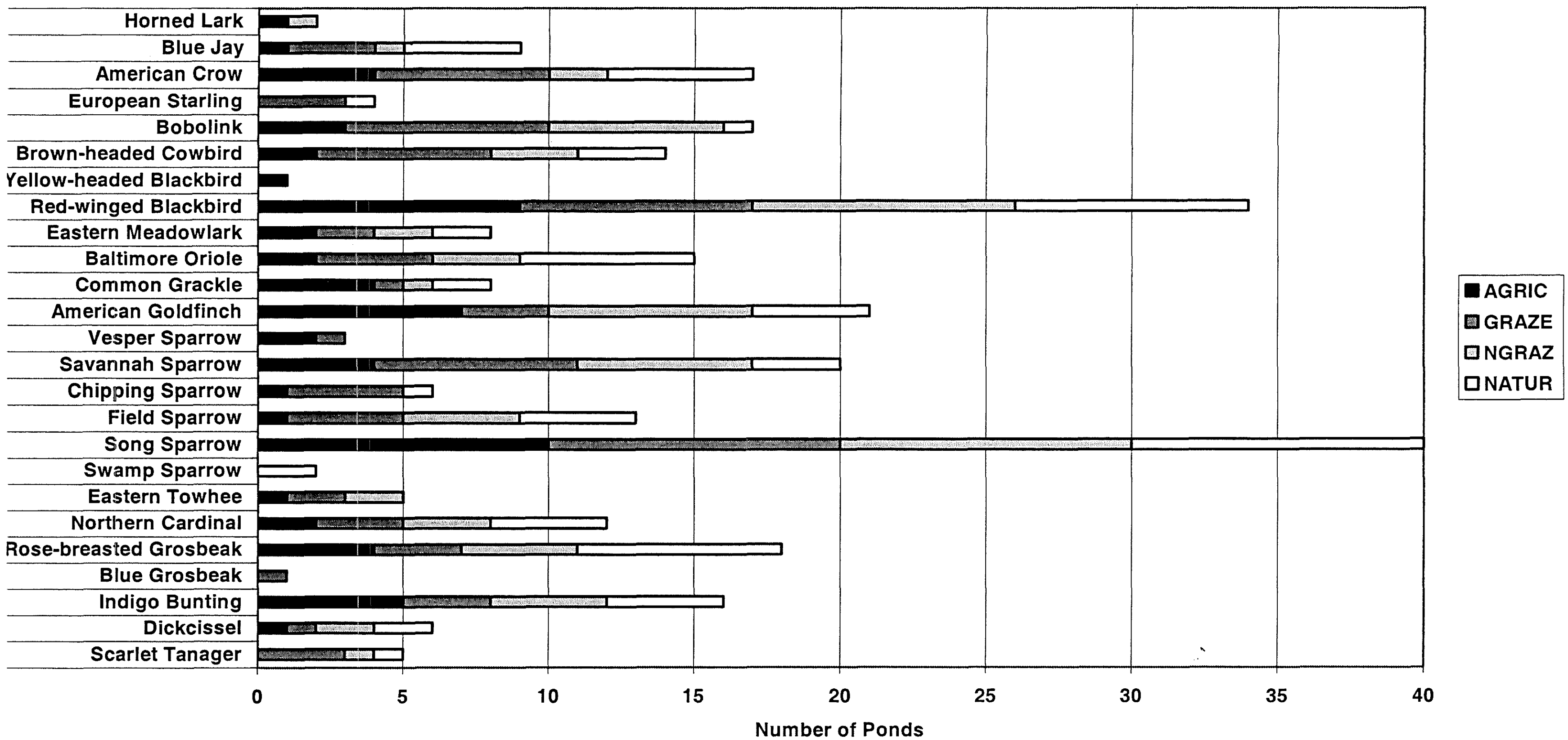


Figure 6C. Bird species present within four types of surrounding land uses, based on detection by point counts, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

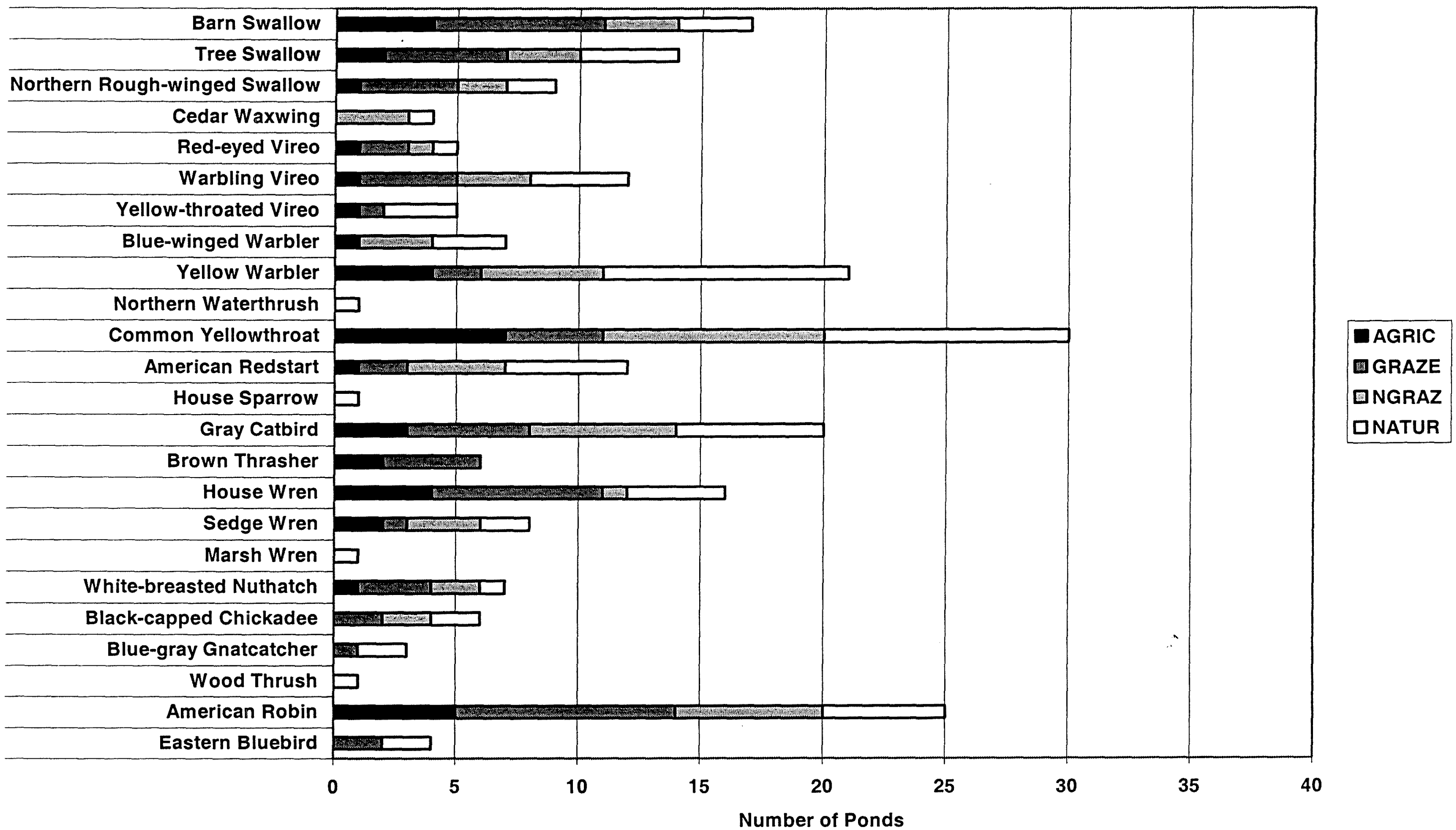


Figure 6D. Bird species present within four types of surrounding land uses, based on detection by point counts, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

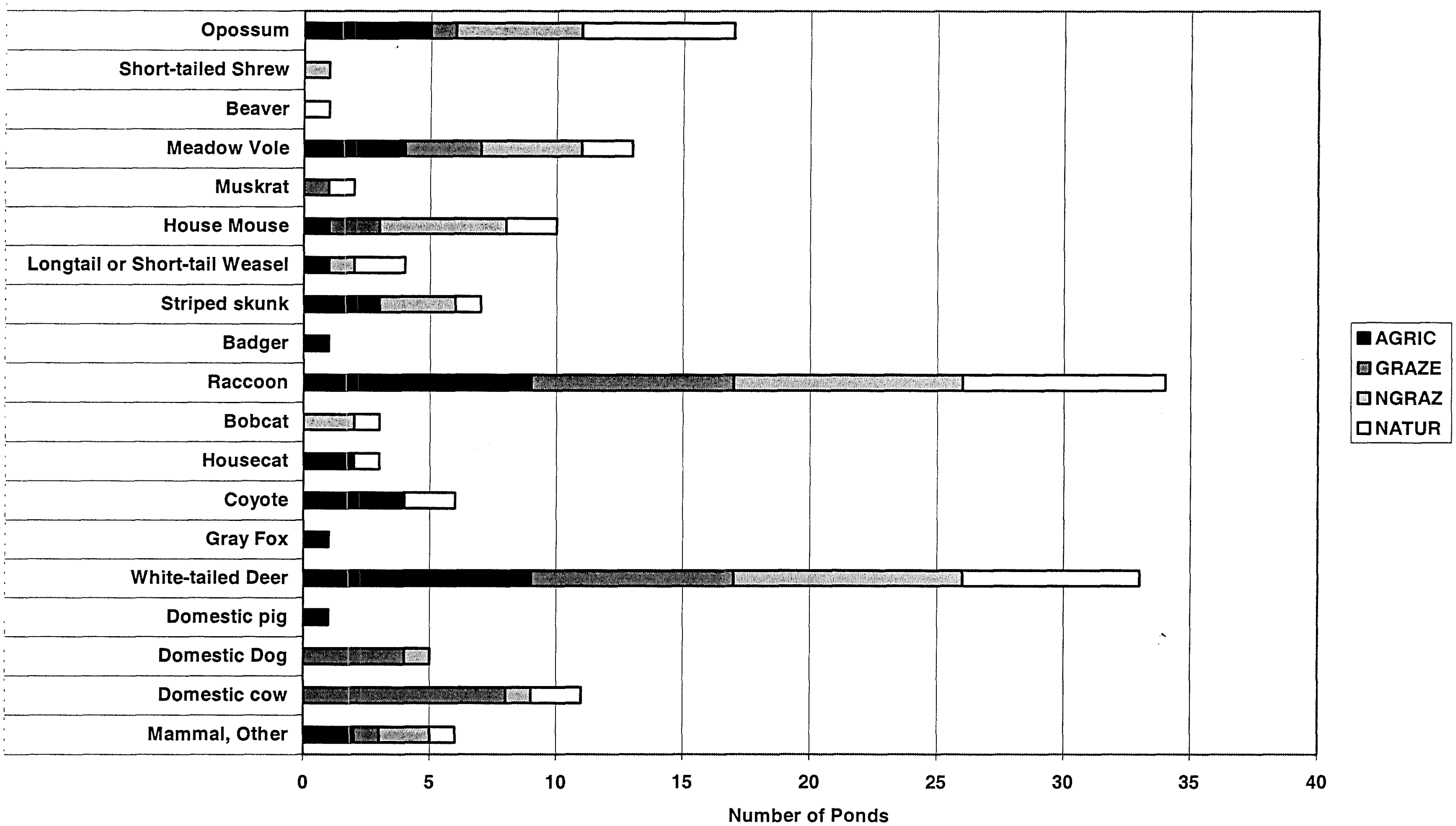


Figure 7. Mammal species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001 (scent stations excluded grazed ponds, but incidental observations included all ponds. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

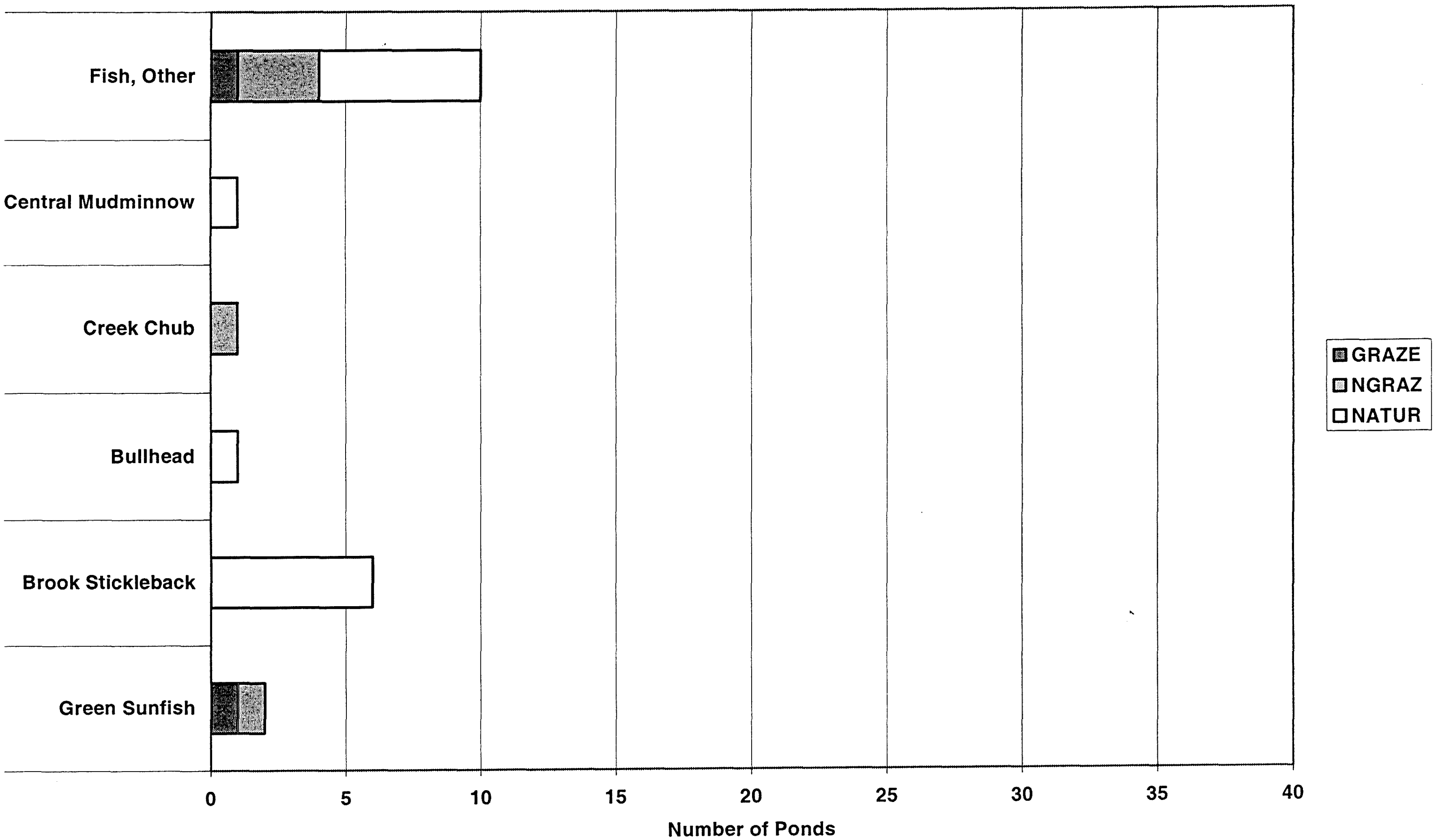


Figure 8. Fish species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

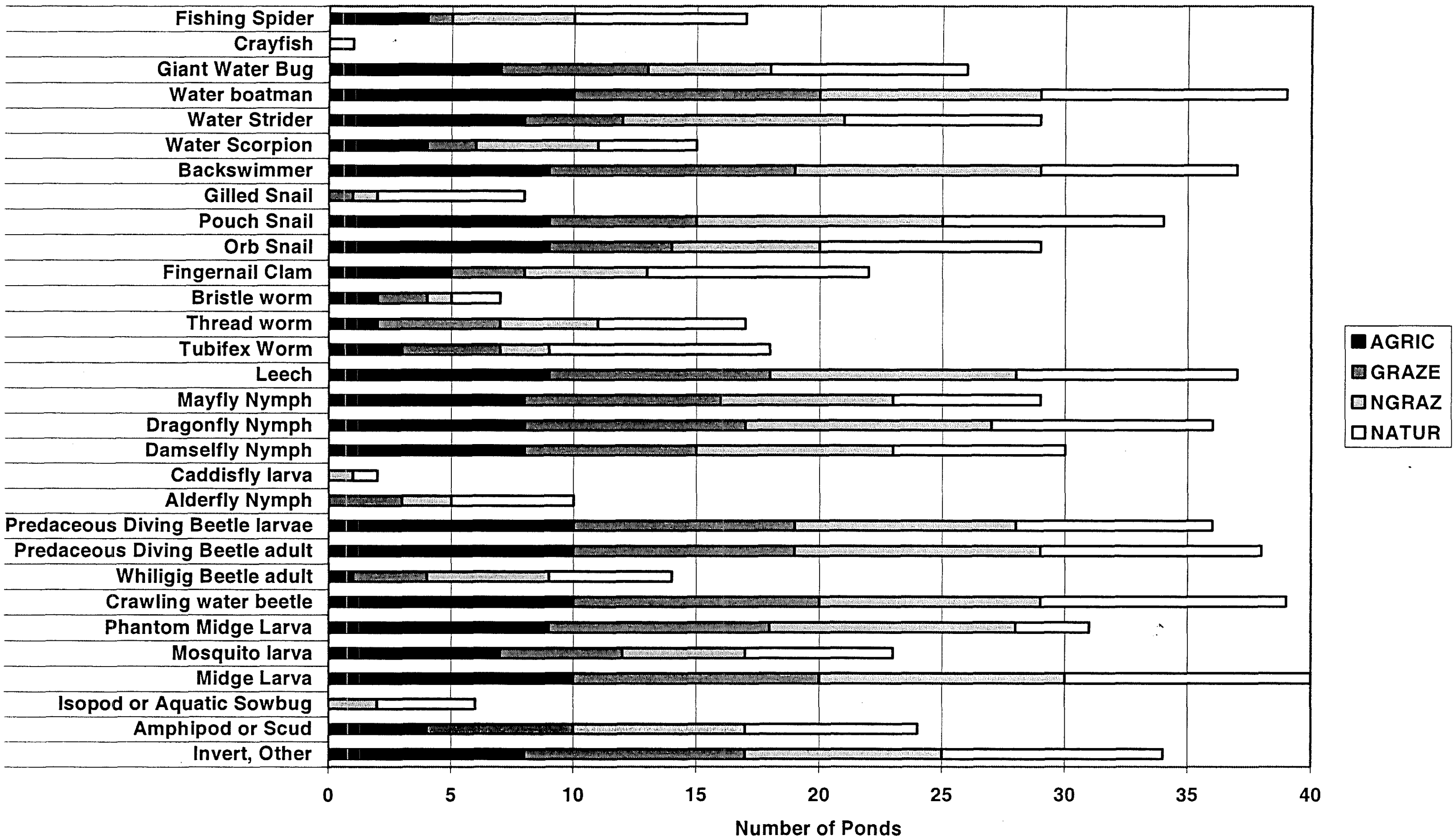


Figure 9. Invertebrate species present within four types of surrounding land uses, based on detection by all survey methods, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

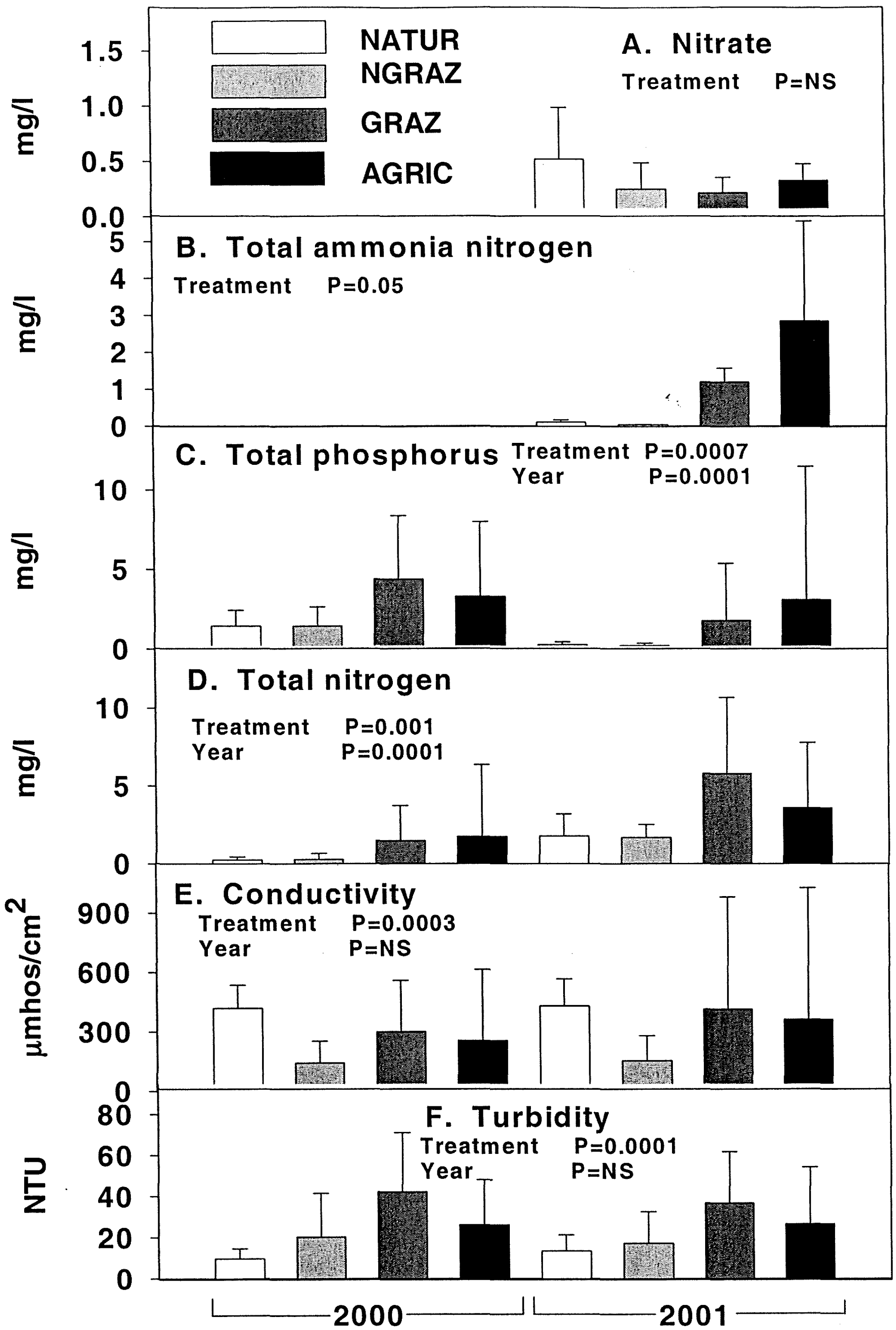


Figure 10. Mean (1 SD of mean) of (A) nitrate, (B) total ammonia nitrogen, (C) total phosphorus, (D) total nitrogen, (E) conductivity, and (F) turbidity for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. NTU=nephelometric turbidity units. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

Appendix A. List of Common and Scientific Names for Species Identified using Farm Ponds in Houston and Winona Counties, Minnesota, 2000-2001.

Common and scientific names for all species are based on the Integrated Taxonomic Information System (ITIS 2002).

Taxa	Common name	Scientific name	Order	Family
Birds	Mallard	*Anas platyrhynchos*	ANSERIFORMES	ANATIDAE
	Blue-winged Teal	*Anas discors*	ANSERIFORMES	ANATIDAE
	Wood Duck	*Aix sponsa*	ANSERIFORMES	ANATIDAE
	Ring-necked Duck	*Aythya collaris*	ANSERIFORMES	ANATIDAE
	Canada Goose	*Branta canadensis*	ANSERIFORMES	ANATIDAE
	American Bittern	*Botaurus lentiginosus*	CICONIIFORMES	ARDEIDAE
	Great Blue Heron	*Ardea herodias*	CICONIIFORMES	ARDEIDAE
	Great Egret	*Ardea alba*	CICONIIFORMES	ARDEIDAE
	Green Heron	*Butorides virescens*	CICONIIFORMES	ARDEIDAE
	Sandhill Crane	*Grus canadensis*	GRUIFORMES	GRUIDAE
	Sora	*Porzana carolina*	GRUIFORMES	RALLIDAE
	Common Moorhen	*Gallinula chloropus*	GRUIFORMES	RALLIDAE
	American Coot	*Fulica americana*	GRUIFORMES	RALLIDAE
	American Woodcock	*Scolopax minor*	CHARADRIIFORMES	SCOLOPACIDAE
	Pectoral Sandpiper	*Calidris melanotos*	CHARADRIIFORMES	SCOLOPACIDAE
	Lesser Yellowlegs	*Tringa flavipes*	CHARADRIIFORMES	SCOLOPACIDAE
	Spotted Sandpiper	*Actitis macularia*	CHARADRIIFORMES	SCOLOPACIDAE
	Killdeer	*Charadrius vociferus*	CHARADRIIFORMES	CHARADRIIDAE
	Ruffed Grouse	*Bonasa umbellus*	GALLIFORMES	PHASIANIDAE
	Domestic chicken	*Gallus gallus*	GALLIFORMES	PHASIANIDAE
	Ring-necked Pheasant	*Phasianus colchicus*	GALLIFORMES	PHASIANIDAE
	Wild Turkey	*Meleagris gallopavo*	GALLIFORMES	PHASIANIDAE
	Rock Dove	*Columba livia*	COLUMBIFORMES	COLUMBIDAE

Taxa	Common name	Scientific name	Order	Family
	Mourning Dove	*Zenaida macroura*	COLUMBIFORMES	COLUMBIDAE
	Turkey Vulture	*Cathartes aura*	CICONIIFORMES	CATHARTIDAE
	Northern Harrier	*Circus cyaneus*	FALCONIFORMES	ACCIPITRIDAE
	Sharp-shinned Hawk	*Accipiter striatus*	FALCONIFORMES	ACCIPITRIDAE
	Red-tailed Hawk	*Buteo jamaicensis*	FALCONIFORMES	ACCIPITRIDAE
	Bald Eagle	*Haliaeetus leucocephalus*	FALCONIFORMES	ACCIPITRIDAE
	American Kestrel	*Falco sparverius*	FALCONIFORMES	FALCONIDAE
	Barred Owl	*Strix varia*	STRIGIFORMES	STRIGIDAE
	Great Horned Owl	*Bubo virginianus*	STRIGIFORMES	STRIGIDAE
	Belted Kingfisher	*Ceryle alcyon*	CORACIIFORMES	ALCEDINIDAE
	Hairy Woodpecker	*Picoides villosus*	PICIFORMES	PICIDAE
	Downy Woodpecker	*Picoides pubescens*	PICIFORMES	PICIDAE
	Yellow-bellied Sapsucker	*Sphyrapicus varius*	PICIFORMES	PICIDAE
	Pileated Woodpecker	*Dryocopus pileatus*	PICIFORMES	PICIDAE
	Red-headed Woodpecker	*Melanerpes erythrocephalus*	PICIFORMES	PICIDAE
	Red-bellied Woodpecker	*Melanerpes carolinus*	PICIFORMES	PICIDAE
	Northern Flicker	*Colaptes auratus*	PICIFORMES	PICIDAE
	Whip-poor-will	*Caprimulgus vociferus*	CAPRIMULGIFORMES	CAPRIMULGIDAE
	Common Nighthawk	*Chordeiles minor*	CAPRIMULGIFORMES	CAPRIMULGIDAE
	Chimney Swift	*Chaetura pelagica*	APODIFORMES	APODIDAE
	Ruby-throated Hummingbird	*Archilochus colubris*	APODIFORMES	TROCHILIDAE
	Eastern Kingbird	*Tyrannus tyrannus*	PASSERIFORMES	TYRANNIDAE
	Great Crested Flycatcher	*Myiarchus crinitus*	PASSERIFORMES	TYRANNIDAE
	Eastern Phoebe	*Sayornis phoebe*	PASSERIFORMES	TYRANNIDAE
	Eastern Wood-Pewee	*Contopus virens*	PASSERIFORMES	TYRANNIDAE
	Acadian Flycatcher	*Empidonax virescens*	PASSERIFORMES	TYRANNIDAE

Taxa	Common name	Scientific name	Order	Family
	Willow Flycatcher	*Empidonax traillii*	PASSERIFORMES	TYRANNIDAE
	Least Flycatcher	*Empidonax minimus*	PASSERIFORMES	TYRANNIDAE
	Horned Lark	*Eremophila alpestris*	PASSERIFORMES	ALAUDIDAE
	Blue Jay	*Cyanocitta cristata*	PASSERIFORMES	CORVIDAE
	American Crow	*Corvus brachyrhynchos*	PASSERIFORMES	CORVIDAE
	European Starling	*Sturnus vulgaris*	PASSERIFORMES	STURNIDAE
	Bobolink	*Dolichonyx oryzivorus*	PASSERIFORMES	ICTERIDAE
	Brown-headed Cowbird	*Molothrus ater*	PASSERIFORMES	ICTERIDAE
	Yellow-headed Blackbird	*Xanthocephalus xanthocephalus*	PASSERIFORMES	ICTERIDAE
	Red-winged Blackbird	*Agelaius phoeniceus*	PASSERIFORMES	ICTERIDAE
	Eastern Meadowlark	*Sturnella magna*	PASSERIFORMES	ICTERIDAE
	Baltimore Oriole	*Icterus galbula*	PASSERIFORMES	ICTERIDAE
	Common Grackle	*Quiscalus quiscula*	PASSERIFORMES	ICTERIDAE
	American Goldfinch	*Carduelis tristis*	PASSERIFORMES	FRINGILLIDAE
	Vesper Sparrow	*Pooecetes gramineus*	PASSERIFORMES	EMBERIZIDAE
	Savannah Sparrow	*Passerculus sandwichensis*	PASSERIFORMES	EMBERIZIDAE
	Chipping Sparrow	*Spizella passerina*	PASSERIFORMES	EMBERIZIDAE
	Field Sparrow	*Spizella pusilla*	PASSERIFORMES	EMBERIZIDAE
	Song Sparrow	*Melospiza melodia*	PASSERIFORMES	EMBERIZIDAE
	Swamp Sparrow	*Melospiza georgiana*	PASSERIFORMES	EMBERIZIDAE
	Eastern Towhee	*Pipilo erythrophthalmus*	PASSERIFORMES	EMBERIZIDAE
	Northern Cardinal	*Cardinalis cardinalis*	PASSERIFORMES	CARDINALIDAE
	Rose-breasted Grosbeak	*Pheucticus ludovicianus*	PASSERIFORMES	CARDINALIDAE
	Blue Grosbeak	*Guiraca caerulea*	PASSERIFORMES	CARDINALIDAE
	Indigo Bunting	*Passerina cyanea*	PASSERIFORMES	CARDINALIDAE
	Dickcissel	*Spiza americana*	PASSERIFORMES	CARDINALIDAE

Taxa	Common name	Scientific name	Order	Family
	Scarlet Tanager	*Piranga olivacea*	PASSERIFORMES	THRAUPIDAE
	Barn Swallow	*Hirundo rustica*	PASSERIFORMES	HIRUNDINIDAE
	Tree Swallow	*Tachycineta bicolor*	PASSERIFORMES	HIRUNDINIDAE
	Northern Rough-winged Swallow	*Stelgidopteryx serripennis*	PASSERIFORMES	HIRUNDINIDAE
	Cedar Waxwing	*Bombycilla cedrorum*	PASSERIFORMES	BOMBYCILLIDAE
	Red-eyed Vireo	*Vireo olivaceus*	PASSERIFORMES	VIREONIDAE
	Warbling Vireo	*Vireo gilvus*	PASSERIFORMES	VIREONIDAE
	Yellow-throated Vireo	*Vireo flavifrons*	PASSERIFORMES	VIREONIDAE
	Blue-winged Warbler	*Vermivora pinus*	PASSERIFORMES	PARULIDAE
	Yellow Warbler	*Dendroica petechia*	PASSERIFORMES	PARULIDAE
	Northern Waterthrush	*Seiurus noveboracensis*	PASSERIFORMES	PARULIDAE
	Common Yellowthroat	*Geothlypis trichas*	PASSERIFORMES	PARULIDAE
	American Redstart	*Setophaga ruticilla*	PASSERIFORMES	PARULIDAE
	House Sparrow	*Passer domesticus*	PASSERIFORMES	PASSERIDAE
	Gray Catbird	*Dumetella carolinensis*	PASSERIFORMES	MIMIDAE
	Brown Thrasher	*Toxostoma rufum*	PASSERIFORMES	MIMIDAE
	House Wren	*Troglodytes aedon*	PASSERIFORMES	TROGLODYTIDAE
	Sedge Wren	*Cistothorus platensis*	PASSERIFORMES	TROGLODYTIDAE
	Marsh Wren	*Cistothorus palustris*	PASSERIFORMES	TROGLODYTIDAE
	White-breasted Nuthatch	*Sitta carolinensis*	PASSERIFORMES	SITTIDAE
	Black-capped Chickadee	*Poecile atricapillus*	PASSERIFORMES	PARIDAE
	Blue-gray Gnatcatcher	*Polioptila caerulea*	PASSERIFORMES	SYLVIIDAE
	Wood Thrush	*Hylocichla mustelina*	PASSERIFORMES	TURDIDAE
	American Robin	*Turdus migratorius*	PASSERIFORMES	TURDIDAE
	Eastern Bluebird	*Sialia sialis*	PASSERIFORMES	TURDIDAE
Amphibians	Blue-spotted salamander	*Ambystoma laterale*	CAUDATA	AMBYSTOMATIDAE

Taxa	Common name	Scientific name	Order	Family
	Tiger salamander	*Ambystoma tigrinum*	CAUDATA	AMBYSTOMATIDAE
	American toad	*Bufo americanus*	ANURA	BUFONIDAE
	Eastern gray tree frog	*Hyla versicolor*	ANURA	HYLIDAE
	Chorus frog	*Pseudacris triseriata*	ANURA	HYLIDAE
	Spring peeper	*Pseudacris crucifer*	ANURA	HYLIDAE
	Green frog	*Rana clamitans*	ANURA	RANIDAE
	Wood frog	*Rana sylvatica*	ANURA	RANIDAE
	Leopard frog	*Rana pipiens*	ANURA	RANIDAE
	Pickerel frog	*Rana palustris*	ANURA	RANIDAE
Reptiles	Snapping turtle	*Chelydra serpentina*	TESTUDINES	CHELYDRIDAE
	Painted turtle	*Chrysemys picta*	TESTUDINES	EMYDIDAE
	Common garter snake	*Thamnophis sirtalis*	SERPENTES	COLUBRIDAE
	Brown snake	*Storeria dekayi*	SERPENTES	COLUBRIDAE
	Redbelly snake	*Storeria occipitomaculata*	SERPENTES	COLUBRIDAE
	Fox snake	*Elaphe vulpina*	SERPENTES	COLUBRIDAE
Invertebrates	Fishing spider		ARANEAE	LYCOSIDAE
	Giant water bug		HEMIPTERA	BELASTOMATIDAE
	Water boatman		HEMIPTERA	CORIXIDAE
	Water strider		HEMIPTERA	GERRIDAE
	Water scorpion		HEMIPTERA	NEPIDAE
	Backswimmer		HEMIPTERA	NOTONECTIDAE
	Gilled snail		GASTROPODA (CLASS)	LYMNAEIDAE
	Pouch snail		GASTROPODA (CLASS)	PHYSIDAE
	Orb snail		GASTROPODA (CLASS)	PLANORBIDAE (HELISOMA)
	Fingernail clam		PELECYPODA (CLASS)	SPHAERIIDAE
	Bristle worm		OLIGOCHAETA (CLASS)	MANY

Taxa	Common name	Scientific name	Order	Family
	Thread worm		OLIGOCHAETA (CLASS)	MANY
	Tubifex worm		OLIGOCHAETA (CLASS)	TUBIFICIDAE
	Leech		HIRUDINEA (CLASS)	HIRUNDINEA, GLOSSIPHONIIDAE, ERPOBDELLIDAE
	Mayfly nymph		EPHEMEROPTERA	EPHEMERIDAE, HEPTAGENIIDAE, BAETIDAE
	Dragonfly nymph		ODONATA	ANISOPTERA (SUBORDER)
	Damselfly nymph		ODONATA	ZYGOPTERA (SUBORDER)
	Caddisfly larva		TRICHOPTERA	MANY
	Alderfly nymph		MEGALOPTERA	SIALIDAE
	Predaceous diving beetle larva		COLEOPTERA	DYTISCIDAE
	Predaceous diving beetle adult		COLEOPTERA	DYTISCIDAE
	Whiligig beetle adult		COLEOPTERA	GYRINIDAE
	Crawling water beetle		COLEOPTERA	HALIPLIDAE
	Phantom midge larva		DIPTERA	CHAOBORIDAE
	Mosquito larva		DIPTERA	CULICIDAE
	Midge larva		DIPTERA	TENDIPEDIDAE (CHIRONOMIDAE)
	Isopod or aquatic sowbug		ISOPODA	ASELLIDAE
	Amphipod or scud		AMPHIPODA	TALITRIDAE, GAMMARIDAE
Fish	Central mudminnow	*Umbra limi*	ESOCIFORMES	UMBRIDAE
	Creek chub	*Semotilus atromaculatus*	CYPRINIFORMES	CYPRINIDAE
	Brook stickleback	*Culaea inconstans*	GASTEROSTEIFORMES	GASTEROSTEIDAE
	Green sunfish	*Lepomis cyanellus*	PERCIFORMES	CENTRARCHIDAE
Mammals	Opossum	*Didelphis marsupialis*	DIDELPHIMORPHIA	DIDELPHIDAE
	Gray fox	*Vulpes cinegeoargenteus*	CARNIVORA	CANIDAE
	Coyote	*Canis latrans*	CARNIVORA	CANIDAE
	Domestic dog	*Canis familiaris*	CARNIVORA	CANIDAE

Taxa	Common name	Scientific name	Order	Family
	Raccoon	*Procyon lotor*	CARNIVORA	PROCYONIDAE
	Badger	*Taxidea taxus*	CARNIVORA	MUSTELIDAE
	Striped skunk	*Mephitis mephitis*	CARNIVORA	MEPHITIDAE
	Longtail or short-tail weasel	*Mustela frenata or Mustela erminea*	CARNIVORA	MUSTELIDAE
	Housecat	*Felis catus*	CARNIVORA	FELIDAE
	Bobcat	*Felis rufus*	CARNIVORA	FELIDAE
	Beaver	*Castor canadensis*	RODENTIA	CASTORIDAE
	Meadow vole	*Microtus pennsylvanicus*	RODENTIA	MURIDAE
	White-tailed deer	*Odocoileus virginianus*	ARTIODACTYLA	CERVIDAE
	Domestic cow	*Bos taurus*	ARTIODACTYLA	BOVIDAE

Amphibian Reproductive Success as an Indicator of Habitat Quality in Agricultural Farm Ponds

by

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Brian R. Gray, Jeffrey R. Parmelee, and Shawn E. Weick

Farm Ponds as Critical Habitats for Native Amphibians: Final Report

Submitted to

Legislative Commission on Minnesota Resources
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12. Box and whisker plots showing median (bar inside box), 25^{th} and 75^{th} quartiles (box surrounding bar), and range (excluding outliers) for (A) total nitrogen, (B) nitrate, (C) ammonia, and (D) turbidity in farm ponds with different adjacent land uses, Winona and Houston counties, southeast Minnesota. The data are presented as box plots with the median bar and the first and third interquartile ranges identified, representing the central 50% of the values. The whiskers show the range of values falling within the inner fence (1.5*quartile spread). Circles represent values outside the inner fence. Extreme values, values outside the outer (3*quartile spread), are plotted with asterisks. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

Amphibian Reproductive Success as an Indicator of Habitat Quality in Agricultural Farm Ponds

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Abstract

We studied small, constructed farm ponds in southeastern Minnesota to assess their value as amphibian breeding sites. Our study examined habitat factors associated with amphibian reproduction at two spatial scales: the pond and the landscape surrounding the pond. We found that small farm ponds in southeastern Minnesota support reproduction for at least seven species of amphibians. Indices of reproductive success were most closely associated with pond variables relative to landscape scale variables. We did not find support for the idea that amphibian communities in ponds surrounded by row crops exhibit reduced reproductive success relative to natural or nongrazed ponds. Ponds used for watering of cattle had consistently elevated concentrations of nitrogen, higher turbidity and possibly reduced amphibian reproductive success. Reproductive success was reduced in ponds with elevated nitrogen concentration, dense emergent vegetation, and those containing fish. Individual amphibian species varied in the habitat factors that were associated with higher reproductive success. In southeastern Minnesota, natural wetlands are rare, due to both glacial history and agricultural practices. Agricultural practices and disturbance may interact to reduce habitat quality from a theoretical optimum, but the ponds are apparently satisfactory for amphibian reproduction and comparable in this regard with natural wetlands in the region. Human-created ponds, designed to serve the needs of farmers, can be managed to provide valuable aquatic breeding habitat for amphibians in this region.

Key words: agriculture, amphibian, aquatic predators, aquatic vegetation, farm pond, fish, grazing, habitat, landscape, morphometry, nitrogen pond design, pond management, water quality.

Introduction

Global declines in amphibian populations are of concern to biologists and the public (Houlahan et al. 2000). In the Midwestern USA, the Northern Cricket Frog (*Acris crepitans*) has experienced a range contraction, largely disappearing from Minnesota, most of Wisconsin, and northern Iowa (Hay 1998). The reasons for this widespread decline in a formerly common species are unknown (Lannoo 1998a). Minnesota is also an epicenter for the phenomenon of frog malformations, another environmental puzzle demanding a solution (Helgen et al. 1998; Rosenberry 2001). These factors have made amphibian conservation a high priority in the Midwestern USA (Lannoo 1998b). Effective management of amphibian populations in the Midwestern USA requires an understanding of factors in predominantly agricultural landscapes that influence amphibian populations (Knutson et al. 1999; Knutson et al. 2000; Semlitsch 2000).

Amphibian communities respond to habitat factors at more than one spatial scale. Lehtinen et al. (1999) studied amphibian communities in wetlands of central and southwestern Minnesota, including two ecoregions. They found that amphibian species richness was lower with greater wetland isolation and road density at all spatial scales, and lower near urban areas. Hecnar and M'Closkey (1998) studied amphibian communities in Ontario, Canada and found that species richness was highly correlated with local variables related to fish predation and to regional variables related to forest cover. Knutson et al. (1999; 2000) found that species richness and abundance were positively associated with agricultural land use in Wisconsin, but not in Iowa.

In this study, we were interested in habitat factors that may contribute to successful amphibian reproduction and are subject to management actions. Our goals were to test the following research questions and hypotheses:

1) Are land uses adjacent to the breeding pond, such as row crops, grazed grassland, and

nongrazed grassland related to amphibian reproductive success?

   a) We hypothesize that breeding ponds surrounded by row crops (corn or soybeans) and grazing have poorer amphibian reproduction compared with natural wetlands and ponds surrounded by nongrazed grassland (Hecnar 1997; Bishop et al. 1999; Knutson et al. 1999).

2) What is the appropriate spatial scale for amphibian habitat management: the landscape surrounding the pond or the pond itself?
   a) We hypothesize that amphibian reproductive success is most closely associated with pond variables than with landscape variables (Bonin et al. 1997; Hecnar 1997).
   b) Furthermore, features of the landscape closest to the pond are the most closely associated with amphibian reproductive success.
3) What aspects of pond design or management will improve amphibian breeding habitat quality?
   a) We hypothesize that ponds with moderate amounts of vegetative cover, no fish, and at least medium water quality will have higher reproductive success (Lannoo 1996) (Lannoo 1998a).

Study Area

Our study ponds were located in Houston and Winona counties in the state of Minnesota, USA. The study area is part of the Driftless Area Ecoregion of southeastern Minnesota, western Wisconsin, and northeastern Iowa (McNab and Avers 1994; Fig. 1). This ecoregion was not covered by ice during the last (Wisconsin) glaciation, a feature that distinguishes it from other ecoregions in the agricultural (Mickelson et al. 1982). The landforms are characterized by maturely dissected, upland plateaus with steep bedrock ridges descending to river drainages that flow to the Mississippi River (McNab and Avers 1994). Prior to European settlement, the ecoregion was covered by an oak savanna complex (*Quercus* spp.) of mixed grasslands with forests in areas protected from fire. Forests today are mixed oak and maple hardwoods and are interspersed with pastures, hay fields, small towns, and cities. Natural wetlands are found in the floodplains of rivers and streams; most natural fen wetlands were drained and tiled to convert the land to agriculture. Complex topography and erosive soils support less intensive agriculture than in many parts of the Midwest, with agriculture occupying only 30–40% of the landscape.

Small constructed farm ponds represent nearly all the available lentic wetlands in this well-drained landscape dominated by small streams and rivers; they are potentially significant habitats for amphibians (Hall 1997). The region contains thousands of small farm ponds designed to prevent soil erosion. Most farm ponds are privately owned and adjacent land uses are row crops, livestock grazing, and forestry. Some ponds are surrounded by fallow grasslands enrolled in the U.S. Department of Agriculture's Conservation Reserve Program (CRP). To our knowledge, no studies have evaluated how the ponds benefit wildlife. Informal surveys of the ponds indicate an abundance of frogs and toads, despite intensive agricultural use adjacent to the ponds.

Methods

We examined a large number of habitat variables believed to have potential landscape and environmental effects on amphibians, including land uses adjacent to the breeding pond, pond vegetation and morphometry, water quality, and the potential aquatic predator community. We examined amphibian habitat variables at two scales (the landscape surrounding the pond and the pond itself) and associated them with amphibian reproductive success.

We used a randomized block design to select our 40 study ponds. We randomly placed a 10-km grid over Houston and Winona counties and selected ten random intersection points as our blocking factors (Fig. 1). We selected four contrasting amphibian breeding habitats in close proximity to each random point, based on adjacent land uses and wetland type: constructed farm ponds adjacent to (1) row crop agriculture, (2) grazed grassland, and (3) nongrazed grassland, and (4) a natural wetland. These four types of breeding habitats (hereafter referred to as ponds) were considered treatments in the randomized block design for purposes of data analysis. We used USFWS National Wetland Inventory (NWI) maps (1979-1988, 1:24,000) overlaid on USGS Digital Orthophoto Quarter Quad (DOQQ) maps (1991) (http://deli.dnr.state.mn.us/metadata/index_th.html) for pond selection. Our constructed ponds were NWI-classified as diked or impounded and the natural wetlands were classified as palustrine, unconsolidated bottom, and intermittently flooded (Cowardin et al. 1979). Ponds identified on the DOQQ maps but not on the NWI maps (constructed after 1988) were included in the set of possible study ponds.

The land uses immediately surrounding the pond determined the treatment class of the pond. The width of the grass buffer surrounding the pond differentiated our row crop and nongrazed pond types. If the grass buffer was < 30 m wide and adjacent to row crop agriculture (corn or soybeans) the pond was considered agricultural. If the buffer strip was ≥ 30 m wide and had no cattle grazing, the pond was considered nongrazed. If domestic livestock (cattle or horses) had direct access to the pond, it was considered grazed. Our natural wetlands represented wetland habitats available in the Driftless Area Ecoregion in the absence of constructed farm ponds. We were unable to control for land uses surrounding the natural wetlands because natural wetlands were scarce. Ephemeral wetlands (those that are dry most of the year) and ponds within 80 m of barnyards or livestock confinement areas were excluded. Most ponds were privately owned and written permits for access were obtained from all landowners and public land managers.

Amphibian Reproductive Success

We surveyed amphibians using egg mass and larval surveys. We made a total of 1644 visits to ponds in 2000 (842 visits) and 2001 (802 visits), visit frequency per pond was ~ every 2 weeks. Northern Leopard Frog and Pickerel Frog larvae could not be reliably differentiated in the field, so these species were considered together. The Wood Frog and the Blue-spotted Salamander were identified at too few ponds to include in our analyses.

We determined the presence of amphibian eggs by conducting egg mass surveys at each pond every 2-3 weeks from April to August in 2000 and 2001. The littoral zone of each pond was searched for egg masses of all species (Crouch and Paton 2000). We also conducted larvae and metamorph dipnet and visual encounter surveys at each pond once every 2-3 weeks (Thoms et al. 1997). We estimated the abundance of larvae or metamorphs by species in the following classes: (1) 1-10, (2) 11-99, and (3) $\geq$100.

Measures of reproduction and survival are the most sensitive indicators of habitat quality for wildlife species (Van Horne 1983), therefore we developed an index of reproductive success as our response variable. We defined categorical indices of reproductive success for amphibians at each pond based on observations from the egg mass, larvae, and metamorph surveys. For each species, reproductive success was ranked: high at ponds where the abundance class of larvae or metamorphs was $\geq$ 2 on at least 3 visits, medium at ponds where the abundance class of larvae or metamorphs was $\geq$ 2 on 2 or fewer visits or the abundance class of larvae or metamorphs was = 1 on at least 3 visits or egg masses were detected, and low at ponds not meeting the previous criteria. Each pond was assigned a ranking for multi-species reproductive success: 'overall high' included ponds with 2 or more species with high reproductive success; all other ponds were ranked as 'overall low'. Calling data were not used to rank reproductive success; we observed amphibian species calling at many sites where we never observed any evidence of reproductive success (larvae or metamorphs) for that species.

Amphibian voucher specimens were collected to aid accurate identification of specimens and as a permanent public record. Voucher specimens were deposited at the Bell Museum of Natural History, Minneapolis Minnesota, and were collected under Special Permit No. 9516 from the Minnesota Department of Natural Resources. We initially examined eggs and larvae under a dissecting microscope to verify field identifications. Common names of species follow Crother (2001).

Habitat variables

We measured five sets of related habitat predictors, including 61 individual variables representing aspects of the landscape surrounding the pond, pond morphometry, pond vegetation, predators who prey on amphibian eggs and larvae, and water quality (Table 1).

Landscape

We used International Coalition Land Use Land Cover maps (1990, 1:24,000 scale, http://deli.dnr.state.mn.us/metadata/index_th.html) to measure the proportion and number of patches of land in different cover classes, the densities of roads, area of urban development, and nearest neighbor distances to wetlands, forests, and row crops (corn, soybeans) within 500, 1000, and 2500 m of the breeding pond (Table 1). This range of distances corresponds to home range sizes for many amphibian species (Stebbins and Cohen 1995) and other landscape studies of amphibian habitat have used this range of distances (Vos and Stumpel 1995; Knutson et al. 1999; Lehtinen et al. 1999; Knutson et al. 2000). We used National Wetland Inventory maps to measure the area of wetlands surrounding the breeding ponds.

Pond Morphometry

We measured the perimeter and area of each pond from the digital land use land cover maps (Table 1). We measured the maximum water depth in each pond to the nearest 10 cm at each visit. We calculated the maximum difference between lowest and highest water levels over the entire season. We estimated the percent of shoreline < 1 dm deep at the time of the vegetation surveys and noted whether or not the pond was receiving runoff from any confined animal feeding areas.

Pond Vegetation

We measured vegetation in 2000 using a modification of aquatic plant sampling developed by Yin et al. (2000). We collected 6 samples (1.5 m X 0.36 m) with a modified garden rake, spaced evenly around the perimeter (littoral zone) of each pond in 2000. We estimated the percent cover of each aquatic plant species, the percent cover of shoreline emergent vegetation, and visually estimated percent cover of different land uses within 200 m of the pond (Table 1).

Predator Community

We assessed the presence of aquatic predators on amphibian eggs and larvae at each pond in 2000 and 2001 (Table 1). We identified the presence of fish using visual encounter and dipnet surveys at each pond visit, in conjunction with the amphibian surveys. Fish were also surveyed using funnel traps and identified (Peterka 1989). Potential macroinvertebrate predators on amphibian larvae, particularly odonates, hemipterans, and crayfish, were sampled at 2 locations in the littoral zone of each pond with 3 sweeps of a long-handled benthos net. We collected the 2 samples in contrasting vegetation types, if vegetation varied around the perimeter of the pond. We targeted riparian vegetation and shallow open sediments for sampling, habitats known to harbor most predatory macroinvertebrate species (Merritt 1984; Thorpe and Covich 1991). We sampled each pond 3 times, twice in June and once in July in each year. Our goal was to determine the presence of potential invertebrate and fish predators. We did not attempt to estimate abundances.

Water Quality

We collected water for chemical analysis once every two weeks (seven times) from 24 April through 24 July 2000 at 26 selected ponds. The remaining 14 ponds were sampled once for chemical analysis during the week of 22 May 2000 (193 total pond samples). In 2001, water samples were collected once a month (four times) at all ponds (except pond 15 which dried) for chemical analysis from 23 April through 25 July (156 total pond samples). Each composite sample was comprised of separate water samples collected from 4 equidistant locations along the pond perimeter. Water samples were collected approximately 1 m from the shoreline at mid-depth. All water samples were labeled and immediately placed in coolers on ice and then refrigerated. Sample numbers and codes were assigned to each sample to ensure blind testing by laboratory staff.

Nutrient analyses were conducted within 30 days of collection at the Upper Midwest Environmental Sciences Center Water Quality Laboratory. Unfiltered water samples from both 2000 and 2001 were analyzed for total nitrogen and total phosphorus following standard methods (APHA 1998) after digestion (persulfate method; APHA, 1998). In 2001, water was also filtered (Whatman CA 0.45 μm) and analyzed for nitrate and ammonium concentrations. Nutrient analyses were completed on a Bran+Luebbe TrAAcs 800 Continuous Flow Analysis System. Quality assurance for nutrient analyses included sample splits, spike recovery, and routine evaluation of external standards.

At each study site we also measured dissolved oxygen concentration, pH, conductivity, and turbidity in the field with calibrated water quality probes (e.g., YSI Model 57 multiparameter probe, Hach Model 2100P Turbidimeter) according to standard methods (APHA 1998) and UMSC standard operating procedures.

Statistical Analysis

The statistical models were built using 2000 data. We first assessed whether reproductive success was associated with the design components of our study. In this step, we regressed reproductive success on treatment (the four land use classes: grazed, nongrazed, agricultural, and natural) and block (10 random point locations) using logistic regression.

We formulated *a priori* hypotheses about expected relationships between amphibian reproductive success and habitat variables based on published literature. We expected that our treatments would influence reproductive success because of differences in origin and disturbance; natural ponds would have the highest reproductive success, followed in rank order by nongrazed, grazed, and agricultural. We expected that reproductive success would be higher where habitat patch diversity and the edge density of wetlands in the surrounding landscape, and vegetation cover in the pond were higher (Knutson et al. 1999; Knutson et al. 2000). We expected that reproductive success would be lower where the abundance of predatory invertebrates, total nitrogen, and turbidity of the water were higher and fish were present (Skelly and Werner 1990; Hecnar and M'Closkey 1997a; Rouse et al. 1999; Van Buskirk 2001). We expected that reproductive success for grassland-associated amphibians would be higher where the proportion of the landscape in grassland was higher and a similar relationship was expected between forests and forest-associated amphibians (Vogt 1981; Christiansen and Bailey 1991; Oldfield and Moriarty 1994; Harding 1997; Knutson et al. 1999; Knutson et al. 2000). In addition, associations with species-specific life history traits such as requiring permanent vs. temporary water were expected (Knutson et al. 1999).

We also assessed associations between reproductive success and groups of habitat predictors. Using logistic regression, we regressed reproductive success on water quality, pond vegetation, pond morphometry, predator community, and landscape variables within 3 different buffer distances (500, 1000, and 2500 meters). All of the predictor variables within each group were included in the models, unless complete or quasi-complete separation of the data occurred (Allison 2000). In that case, we removed the relevant predictor using a standard error criterion. We then ranked the resulting models using Akaike's information criterion, as modified for small sample sizes (AIC_c) (Akaike 1973; Burnham and Anderson 1998). Smaller AIC_c values are considered indicative of models that contain more information about response metrics. For comparison with a traditional linear model we also provide estimates of the proportion of variance explained (R^2) (Nagelkerke 1991).

Next, we assessed the predictors within each group of variables to find those that explained the most variance. We used logistic regression with stepwise selection within each habitat group (e.g. landscape 500, 1000, 2500, pond morphometry, pond vegetation, water quality, predator community). Because the three landscape buffers are overlapping and therefore not independent, we selected the 500-m buffer distance as the single, 'best' landscape buffer distance for all species based on the all-species model and the AIC_c criterion. The 1000 and 2500 m buffer groups were dropped from further analysis.

In the final step of the predictor-reduction process, the significant predictor variables from each group were pooled and entered into a separate final stepwise logistic regression model for each species and all species pooled. Treatment was also included in the final stepwise model runs. Interactions between the final variables remaining in the models were tested.

We evaluated our final models using 2001 data. Models were evaluated using % Concordant and Somer's D statistics (Guisan and Harrell 2000; Mitchell et al. 2001).

To further investigate variables appearing in our final models, we used simple two-way analysis of variance (General Linear Models, Littell et al. 1991) to determine differences in means of nutrient concentrations and water quality variables across treatments and years. Where necessary, natural log transformations were used to stabilize variance and induce homogeneity of variance. Nutrient concentrations of several ponds were repeatedly abnormally high; these data were excluded from analyses. The data are presented as box plots with the median bar and the first and third interquartile ranges identified, representing the central 50% of the values. The whiskers show the range of values falling within the inner fence (1.5*quartile spread). Circles represent values outside the inner fence. Extreme values, values outside the outer (3*quartile spread), are plotted with asterisks. A significance level of 0.05 was used for stepwise selection and ANOVA procedures. All computations were performed using SAS® (SAS Institute 1999-2001).

Results

We identified 10 species of amphibians in the study ponds, including the Tiger Salamander (*Ambystoma tigrinum*), American Toad (*Bufo americanus*), Gray Treefrog (*Hyla versicolor*), Western Chorus Frog (*Pseudacris triseriata*), Spring Peeper (*Pseudacris crucifer*), Green Frog (*Rana clamitans*), Wood Frog (*Rana sylvatica*), Northern Leopard Frog (*Rana pipiens*), and Pickerel Frog (*Rana palustris*) (Fig. 2). Larval Blue-spotted Salamanders (*Ambystoma laterale*) were identified at a single natural wetland.

Adjacent Land Uses

Indices of reproductive success were similar between years for all species (Fig. 3A), and for most individual species (Figs. 4A-10A). Treatment and block were not statistically associated with reproductive success for any species (P>0.05), except for the Gray Treefrog, which had lower reproductive success in natural ponds compared with nongrazed ponds; there were no differences among the other pond types for this species (Table 3, Fig. 6B).

For all species combined and the Gray Treefrog, water quality variables as a group had the lowest AIC_C statistics and explained the most variation in reproductive success (Table 2). Pond morphometry variables were most closely associated with reproductive success for the American Toad, Western Chorus Frog, and Green Frog. Northern Leopard/Pickerel Frog and Spring Peeper reproductive success was associated with Landscape 500, 1000, and pond morphometry. Tiger Salamander reproductive success was associated with pond vegetation. Pond factors were collectively more indicative of overall multi-species reproductive success at a pond than the landscape variables (Table 2). This was true for Western Chorus Frogs, Gray Treefrogs, and Green Frogs. However, for American Toads, Northern Leopard/Pickerel Frogs, Spring Peepers, and Tiger Salamanders, landscape variables rank as one of the top two models.

Spatial scale: Landscape vs. Pond

The issue of what spatial scale is most appropriate for measurement of landscape variables was not clearly resolved. For several species, the 3 sets of landscape variables (500, 1000, 2500) were closely ranked, with minor differences in AIC_C (Table 2). For most species (except American Toad), landscape 500 or 1000 were ranked higher than landscape 2500, supporting the idea that landscapes closer to the pond are the most important.

Pond Design and Management

Habitat Factors

For all species combined, the final (summary) model includes total nitrogen, fish, and emergent vegetation cover (Table 3, Fig. 11). The probability of high reproductive success for all species combined, based on the logistic regression model, was estimated by:

$$\text{prob} = 1/(1+\exp(-7.7040+3.3201*\text{cover\_emer}+4.9627*\text{fish}+17.7332*\text{totnitr}))$$

Water quality variables also appear in final models for the Gray Treefrog (total nitrogen), Green Frog (turbidity), and Northern Leopard or Pickerel Frog (conductivity) (Table 3). This corresponds with the high relative importance of water quality variables in the variable group analysis (Table 2). Predators appear in the all-species model (fish), and the American Toad model (backswimmer) (Table 3). Landscape appears in the Tiger Salamander model (distance to the nearest forest). Pond vegetation appears in the all-species model (emergent cover) and the Gray Treefrog (index of total vegetation cover). Treatment was also significant for Gray Treefrogs (Table 3). None of the 61 measured variables were associated with reproductive success for

Western Chorus Frogs or Spring Peepers (Table 3). No interactions of the variables in the final models were statistically significant at the 5% level.

Models based on 2000 data showed predictive ability with the 2001 data (D>0.20, Mitchell et al. 2001) for all species combined, the American Toad, Gray Treefrog, Green Frog, and Tiger Salamander, although the explanatory power of the models was generally lower than in 2000 (Table 4). However, the Northern Leopard/Pickerel Frog model fit in 2001 was poor (D=0.14). This indicates that for most species the models were relevant at the same sites over two years. For example, the model derived from the 2000 data for all species was 75% concordant between predicted probabilities and observed responses for the data collected in 2001. Our models explained 17-80% of the variability in the data sets in 2000 and 4-34% of the variability in 2001.

Average total nitrogen (TN) concentrations pooled across years were higher in the grazed (3.7 mg/l) and agricultural (2.7 mg/l), than in the natural (1.0 mg/l) and nongrazed (1.0 mg/l) ponds. However, TN mean concentrations varied between years (Figure 12a); during 2000 there were no significant differences between grazed (1.5 mg/l) and agriculture (1.8 mg/l) ponds (although both were significantly higher than natural (0.3 mg/l) or nongrazed (0.3 mg/l) ponds). During 2001 TN in grazed ponds (mean= 5.7 mg/l) was significantly higher than all other pond types. Nitrate concentrations (measured only in 2001) were generally low in all ponds, ranging from 0.2 mg/l in the grazed to 0.5 mg/l in the natural ponds (Figure 12b). Ammonia concentrations were higher in agriculture (2.9 mg/l) and grazed (1.2 mg/l) ponds than nongrazed (0.05 mg/l) and natural (0.1 mg/l) ponds (Figure 12c). Averaged across years, turbidity was higher in grazed (39.7 NTU) and agricultural (22.7 NTU) then in the natural (11.7 NTU) or nongrazed (18.7) ponds. Turbidity levels were relatively constant across years (Figure 12d).

Fish species commonly collected during this study included the brook stickleback (*Culea inconstans*), creek chub (*Semotilus atromaculatus*), green sunfish (*Lepomis cyanellus*) and central mud minnows (*Umbra limi*). Fish presence and land use category were associated (Fisher's exact test, P=0.0004), with 8 out of 10 natural ponds containing fish, and only 3 nongrazed, 1 grazed, and 0 agricultural ponds out of 10 containing fish. Sunfish were only found in the grazed and nongrazed ponds, while sticklebacks, creek chubs and mud minnows were found only in the natural and nongrazed ponds.

Our natural ponds were heavily vegetated, while the grazed ponds had little aquatic or emergent vegetation, due to frequent disturbance. Agricultural and nongrazed ponds were intermediate in aquatic vegetative cover. Natural ponds were 10.9 times more likely to be in a higher emergent vegetation cover category than grazed ponds.

Discussion

Adjacent Land Uses

We did not find support for our hypothesis that breeding ponds surrounded by row crops (corn or soybeans) or grazing are less likely to support amphibian reproduction compared with natural wetlands and ponds surrounded by nongrazed grassland. Previous studies have shown that land uses surrounding the breeding site can affect amphibian mortality and populations (Dodd and Cade 1998). Intensive row crop agriculture has been shown to decrease diversity (Bonin et al. 1997; Hecnar and M'Closkey 1997b) and remaining habitats for amphibians in the Midwest are frequently in close proximity to agricultural land (Knutson et al. 1999; Knutson et al. 2000). Roads and urban development near breeding ponds and long distances to the next nearest pond can also affect amphibian species richness and abundance (Vos and Stumpel 1995; Findlay and Houlahan 1997; Knutson et al. 1999; Lehtinen et al. 1999).

Spatial scale: Landscape vs. Pond

We found support for the idea that pond factors are more closely associated with amphibian reproductive success than landscape factors in our study area. Also, we found weak evidence that landscape factors within 500-1000 m of the pond were most associated with habitat quality. These results compare with Lehtinen et al. (1999); they found that landscape factors at the full range of spatial scales from 500-2500 m away from the pond influenced species richness. Other studies have found that landscape variables explained < 35% of the statistical variation in their data sets (Bonin et al. 1997; Hecnar 1997). In contrast, Beebee (1985) found that pond characteristics, including water chemistry, were not as predictive of amphibian diversity as were landscape variables. Our study ponds were located within a single ecoregion, with presumably less variation in the landscape context among them than would be observed if study areas were located in multiple ecoregions. Landscape factors may play a larger role in amphibian reproductive success where there are larger variations in the landscape features, for example, if some study areas were very isolated from other suitable breeding habitats and others were not.

The relative influence of landscape vs. pond variables has important implications for modeling amphibian habitat quality across larger spatial scales. If detailed information on water quality is needed to assess habitat suitability, GIS-based models will not be sufficient to identify high quality amphibian breeding sites if water quality information is lacking.

Pond Design And Management

Multi-species factors

Our final multi-species model shows that the best-case scenario for overall amphibian reproductive success in the Driftless Area is found in ponds with no fish, low amounts of vegetation and low nitrogen (Fig. 11). The presence of fish interacted synergistically with emergent vegetation and total nitrogen concentrations to reduce the probability of high reproductive success in ponds. When fish and vegetation were absent from a pond, the probability of two or more amphibian species exhibiting high reproductive success was significantly higher at a given nitrogen concentration than when fish were present. For example, amphibians in a pond with no fish or vegetation would have a 0.5 probability of attaining high reproductive success with total nitrogen concentrations of 0.45 mg/l (Fig. 11). With fish present, but no vegetation, the same reproductive success would occur at a total nitrogen concentration of 0.16

mg/l. With both fish and high density of vegetation, the model predicts that reproductive success would not reach 0.1, regardless of total nitrogen concentrations. The incremental effects of vegetation appear greater than those of the presence of fish, but only with fish present is the likelihood of no reproduction feasible. Clearly, and unexpectedly, these three factors combined synergistically to limit reproductive success in these ponds. These results compare with Hecnar and M'Closkey (1998) who found anuran species richness to be more strongly related to the presence of predatory fish and surrounding landscape variables (forest cover) than to water chemistry.

We expected that more vegetation in the pond would be positive for amphibian reproduction, providing more attachment sites for eggs and refuges from predators, but our data indicate the opposite was true. Vegetation variables when they appeared in the models were always negative. We reasoned that perhaps our natural ponds were more likely to have both fish and abundant vegetation and that the vegetation relationships were confounded by the presence of fish. Examination of the data shows that natural ponds were more likely to have fish, but analyses controlling for fish presence still resulted in vegetation variables with a negative relationship with reproductive success. Another possibility is that abundant vegetation causes detection problems, reducing the apparent abundance of larvae and metamorphs. We cannot rule this out as one explanation of these results. However, it is also possible that some of the amphibians we studied are attracted to breeding sites with moderate or low amounts of vegetation rather than heavily vegetated sites (Vogt 1981). This may be especially true for American Toads and Gray Treefrogs.

Predation is an extremely potent factor in the ecology of amphibians -- determining the distribution of many species on both local and regional scales, affecting life history characteristics, and development of noxious dermal and egg membrane secretions (Petranka 1983; Kats et al. 1988; Semlitsch et al. 1988; Broenmark and Edenhamn 1994; Lannoo 1998a). Soft bodies (lack of armouring), slow rates of movement, and propensity to feed in exposed shallow regions of ponds and creeks create a suite of characteristics placing amphibians at particularly high predation risk by predatory fishes (Kats et al. 1988; Broenmark and Edenhamn 1994; Lannoo et al. 1994; Gamradt and Kats 1996; Kiesecker and Blaustein 1998) (Adams 2000).

Biogeographic patterns of salamander and frog distributions in the eastern U.S. have been correlated to the susceptibility of the amphibians to fish predators and the distribution of these predators (Petranka 1983; Kats et al. 1988; Semlitsch et al. 1988). Because of the high risk of predation by fish, most amphibians require fishless habitats to breed and survive. Historically, wetlands and prairie potholes have provided such habitats, remaining fishless due to drought-induced drying and hypoxia with resultant summer- and winter-kills. Recent introductions of fish into many ponds and wetlands has been linked to the decline of several once common amphibians (Broenmark and Edenhamn 1994; Lannoo 1998a). Introductions of American bullfrogs (*Rana catesbeiana*) have also caused declines of amphibians in parts of western U.S. and possibly in the east, where their range has been extended by accidental release (Kiesecker and Blaustein 1998).

In the eastern U.S., several taxa of amphibians do co-occur with fish (e.g., *Rana catesbeiana, Rana clamitans, Bufo americanus,* and *Notophthalmus viridescens*); these species contain either unpalatable eggs or larvae (Kats et al. 1988). Unpalatable species are also the only amphibians found in great abundance in permanent water bodies supporting fish (Petranka 1983; Semlitsch et al. 1988).

Invertebrate predators (e.g., dragonfly, dipteran larvae, and crayfish) also affect microhabitat distribution and competitive interactions of amphibian larvae (Woodward 1983; Van Buskirk 1988; Fauth 1990; Gamradt et al. 1997). There is no indication, however, that under endemic conditions invertebrate predators are as potent as fish at excluding amphibians from either temporary or permanent aquatic habitats. Non-native crayfish have been linked to the decline of salamanders in California (Gamradt et al. 1997), suggesting that under certain conditions the potency of invertebrate predation could match that of fish predators.

Water quality characteristics, such as water temperature, pH, dissolved oxygen concentration, conductivity, turbidity, and nitrogen (total nitrogen, nitrate, and ammonia) and phosphorus can directly (e.g., anoxia, ammonia toxicity) and indirectly (e.g., food web effects, development of noxious algae, etc.) effect amphibian survival, growth, and reproduction. Commonly applied fertilizers, containing nitrogen and phosphorus stimulate the growth of primary producers in aquatic systems.

Several principle outcomes arise in ponds from nutrient enrichment that can affect the health of amphibians. First, because most anuran larvae are herbivores until metamorphosis, grazing on attached algae and phytoplankton, some level of nutrient enrichment may be beneficial to the growth and survival of tadpoles. Large, fast-growing tadpoles metamorphose more rapidly into large frogs than small, slow growing tadpoles. Shorter larval periods result in reduced exposure to predation and competition and result in greater number of adult frogs (Werner 1986). Over-enrichment, however, can pose an environmental hazard for aquatic organisms. Highly productive ponds experience wide swings in dissolved oxygen and pH. Low oxygen and excessively high or low pH can be detrimental to the survival of eggs and larvae (Freda and Gonzalez 1986). In some instances, if nitrate concentrations are high enough, adverse sublethal effects or even mortality may be realized (Baker and Waights 1994; Hecnar 1995). Stress placed on eggs and larvae may not cause direct mortality, but in combination with other stressors (Howe et al. 1998) such factors may prove lethal.

In our study, ponds situated in agricultural and grazed landscapes contained more turbid waters and higher concentrations of dissolved nutrients than those in nongrazed or natural landscapes (Fig. 12). Negative effects of nitrogen on anuran reproductive success observed in this study were not unprecedented (Bishop et al. 1999); what was unusual were the relatively low concentrations that resulted in negative effects. Data summarized by Rouse et al. (1999) show lethal effects of nitrate for a variety of anurans ranged from 14-385 mg/l, while sublethal developmental effects on larvae ranged from 2.5-10 mg/l nitrate. These responses were species and life-stage specific, with early life stages always being more sensitive than adults, and bufonid adults tending to be the least sensitive species and life stage.

In a study combining field surveys and laboratory exposures Bishop et al. (1999) documented reduced amphibian diversity and density in an Ontario, Canada wetland-agricultural complex relative to nearby non-agricultural wetlands. Amphibian diversity, density, and reproductive success were negatively correlated with proximity to nutrient-laden runoff. The proximate causal factor appeared to be reduced reproductive success and abnormalities during early life stage development. Water from agriculturally impacted zones contained relatively high phosphorus (reactive phosphorus: 0.8 mg/l), nitrogen (total Kjeldahl nitrogen: 4.2 mg/l), and ammonia (total ammonia nitrogen: 0.2 mg/l). It is difficult to determine which, if any, of these constituents were causally responsible for the effect seen by Bishop et al. (1999). Despite the uncertainty of causal mechanisms in the field, it is clear from many other studies that nitrogenous compounds have potent negative effects on amphibian development, growth, and survival (Baker and Waights 1993; Baker and Waights 1994) (Huey and Beitinger 1980b, a) (Marco and Blaustein 1999).

Cattle grazing and loafing in water bodies has been long recognized as the cause of negative geomorphological (Trimble and Mendel 1995) and water quality (Waters 1995) conditions. Most attention has been given to impacts of cattle grazing on stream fishes and very little data exists for such impacts on amphibians. Corn and Bury (1989) reported reduced biomass and density of amphibians inhabiting streams from logged compared to unlogged watersheds. These effects were attributed to the increased filling of interstitial spaces in stream sediments critical for the development of larvae. Elevated concentrations of nitrogenous compounds and turbidity in the grazed ponds relative to all other pond clearly indicates cattle as the source of these potential stressors.

Weather patterns during the amphibian breeding season in 2000 and 2001 were contrasting. The spring of 2000 was relatively dry, followed by frequent rains beginning the end of May and continuing through July (NOAA 2000). In 2001, the spring was unusually cool and wet, followed by dry weather from June to August (NOAA 2001).

We found little evidence that pond area or depth were related to amphibian reproductive success. While pond morphometry variables as a group ranked high in the group analysis (Table 2) and in a few of the intermediate step-wise models (Table 3), none of these variables appeared in any of the final summary models. We observed in the field that amphibians attempted to breed whenever water levels and conditions were suitable, and ceased breeding during time intervals when conditions were unsuitable. In some instances, amphibians laid eggs at a pond, the pond dried and the eggs were observed dessicated and dead. Later in the same season, the same species returned to the pond and resumed breeding activities.

Individual Species Factors

We found support for the hypothesis that the Tiger Salamander would be found farther rather than closer to forests (Table 3). Our model for the

American Toad indicates that toads were positively associated with at least one invertebrate predator. This is not too surprising, in that right habitat conditions might support populations of both amphibians and some aquatic invertebrates, who also happen to be larval amphibian predators. The Gray Treefrog model contained the most information of all the individual species models (Table 3). Habitat associations generally followed the all-species model. This suggests that Gray Treefrogs may be a useful representative species for habitat quality in our study area.

Our models did not identify any individual variables associated with pond depth or permanent water for the Green Frog, but the group model indicated that pond morphometry variables were associated with Green Frog reproductive success (Table 2). The Green Frog is a 'sit and wait' predator (Harding 1997), and turbid waters may be less desirable as breeding sites if food resources are difficult to see (Table 3). Landscape variables were associated with Northern Leopard/Pickerel Frog reproduction in the group analysis (Table 2), but only one water quality variable composed the final model (Table 3). We were disappointed that no individual variables were associated with reproductive success for either the Western Chorus Frog or the Spring Peeper. We can only conclude that some factor(s) that we did not measure are more indicative of habitat quality for these species. The group analysis indicates that pond morphometry, pond vegetation, and landscape variables may be important for the Western Chorus Frog, and the landscape and pond morphometry are important for the Spring Peeper, but the functional relationships are difficult to identify without reference to individual variables.

Design and Management

Our findings support the idea that informed farm pond design and management could improve breeding habitat quality for some species of amphibians. The USDA has published engineering guidelines for building farm ponds (Deal et al. 1997). Specific design elements to support amphibian populations would include providing gently sloping shorelines, to provide breeding sites for amphibians, regardless of water levels. Another design consideration is the establishment of aquatic vegetation in the pond. Our data indicates that less, rather than more vegetation is desirable, at least for the set of species we studied.

Pond management guidelines that derive from our results include limiting cattle access to the pond to improve water quality, and avoiding the introduction of fish. If fish populations are already established and removing them is not an option, increasing habitat diversity may help provide refuges for amphibian breeding (Kats et al. 1988; Sih et al. 1988). Wide grassed buffer strips help reduce sediment and water flow into ponds during storm events. Wide buffer strips should also reduce nitrogen input into the ponds, another factor that may suppress amphibian populations.

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Table 1. Habitat predictor variables used in regression analyses based on data collected at farm ponds in Houston and Winona Counties, Minnesota, 2000-2001.

Group	Variable name	Description
Landscape	FOREST500	Total area (ha) of forests within 500 meters of the pond center.
	FOREST1000	Total area (ha) of forests within 1000 meters
	FOREST2500	Total area (ha) of forests within 2500 meters
	GRASSLAND500	Total area (ha) of grassland within 500 meters
	GRASSLAND1000	Total area (ha) of grassland within 1000 meters
	GRASSLAND2500	Total area (ha) of grassland within 2500 meters
	WET_AREA500	Total area (ha) of permanent and temporary wetlands within 500 meters
	WET_AREA1000	Total area (ha) of permanent and temporary wetlands within 1000 meters
	WET_AREA2500	Total area (ha) of permanent and temporary wetlands within 2500 meters
	ROAD_LENGTH500	Total km of all roads within 500 meters
	ROAD_LENGTH1000	Total km of all roads within 1000 meters
	ROAD_LENGTH2500	Total km of all roads within 2500 meters
	STREAM_LENGTH500	Total km of all streams within 500 meters
	STREAM_LENGTH1000	Total km of all streams within 1000 meters
	STREAM_LENGTH2500	Total km of all streams within 2500 meters
	NEAR_WET	Distance (m) to next nearest wetland (all types)
	NEAR_FOREST	Distance (m) to next nearest forest
	SHDI500	Shannon Diversity Index (SHDI) for patch diversity within 500 meters
	SHDI1000	Shannon Diversity Index (SHDI) for patch diversity within 1000 meters
	SHDI2500	Shannon Diversity Index (SHDI) for patch diversity within 2500 meters
	ED_WET500	Edge density (m/ha) of wetland within 500 meters
	ED_WET1000	Edge density (m/ha) of wetland within 1000 meters
	ED_WET2500	Edge density (m/ha) of wetland within 2500 meters
	BARN	Index of distance to nearest confined animal feeding area (within watershed and topographically higher than pond)
Pond morphometry	W_DEPTH_MEAN	Pond depth (decimeters)
	W_DEPTH_DIFF	Maximum difference between minimum and maximum water depths for a pond within a year (decimeters):
	POND_AREA	Pond area (ha) (permanent water directly

Group	Variable name	Description
		associated with study site)
	SLDEEP	Index of the % of shoreline < 1 dm in depth: VEG
	POND_PERIM	Pond perimeter (m) (permanent water directly associated with study site)
	OVER_FLOW	1=observed source of overland flow from confined animal feeding area, 0 = no observed overland flow
Pond vegetation	TREE	Index of % of shoreline composed of trees
	SHRUB	Index of % of shoreline composed of shrubs
	EMER	Index of % of shoreline composed of emergent vegetation
	COVER_ROOT	Index of % cover of non-rooted floating vegetation
	COVER_FLOAT	Index of % cover of rooted floating vegetation
	COVER_EMER	Index of % cover of emergent vegetation
	COVER_SUBM	% cover of submergent vegetation
	ALGAE	Presence or absence of submergent algae
	VEG_SUM	Sum of index values for COVER_ROOT, COVER_FLOAT, COVER_EMER, COVER_SUBM, and ALGAE
Predator community	FISH	Presence or absence of fish in pond
	GRSU	Presence or absence of green sunfish
	BCKSMR	Sum of abundance indices for invertebrates: backswimmer
	DRGFLY	Sum of abundance indices for invertebrates: dragonfly nymph
	LEECHH	Sum of abundance indices for invertebrates: leech
	FSPIDR	Sum of abundance indices for invertebrates: fishing spider
	GWTBUG	Sum of abundance indices for invertebrates: giant water bug
	PDVBTA	Sum of abundance indices for invertebrates: predaceous diving beetle adult
	WATBEE	Sum of abundance indices for invertebrates: water beetle
	WSCRPN	Sum of abundance indices for invertebrates: water scorpion
	AMTRIN	Maximum abundance index for Tiger Salamander larvae
Water quality	TOTNITR	Mean Total nitrogen (mg/L)
	TOTNITR_DIFF	Max difference between min and max TOTNITR for a pond within a year (mg/L)
	TURB	Mean Turbidity (NTU)

Group	Variable name	Description
	TURB_DIFF	Max difference between min and max TURB for a pond within a year (NTU)
	DISOXY	Mean dissolved oxygen (mg/L) (4 subsamples)
	DISOXY_DIFF	Max difference between min and max DISOXY for a pond within a year (mg/L)
	TEMP	Mean temperature (^{o}C)
	TEMP_DIFF	Max difference between min and max TEMP for a pond within a year (^{o}C)
	COND	Mean conductivity (µmhos/cm)
	COND_DIFF	Max difference between min and max COND for a pond within a year (µmhos/cm)
	BIOTIC	Index of Citizen Monitoring Biotic Index for streams and rivers

Table 2. Sets of models from logistic regression model testing for groups of variables for farm ponds in Houston and Winona Counties, Minnesota, 2000. The models are shown ranked by AIC_C.

Species	Models	Number of vriables	AICc	R^2
All Species	Water Quality	11	55.7	0.80
	Pond Morphometry[a]	5	59.1	0.30
	Predator Community[b]	10	60.3	0.64
	Pond Vegetation	8	65.2	0.40
	Landscape (500)	10	68.1	0.51
	Landscape (1000)	10	74.1	0.37
	Landscape (2500)	10	75.0	0.35
American Toad	Pond Morphometry	6	91.2	0.27
	Landscape (2500)	10	93.5	0.51
	Pond Vegetation	8	94.8	0.34
	Landscape (500)	10	99.6	0.40
	Predator Community	11	100.0	0.48
	Landscape (1000)	10	102.2	0.35
	Water Quality[c]	11	114.4	0.18
Western Chorus Frog	Pond Morphometry[a]	5	64.2	0.11
	Pond Vegetation[d]	7	66.7	0.22
	Landscape (1000)[e]	9	69.1	0.34
	Water Quality	11	70.6	0.49
	Landscape (500)[e]	9	70.6	0.30
	Landscape (2500)[e]	9	73.4	0.22
	Predator Community[b]	10	79.0	0.17
Gray Treefrog	Water Quality	11	83.7	0.73
	Pond Morphometry[a]	5	84.4	0.40
	Predator Community[b]	10	92.0	0.57
	Landscape (1000)[e]	9	92.3	0.51
	Landscape (500)[e]	9	96.2	0.44
	Landscape (2500)[e,f]	9	98.4	0.40
	Pond Vegetation[f]	8	103.4	0.22
Green Frog	Pond Morphometry[a]	5	91.2	0.16
	Water Quality	11	96.5	0.51
	Pond Vegetation[f]	8	99.9	0.20
	Landscape (1000)[g]	10	100.9	0.35
	Landscape (500)	10	104.6	0.27
	Landscape (2500)[f]	10	108.8	0.18
	Predator Community	11	109.1	0.27

Species	Models	Number of vriables	AICc	R^2
Northern Leopard or Pickerel Frogs	Landscape (500)	10	94.4	0.49
	Landscape (1000)	10	94.7	0.48
	Pond Morphometry[a]	5	95.4	0.08
	Landscape (2500)	10	95.9	0.46
	Pond Vegetation	8	99.3	0.24
	Water Quality	11	99.6	0.48
	Predator Community[b]	10	105.8	0.27
Spring Peeper	Landscape (500)[e]	9	61.2	0.50
	Pond Morphometry[a]	5	61.5	0.16
	Landscape (2500)[e]	9	64.4	0.43
	Landscape (1000)[e]	9	65.0	0.42
	Pond Vegetation	8	67.6	0.26
	Water Quality	11	70.4	0.48
	Predator Community[b]	10	73.1	0.31
Tiger Salamander	Pond Vegetation[d,h]	6	38.6	0.48
	Landscape (500)	10	42.9	0.75
	Water Quality[i]	10	45.5	0.68
	Pond Morphometry[a]	5	46.1	0.10
	Landscape (2500)	10	46.6	0.65
	Landscape (1000)	10	50.1	0.55
	Predator Community[b,j]	7	50.6	0.16

[a] Variable OVER_FLOW removed to allow maximum likelihood estimates to converge.
[b] Variable GRSU removed to allow maximum likelihood estimates to converge.
[c] Failed the proportional odds assumption, $P < 0.0001$.
[d] Variable COVER_FLOAT removed to allow maximum likelihood estimates to converge.
[e] Variable BARN removed to allow maximum likelihood estimates to converge.
[f] Failed the proportional odds assumption, $P < 0.01$.
[g] Failed the proportional odds assumption, $P < 0.02$.
[h] Variable TREE removed to allow maximum likelihood estimates to converge.
[i] Variable TNITR_DIFF removed to allow maximum likelihood estimates to converge.
[j] Variables AMTRIN, GWTBUG and WSCRPN removed to allow maximum likelihood estimates to converge.

Table 3. Predictor variables significant at $\alpha = 0.05$ from stepwise logistic regression for farm ponds in Houston and Winona Counties, Minnesota, 2000. The summary models resulted from combining the significant individual variables from each group above it in a final model.

Species	Model	Variables	Odds ratio	Param. Est.	SE	Variable P-value	Model P-Value	AICc	R^2
All Species	Pond Vegetation	COVER_EMER	0.2	-1.5	0.7	0.02	0.01	51.9	0.21
	Predator Community	FISH	0.04	-3.2	1.4	0.02	0.0003	43.8	0.51
		BCKSWMR	1.9	0.7	0.3	0.04			
		WATERBEE	4.1	1.4	0.5	0.01			
	Water Quality	TOTNITR	0.003	-5.9	2.7	0.03	<0.0001	37.7	0.59
		COND	1.0	-0.01	0.004	0.005			
	Summary (binary)	COVER_EMER	0.04	-3.3	1.5	0.03	<0.0001	28.4	0.79
		TOTNITR	0.001	-17.7	7.8	0.02			
		FISH	0.01	-5.0	2.0	0.01			
American Toad	Pond Vegetation	SHRUB	0.6	-0.5	0.2	0.01	0.02	82.4	0.15
	Predator Community	BCKSMR	2.2	0.8	0.3	0.002	0.001	76.2	0.30
	Summary (ordinal)	BCKSMR	2.2	0.8	0.3	0.002	0.001	76.2	0.30
Gray Treefrog	Landscape500	ED500	0.9	-0.1	0.04	0.04	0.003	82.4	0.22
	Pond Morphometry	POND_AREA	44.4	3.8	1.6	0.02	0.001	78.8	0.34
		POND_PERIM	1.0	-0.02	0.01	0.004			
	Pond Vegetation	VEG_SUM	0.8	-0.3	0.1	0.04	0.03	86.3	0.13
	Predator Community	FISH	0.02	-3.8	1.2	0.001	<0.0001	82.0	0.23
		WATBEE	2.8	1.0	0.4	0.004			
	Water Quality [a]	TOTNITR	0.02	-3.9	1.6	0.01	<0.0001	59.4	0.65
		COND	1.0	-0.02	0.005	0.0002			
	Summary (ordinal)	TRTMT (NGRAZ vs NAT)	41.9	1.8	0.7	0.01	<0.0001	71.3	0.61
		TRTMT (AGRIC vs NAT)	11.7	0.6	0.6	0.36			
		TRTMT (GRAZ vs NAT)	4.0	-0.5	0.7	0.47			
		VEG_SUM	0.6	-0.5	0.2	0.01			
		TOTNITR	0.02	-4.0	1.6	0.01			

Species	Model	Variables	Odds ratio	Param. Est.	SE	Variable P-value	Model P-Value	AICc	R^2
Green Frog	Landscape500	ROAD_LENGTH500	0.5	-0.6	0.3	0.04	0.04	82.1	0.12
	Pond Vegetation	EMER	1.5	0.4	0.2	0.04	0.04	82.2	0.12
	Predator Community	DRGFLY	1.9	0.6	0.3	0.05	0.04	82.3	0.12
	Water Quality	TURB	1.0	-0.05	0.02	0.02	0.01	78.7	0.21
	Summary (binary)	TURB	0.9	-0.1	0.02	0.01	0.001	49.3	0.31
Northern Leopard or Pickerel Frog	Pond Vegetation	ALGAE	0.2	-1.4	0.7	0.04	0.03	82.8	0.13
	Predator Community	WATBEE	1.8	0.6	0.3	0.04	0.03	82.9	0.13
	Water Quality	COND	1.0	-0.004	0.002	0.04	0.001	81.2	0.17
	Summary (ordinal)	COND	1.0	-0.004	0.002	0.04	0.001	81.2	0.17
Tiger Salamander	Landscape500	NEAR_FOREST	1.0	0.01	0.01	0.04	0.01	31.7	0.26
	Summary (binary)	NEAR_FOREST	1.0	0.01	0.01	0.04	0.01	31.7	0.26
Western Chorus Frog	ALL	NULL							
Spring Peeper	ALL	NULL							

[a] The variables in this model were collinear. The summary model avoids collinearity and is therefore the better model, even though AIC is larger.

Table 4. Validation results, 2001 data analyzed using 2000 models.

	Model Predictors	2000[a] Sommers' D	2000[a] % Conc.	2000[a] R^2	2001 Sommers' D	2001 % Conc.	2001 R^2
All Species (binary)	COVER_EMER TOTNITR FISH	0.93	96	0.79	0.49	75	0.22
American Toad (ordinal)	BCKSMR	0.46	65	0.30	0.23	51	0.07
Eastern Gray Tree Frog (ordinal)	VEG_SUM TOTNITR TRTMT (AGRIC vs NATUR) TRTMT (GRAZ vs NATUR) TRTMT (NGRAZ vs NATUR)	0.86	88	0.61	0.52	76	0.34
Green Frog (binary)	TURB	0.45	72	0.31	0.24	62	0.11
Northern Leopard or Pickerel Frog (ordinal)	COND	0.48	66	0.17	0.14	56	0.04
Tiger Salamander (binary)	NEAR_FOREST	0.75	87	0.26	0.54	77	0.17
Western Chorus Frog (binary)	NULL						
Spring Peeper (binary)	NULL						

[a]Statistics for 2000 provided for comparison with 2001. Models were built from 2000 data.

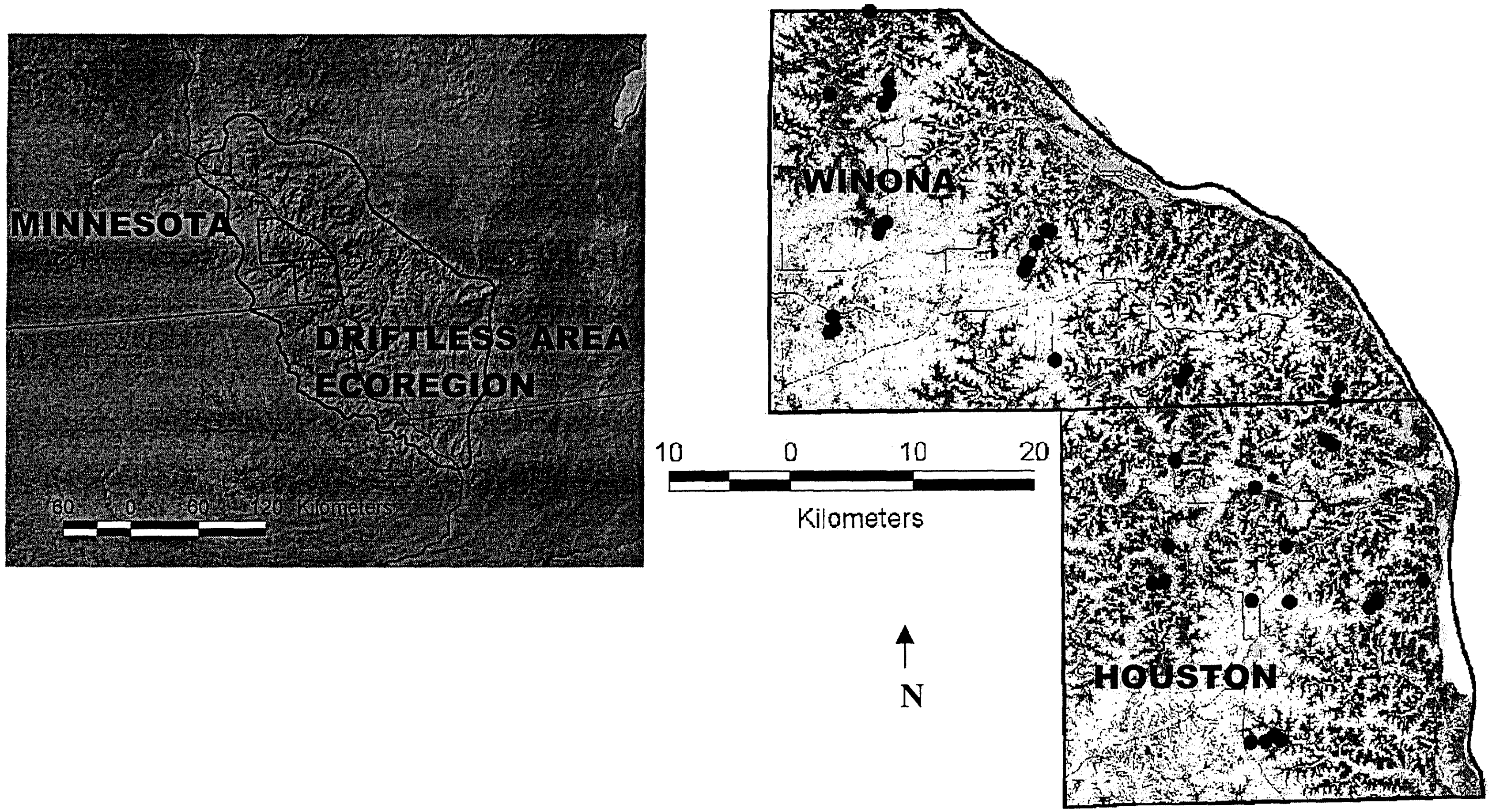


Figure 1. Farm pond study sites in southeastern Minnesota, Houston,and Winona counties.

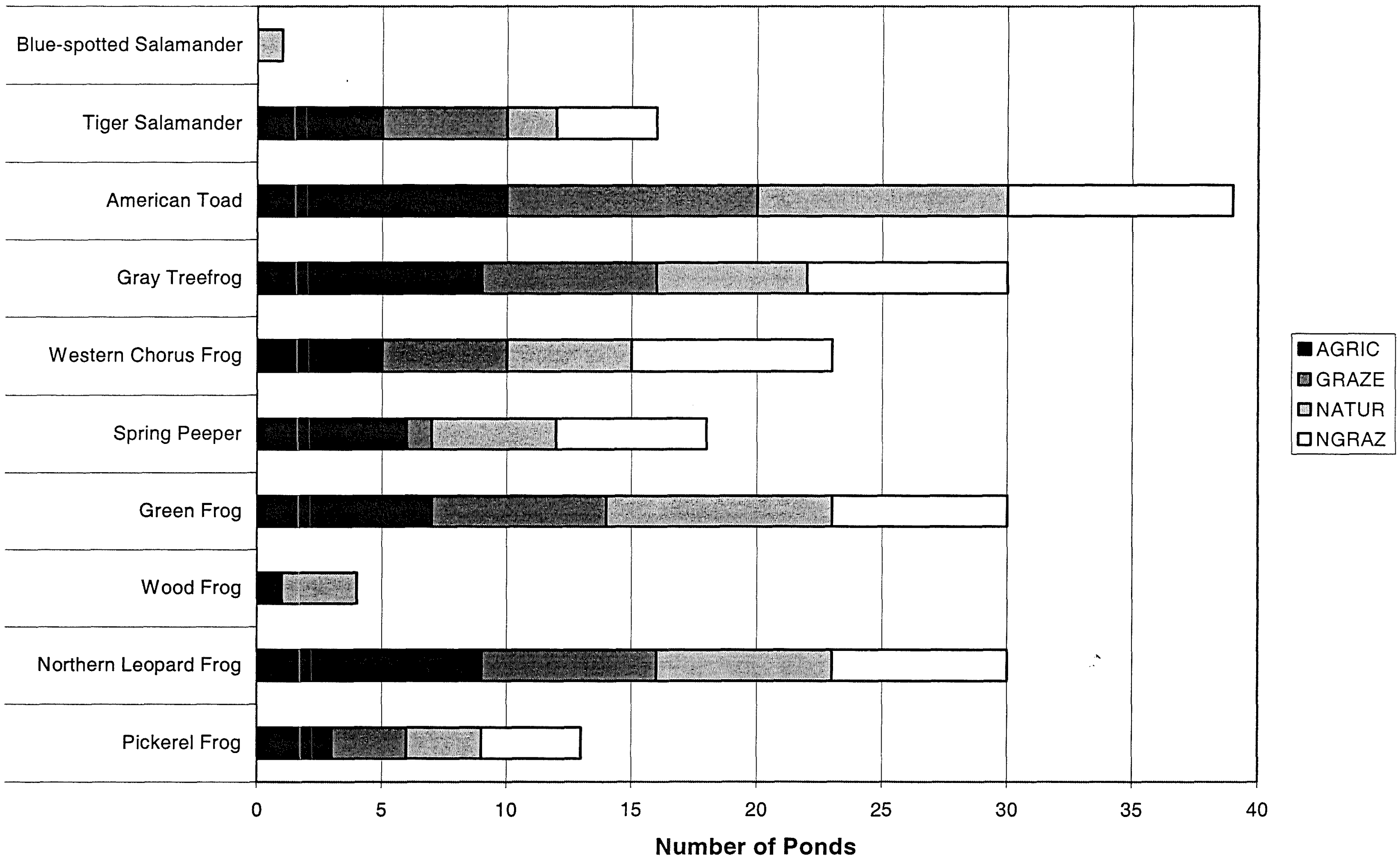


Figure 2. Amphibian species present in 40 ponds and four types of surrounding land uses, based on amphibian egg mass and larval dipnet surveys, for farm ponds in Houston and Winona Counties, Minnesota, 2000-2001. Pond types include natural (A_NATUR), nongrazed (B_NGRAZ), grazed (C_GRAZE), and agricultural (D_AGRIC).

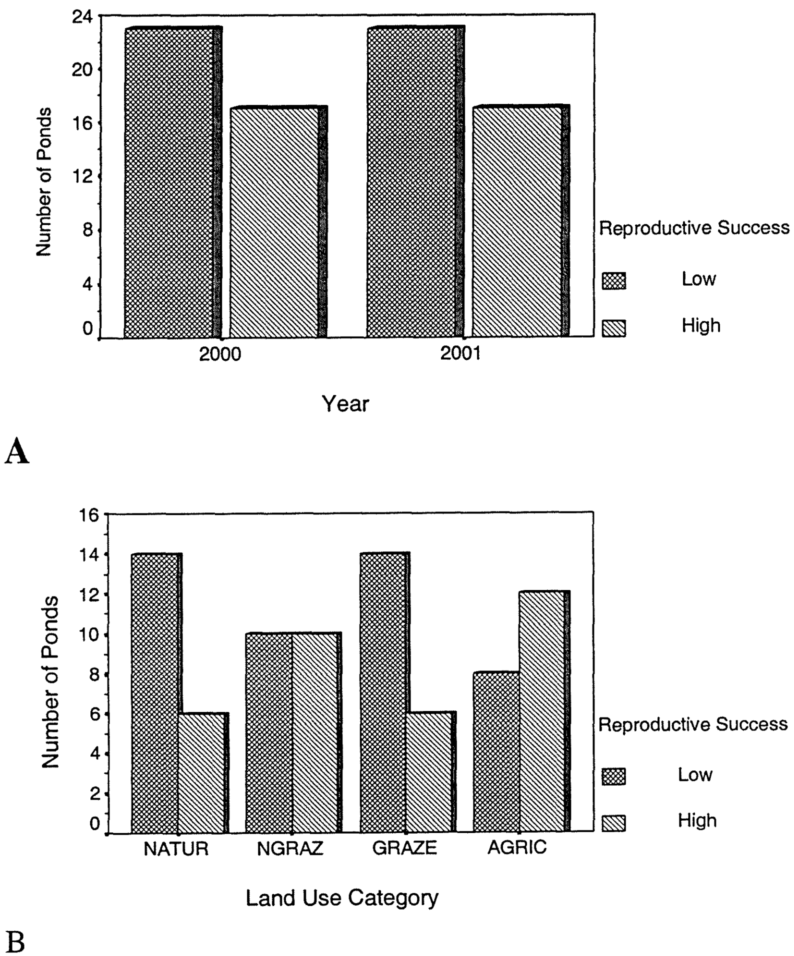


Figure 3. Number of ponds with low and high reproductive success for all species combined by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

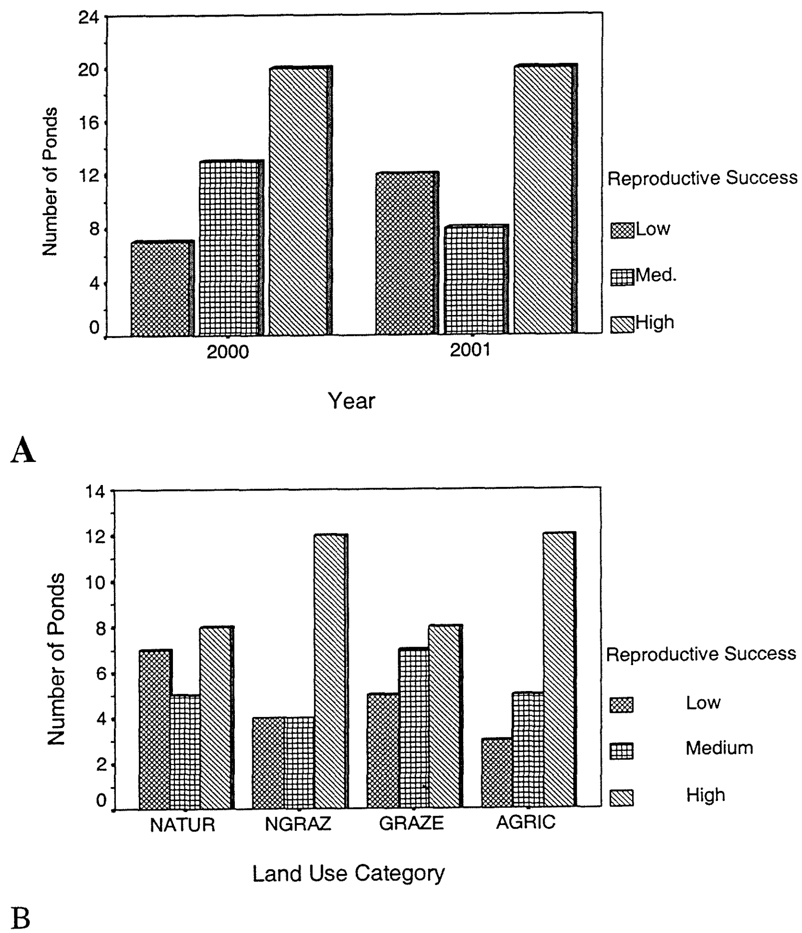


Figure 4. Number of ponds with low, medium, and high reproductive success for the American Toad, by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

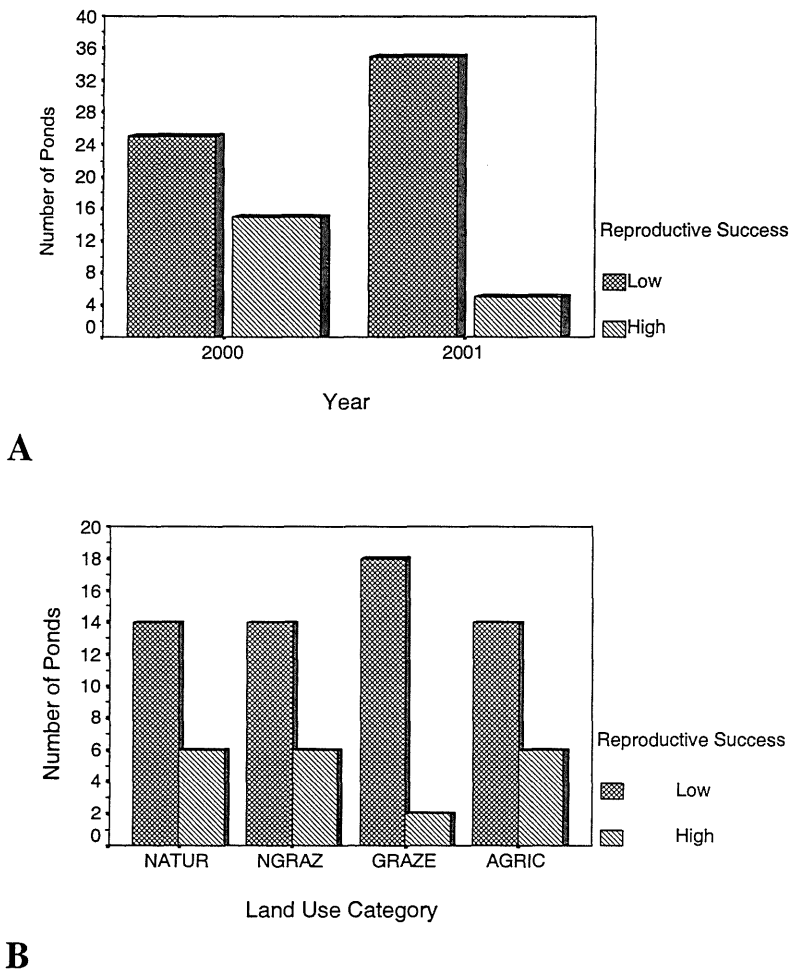


Figure 5. Number of ponds with low and high reproductive success for the Western Chorus Frog by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

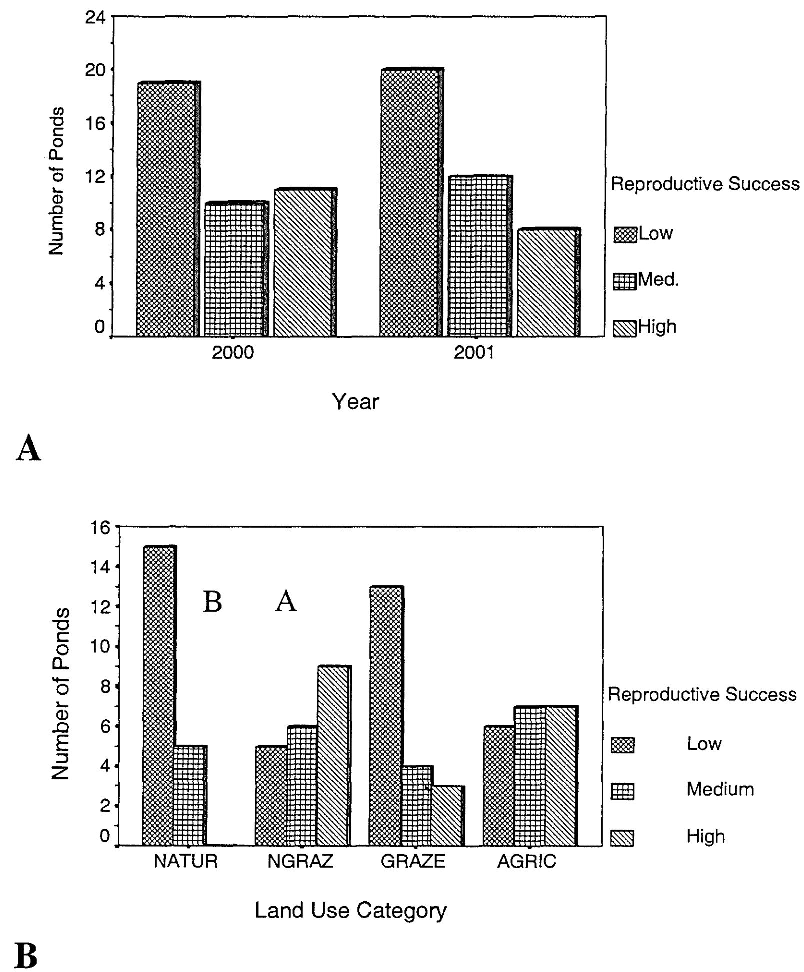


Figure 6. Number of ponds with low, medium, and high reproductive success for the Gray Treefrog, by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

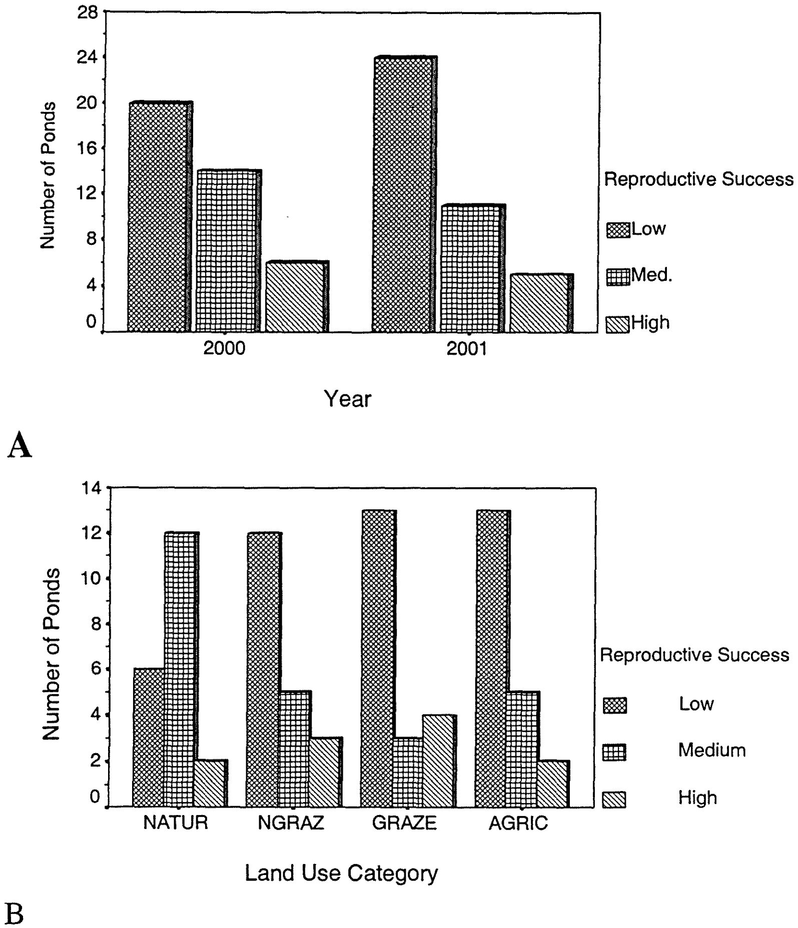


Figure 7. Number of ponds with low, medium, and high reproductive success for the Green Frog, by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

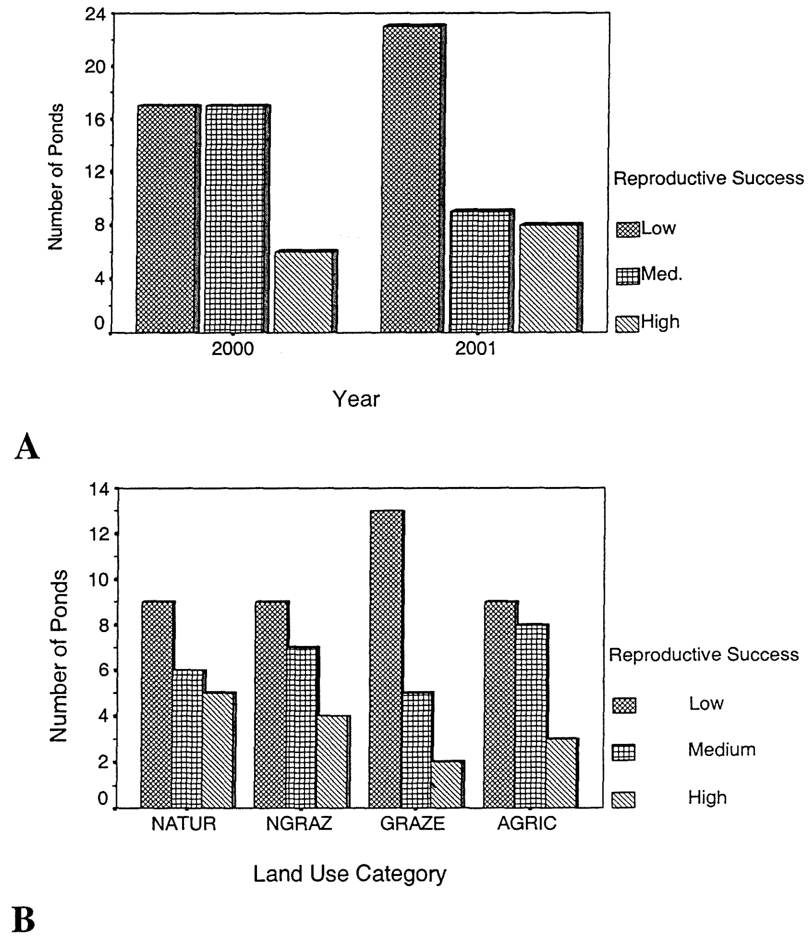


Figure 8. Number of ponds with low, medium, and high reproductive success for the Northern Leopard/Pickerel Frog, by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

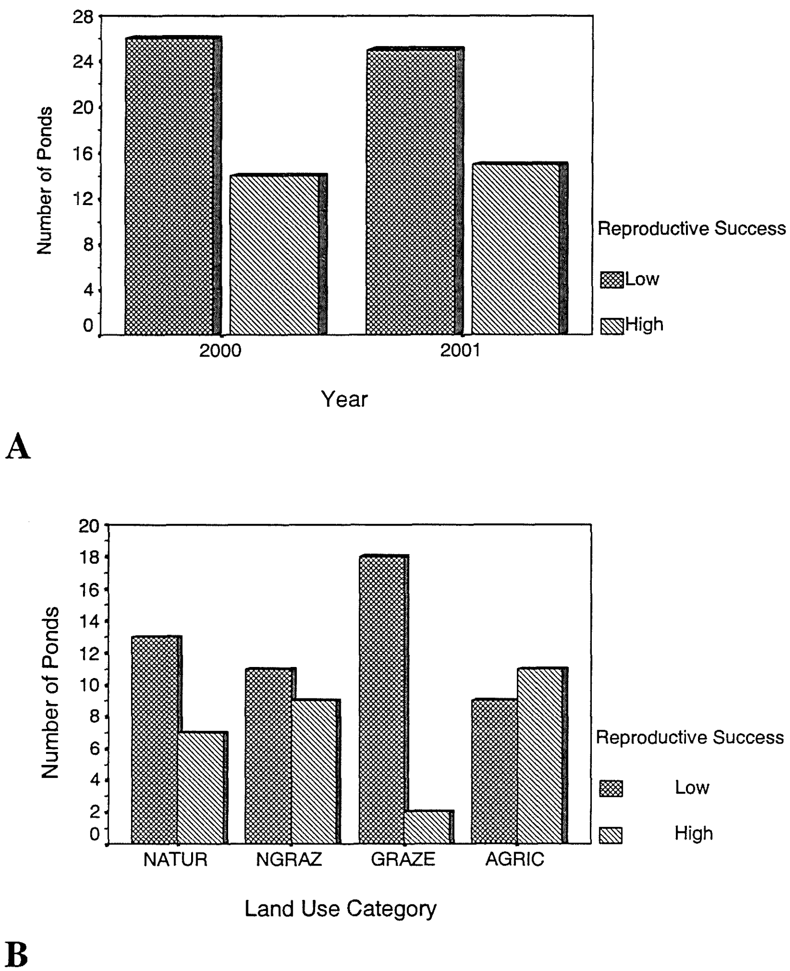


Figure 9. Number of ponds with low and high reproductive success for the Spring Peeper by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

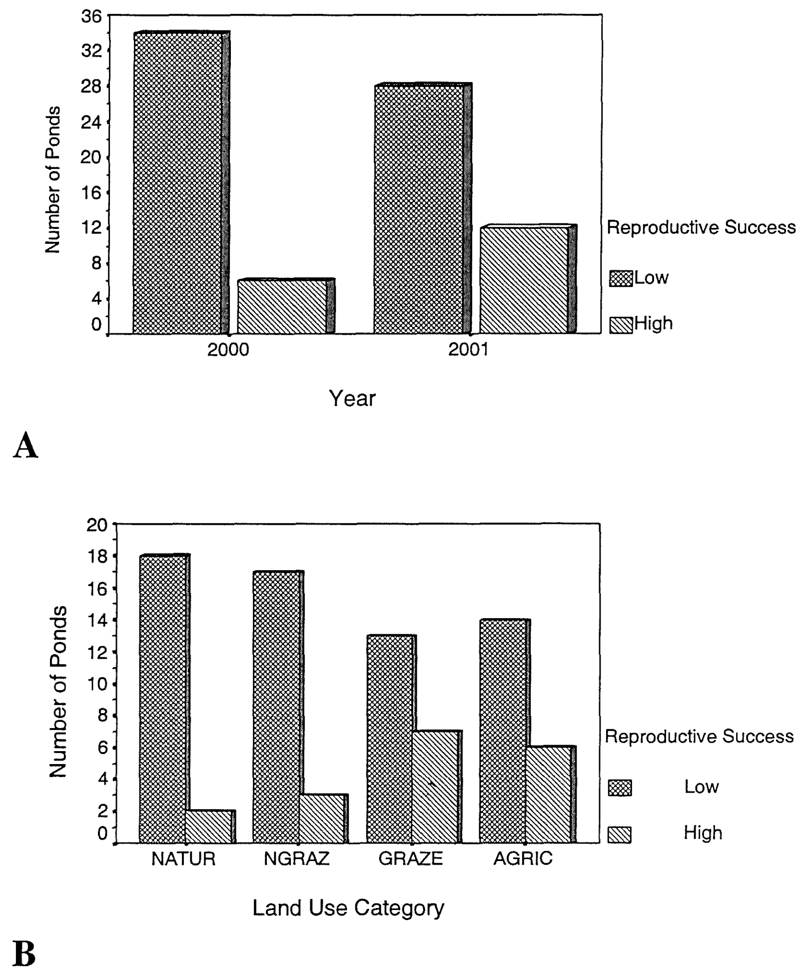


Figure 10. Number of ponds with low and high reproductive success for the Tiger Salamander by year (A) and by surrounding land use (B) in Houston and Winona Counties, Minnesota, 2000 and 2001, pooled. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

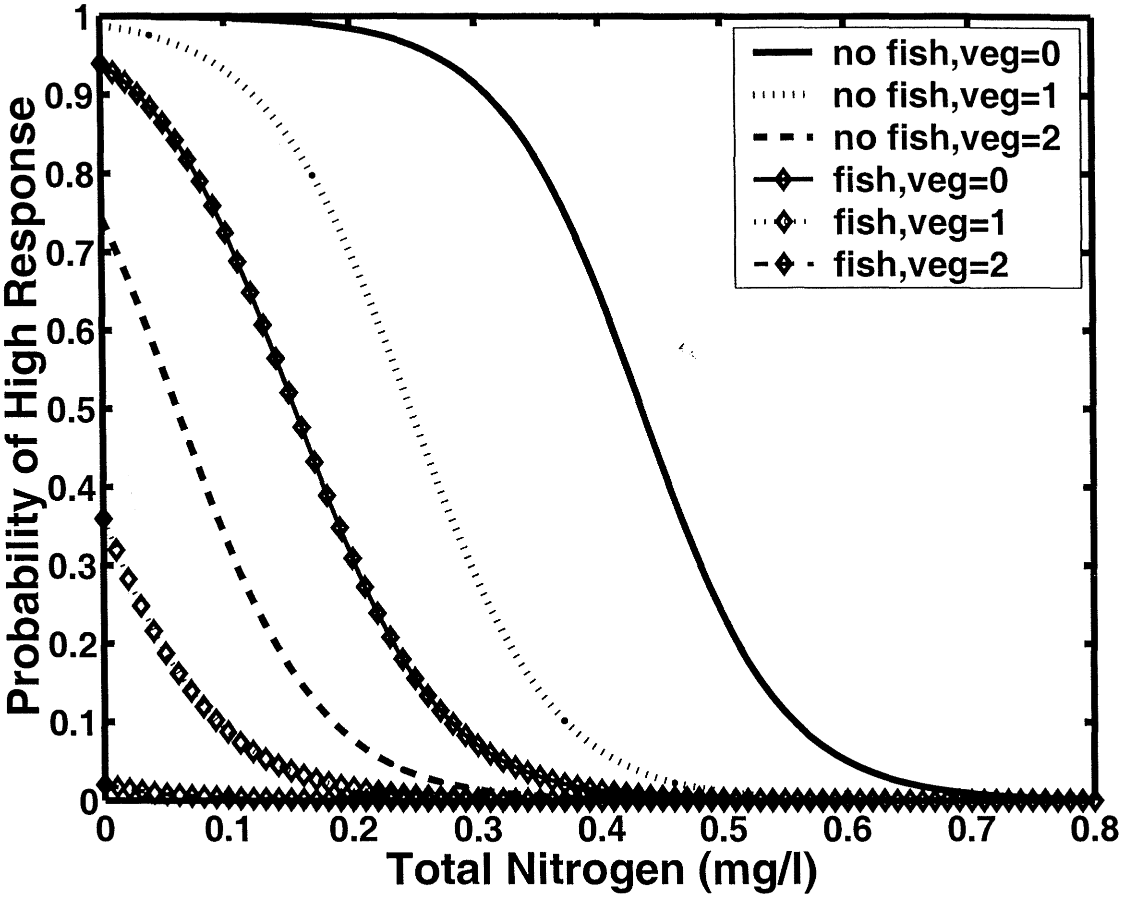
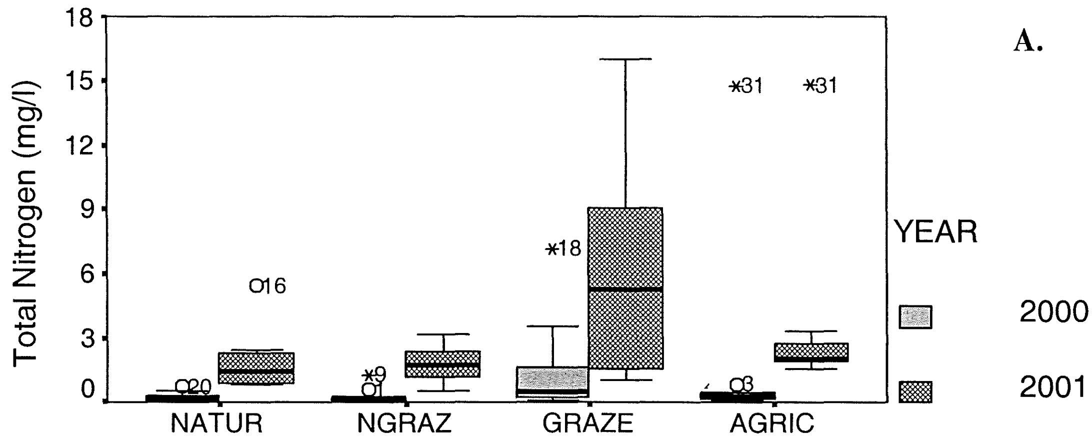


Figure 11. Effects of total nitrogen, adjusted for vegetation and fish presence, on the probability of high reproductive success for 2 or more amphibian species in farm ponds in Houston and Winona Counties, Minnesota, 2000.



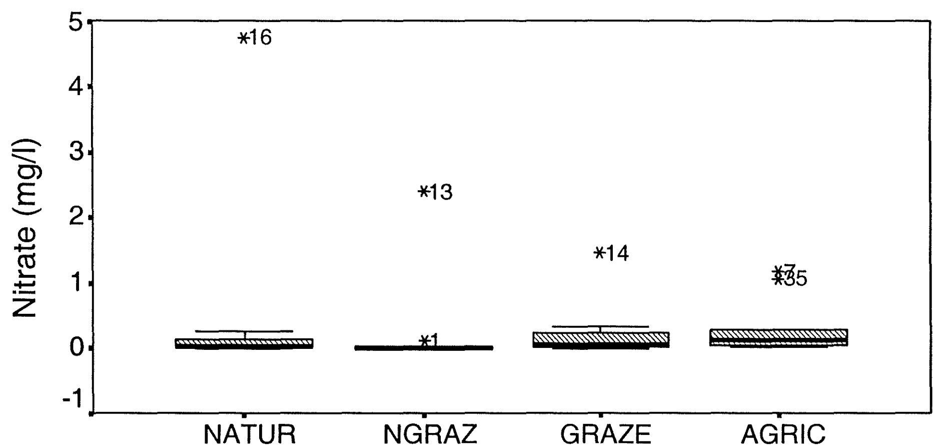
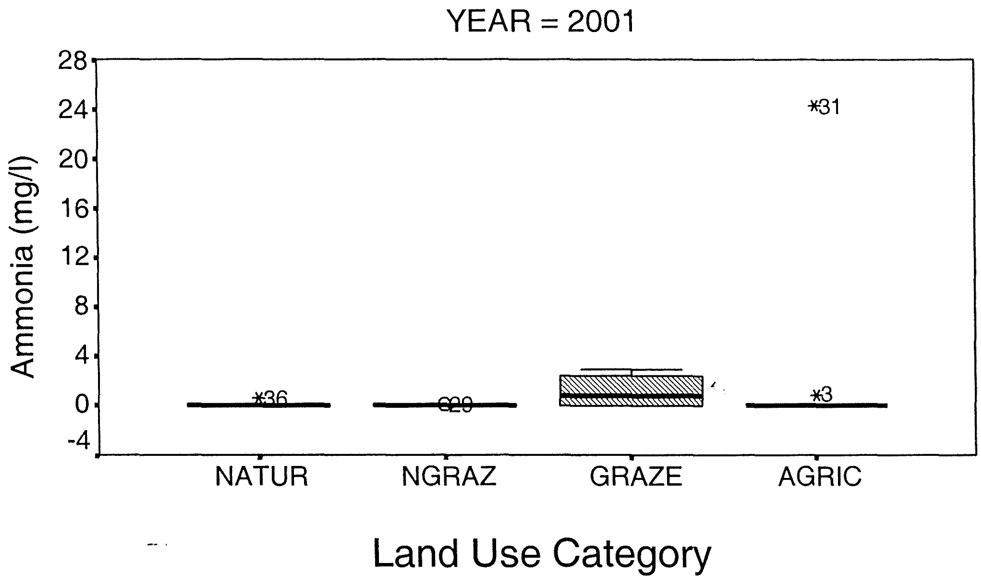
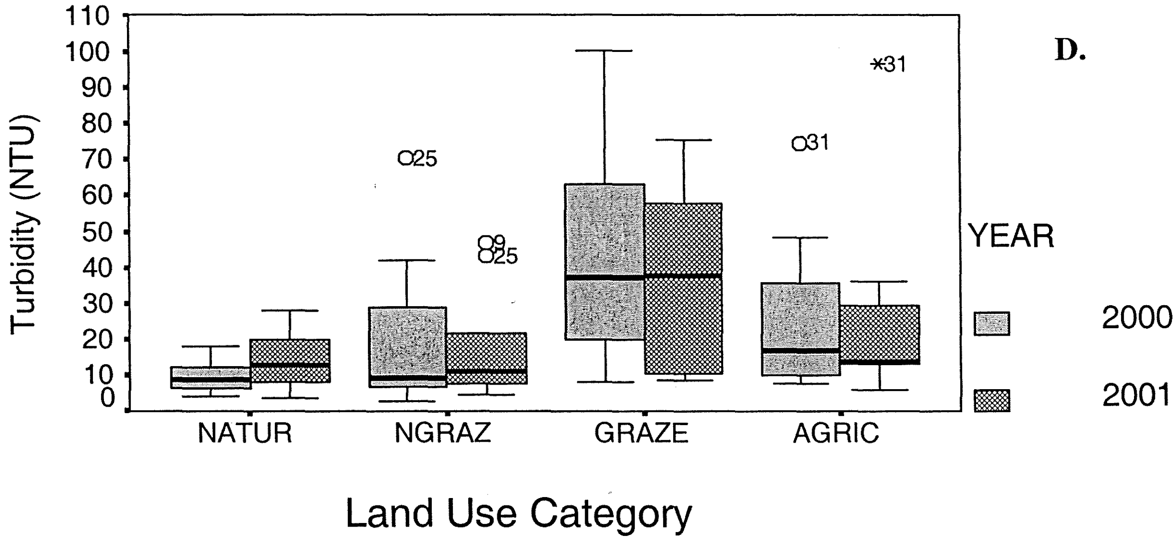


Figure 12. Box and whisker plots showing median (bar inside box), 25th and 75th quartiles (box surrounding bar), and range (excluding outliers) for (A) total nitrogen, (B) nitrate, (C) ammonia, and (D) turbidity in farm ponds with different adjacent land uses, Winona and Houston counties, southeast Minnesota. Nitrate and ammonia were measured in 2001 only. The data are presented as box plots with the median bar and the first and third interquartile ranges identified, representing the central 50% of the values. The whiskers show the range of values falling within the inner fence (1.5*quartile spread). Circles represent values outside the inner fence. Extreme values, values outside the outer (3*quartile spread), are plotted with asterisks. Land use categories include natural (NATUR), nongrazed (NGRAZ), grazed (GRAZE), and agricultural (AGRIC).

C.



D.



(Site ID is displayed for outliers and extreme values)

Figure 12. Continued.

Effects of Agricultural Land Use on the Survival of Anuran Larvae in Constructed and Natural Ponds in the Upper Midwest

by

Joshua M. Kapfer

Farm Ponds as Critical Habitats for Native Amphibians: Final Report

A Thesis Submitted to the
Department of Biology and the
Faculty of the University of Wisconsin-La Crosse
La Crosse, Wisconsin
In Partial Fulfillment of the Requirements for the
Degree of Master of Science in Biology
(August 2002)

and to the

Legislative Commission on Minnesota Resources
100 Constitution Avenue, Room 65
St. Paul, Minnesota 55155-1201

June 2002

U.S. Geological Survey
Upper Midwest Environmental Sciences Center
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La Crosse, Wisconsin 54603

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Effects of Agricultural Land Use on the Survival of Anuran Larvae in Constructed and Natural Ponds in the Upper Midwest

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Abstract

Global declines in amphibian populations are, in part, because of loss of habitat. Consequently, artificially constructed habitats, such as farm ponds, may be important for maintaining regional amphibian populations. The objective of this study was to assess the potential toxicity of water from agricultural ponds on anuran eggs and larvae. Mesocosms were placed in six constructed ponds adjacent to row crops (primarily corn and soybeans) and in four natural ponds. Mesocosms were stocked with embryos of the northern leopard frog (*Rana pipiens*) that were allowed to develop through metamorphosis. Differences in mortality of leopard frogs between constructed ponds adjacent to row crops and natural ponds were assessed. Concurrently, the Frog Embryo Teratogenesis Assay *Xenopus* was used to assess effects of pond water from agricultural, grazed, non-grazed, and natural ponds (2000), and agricultural and natural ponds (2001) on development and survival of the African clawed frog (*Xenopus laevis*). Additionally, concentrations of total nitrogen, total phosphorus, ammonia, and selected pesticides (e.g., atrazine and alachlor) were measured in ponds and compared to survival of *X. laevis*. There were no significant differences in survival of *X. laevis* larvae among agricultural, grazed, non-grazed, and natural ponds during 2000. In addition, no significant difference in the survival of *X. laevis* larvae between agricultural and natural ponds was detected during 2001. Finally, no significant difference in the survival of *R. pipiens* held in mesocosms from agricultural and natural ponds was detected during 2001. While concentrations of pesticides and nutrients detected were generally higher in agricultural ponds than other ponds, neither nutrients nor pesticides had a biologically significant effect on larval survival. Results suggested that, from a standpoint of water quality, farm ponds within the Driftless Area Ecoregion of Minnesota make suitable habitats for larval anurans.

Key words: amphibian, agriculture, farm pond, FETAX, mesocosm, *Rana pipiens*, *Xenopus laevis*

Introduction

Worldwide declines in amphibian populations and observations of malformed anurans have increased concerns about the global health and local status of amphibian populations and have provided the impetus for a substantial amount of research within the past several decades (Souder 2000). This research has primarily focused on determining mechanism(s) causing declines and malformations in amphibians. Several hypotheses have been proposed to explain these events, including both natural (e.g., parasitic infections or disease) and anthropogenic causes (e.g., toxicants and habitat destruction). However, a causative factor for declining amphibian populations and malformed anurans has yet to be determined (Rosenberry 2001; Souder 2000).

Expansion and persistence of agricultural tracts and urbanized areas negatively affect amphibian populations by eliminating habitats and generating toxicants. For example, significantly fewer species of anurans were found near an agricultural site in Ontario than in areas upstream or downstream from the site (Bishop et al. 1997). In addition, American toads (*Bufo americanus*) and green frogs (*Rana clamitans*) grew abnormally and had reduced reproductive success in areas downstream from these same agricultural sites (Bishop et al. 1997). These adverse effects may have been because of toxicosis from organophosphate and organochlorine pesticides, as well as ammonia from agricultural run-off (Bishop et al. 1997). Knutson et al. (1999) reported that the abundance of anuran species in Iowa was negatively associated with the presence of agricultural land, but the abundance of anuran species in Wisconsin was positively associated with agricultural land. They suggested that this may be because of a greater amount of refuge habitats and less cropland within Wisconsin than in Iowa. For example, an estimated 13,000,000 ha or 91.7% of the total surface area of Iowa was used for agriculture in 2000 (Sands and Parks 2001; United States Census Bureau 2002). Conversely, the total area used for agriculture within Wisconsin during 2000 was more than 6,000,000 ha, or only 46.6% of the total area of the state (Wisconsin Agricultural Statistics Service 2001; United States Census Bureau 2002).

Agriculture may negatively affect amphibians because of the release of chemicals used for crop production into the aquatic habitats used by anurans for breeding and overwintering by adults, as well as terrestrial habitats required for dispersal and feeding (Beebee 1996; Burkhart et al. 1998; Carey and Bryant 1995; Fort et al. 1999; Helgen et al. 1998). For example, Allran and Karasov (2001) found atrazine, a triazine herbicide that is commonly used in agriculture, to have lethal and sublethal effects on several species of anurans native to the Upper Midwest. Furthermore, aquatic herbicides may reduce the abundance of vegetation used by tadpoles as a source of food and as cover from predators.

Because anurans and caudates have complex life cycles, exposure to environmental contaminants during critical periods of growth, such as embryo development and tadpole metamorphosis, must be considered. The high permeability of the amphibian integument, as well as a need to regularly absorb water to avoid desiccation, can greatly contribute to absorption of toxic substances that may exist in aqueous solutions during developmental stages (Duellman and Trueb 1986; Pough et al. 1998; Stebbins and Cohen 1995).

Amphibians are also at risk from several types of fertilizers. An estimated 24.8×10^9 kg of nitrogen fertilizers are annually produced in the United States and Canada, and they are a major source of pollution in both water and soil

(Hecnar 1995). Several studies have found that amphibians are affected by nitrogen at concentrations below those that occur in public water supplies and agricultural areas (Hecnar 1995; Marco et al. 1999). Concentrations of nitrate in North American watersheds can range from less than 1 mg/L to more than 100 mg/L. Though the sensitivity of species to nitrates differs, 2.4 to 100 mg/L nitrate have lethal and sublethal effects on amphibians (Marco et al. 1999; Rouse et al. 1999). However, some studies suggest that anurans are not as sensitive to certain nitrogen compounds (e.g., ammonia) as several fish species; therefore, water quality regulations established for fish may also protect anurans (Jofre and Karasov 1999).

Artificial farm ponds, constructed with cost-sharing dollars from the U.S. Department of Agriculture for the purpose of preventing soil erosion, may act as suitable habitat for local wildlife, including amphibians. However, few studies have been conducted within the Upper Midwest on the suitability of agricultural ponds as habitats for local fauna.

Several laboratory assays, such as the Frog Embryo Teratogenesis Assay *Xenopus* (FETAX), have been used to determine whether water collected from sites with large numbers of malformed anurans can induce malformations or mortality in the African clawed frog (*Xenopus laevis*). In these studies, embryos of *X. laevis* cultured in the pond water and in sediment extracts developed malformations and displayed high mortality (Burkhart et al 1998; Fort et al. 1999; but see Tietge et al. 2000). However, few field studies have been conducted with *in situ* field methods or mesocosms to study the effects of agricultural pond water on amphibians.

Mesocosms have been suggested as an appropriate means of obtaining data from field experiments that possess greater ecological relevance than that obtained from laboratory studies alone (Caquet et al. 1996; Odum 1984; Rowe and Dunson 1994; however, see Carpenter 1996). Odum (1984) described mesocosms as "partly enclosed outdoor experimental setups..falling between laboratory *microcosms* and the large, complex real world *macrocosms.*" He also suggested that mesocosms are a suitable means for bridging the gap between laboratory and field studies. Furthermore, mesocosms may accurately represent small water bodies, such as ponds (Williams et al. 2002). However, others suggested that as attempts are made to better mimic nature within a mesocosm, complexity is increased and replicability becomes harder to achieve (Caquet et al. 1996; Crossland and La Point 1992).

Although many toxicological studies have incorporated mesocosms, few have used amphibian test subjects. Because of time and personnel constraints, previous studies used small enclosures (<1 m in diameter), or enclosures that did not allow developing larvae access to pond sediments (Bishop et al. 2000; Cooke 1981; Harris and Bogart 1997; T. Edblom, Department of Natural Resources, Madison, Wisconsin, personal communication; N. Shappell, U.S. Department of Agriculture, Fargo, North Dakota, personal communication). Amphibians that hibernate or aestivate in contaminated sediments may be susceptible to toxicant-induced mortality; therefore, exposure to contaminated sediments may affect mortality and malformation (Fort et al.

1999; Helgen et al. 1998; Rowe and Dunson 1994). In addition, mesocosms used in several studies were simulated ponds created from basins (such as cattle tanks) and not enclosures placed within previously existing wetlands (Caquet et al. 1996; Morin 1986; Rowe and Dunson 1994).

Therefore, because of (1) the potentially negative effect of agriculture on amphibians, (2) the high amount of amphibian malformations and mortality observed in many areas of the United States, and (3) a limited number of *in situ* field studies to determine the effects of agricultural pond water on amphibian survival (Souder 2000), this study was designed to determine the effects of agricultural pond water on amphibian survival. The objective was to determine (1) if water from agricultural ponds in two southeastern Minnesota (USA) counties had any effect on the survival and development of larval anurans and (2) if there was an association between amphibian survival and nutrient and pesticide concentrations in the pond water.

Methods

Study Area

The Driftless Area Ecoregion of the Upper Midwest is about 41,986 km^2 and represents an area that was not glaciated during the Wisconsin glaciation (Albert 1995; McNab and Avers 1994). This area is mostly in southwestern Wisconsin, but also contains portions of southeastern Minnesota, northeastern Iowa, and northwestern Illinois, and has topography with relatively large relief and bedrock composed of mostly limestone, sandstone, and dolomite. Using geographical information systems, 10 points in Houston and Winona counties (Minnesota) were randomly selected. From each random point, the nearest ponds falling into several treatment categories were selected, and, if landowner permission was granted, these ponds were included in this study. In 2000, the nearest agricultural (n=10), natural (n=6), grazed (n=5), and nongrazed (n=5) ponds were used in this study, while in 2001, only agricultural (n=9) and natural (n=10) ponds were used. Ponds were considered agricultural if they were artificial and a grass buffer of <30 m existed between it and row crops. Ponds were considered nongrazed if they were artificially constructed, yet had no row crops, or livestock confinements, and were not grazed by cattle. Finally, ponds were considered grazed if livestock were allowed free access to the water's edge. Conversely, ponds were considered natural if they were not artificial and were >80 m from barnyards or livestock confinements. Although an attempt was made to include an equal number of ponds within each treatment category, limited resources and pond desiccation did not make this possible. Because of their scarcity, several of the 'natural' ponds in this study were either lightly grazed or adjacent to cropland. In addition, distances to roads were not considered in selecting ponds for study. The area, average maximum depth, and dominant land cover associated with each pond were recorded for both years (Tables 1 and 2).

Test Species

The *X. laevis* was used during laboratory assays, and *Rana pipiens* (northern leopard frog) was used during field studies. *Xenopus laevis* was chosen because it is the species suggested for use in FETAX (American Society for Testing and Materials 1998). Although commonly found throughout the Upper Midwest, *Rana pipiens* was selected because its populations may be declining (Harding 1997; Helgen et al. 1998; Oldfield and Moriarty 1994). In addition, because *R. pipiens* is more closely related than *Xenopus laevis* to other native amphibians within the Upper Midwest, its use is more ecologically relevant.

Laboratory Study

In 2000 and 2001, water from ponds was collected every other week from April 15 to July 20 (six sampling periods) to assess amphibian toxicity. Water was collected from each pond by extending an inverted 250-mL beaker, attached to the end of an aluminum rod, about half way into the water column. At middepth, the beaker was everted and allowed to fill with water. The sample was poured into a 1-L amber bottle. This procedure was repeated until the amber bottle was filled. Samples were transported on ice to the laboratory where they were stored in a conventional refrigerator. All glassware used for collection and storage of water was washed with soap and tap water and rinsed with reverse osmosis water and pesticide-grade acetone (Fisher Scientific, Inc., Chicago, Illinois).

Using standard techniques (American Society for Testing and Materials 1998), FETAX was performed with collected water samples. However, instead of using known compounds as test solutions (e.g., pure atrazine), water samples taken directly from ponds were used. The FETAX assay is a standardized static renewal test used to determine if water or chemicals can elicit malformations or mortality in *X. laevis* (American Society for Testing and Materials 1998). During assays, 25 embryos were placed in 60-mm diameter petri dishes with 10 mL of test solution for 96 h. For each water sample, two petri dishes were used. In addition, positive and negative control solutions were used. The negative control, FETAX solution, was a specifically formulated solution that is reported to have no effect on the survival of *X. laevis* (American Society for Testing and Materials 1998). The positive control solution, 6-aminonicotinamide (Fisher Scientific, Inc., Chicago, Illinois), is a known teratogen. During the assay, petri dishes were held in an environmental chamber at 25-28° C, with a light regimen of 12 h light: 12 h dark. At the end of 96 h, numbers of surviving larvae were recorded and percent survival was determined.

In 2000, a pilot FETAX study was performed that used pond water from four different treatment categories: grazed, nongrazed, agricultural, and natural. However, in 2001, all water collection efforts focused on agricultural and natural ponds only.

Water Quality Characteristics

Personnel from U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (Bill Richardson and staff, La Crosse, Wisconsin) collected and analyzed pond water for ammonia, total nitrogen, and total phosphorus with standard methods (American Public Health Association et al. 1995). Water samples for nutrient analyses were collected the same day as water collected for FETAX assays. In addition, water samples collected in June

11-13 from seven ponds (three natural and four agricultural) were analyzed for pesticides by USGS Water Resources (John Elder and staff, Middleton, Wisconsin). Water chemistry and pesticide data were compared with data from FETAX assays.

Field Study

In 2001, differences in survival of developing *Rana pipiens* between agricultural and natural ponds were assessed with field enclosures (mesocosms; Figure 1). *Rana pipiens* tadpoles were held and cultured through metamorphosis in 12 ponds (seven agricultural and five natural ponds). Ponds were chosen on the basis of (1) presence of *R. pipiens* eggs, (2) ease with which mesocosms could be constructed on-site, (3) the pond's tendency to desiccate on the basis of data collected in summer 2000, and (4) whether the pond was agricultural or natural. An attempt was made to distribute mesocosms evenly between agricultural and natural ponds; however, the relative difficulty of constructing enclosures on-site and the absence of *R. pipiens* at certain ponds did not allow this, resulting in mesocosms being placed in a greater number of agricultural ponds than natural ponds.

Individual mesocosms were constructed of a welded aluminum frame (3.65 m long × 0.92 m wide × 1.21 m high) with sides made from two layers of screening—an outer rigid plastic mesh with 1.3-cm openings and an inner layer of plastic window screen with 1-mm openings. This double screen was sufficient to keep out aquatic predators, yet allowed water flow that supplied a natural source of algae as food for developing tadpoles. Screens were held in place with silicone sealant and aluminum strips secured to the frame with screws. Aluminum flashing was fastened onto the bottom 30.6 cm of the frame sides with screws, and sides of the mesocosm were bolted together with stainless-steel stakes that extended 45.8 cm below the bottom edge of the enclosures (Figure 1).

Mesocosms were placed into each pond as soon as either embryos or tadpoles of *R. pipiens* were collected. Each mesocosm was pressed into the sediments so that at least 15 cm of the aluminum flashing was buried. This helped ensure that tadpoles had access to sediments, but could not escape through gaps between the bottom edge of the enclosure and sediments. Each enclosure was placed near the deepest point of the pond to avoid mesocosm desiccation if water levels receded. Care was also taken to avoid including large emergent macrophytes within enclosures as they often contain overwintering eggs of several odonate species that might prey upon young tadpoles (personal observation). Where possible, mesocosms were placed near emergent vegetation that offered some shade. The sides and top of each mesocosm also provided some shade. The top of each mesocosm was covered with a mist net (1.5 cm mesh) to exclude mammalian or avian predators. Netting was cut into 3.56 m long × 0.92 m wide segments that were stretched tight and held in place with plastic ties.

After placement in the pond, dip nets were used to remove potential predators or other tadpoles from the mesocosms (Figure 2) and 100 *R. pipiens* embryos. In four ponds (two agricultural and two natural), embryos were collected and held in small hatching chambers (0.3 m long × 0.1 m wide × 0.1 m high) until reaching about Gosner stage 22 (Gosner 1960). At stage 22, these tadpoles were removed from hatching chambers and placed into mesocosms. Because of low availability of egg masses, remaining mesocosms were supplied with tadpoles (about Gosner stage 25) that had been collected post-hatch from on-site (two mesocosms), or from two reference sites (six mesocosms). All enclosures were in place and stocked with tadpoles by June 1, 2001.

Enclosures were usually checked each week. After tadpoles had developed hind limbs (Gosner stages 39 to 41), two sheets of styrofoam (30.6 cm × 30.6 cm) were placed into each mesocosm to provide a substrate onto which metamorphosing individuals could climb. As newly metamorphosed frogs and tadpoles with four limbs (Gosner stages 42 to 46) were observed, they were removed from enclosures, checked for malformations, and counted. After metamorphosing individuals were first observed, mesocosms were checked more frequently to ensure that frogs were removed as soon as metamorphosis was complete. Larval survival was calculated after all surviving tadpoles had metamorphosed (about 75 to 90 days). Survival was defined as the proportion of metamorphic frogs surviving in mesocosms (Gosner stage 42 to 46) / number of tadpoles initially placed into the mesocosm. Because of desiccation, in one pond, tadpoles were removed before metamorphosis and mortality was determined regardless of developmental stage. Tadpole survival was compared to results from the FETAX assay.

Data Analysis

Difference in survival of *Xenopus laevis* between pond types (nongrazed, grazed, agricultural, and natural in 2000; agricultural and natural in 2001) was analyzed with repeated-measures analysis of variance (ANOVA). An additional repeated-measures ANOVA was also performed in 2000 to determine differences in the survival of *X. laevis* cultured in artificially constructed ponds only (nongrazed, grazed, and agricultural). The difference in survival of *Rana pipiens* in mesocosms from agricultural and natural ponds was analyzed with an independent sample t-test. Multiple regression was used to assess the relations among ammonia, total nitrogen, total phosphorus, and amphibian survival. Data for repeated-measures ANOVA and multiple regression were rank transformed before analysis because of violations of model assumptions by untransformed data. A type I error (α) of 0.05 was used to judge the significance of statistical tests.

Results

There were no significant differences detected in survival of *Xenopus laevis* in water from agricultural, natural, grazed, or nongrazed ponds in 2000 ($F_{3,22}$ = 2.522, $df = 3$, $P = 0.083$, observed power = 0.543, Fig. 3). The additional repeated-measures ANOVA performed on artificially constructed ponds only (nongrazed, grazed, and agricultural) yielded no significant difference in survival as well ($F_{2,17}$ = 3.278, $df = 2$, $P = 0.063$, observed power = 0.544). A significant difference in *X. laevis* survival in dates in 2000 was detected ($F_{5,77}$ = 4.796, $df = 5$, $P = 0.001$, observed power = 0.972); however, none was detected in 2001 ($F_{5, 84} = 0.018$, $df = 5$, $P = 0.691$, observed power = 0.054).

No significant difference in survival of *X. laevis* in water from agricultural and natural ponds was detected in 2001 ($F_{1, 17} = 0.462$, $df = 1$, $P = 0.506$, observed power = 0.098; Fig. 4). Mean survival of *X. laevis* in water from natural ponds was only 7.3% greater than that of *X. laevis* in water from agricultural ponds. Likewise, in 2001 mean survival of *Rana pipiens* in mesocosms within natural ponds was 6.2% greater than those in agricultural ponds; however, in two agricultural ponds no tadpoles survived for unknown reasons (see discussion; Fig. 4). Furthermore, mean survival of *Xenopus laevis* cultured in water from agricultural ponds during FETAX assays was 9.9% greater mean survival of *Rana pipiens* raised in mesocosms housed within agricultural ponds (Fig. 4). Likewise, mean survival of *Xenopus laevis* cultured in water from natural ponds during FETAX assays was 11.0% greater than mean survival of *Rana pipiens* raised in mesocosms housed in natural ponds during 2001 (Fig. 4).

In the field study (2001), water levels receded in two ponds (one agricultural and one natural), leaving enclosures dry before tadpoles could be removed. This resulted in a lower sample size for each pond type (six agricultural and four natural). However, no significant difference in *R. pipiens* survival was detected between agricultural and natural ponds in 2001 ($t = 0.934$, $df = 1$, $P = 0.378$; Fig. 4).

The overall power of the repeated-measures ANOVA was greater for data on the survival of *Xenopus laevis* than survival of *Rana pipiens*. There was an 85% probability of detecting a 20% difference in the survival of *Xenopus laevis* between agricultural and natural ponds with a Type I error (α) of 0.05. However, there was only a 13% probability of detecting a 20% difference in the survival of *Rana pipiens* between agricultural and natural ponds with a Type I error (α) of 0.05.

Concentrations of ammonia, total nitrogen, and total phosphorous were lower in natural ponds than in agricultural ponds (Fig. 5). Ammonia concentrations ranged from 0.026 to 24.27 mg/L, while total nitrogen concentrations ranged from 0.79 to 14.81 mg/L, and total phosphorous levels ranged from 0.028 to 25.53 mg/L. There was a weak negative relation between water chemistry and anuran survival ($R^2 = 0.067$). Average concentrations of the three nutrients were generally low, and only total phosphorus contributed significantly to the relation ($P = 0.047$; Fig. 5). Likewise, relatively small concentrations of pesticides were detected in ponds. Of those chemicals measured, atrazine was detected in the highest concentration (0.026-0.55 μg/L). Concentrations of all other chemicals were lower than that of atrazine (Fig. 6).

Malformed frogs were rare during the field experiment. Only one malformed *R. pipiens* metamorph was collected from a mesocosm in an agricultural pond. Three of its appendages (both hind limbs and one front limb) were stunted. Malformations in *Xenopus laevis* were not assessed because of the subjective nature and discrepancy among researchers in determining what is, in fact, a malformed individual in the FETAX assay.

Discussion

In this study, the relative exposure of ponds to agricultural land use had no apparent effect on larval anuran survival or malformations. Furthermore, larval survival rates were high in all pond types in both years, frequently exceeding 75-85%, which was greater than expected. Likewise, despite a weak relation between total phosphorous and anuran survival, water quality (nutrients and pesticides) in the ponds did not seem to alter survival of anuran larvae. Consequently, on the basis of biological end points (survival and malformations) and variables measured, the water quality in agricultural ponds seems suitable for developing larval anurans within the Driftless Area Ecoregion of the Upper Midwest.

While no statistical difference was found among the survival of *X. laevis* larvae in different pond types in 2000, my results may have biological implications. When natural ponds were removed from the repeated-measures ANOVA, leaving only artificially constructed ponds (nongrazed, grazed, and agricultural), the degree of significance drew nearer to the predetermined level of 0.05. Because of this, I suggest that while artificially constructed ponds do not affect larval anuran survival differently than natural ponds, some types of artificial ponds make more suitable habitats for developing anurans than others. Grazed ponds, e.g., generally had less vegetated terrestrial buffer zones, less aquatic vegetation, and experienced more disturbances (from livestock) than did nongrazed ponds (personal observation). The survival of *X. laevis* larvae cultured in water from grazed and agricultural ponds was more than 10% less than those larvae cultured in water from nongrazed ponds. Futhermore, a <1% difference existed between the survival of *X. laevis* from nongrazed ponds and natural ponds.

In two mesocosms within agricultural ponds that were not subject to desiccation, no larvae survived. This may be an artifact of the animals being confined within the enclosures. In one pond, all individuals within the mesocosm died, but many free-ranging *R. pipiens* tadpoles survived through metamorphosis. This situation could be because of the stress of confinement within a smaller, restricted population (being housed within the mesocosm) compared to a larger, free-ranging population. In laboratory settings, overcrowding of test subjects can induce stress-related illness and mortality (American Society for Testing and Materials 1998), and it is possible that similar results could occur in animals confined in outdoor enclosures. In the second mesocosom with 100% mortality, tadpoles were heavily parasitized by leeches (Hirudinea). No free-ranging *R. pipiens* tadpoles were found within this pond. However, I observed several other species in this pond (*Rana clamitans, Pseudacris crucifer, Hyla versicolor, Bufo americanus*) and found that they were also parasitized by leeches, albeit not as heavily as *Rana pipiens* within the mesocosm. Individuals of other species did not seem to be affected to the same degree as the tadpoles within the enclosure. The effect of leeches on anurans has been recently documented for wood frog (*Rana sylvatica*) tadpoles that are much smaller than *Rana pipiens* tadpoles (Berven and Boltz 2001). However, the effects of leeches on *R. pipiens* are not available. Several other ponds (both agricultural and natural) that contained leeches and anurans did not experience the same degree of mortality.

Recently, it has been reported that Minnesota contains an unusually large number of anuran malformation "hot spots" (malformation rate $\geq 5\%$), as well as several sites where anuran populations have severely declined (Souder 2000). However, the majority of these sites do not exist within the Driftless Area Ecoregion.

It is unclear why the Driftless Area Ecoregion of Minnesota has fewer sites with malformed anurans or declining anuran populations. While Minnesota contains more than 11,000,000 ha of agricultural land, agriculture constitutes only 56.1% of the total area within the state (Hunst and Howse 2001; United States Census Bureau 2002). In addition, much of the agricultural land found in Minnesota is concentrated in the western portion of the state and not within the Driftless Area Ecoregion (Hunst and Howse 2001). Furthermore, historically low numbers of malformed anurans within the region may be a function of unique topography and geology. For example, the bedrock within the Driftless Area Ecoregion can efficiently allow passage of water and facilitate removal of possible chemical impurities. Conversely, subsoil glacial till that is prevalent in western Minnesota and Iowa, often results in an impermeable clay substrate that does not readily allow water absorption. Instead, water and potential contaminants are forced to flow over the surface layer of soil and directly into water catchment areas that are often existing ponds. While geology may be important for filtering groundwater that enters natural ponds within the Driftless Area Ecoregion, agricultural ponds are typically clay lined and exist at higher elevations, resulting in only a small amount of water coming from groundwater, and a larger amount coming from run-off.

Topography within this region is less conducive to the existence of large continuous tracts of agricultural land. For example, the larger relief associated with the karst (or limestone) geology, typical of the Driftless Area Ecoregion, is not as favorable for row crops as topography in areas of flat terrain, such as western Minnesota and Iowa. Furthermore, unlike cropland in glaciated areas, cropland within the Driftless Area Ecoregion is often divided by large vegetative buffer zones, such as forests that cover hillsides.

Agricultural practices within the Driftless Area Ecoegion may also be more conducive to amphibian survival than in glaciated regions. Crop rotation, strip farming, and use of animal manure as fertilizer are common in the Driftless Area Ecoregion. Conversely, croplands in areas of western Minnesota consist mostly of monoculture crops that receive heavy applications of inorganic fertilizers and pesticides (P. Stoelting, University of Wisconsin-La Crosse, Wisconsin, personal communication). Many of these agricultural chemicals have lethal or sublethal effects on amphibians (Allran and Karasov 2000; Allran and Karasov 2001; Howe et al. 1998; Tavera-Mendoza et al. 2002).

Agricultural practices and subsoil geology may explain the low levels of nutrients and pesticides detected in this study. Although there was a statistically significant relation between *Xenopus laevis* survival and concentration of total phosphorous, this relation was weak and its biological significance is questionable. Because studies involving the effects of total phosphorus and total nitrogen on amphibians are few, or none, I am unsure if levels of these particular nutrients detected were within the range that is detrimental to amphibians (Table 3). Levels of ammonia detected were occasionally in the range found to adversely affect amphibians and did not result in a statistical relation with anuran survival (Table 3; Jofre and Karasov 1999). Our overall average nutrient levels detected in agricultural ponds were skewed because of atypical concentrations (>14 mg/L) found in a single pond near a barnyard confinement.

Atrazine was the herbicide detected in the highest concentration in all ponds. However, the levels of atrazine were lower than minimum levels found to have lethal and sublethal effects on amphibians (Table 3; Allran and Karasov 2001; Allran and Karasov 2000; Howe et al. 1998; Tavera-Mendoza et al. 2002; but, see Hayes et al. 2002). These low concentrations also coincide with the relatively small amount of pesticides applied to crops within Minnesota. For example, atrazine was applied to less than 40% of the land planted with corn in the state during 2000 (Hunst and Howse 2001).

Other factors—such as predation, pond desiccation, and infectious diseases—may affect larval anuran survival. These factors may have been significantly different between agricultural and natural ponds; however, they were not assessed in this study. For example, agricultural ponds frequently harbor high densities of larval eastern tiger salamanders (*Ambystoma tigrinum tigrinum*; personal observation). Larval tiger salamanders are voracious predators that consume many types of aquatic organisms, including anuran tadpoles (Lannoo and Bachmann 1984). Their presence may have a negative effect on larval anuran survival that is not related to water quality. Likewise, it is possible that many artificially constructed ponds are smaller than natural ponds and are more susceptible to desiccation. Artificially constructed impoundments (such as farm ponds) are often eutrophic that may increase the amount of suitable habitat available for disease vectors and possibly increase infection rates (Johnson et al. 2002). Finally, in addition to mortality and malformation, there may be other biological end points relevant to amphibian conservation (i.e., sexual development and sex ratios) that were not examined in this study (Hayes et al. 2002).

Finally, field and laboratory experiments in studies involving amphibian ecotoxicology are important. Because of resource constraints, a small number of mesocosms were incorporated into this study resulting in low statistical power. However, to my knowledge this is the first time that enclosures of this size and construction have been used in amphibian field experiments. Therefore, with a larger number of enclosures (yielding greater statistical power) and more experience in proper use of these mesocosms, further studies may yield ecologically important information about the amphibian survival in natural and artificial habitats.

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Table 1. Characteristics of agricultural and natural ponds in southeastern Minnesota, Houston and Winona Counties, 2000.

Average maximum depth (m)	Area (ha)	Dominant land cover within watershed	Pond name
	Nongrazed Ponds		
2.65	0.41	Forest	Alt-nongraz
0.74	0.19	Grassland	Uti-nongraz
2.52	0.38	Corn/soybean	StCh-nongraz
1.25	0.04	Corn/soybean	She-nongraz
1.48	0.08	Forest/grassland	Eit-nongraz
	Grazed Ponds		
2.76	0.26	Corn/soybean	Alt-graz
0.90	0.04	Grassland	Uti-graz
1.07	0.10	Grassland	StCh-graz
1.78	0.24	Forest	She-graz
0.56	0.23	Grassland	Eit-graz
	Agricultural Ponds		
1.40	0.48	Corn	Alt-ag
1.66	0.12	Corn/soybean	Uti-ag
1.07	0.32	Corn/soybean	Lew-ag
0.71	0.39	Corn	StCh-ag
1.62	0.53	Corn	Hou-ag
1.06	0.09	Corn/soybean	Mou-ag
0.70	0.05	Corn/soybean	She-ag
0.63	0.10	Corn/soybean	Cal-ag
1.33	0.08	Corn	Bro-ag
0.71	0.15	Corn/soybean	Eit-ag
	Natural Ponds		
0.71	5.58	Forest	Alt-nat
0.82	0.30	Forest	Uti-nat
1.09	1.60	Grassland	Stch-nat
0.42	0.33	Forest	Cal-nat
0.79	0.76	Forest	Eit-nat

Source: Knutson et al. (2002)

Table 2. Characteristics of agricultural and natural ponds in southeastern Minnesota, Houston and Winona Counties, 2001.

Average maximum depth (m)	Area (ha)	Dominant land cover within watershed	Pond name
	Agricultural Ponds		
1.03^{+}	0.48	Corn	Alt-ag
1.95^{+}	0.12	Corn or soybean	Uti-ag
0.54^{+}	0.32	Corn	Lew-ag
1.42	0.53	Corn	Hou-ag
0.99	0.09	Corn or soybean	Mou-ag
0.90^{+}	0.05	Corn or soybean	She-ag
0.23	0.10	Corn or soybean	Cal-ag
1.46^{+}	0.08	Corn	Bro-ag
0.58^{+}	0.15	Corn or soybean	Eit-ag
	Natural Ponds		
0.39	5.58	Forest	Alt-nat
0.78^{+}	0.30	Forest	Uti-nat
0.25	0.54	Grassland (grazed)	Lew-nat
1.42^{+}	1.60	Grassland	Stch-nat
0.19^{+}	0.27	Forest	Hou-nat
0.28	0.10	Grassland (grazed)	Mou-nat
0.24	0.27	Grassland (grazed)	She-nat
0.39	0.33	Forest	Cal-nat
0.49^{+}	0.38	Forest	Bro-nat
2.46	0.76	Forest	Eit-nat

$^{+}$Pond used for assessment of survival in *Xenopus laevis* and *Rana pipiens*
Source: Knutson et al. (2002)

Table 3. Toxicant levels found to affect amphibians in previous studies compared to concentrations found in farm ponds from study area and reference concentrations in the Upper Midwest.

	Previous studies				
Toxicant	**Level of effect for amphibians**	**Species**	**Biological end point**	**Levels found in farm ponds from study area**	**Concentration in Upper Midwest**
Ammonia	0.6 mg/L[a] 1.5 mg/L[a]	*Rana clamitans* larvae *Rana pipiens* larvae	survival survival	0.032-24.27mg/L[b]	<0.1->0.7mg/L[c] (downstream of Minnesota River)
Nitrate	13-40 mg/L[d]	variable	survival	0.0-4.8 mg/L[b]	1.5-2.1 mg/L[c] (downstream of Minnesota River)
Total nitrogen	n/a	n/a	n/a	0.79-14.81mg/L[b]	<2.0->7.0 mg/L[c] (downstream of Minnesota River)
Phosphorus	n/a	n/a	n/a	0.028-25.531 mg/L[b]	<0.2-0.4mg/L[c] (downstream of Minnesota River)
Alachlor	3.3 mg/L[e]	*Bufo americanus* larvae	survival	0.0035-0.0063 µg/L[f]	0.27 µg/L[g] (reservoir outflows)
Atrazine	47.6 mg/L[e] 0.1µg/L[h]	*Rana pipiens* larvae *Xenopus laevis* larvae	survival sexual development	0.026-0.55 µg/L[f]	1.36 µg/L[g] (reservoir outflows)
	21 µg/L[i]	*X. laevis* larvae	sexual development		<0.5 µg/L[j]

[a]Jofre and Karasov (1999) [b]Knutson et al. (2002) [c]Kroening and Andrews (1997) [d]Rouse et al. (1999) [e]Howe et al. (1998) [f]J. Elder (USGS Water Resources, Middleton, Wisconsin, unpublished data, June 2001) [g]Stamer et al. (1998) [h] Hayes et al. (2002) [i] Tavera-Mendoza et al. (2002) [j]Fallon et al. (1997)

Figure 1. Construction of a mesocosm near an agricultural pond, in southeastern Minnesota, Winona County, 2001.

Figure 2. Dip netting a mesocosm after placement within a natural pond in southeastern Minnesota, Houston County, 2001.

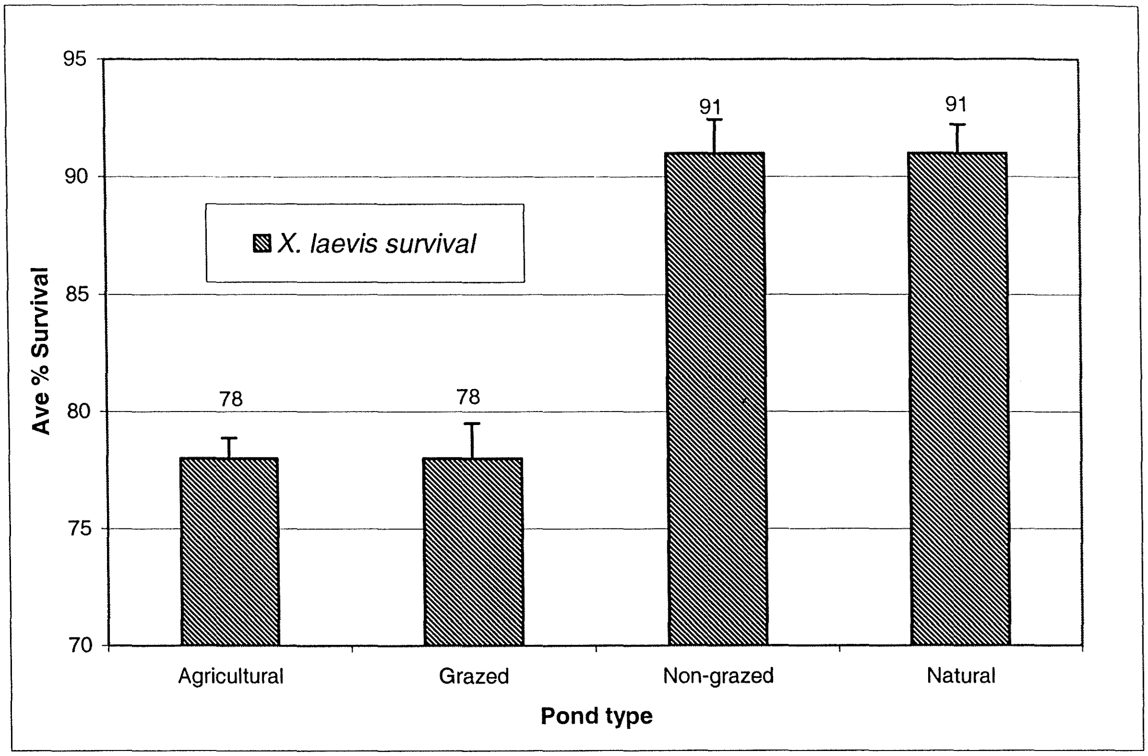

Figure 3. Survival of *Xenopus laevis* in different pond types from southeastern Minnesota, Houston and Winona Counties, 2000 (bars represent standard error).

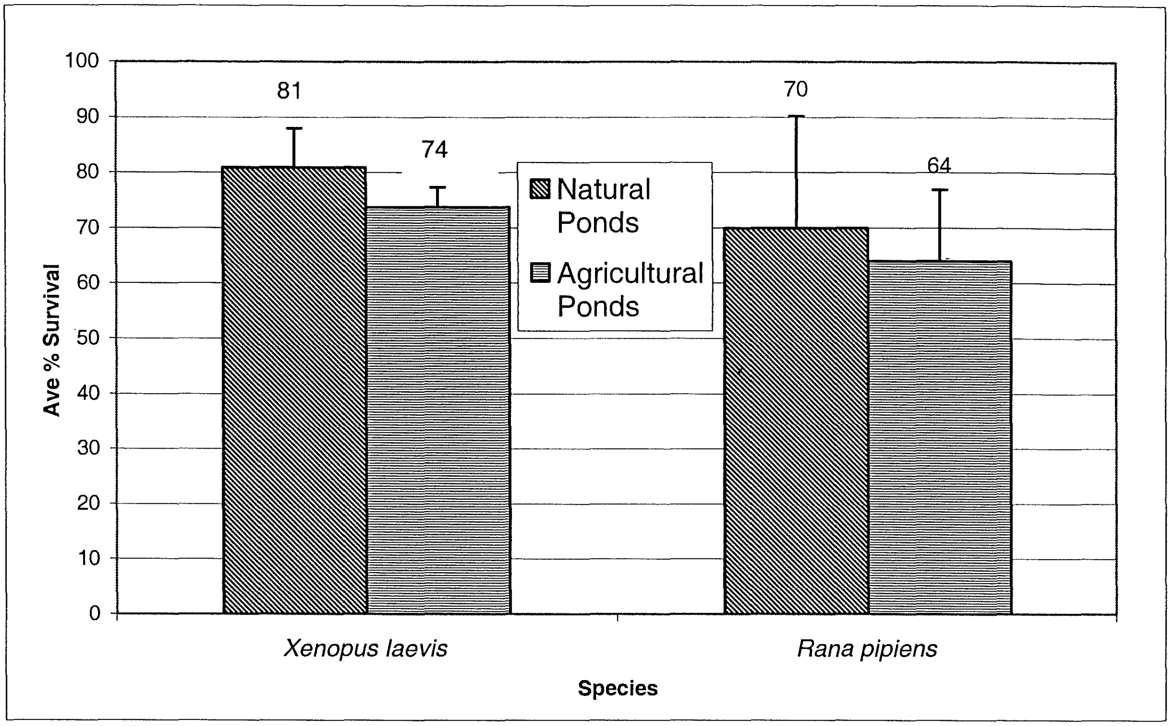

Figure 4. Survival of *Xenopus laevis* and *Rana pipiens* in agricultural and natural pond water from southeastern Minnesota, Houston and Winona Counties, 2001 (bars represent standard error).

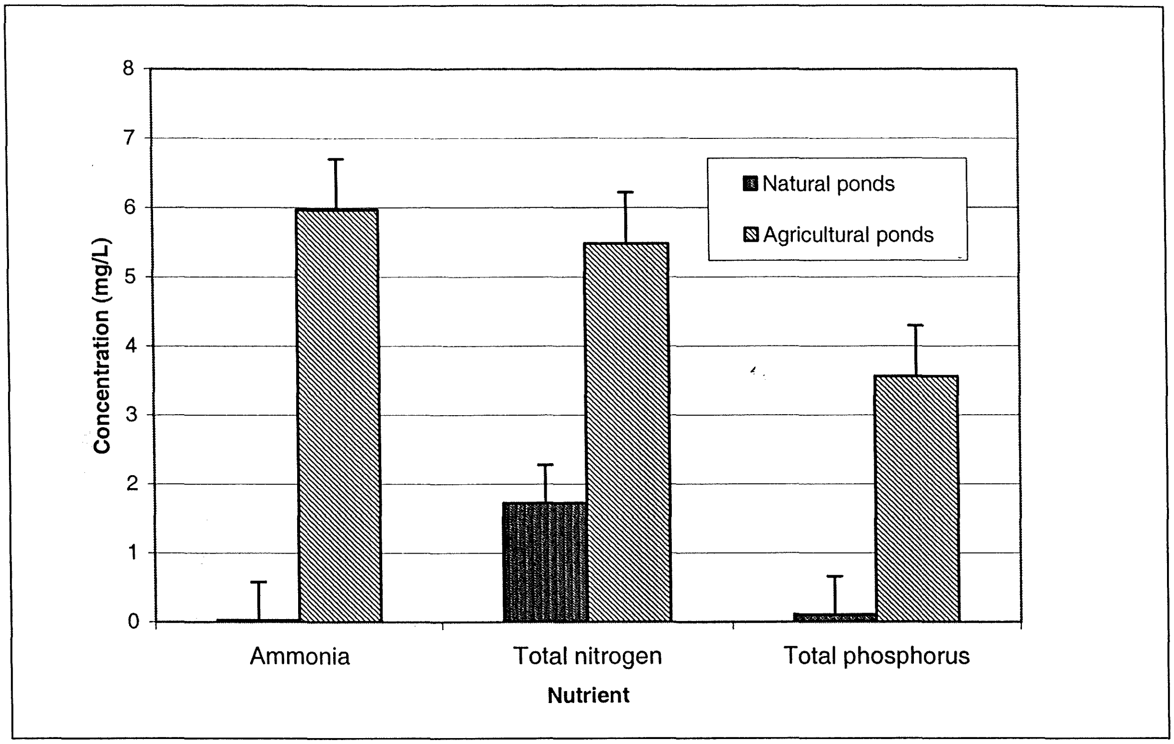

Figure 5. Concentrations of nutrients in agricultural and natural ponds from southeastern Minnesota, Houston and Winona Counties, 2000 and 2001 (bars represent standard error; Knutson et al. 2002).

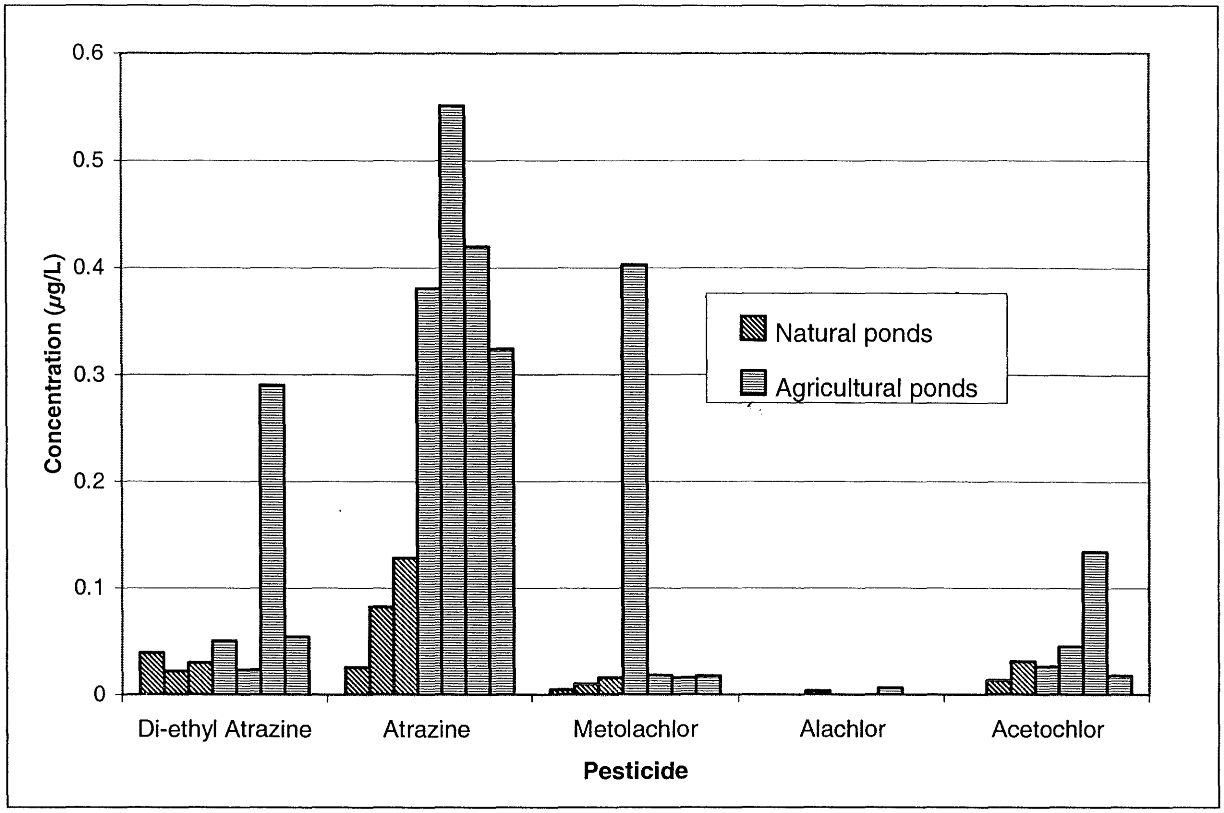

Figure 6. Pesticides in selected agricultural and natural ponds from southeastern Minnesota, Houston and Winona Counties, June 11-13, 2001 (J. Elder, USGS Water Resources, Middleton, Wisconsin, unpublished data).

Effects of Agricultural and Urban Land Use on Movement and Habitat Selection by Northern Leopard Frogs (*Rana Pipiens*)

by

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Farm Ponds as Critical Habitats for Native Amphibians: Final Report

Submitted to

Legislative Commission on Minnesota Resources
100 Constitution Avenue, Room 65
St. Paul, Minnesota 55155-1201

June 2002

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Effects of Agricultural and Urban Land Use on Movement and Habitat Selection by Northern Leopard Frogs (*Rana Pipiens*)

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Abstract

Southeastern Minnesota is a landscape dominated by agriculture, with few natural wetlands apart from sloughs and oxbows associated with streams and rivers. However, many small farm ponds have been built to control soil erosion. Many species of amphibians breed in these ponds. Small cities in the region are expanding and communities along rivers are often adjacent to wetlands that are prime breeding areas for amphibians. Little is known about amphibian movement patterns and habitat selection in either agricultural or urban edge settings. The objective of this study was to compare the movement patterns and habitat selection of anurans in agricultural, urban, and natural ponds in southeastern Minnesota using radio-telemetry. After failing to track anurans with transmitters attached via external harnesses in 2000, we switched to surgical implantation of transmitters in 2001. We surgically implanted transmitters into the peritoneal cavity of 44 Northern Leopard Frogs (*Rana pipiens*) from three sites and tracked them from May to October 2001. Home range sizes and habitat use were investigated. *R. pipiens* at the agricultural site used areas of grassland and forests adjacent to the breeding ponds and these habitats represented most of their home ranges. At the natural and urban sites, *R. pipiens* selected wetland habitats representing only 2% of the available habitat. At the urban site, most frogs remained in a wetland adjacent to the industrial park. We suggest that the amount of high-quality habitat adjacent to the pond is an important influence on amphibian home range size and movement rates. The natural and urban sites were associated with high quality wetlands and grasslands, which provided abundant food, shelter, and over-wintering habitats and allowed smaller home ranges and movements. Frogs at the agricultural pond had to move more and required larger home ranges to meet the same needs. To support *R. pipiens* populations, managers should increase the amount and quality of amphibian feeding and wintering sites adjacent to breeding sites, reducing the hazards encountered and the energy required to move long distances.

Key words: amphibian, habitat selection, radio telemetry, agriculture, *Rana pipiens*, home range, movement distance.

Introduction

The Northern Leopard Frog (*Rana pipiens*) is an anuran commonly found in southeastern Minnesota (Oldfield and Moriarty 1994). *R. pipiens* over winters in permanent water (bottoms of lakes and streams) and breeds early in the spring in southeastern Minnesota. Male frogs begin calling when the water temperature warms to 20°C (Oldfield and Moriarty 1994), typically in April or May. Because it has a close association with wetland habitats, this species is considered a bioindicator for wetlands (Lannoo 1996) and is frequently used in toxicity testing for agricultural chemicals (Cheek et al. 1999; Allran and Karasov 2000).

The Driftless Area Ecoregion of southeastern Minnesota was unglaciated in the most recent glacial periods and is now a well-drained landscape with few natural wetlands apart from sloughs and oxbows associated with rivers and streams. In an effort to control soil erosion, thousands of farm ponds have been created with cost-sharing money from the US Department of Agriculture. The dominant row crops in the area are corn and soybeans, and grasslands are dominated by alfalfa and livestock grazing.

Agricultural practices adjacent to these ponds may influence the habitat quality of these areas for anurans. The dominant row crops in the region are corn and soybeans, crops that receive annual tillage as well as fertilizer and pesticide applications. Other agricultural practices that may affect amphibians include haying practices and livestock grazing. A previous study found that amphibian populations associated with intensive agricultural land use in Canada were negatively affected (Bonin et al. 1997; Hecnar 1997).

Small cities in southeastern Minnesota are growing; urban communities along rivers are often adjacent to wetlands that are prime breeding areas for amphibians. Urban land use was associated with lower anuran abundance and species richness in Wisconsin and Iowa (Knutson et al. 1999; Knutson et al. 2000). Research has demonstrated a number of negative effects on amphibians from urban life, including habitat loss and increased road mortality (Findlay and Houlahan 1997; Lehtinen et al. 1999; Findlay and Bourdages 2000). Furthermore, urbanization may act as a barrier to anurans attempting to migrate between suitable habitats. Ashley and Robinson (1996) reported that anurans experienced high mortality at road crossings while migrating between breeding and nonbreeding areas. Paved road density within 2 km of a wetland was also found to negatively affect species diversity of herptiles (Vos and Chardon 1998).

Though evidence suggests agriculture and urbanization may be detrimental to anuran populations, the specific effects of these factors on amphibians, particularly movement and habitat use, are poorly understood. The objectives of this study were to (1) evaluate, and refine as needed, existing radio transmitter attachment techniques appropriate for *R. pipiens*, and (2) evaluate how agriculture and urbanization affect *R. pipiens* post-breeding habitat use, movement patterns, mortality in southeastern Minnesota.

Methods

Transmitter Attachment

External Attachment

We attached Holohil BD-2GHX transmitters with a whip antenna (1.85 g) to *R. pipiens* using a variety of external harnesses. Transmitters did not exceed 5% of the frog's total weight (Richards et al. 1994). We tested several types of harness materials to find one that did not restrict frog movements, become entangled in vegetation, or fail in wet conditions. We tested nickel bead-chain, aluminum bead-chain, plastic zip-ties, and sewing elastic as harness materials. Beginning August 1, 2000, frogs fitted with external transmitters were released near the site of capture and observed to assess transmitter loss, transmitter entanglement in vegetation, and behavioral changes. Because frogs frequently slipped out of their harnesses, we confined some frogs (11) in three 1.8 m x 1.8 m enclosures placed along the edge of a wetland to observe the effects of attachment methods. Four individuals were released from the enclosures and tracked from October 12 to October 26, 2000.

Surgical Implantation

We tested surgical implantation (peritoneal and subcutaneous placements) in the laboratory in winters 2000 and 2001. Our methods were similar to those used by Lamoureux and Madison (1999) with *Rana clamitans*. We also benefited from the experiences of other scientists working with anuran surgical implantation (personal comm., G. Birchfield, University of Missouri, Columbia; C. Goldberg and M. Goode, University of Arizona, Tempe; and S. Heppell, US Environmental Protection Agency). Procedures for anesthesia and euthanasia followed Green (2001). We inserted the transmitter through the left ventro-lateral abdominal wall. Incisions in the skin and muscle wall were sutured with nonabsorbable 5-0 sutures (Appendix A).

In the first laboratory test, *R. pipiens* received either subcutaneous implants or peritoneal implants. Peritoneal implantation only was used during a second laboratory test. Laboratory frogs were observed for up to 19 weeks post-surgery to assess health and behavior. In 2001, all frogs in the field study received peritoneal surgical implantation of radio transmitters.

Field Study

In 2001, breeding *R. pipiens* weighing >37 g were caught at each study site and surgically fitted with Holohil BD-2GHX radio transmitters (peritoneal implants, 165 MHz band, 20-week lifetime, 1.85g, loop antenna). Transmitters did not exceed 10% of the frog's total weight (Richards et al. 1994). Surgeries were performed in the field near the capture site; frogs were allowed to recover for up to 4 hours before release. Frogs were tracked from the ground with an Advanced Telemetry System receiver and a hand-held Yagi antenna. A hand-held global positioning system (GPS, Rockwell Collins, Inc., Cedar Rapids, Iowa; accuracy ~ 6.4 m) was used to determine geographical coordinates of tracked frogs. Individuals were located four to five times per week and geographic location, habitat type, environmental parameters, and frog health were recorded. Frogs were tracked from the time of implant to October, or until frogs were lost or transmitters failed. Our goal was to catch and radio-tag 15 *R. pipiens* from each of the three study sites.

Study Sites and Statistical Analysis

Field studies of *R. pipiens* movements and habitat use were conducted at an agricultural, urban, and natural wetland in southeastern Minnesota. The natural site was a pond created by damming a small stream; this site was a 6.3 ha wetland (grass, sedges, and emergent vegetation) surrounded by agricultural row crops (Fig. 1; Winona County, St. Charles Township, Minnesota). The agricultural site was a constructed farm pond (0.6 ha) surrounded by a narrow grass buffer and agricultural row crops (Fig. 2; Houston County, Sheldon Township, Minnesota). The urban site was part of the Mississippi River floodplain adjacent to Winona, Minnesota. It was a large natural wetland (121.0 ha) adjacent to an industrial park (Fig. 3; Winona County, Winona Township, Minnesota). This site was part of the Upper Mississippi River National Wildlife and Fish Refuge managed by the US Fish and Wildlife Service.

We evaluated home range size, habitat use, and movement patterns of *R. pipiens* using land-use maps and frog GPS locations. Digital maps of land uses (1-km radius circles centered on breeding pond) were interpreted from infrared aerial photographs (summer 2001) using a geographical information system (GIS; ArcView 3.2, ESRI, Inc.). This area corresponded to the maximum movement of radio-tagged frogs (Figs. 1-3). We interpreted major land-use types, including open water, emergent aquatic vegetation, flooded grass and wet meadow, natural grass, roadside grass, forest, shrub, developed urban, and farmsteads. Agricultural land-use types included pasture, corn, soybeans, alfalfa, oats, and clover. For the habitat use analyses, land-use maps were collapsed into five habitat types including forest/shrub-scrub, grass, developed, cropland, and wetland. Developed habitats made up only a small proportion (<1%) of the agricultural and natural sites and were considered part of the grass habitat type. Grass areas at the urban site were considered developed because they were frequently mowed.

Home range sizes were calculated with the MOVEMENT program (Hooge and Eichenlaub 1997). This program uses the minimum convex polygon (White and Garrott 1990) and the kernel (Garton 2001) methods to determine home range and core area size for each individual. Individual home ranges were calculated by incorporating 95% of the GPS data points for individual frogs (White and Garrott 1990). Core areas were plotted similarly, but incorporated 65% of the data points for an individual frog (Garton 2001).

We also used the computer program Home Range to determine habitat selection by frogs at each site (Ackerman et al. 1990). This program incorporates several methods, including Johnson's (Johnson 1980) and Friedman's (Friedman 1937) to compare habitat use with habitat availability to assess habitat selection. For each frog, use of a particular habitat type was the percentage of locations in that habitat type divided by the total number of locations in all habitat types. Habitat availability was defined as the percentage of each habitat type within the study site based on the interpreted land-use maps. The Friedman method tests the hypothesis that habitat types chosen by an individual are not differentially selected (Alldredge and Ratti 1986). The habitat type with the highest sum of the difference rankings was the most selected and the habitat type with the lowest sum of the difference rankings was the least selected. If the sums of difference rankings between habitat types were significantly different, then the MOVEMENT program calculated multiple t-tests to determine which habitat types differed from one another.

The Johnson method determines habitat selection by comparing the ranks of observed habitat use (the highest use received the lowest numerical rank) for each habitat type with the ranks of availability (the highest availability received the lowest numerical rank) of each habitat type for each frog (Alldredge and Ratti 1986). The differences in use and availability ranks for each habitat type are averaged across all frogs to determine a selection index. The habitat type with the lowest average difference is the one most selected for relative to other habitat types. Hotelling's T^2 statistic is used to test the hypothesis that the relative selection for all habitats is equal. If this hypothesis is rejected, the program runs Waller-Duncan multiple comparison tests to determine which habitat types are differentially selected. The Kruskal-Wallis test was used to compare home range sizes, movement distances, and movement rates among study sites.

Frogs were considered blocks in all three methods because frogs, not individual locations of frogs, are more likely to be independent.

Field Study Continues in 2002

We obtained funding from the USGS Amphibian Research and Monitoring Initiative (ARMI) to continue the telemetry study for an additional field season (2002). This work is planned as the thesis component of Brian Pember's M.S. degree from the University of Wisconsin-La Crosse.

Results and Discussion

Transmitter Attachment

We observed 26 frogs fitted with transmitters attached to external harnesses from August 1 to October 12, 2000. All external attachment techniques were deemed inadequate for our needs because the frogs slipped out of the harnesses, and lesions developed where harnesses contacted the skin (Table 1). Seven of the frogs fitted with transmitters on August 1, 2000 shed their transmitters by August 3. Fifteen frogs were continuously refitted with transmitters as escapes occurred. By August 29, 14 of the original 26 frogs had either escaped or were released because of skin lesions.

Nickel Bead Chain

Frogs with nickel bead chains frequently developed skin erosions and escaped from their harnesses (Table 1). We initially glued the chains onto the transmitters using 2-ton epoxy, as recommended by the transmitter manufacturer. The glue failed on several harnesses. The transmitters were all re-fitted with tubes and the harness material was passed through the tube, eliminating the need to glue harnesses directly to the transmitter. The nickel harnesses also rusted and discolored the skin of the frogs, usually within 2-7 days, with one chain rusting to the point of breaking after 7 days. These harnesses usually remained attached for about 12 days; when transmitter loss occurred in nickel bead chains, it usually took place within the first three days (with two exceptions).

Aluminum Bead Chain

Frogs with aluminum bead chains had the least problems with skin erosion, but experienced excessive transmitter loss (Table 1). One frog carried a transmitter 13 days; however, two other frogs lost transmitters multiple times. One frog experienced skin erosion after nine days and was released four days later. One frog carried a transmitter without developing skin erosions 13 days until the transmitter was shed. Another frog carried a transmitter 54 days without developing skin erosions. However, this frog slipped out of the harness five times in that period. The first escape occurred after two days.

Elastic Harness

All four frogs fitted with these harnesses lost their transmitters one or more times and three developed lesions (Table 1). In addition, elastic harnesses were difficult to work with and required two people to fit the harness. In one case, this attachment method was successful for 13 days.

Plastic Cable Tie

Four frogs fitted with plastic cable ties developed the first signs of skin erosion an average of 15 days after attachment and also shed their transmitters (Table 1). The fifth frog was not recovered after 2 days, but appeared to have gone into an underground burrow.

Surgical Implantation

In the first laboratory test, *R. pipiens* received either subcutaneous implants (N=3) or peritoneal implants (N=4). We used peritoneal implantation during a second laboratory test (N=12). The subcutaneous transmitter was unsatisfactory; it created a lump on the frog's ventral surface, pressed against the sutures, and led to transmitter loss.

Frogs in the laboratory surgical trials (winter 2001) began feeding within four days and most increased their weight one week after surgery (Table 2). The suture sites healed within two weeks. Sores developed near the suture site of eight frogs approximately six weeks post-surgery, but did not affect the frog's ability to feed or move. These sores healed in four frogs. Three frogs with peritoneal implants passed the transmitter through their digestive system (gastrointestinal capture) at 34, 89, and 98 days post-surgery.

Field Surgery

Forty-four frogs were surgically implanted with radio transmitters and released at the collection site from May to July 2001. Thirty-four of the 44 frogs survived the procedure (natural: N=17, agricultural: N=7, urban: N=10; Table 3). Three frogs had small sores at the suture site, but these individuals continued to gain weight and the lesions did not seem to adversely affect their behavior.

Test subjects at the natural site were an average weight of 47 g when tagged, and frogs recaptured for weighing (N=11) increased their weight by 15% during the study (Table 3). (Some individuals were lost or experienced mortality before they could be reweighed.) Test subjects at the agricultural site averaged 56 g when tagged, and those recaptured for weighing (N=6) increased their weight by 24%. Test subjects at the urban site weighed an average 41 g when tagged and increased their weight by 30% (N=5). Individual frogs were tracked from six to 119 days (mean=52 days) during the study. Those frogs that successfully recovered from surgery and were released into the field had good survival and experienced generally good health, as evidenced by weight gain.

Most surgical failures were failures to recover from anesthesia (eight of 10 frogs). We deemed this over-exposure to the anesthetic tricaine methanesulfonate (MS-222). *R. pipiens* was highly sensitive to the temperature of the MS-222, probably because increased metabolic rates are induced by higher temperatures. We were not alerted to this problem from our laboratory studies, because the laboratory anesthesia solution was room temperature at the time of surgery. We recommend cooling the frogs and anesthetic solution to $\leq 25^{o}C$ to slow down the metabolic rate of the frog and reduce the toxicity of MS-222. This follows the recommendations of Green (2001). The causes of the remaining two surgical mortalities were unknown. As a result of these experiments, standard operating procedures for implanting transmitters into *R. pipiens*, and holding adult leopard frogs in a laboratory setting were developed and refined (Appendices A and B).

We chose to perform surgeries in the field to reduce transport stress and time. We also wanted to prevent accidental transfer of diseases from the laboratory to native populations. However, more controlled surgical conditions may have reduced surgical mortalities. We are now conducting surgeries in a van parked near the study area. This has reduced surgical area exposure to wind and rain and improved surgical survival (S. Weick, USGS, La Crosse, Wisconsin, pers. comm.).

Lamoureux and Madison (1999) performed 23 surgeries with no reported mortality; they used a 0.5% solution of 3-ethyl-m-aminobenzoate to anesthetize the frogs, but did not report exposure times. They released implanted frogs within 24 hours of collection. Although unstated, it seems likely the surgeries were conducted in a laboratory. Werner (1991) implanted radio transmitters into six *Bufo americanus* with no reported mortality. The toads were anesthetized in a 0.01% solution of MS-222 for 15-20 minutes. Individuals were returned to their point of collection within 24 hours.

Habitat Use

The maximum distances frogs moved and their movement rates varied among the study sites (Table 4). Frogs at the agricultural site had the greatest mean total distance traveled (1,190 m) compared with the natural site (637 m) and the urban site (422 m; P<0.002, Kruskal-Wallis Test). Mean daily movement rate at the three sites ranged from 6.2 to 47.4 m/day (Table 4) and frogs at the agricultural site had a higher movement rate than frogs at the other study sites ($P < 0.07$, Kruskal-Wallis Test). There were no differences in the mean distances traveled by frogs among sites ($P < 0.12$, Kruskal-Wallis Test). Four individuals (three at the urban site and one at the natural site) crossed roads while migrating from the breeding pond. Three frogs (two at the agricultural site and one at the natural site) migrated from the pond where they were tagged to another pond, presumably for overwintering. Movement paths used by these three frogs were direct (e.g., see Fig. 4) and did not follow any apparent physical corridors (e.g., fence lines or waterway). We observed that increased movement was associated with rainfall events. We lost radio contact with as many as 10 frogs after rain events.

Home range size was larger at the agricultural site than at the natural and urban sites ($P<0.05$, Kruskal-Wallis Test) (Table 5). However, there were no differences in the size of core areas among the study sites ($P<0.47$, Kruskal-Wallis Test). Urban frogs did not exhibit the long distance movements and subsequent use of outlying areas as noted for several frogs at the agricultural and natural sites.

The proportions of the five habitat types and habitat use by frogs varied among the study sites (Table 6). For example, at the natural site, the majority of frog locations occurred in wetlands (64%) followed by grasses (18%) and forest (17%). Habitats used by radio-tagged frogs at the agricultural site included grasses (42%), forest/shrub (40%), and row crops (13%). Agricultural crops in which radio-tagged frogs were found included alfalfa (8%), corn (4%), soybeans (2%), and oats (1%) (Fig. 5). Alfalfa was cut four times, killing four of the eight frogs that used alfalfa during the study. At the urban site, frogs were found primarily in wetlands (59%), followed by forest/shrub (20%), and developed (14%; Table 6). Six out of 10 frogs at the urban site used developed areas at some time. Three frogs spent the majority of their time in an abandoned lot located across the road from the wetland where they were released and an additional three frogs used other developed areas (Fig. 6)

The Friedman method indicated that radio-tagged frogs at the urban and agricultural sites did not differentially select habitat types ($P > 0.05$; Table 7). However, at the natural site, radio-tagged frogs did select certain habitat types ($P=0.0001$; Table 7). Habitat types at the natural site, ranked from most to least selected, were wetland, forest, grasses, and row crops. Multiple comparisons revealed significant differences in selection between wetlands and all other habitat types except forest, and also between forest and grasses at the natural site ($P < 0.05$).

The Johnson method indicated that radio-tagged frogs at both the urban and natural sites selected for certain habitats, but not frogs at the agricultural site ($P < 0.05$; Table 8). At the natural site, habitat types ranked from most to least selected were wetland, forest, grasses, and row crops. The Waller-Duncan tests revealed significant differences between wetlands and all other habitat types except forest, and also between the forest and grass ($P < 0.05$). At the urban site, habitat types from most to least selected were forest, wetland, and developed. Waller-Duncan tests revealed significant differences between the developed habitat type and the other two habitats (forest and wetland; $P < 0.05$).

We observed that breeding in agricultural landscapes presents numerous hazards to *R. pipiens*. We confirmed that haying of clover and alfalfa induced mortality for frogs occupying hayfields. Radio-tagged frogs frequently crossed potentially hazardous areas, such as roads, parking lots, and tilled agricultural fields that increase the likelihood of mortality.

Frogs at the agricultural site used the surrounding habitat according to availability. For example, frogs were found in forest and grass habitats 80% of the time and these habitats constituted >90% of the available habitat. In addition, frogs at the agricultural site had the largest home ranges. Relatively small ponds with small grass buffer strips may force individuals to travel longer distances because of competition for breeding or food resources or to accommodate dispersal to other wetlands, whereas natural ponds and large wetlands adjacent to an urban area have enough high quality habitat to sustain smaller home ranges.

Frogs at the urban site were found in forest and wetland habitats 73% of the time. However, these habitats represented only 52% of the available habitat. The wetland at the urban site is a mix of open water, cattail (*Typha* spp.), bur-reed (*Sparganium* spp.), and bulrush (*Scirpus* spp.) emergents. These form dense stands of floating aquatic vegetation mats that offer protection, breeding, feeding, and overwintering areas. Frogs at this site are not required to move long distances to find these essentials. This may explain why urban frogs had small home ranges. In addition, because the urban area surrounding the wetland is disturbed more frequently than at the agricultural site, these frogs may have been reluctant to leave the protection of the wetland or forest.

Frogs at the natural site were found in wetland habitats 43% of the time, but wetlands represented only 2% of the total available habitat. The wetland, though small, provides cover, food, breeding, and over wintering areas. In this site, the presence of overwintering habitats may explain why individuals did not travel far. Because it is spring fed, this wetland does not completely freeze during the winter and suitable overwintering habitat is available.

Matthews and Pope (1999) documented *Rana musocsa* movements from summer lakes to overwintering lakes. They studied the movements of 24 frogs from August to October. In August, *R. musocsa* were distributed in 10 of 11 lakes in the study area. Small shallow ponds contained the highest numbers of frogs. Movement increased in September as the frogs moved toward over-wintering lakes and ponds. By October, the frogs had moved several hundred meters to one deep lake and two shallow lakes. This type of movement from breeding ponds to over- wintering ponds was also observed in 3 *R. pipiens* during our study. These frogs moved >1000 m to overwintering ponds.

The movements we observed indicate that frogs are able to move considerable distances to find suitable habitats when environmental or physiological changes occur. Frogs occupying habitats that meet basic life history requirements are less likely to move long distances in search of resources. Reducing distances between essentially habitats should also reduce contacts with hazardous situations that increase mortality. Large, diverse wetlands probably provide all of the requirements needed by *R. pipiens* for survival including food, shelter, breeding and overwintering areas. Wetland complexes consisting of several types of wetlands (shallow and deep water) with maximum distances of 500-1000 m between wetlands are probably ideal habitats for *R. pipiens*. A mix of shallow ponds that warm faster in the spring for breeding and ponds deep enough to avoid freezing over the winter would provide the ideal mix of habitats. Wetlands adjacent to small creeks and streams also benefit overwintering anurans by providing frost-free aquatic habitats.

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Table 1. Outcomes of four different transmitter harness materials used to attach radio transmitters to *R. pipiens* for study sites in Houston and Winona Counties, Minnesota, August to October 2000.

Harness type	# Frogs (N=26[a])	Transmitter loss mean days (SD)	First indication of skin erosion, mean days (SD)	Total days tracked mean days (SD)[a]
Nickel bead chain	15	3.2 (1.2)	8.5 (2.2)	7.3 (2.3)
Aluminum bead chain	4[b]	11.5 (3.2)	9[c]	27 (3.9)
Elastic harness	4[b]	20.8 (2.9)	18.7 (2.7)	31.5 (2.4)
Cable ties	5	5.5 (1.3)	15 (1.4)	24 (2.9)

[a] Column sum is >26 because some frogs were fitted with more than one type of harness.
[b] One frog was switched from aluminum bead chain to elastic harness after 13 days.
[c] One frog with an aluminum harness developed a skin erosion.

Table 2. Fate of animals subjected to laboratory surgeries, 2001.

Frog ID	Implant location	Surgical Weight (g)	Surgical Recovery	Final weight (g)	Fate	Days post-surgery	Comments
1	Peritoneum	31.3	yes	55.0	Survived[a]	103	Transmitter passed on day 89 (gastrointestinal capture)
2	Peritoneum	47.5	yes	66.9	Escaped	95	Transmitter passed on day 34 (gastrointestinal capture)
3	Peritoneum	42.9	yes	47.9	Survived[a]	134	
4	Peritoneum	28.9	yes	49.7	Survived[a]	134	
5	Peritoneum	25.6	yes	53.3	Survived[a]	134	Transmitter passed on day 98 (gastrointestinal capture)
6	Peritoneum	27.9	yes	49.3	Survived[a]	134	
7	Peritoneum	28.1	yes	45.3	Survived[a]	134	
8	Peritoneum	25.6	yes	42.4	Survived[a]	134	Feeding decreased days 92-105
9	Peritoneum	25.7	yes	.	Died	3	Frog emaciated, post-surgical complications
10	Peritoneum	25.0	yes	44.3	Survived[a]	134	Small sore on skin noted at necropsy
11	Peritoneum	23.9	yes	19.3	Died	9	Frog emaciated, post-surgical complications
12	Peritoneum	24.5	yes	-	Died	6	Post-surgical complications
13[b]	Peritoneum	24.7	yes	-	Died	49	Infection, source unknown.
14[b]	Peritoneum	23.6	yes	-	Died	14	Cause unknown, presumed post-surgical complications
15[b]	Peritoneum	29.3	yes	47.6	Survived[a]	116	Transmitter appears to be in a gastrointestinal capture
16[b]	Sub-Q	19.9	yes	40.9	Survived[a]	116	Transmitter covered in tissue
17[b]	Sub-Q	23.3	yes	-	Survived[a]	24	Transmitter broke through sutures. Skin healed in < 1 week.
18[b]	Peritoneum	15.1	yes	-	Died	6	Post-surgical complications, transmitter broke through suture on day 4
19[b]	Sub-Q	20.8	no	-	Died	1	Surgical complications

[a] Survived to end of study.

[b] Animals in the first surgical group.

Table 3. Transmitter implant and tracking data by individual *R. pipiens* for study sites in Houston and Winona Counties, Minnesota, 2001.

Frog ID	Date of surgery	Weight (g)	SVL[a] (cm)	Number of locations	Tracked Start	Tracked End	Total days tracked	Final Weight (Date)	Fate
Agriculture									
6495	31-May	55	9.1	37	1-Jun	20-Jul	49	58.2 (6/28)	Missing 7/23
6520	11-Jun	78.7	10.1	64	12-Jun	6-Sep	86	110.9 (8/27)	Transmitter failure 9/7
6630	5-Jul	44.3	8.6	21	5-Jul	2-Aug	28		Missing 8/3
6646	5-Jul	50.9	8.4	71	5-Jul	15-Oct	102	61.1 (8/27)	Overwintering pond
6198	10-Jul	41.5	7.8	69	10-Jul	18-Oct	100	54.9 (8/27)	Overwintering pond
6183	10-Jul	55.6	9	30	10-Jul	22-Aug	43	63.6 (8/1)	Trampled 8/22
6558	10-Jul	66.5	9.7	44	10-Jul	6-Sep	58	83.3 (8/1)	Missing 9/7
Natural									
6504	14-May	42.1	8.9						Did not recover
6520	14-May	37.6	8.3						Did not recover
6478	14-May	39	7.7	38	15-May	2-Jul	48	43 (6/28)	Missing 7/3
6558	11-Jun	39.1	7.6	4	11-Jun	19-Jun	8		Struck by mower 6/19
6546	11-Jun	37.2	7.7	35	11-Jun	30-Jul	49	45.2 (6/28)	Struck by mower 7/30
6530	11-Jun	46.2	8.3	36	11-Jun	27-Jul	46	44.6 (6/28)	Missing 7/30
6593	14-Jun	61.3	8.8	59	14-Jun	1-Sep	79	79.2 (8/27)	Transmitter failure 9/4
6220	14-Jun	57	9	6	14-Jun	20-Jun	6		Dead 6/20
6358	15-Jun	35.9	7.8	37	15-Jun	3-Aug	49	50.6 (8.2)	Missing 8/6
6620	21-Jun	54.8	8.4	16	21-Jun	13-Jul	22		Missing 7/9
6373	21-Jun	44	8.6	42	21-Jun	18-Oct	119	43.7 (6/28)	Missing 7/27, found 8/24
6383	25-Jun	38.7	8.5						Did not recover
6104	25-Jun	41.9	8.3	65	25-Jun	18-Oct	115	45.4 (8/27)	Overwintering burrow
6121	25-Jun	64.2	9.7	38	25-Jun	21-Aug	57	71.6 (8/2)	Missing 8/22
6147	28-Jun	69.2	9.9	62	28-Jun	18-Oct	112	74.7 (9/12)	Missing 7/9, found 7/30, wintered in stream
6403	28-Jun	37.1	8.5	19	28-Jun	26-Jul	28		Struck by mower 7/26

Frog ID	Date of surgery	Weight (g)	SVL[a] (cm)	Number of locations	Tracked Start	Tracked End	Total days tracked	Final Weight (Date)	Fate
6155	28-Jun	40.6	7.7	12	28-Jun	16-Jul	18		Missing 7/17
6133	28-Jun	45.4	8.6	67	28-Jun	15-Oct	109	49.1 (8/31)	Missing 8/7, found 8/13
6383	28-Jun	37.1	7	29	28-Jun	10-Aug	43	52.5 (8/2)	Underground 8/22 (predated?)
6207	16-Jul	53	7.9	12	16-Jul	31-Jul	15		Struck by mower 7/31
Urban									
6530	15-May	42.1	8.6						Did not recover
6373	15-May	46.2							Did not recover
6358	15-May	39.8	8.2						Did not recover
6220	15-May	47.9	7.7						Did not recover
6495	15-May	55.5	9.1						Did not recover
6546	15-May	58.2	9.5						Did not recover
6504	31-May	47.9	9	43	31-May	7-Aug	68	48.1 (6/20)	Missing 8/8
6574	12-Jun	38.6	8.7	15	12-Jun	3-Jul	21	39.5 (6/21)	Missing 7/5
6593	13-Jun	42.5	9						Did not recover
6582	13-Jun	37.3	8.8	24	13-Jun	17-Jul	34	38.7 (6/21)	Missing 7/18
6608	15-Jun	41.4	8.4	66	15-Jun	1-Oct	108	58.9 (9/5)	Transmitter failure
6423	28-Jun	37.8	8.4	7	28-Jun	10-Jul	12		Predated 7/10
6167	28-Jun	49.7	9.5	13	28-Jun	17-Jul	19		Missing 7/18
6655	10-Jul	49.2		6	10-Jul	17-Jul	7		Missing 7/18
6446	16-Jul	35.1	8.1	9	16-Jul	27-Jul	11		Missing 7/30
6433	16-Jul	36	8.6	10	16-Jul	30-Jul	14		Predated 7/30 (owl)
6232	16-Jul	41.6	8.5	58	16-Jul	9-Oct	85	83.8 (8/31)	Missing 10/10

[a] SNV= Snout-vent length.

Table 4. Rates of movement (m/day) and distance traveled (m) by *R. pipiens* at study sites in Houston and Winona Counties, Minnesota, 2001.

Site	Frog ID	N	Min. dist. (m)	Max. dist. (m)	Total dist. (m)	Mean dist. (m)	Min. date	Max. date	Dur.	Min. move. rate (m/day)	Max. move. rate (m/day)	Mean move. rate (m/day)
Agric	6183	30	0.00	225.24	798.47	27.53	20010710	20010822	43	0.00	112.62	18.57
Agric	6198	69	0.00	154.21	1592.64	23.42	20010710	20010710	100	0.00	137.00	15.93
Agric	6495	37	1.00	396.01	1634.00	45.39	20010601	20010720	49	1.00	396.01	33.35
Agric	6520	64	1.00	134.95	1131.39	17.96	20010612	20010906	86	0.47	80.99	13.16
Agric	6558	44	0.00	276.72	1113.27	25.89	20010710	20010906	58	0.00	101.83	19.19
Agric	6630	21	2.83	147.62	755.48	37.77	20010705	20010802	28	1.41	104.81	26.98
Agric	6646	71	0.00	102.59	1302.71	18.61	20010705	20011015	102	0.00	66.71	12.77
min			0.00	102.59	755.48	17.96				0.00	66.71	12.77
max			2.83	396.01	1634.00	45.39				1.41	396.01	33.35
mean			0.69	205.33	1189.71	28.08				0.41	142.85	19.99
Natural	6104	65	0.00	145.77	736.48	11.51	20010625	20011018	115	0.00	72.89	6.40
Natural	6121	38	1.00	121.76	779.37	21.06	20010625	20010821	57	0.33	114.12	13.67
Natural	6133	67	0.00	151.64	837.00	12.68	20010628	20011015	109	0.00	151.64	7.68
Natural	6147	62	0.00	418.59	1032.91	16.93	20010628	20011018	112	0.00	30.41	9.22
Natural	6155	12	8.25	153.32	567.94	51.63	20010628	20010716	18	4.12	153.32	31.55
Natural	6207	12	0.00	86.09	246.71	22.43	20010716	20010731	15	0.00	41.98	16.45
Natural	6220	6	0.00	20.62	31.25	6.25	20010614	20010620	6	0.00	20.62	5.21
Natural	6358	37	1.41	61.07	488.04	13.56	20010615	20010803	49	1.41	61.07	9.96
Natural	6373	42	0.00	304.33	1540.39	37.57	20010621	20011018	119	0.00	262.60	12.94
Natural	6383	29	0.00	94.05	778.74	27.81	20010628	20010810	43	0.00	94.05	18.11
Natural	6403	19	1.41	179.10	647.78	35.99	20010628	20010726	28	1.41	141.94	23.13
Natural	6478	38	0.00	91.02	523.87	14.16	20010515	20010702	48	0.00	91.02	10.91
Natural	6530	36	2.00	103.82	423.14	12.09	20010611	20010727	46	1.58	103.82	9.20
Natural	6546	35	2.83	164.24	848.29	24.95	20010611	20010730	49	1.80	82.12	17.31
Natural	6558	4	47.51	155.86	258.84	258.84	20010611	20010619	8	27.73	47.51	32.35

Site	Frog ID	N	Min. dist. (m)	Max. dist. (m)	Total dist. (m)	Mean dist. (m)	Min. date	Max. date	Dur.	Min. move. rate (m/day)	Max. move. rate (m/day)	Mean move. rate (m/day)
Natural	6593	59	1.41	80.89	790.52	13.63	20010614	20010901	79	0.75	61.91	10.01
Natural	6620	16	2.24	80.78	296.10	19.74	20010621	20010713	22	2.24	80.78	13.46
min			0.00	20.62	31.25	6.25				0.00	20.62	5.21
max			47.51	418.59	1540.39	258.84				27.73	262.60	32.35
mean			4.00	141.94	636.90	35.34				2.43	94.81	14.56
Urban	6167	13	2.24	24.74	116.87	9.74	20010628	20010717	19	2.24	24.74	6.15
Urban	6232	58	1.00	83.95	938.92	16.47	20010716	20011009	85	0.94	83.95	11.05
Urban	6423	7	2.00	43.42	100.29	16.71	20010628	20010710	12	2.00	19.72	8.36
Urban	6433	10	4.47	502.36	663.04	73.67	20010716	20010730	14	4.47	167.45	47.36
Urban	6446	9	0.00	184.83	339.62	42.45	20010716	20010727	11	0.00	84.29	30.87
Urban	6504	43	2.24	78.87	674.35	16.06	20010531	20010807	68	2.00	27.80	9.92
Urban	6574	15	6.40	29.27	195.92	13.99	20010612	20010703	21	2.54	26.02	9.33
Urban	6582	24	1.00	55.36	360.04	15.65	20010613	20010717	34	1.00	55.36	10.59
Urban	6608	66	1.00	41.72	772.04	11.88	20010615	20011001	108	0.67	41.72	7.15
Urban	6655	6	2.24	19.10	57.13	11.43	20010710	20010717	7	2.03	19.10	8.16
min			0.00	19.10	57.13	9.74				0.00	19.10	6.15
max			6.40	502.36	938.92	73.67				4.47	167.45	47.36
mean			2.26	106.36	421.82	22.81				1.79	55.02	14.89

Table 5. Average kernel home range and core area (m^2) for study sites in Houston and Winona Counties, Minnesota, 2001.

	Pond	N	Developed	Forest	Grass	Crop	Wetland	Total
95% Home Range Area	Agriculture	7	N/A	3701	3060	868	335	7964
	Natural	11	N/A	1152	965	447	2939	5503
	Urban	4	1701	745	N/A	N/A	2300	4746
65% Core Area	Agriculture	7	N/A	392	501	160	9805	1151
	Natural	11	N/A	237	141	0.49	868	1246
	Urban	4	419	361	N/A	N/A	732	1512

Table 6. Proportion of area occupied by habitat types within 1,000 m of the breeding site and proportion of use by *R. pipiens* for study sites in Houston and Winona Counties, Minnesota, 2001.

	Site	Developed	Forest	Grasses	Row Crop	Wetland
Available	Agricultural	0.0	54.9	36.0	8.9	0.2
	Natural	0.0	10.8	43.8	43.4	2.0
	Urban	47.5	13.3	0.0	0.0	39.2
Used	Agricultural	0.0	39.5	41.5	12.6	6.4
	Natural	0.0	17.3	18.7	0.5	63.6
	Urban	14.4	20.4	6.6	0.03	58.5

Table 7. Differences between habitat use and availability based on Friedman sums of difference rankings for study sites in Houston and Winona Counties, Minnesota, 2001.

Site	N	Developed	Forest	Grasses	Crop	Wetland	P-value
Agricultural	7	N/A	13	19	18	20	0.50
Natural	17	N/A	49	31	24	66	0.0001
Urban	10	16	23	N/A	N/A	21	0.29

Table 8. Differences between habitat use and availability based on the Johnson method (1980) for study sites in Houston and Winona Counties, Minnesota, 2001.

Site	N	Developed	Forest	Grasses	Crop	Wetland	P-value
Agricultural	7	N/A	1.2	-0.4	-0.1	-0.7	0.19
Natural	17	N/A	-0.5	1.5	1.6	-2.7	0.0001
Urban	10	1.2	-0.7	N/A	N/A	-0.6	0.05

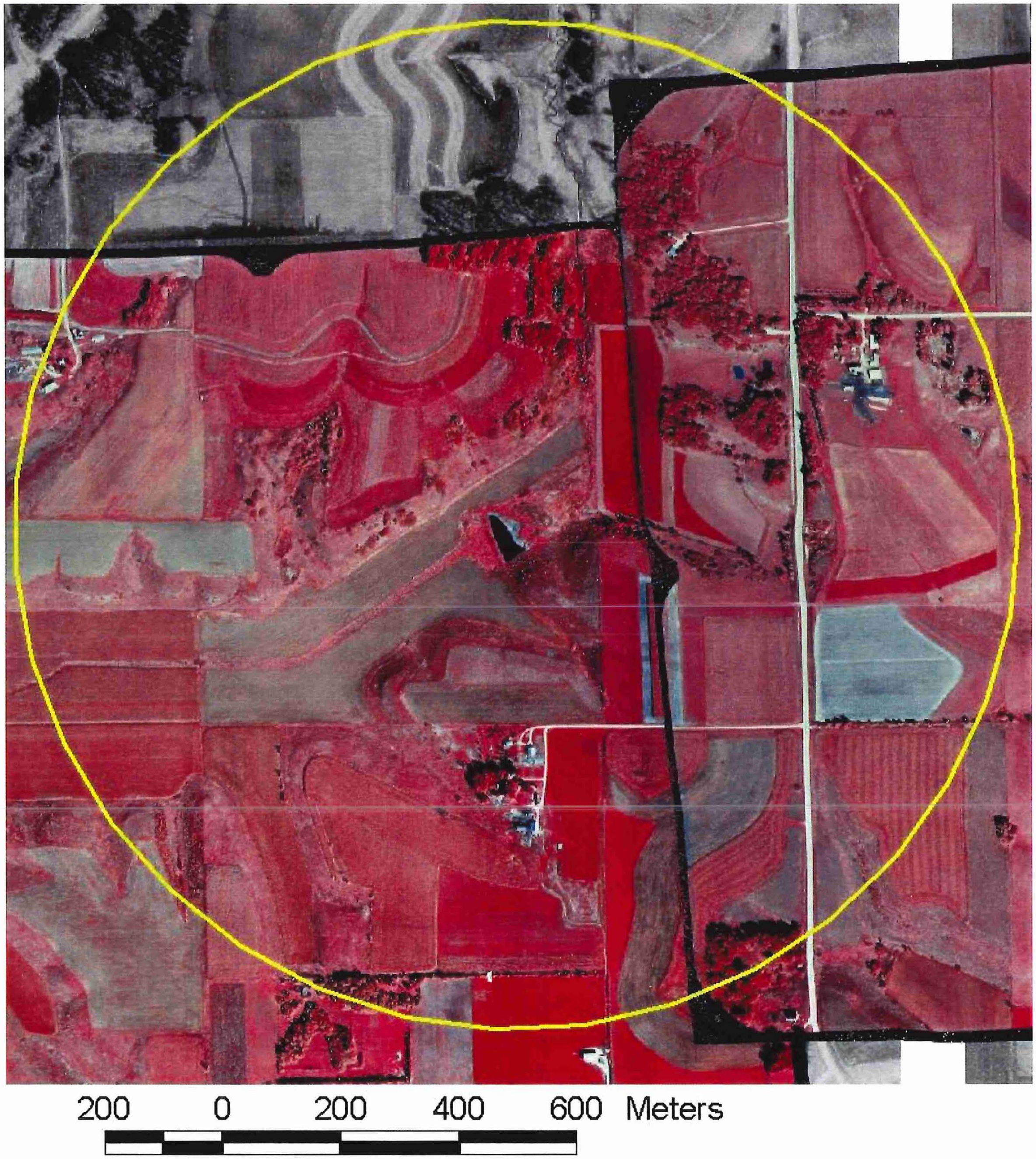

Figure 1. Color infrared photograph of the natural site with a 1,000-m study site boundary, Winona County, Minnesota, September 2001.

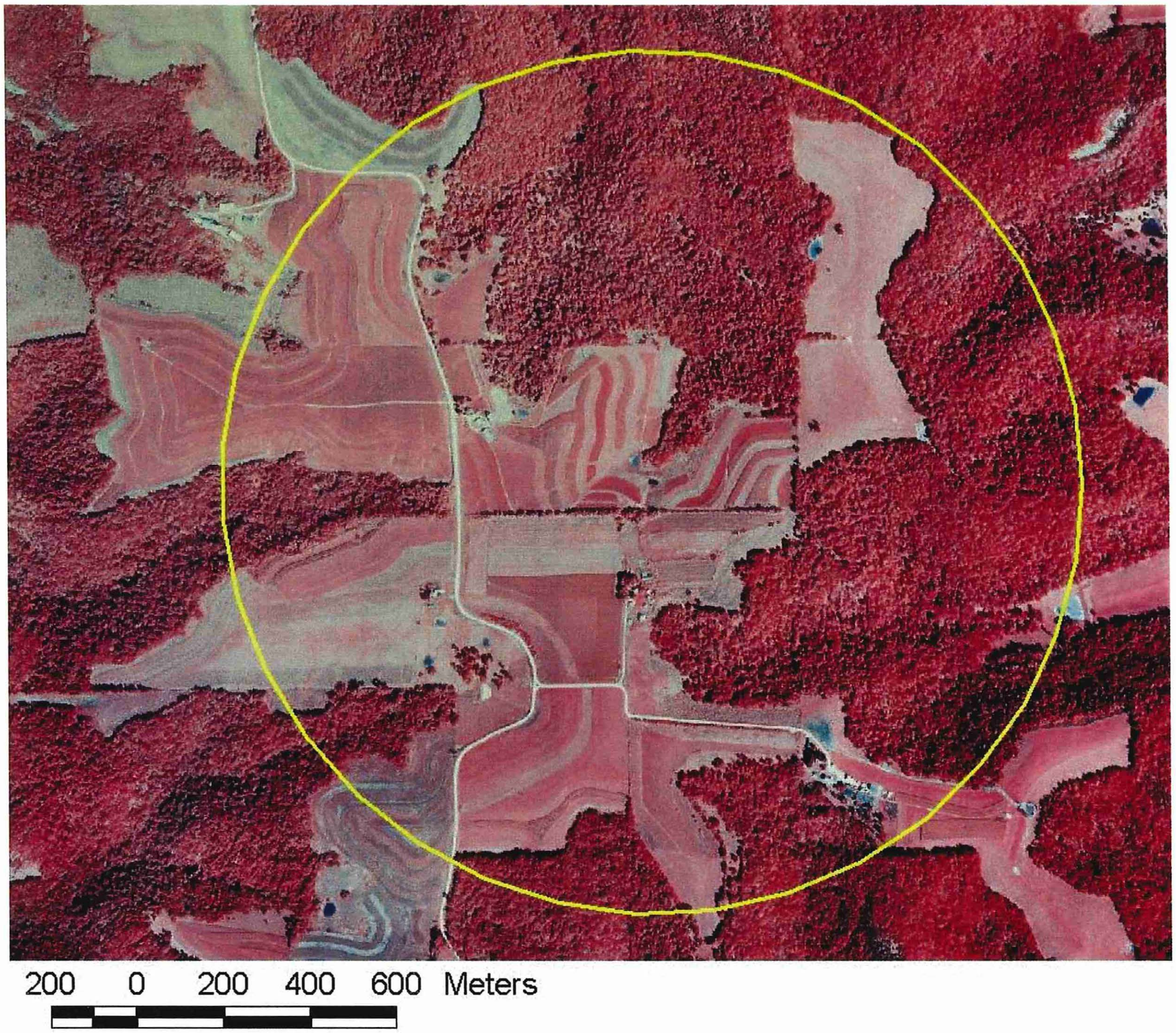

Figure 2. Color infrared photograph of the agricultural site with a 1000-m study site boundary, Houston County, Minnesota, September 2001.

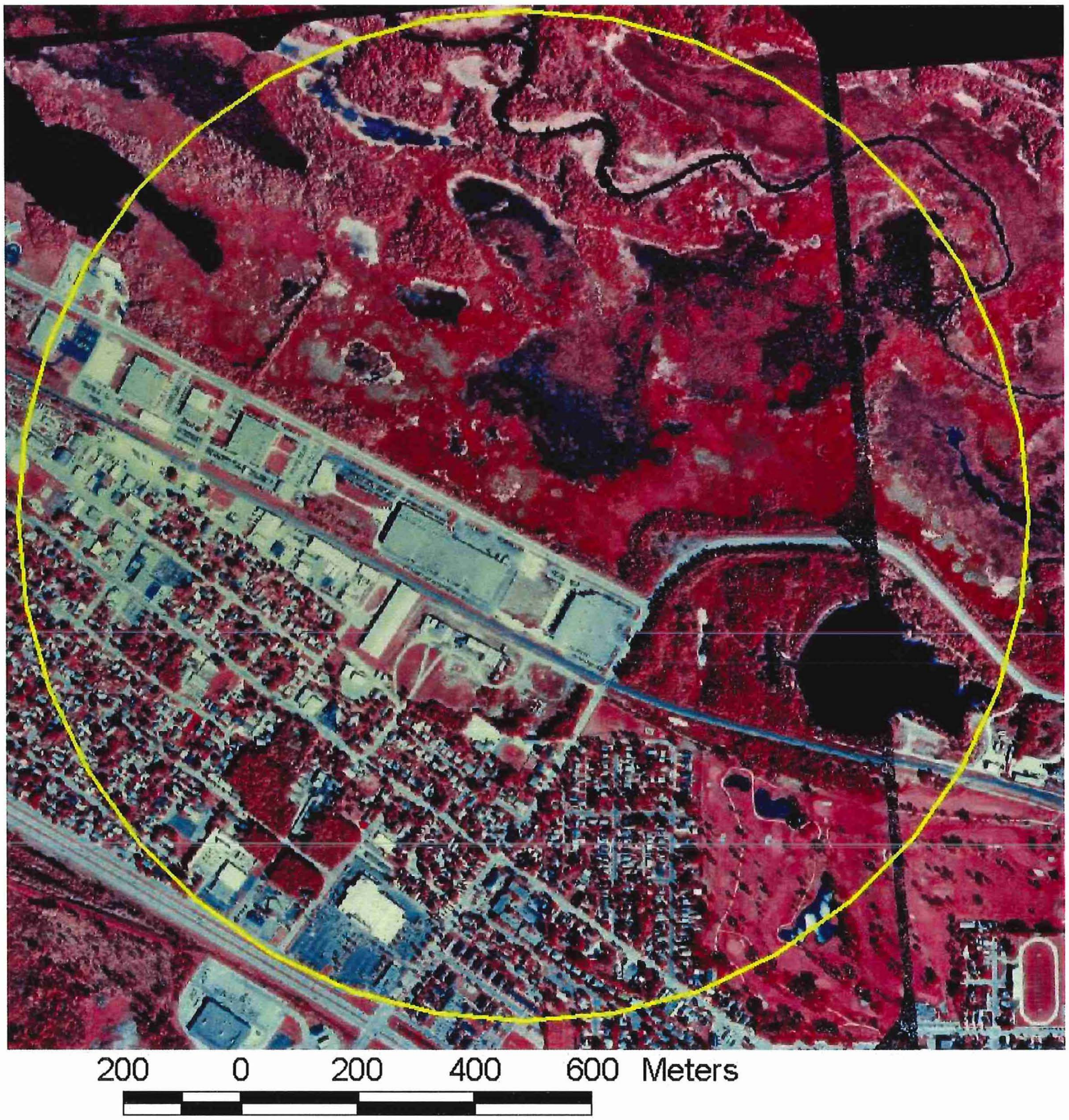

Figure 3. Color infrared photograph of the urban site with a 1000-m study site boundary, Winona County, Minnesota, September 2001.

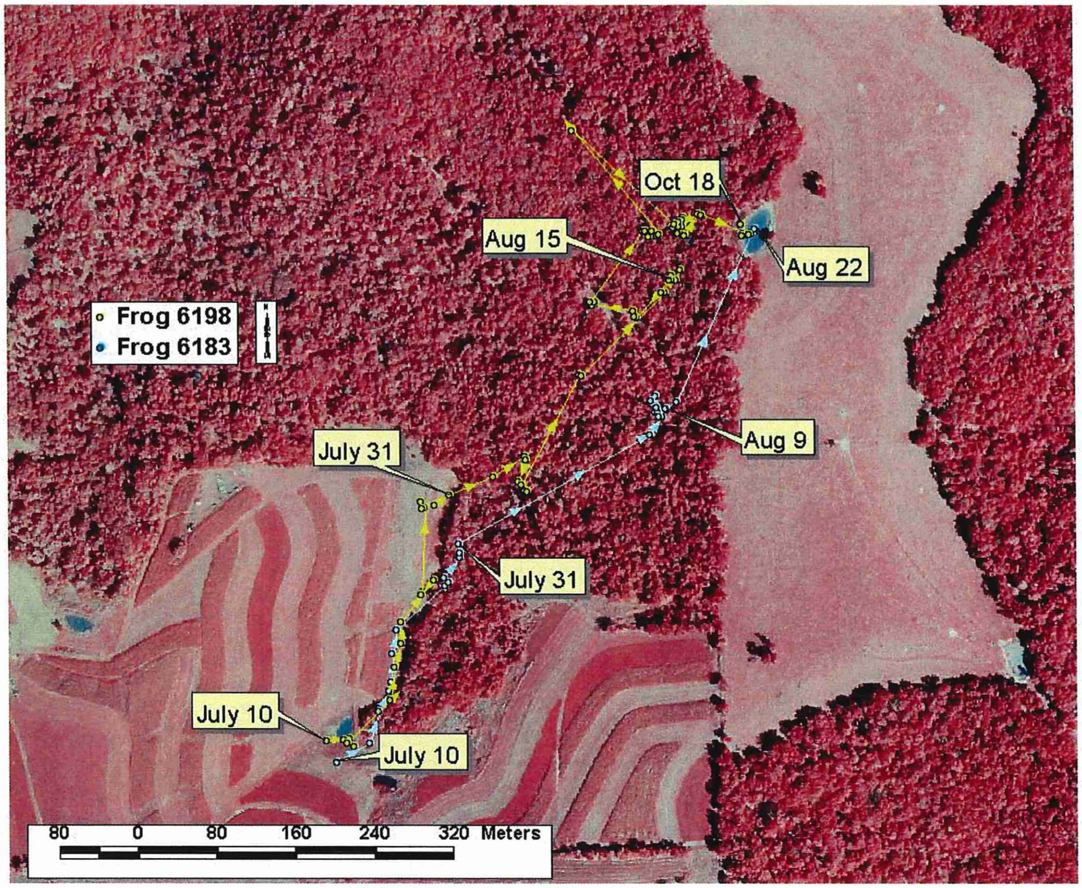

Figure 4. Movement patterns of *Rana pipiens* post-breeding in an agricultural landscape of Houston County, Minnesota, 2001. The movement is from one agricultural farm pond to another pond, requiring movement down-slope, then upslope through forest to the second pond. Evidence indicates frog #6198 will overwinter at the second pond.

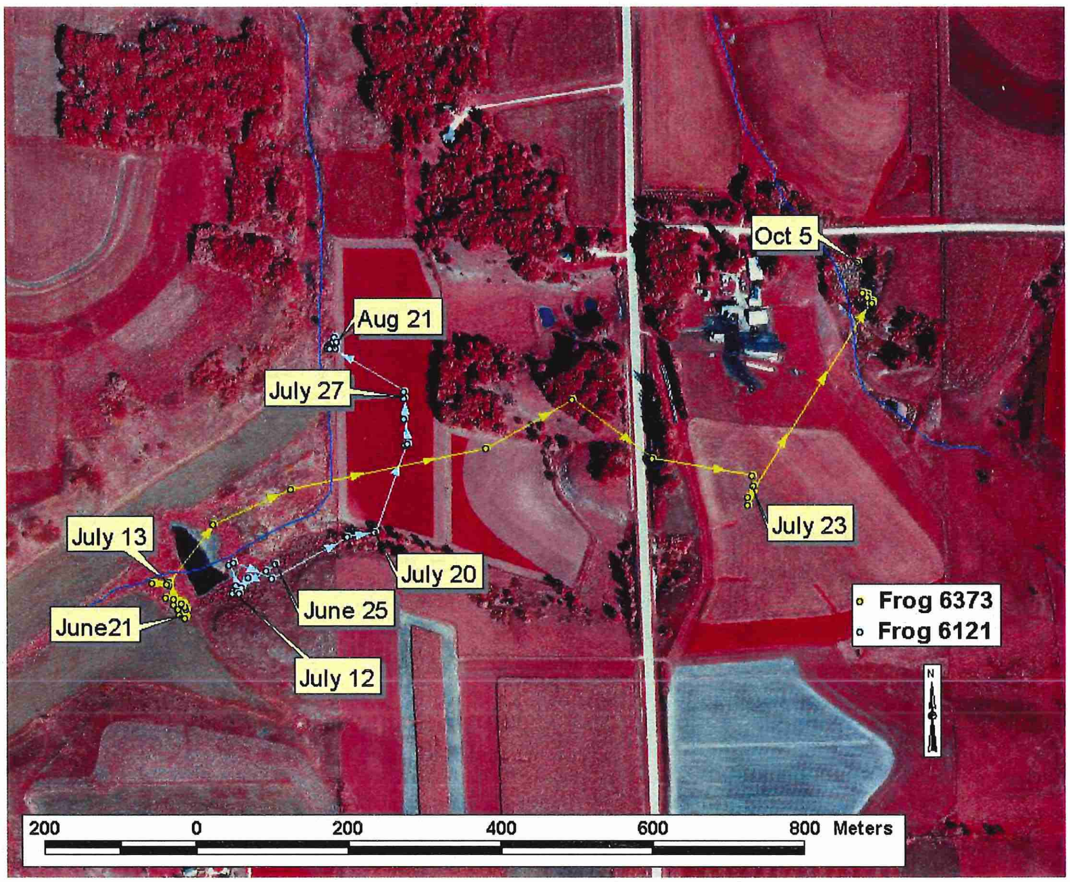

Figure 5. Movement patterns of *Rana pipiens* post-breeding in an agricultural landscape of Winona County, Minnesota, 2001. The movement is from a diked natural wetland (lower left of photo) through various types of cropland where harvest of alfalfa proved fatal for four frogs. Frog #6373 crosses a two-lane pavement road and ends in a small patch of trees near a stream.

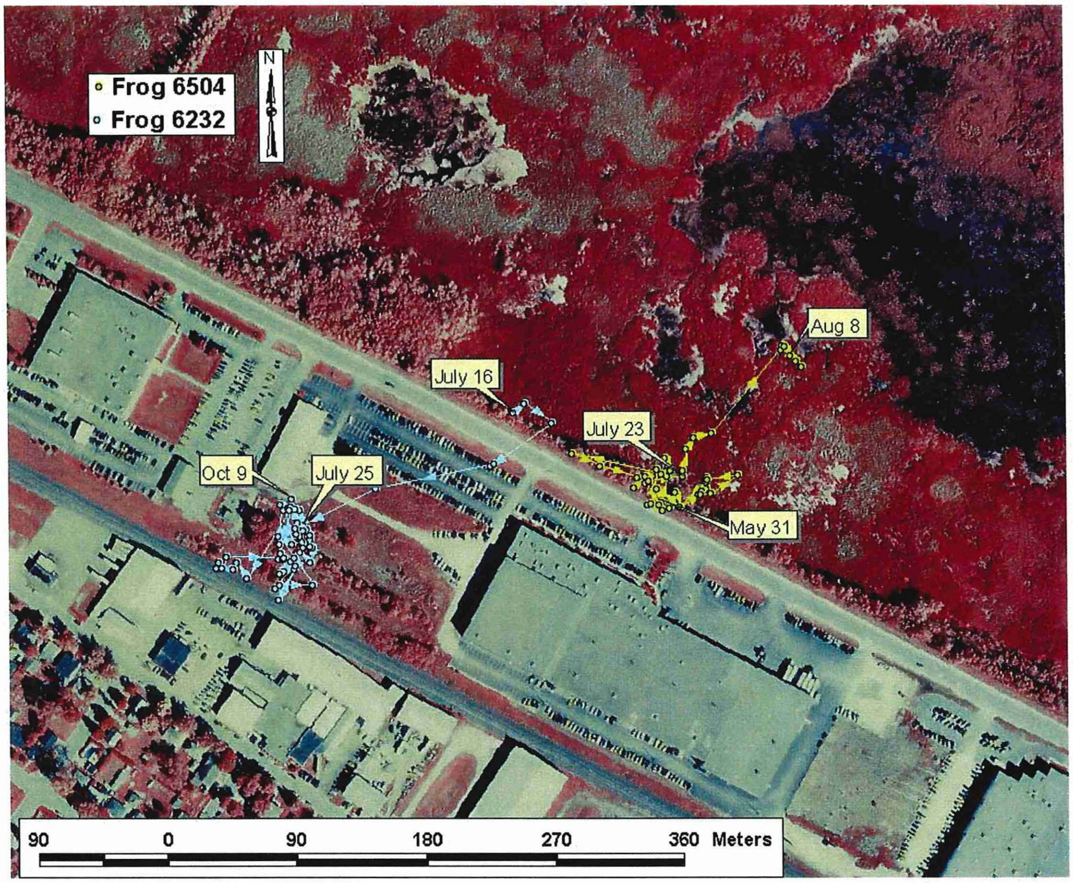

Figure 6. Movement patterns of *Rana pipiens* post-breeding in an urban landscape of Winona County, Minnesota, 2001. Movements of frog #6504 are all within backwaters of the Mississippi River, bordering Federal land owned by the U. S. Fish and Wildlife Service, Upper Mississippi River National Wildlife and Fish Refuge. Frog #6232 moved out of the refuge and into scrub/shrub urban land, crossing urban industrial parking lots. Frog 6232 was still in this habitat when last tracked on October 9. Frog not found on October 10, 2001.

Appendix A. Standard Operating Procedures for Radio Transmitter Implantation into the Peritoneal Cavity of Northern Leopard Frogs.

Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road, P.O. Box 818
La Crosse, Wisconsin 54603

SOP No. TS416.0
Date: 3-7-2000
Revised 6-17-2001
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STANDARD OPERATING PROCEDURE

PROCEDURE TITLE: Implanting Radio Transmitters into the Peritoneal Cavity of Adult Northern Leopard Frogs (*Rana pipiens*).

APPLICABILITY: Branch of Terrestrial Sciences of Wildlife Ecology, Upper Mississippi Sciences Center

PRINCIPLE: This procedure applies to the implantation of transmitters into the peritoneal cavity of *R. pipiens* and was adapted from established protocols from Caren Goldberg and Mat Goode's implantation methods with barking frogs, Arizona State University, and Gayle Birchfield's work with green frogs, University of Missouri. The frogs will be anesthetized during the surgical procedure and it is assumed that the implantation of the transmitter does not alter the normal behavior of the frog under investigation.

PRECAUTIONS: Precautions will be taken while conducting the surgery to ensure the safety of the personnel involved in the procedure. Read the Material Safety Data sheet for Finquel™ (MS-222). Surgery will take place in a ventilated area with personnel wearing safety goggles and chemical resistant gloves. Transmitter weight shall not exceed 5% (37g) of the frog's weight to minimize effects on the frog. Surgical procedures will be completed as efficiently as possible. Surgeries should not exceed 30 minutes. Collection permits will be obtained as required by the state in which the capturing and tagging will take place.

PROCEEDURE:

A. Equipment and supplies

1. Two-person crew (surgeon and assistant)
2. Hip boots or chest waders
3. Dip nets
4. 5-gallon pail to hold captured frogs
5. Surgery tray, fiberglass, (about 50 cm x 30 cm x 5 cm)
6. Sterile surgical gloves
7. Sponge (about 20 cm x 12 cm x 5 cm)
8. Cut up sponge pillows (about 5cm x 3cm x 2cm), one for each surgery
9. Sterile drop cloth

10. No. 3 scalpel handle with No. 15 scalpel blades
11. Tissue forceps
12. Tissue spreader
13. Needle holder
14. Sterile gauze package
15. Surgical scissors
16. Polysorb 6/0 suture package with reverse cutting needle (1 per frog)
17. 2 squeeze bottles (50-ml)
18. Holohil BDG-2HX transmitter
19. Transmitter receiver
20. Finquel™ (MS-222)
21. Anesthetizing container (1-gallon wide-mouth glass jar)
22. Sodium bicarbonate
23. pH paper
24. Electronic balance
25. Sterile saline solution
26. Recovery container (6"x10"x3", plastic with lid)
27. Instrument disinfectant (Benzal)
28. Instrument container (shallow rectangle Rubbermaid container large enough to hold the surgical instruments)
29. Instrument basket (strainer type of plastic tray large enough to hold the surgical instruments while fitting into the surgical container.
30. Transmitter container (100-ml glass jar)
31. Measuring tape
32. 1-gallon plastic bags
33. Data sheets
34. Container for rinse solutions (1-gallon container)
35. Portable operating table
36. Bleach
37. Holding pen (about 2 m x 2 m x 1 m)
38. Toothbrush to clean instruments
39. Bactine antibiotic spray
40. Plastic container for supplies
41. Benz-all (12.9% benzalkonium chloride solution) 1.35oz bottle of Benz-all mixed in 1 gallon of water makes a solution of 1:750 dilution of Benzalkonium Chloride

B. Preparations to be made before departing for the field.
    1. Mix sterilizing solution (1 bottle of concentrated Benz-all to 1 gallon of clean water)
    2. Sterilize boots and nets with bleach solution
    3. Sterilize surgical supplies for 15 minutes in Benz-all solution, rinse with clean water and air dry.
        a. Surgical tray
        b. Surgical sponges
        c. Sponge pillows

   d. Recovery containers
   e. Clean holding buckets
4. Make sure all supplies are in the plastic container
5. Explain implantation procedure to all personnel
6. Remove the magnet from the surface of the transmitter. Ensure that the transmitter is broadcasting a signal by turning on the receiver and tuning it to the transmitter frequency.
7. Clean the transmitters and surgical equipment with dish soap and rinse with tap water.
8. Place transmitters and surgical equipment in instrument basket. Set the instrument basket in the instrument container filled with Benz-all.
9. Transmitters and equipment should be soaked for a minimum of 15 minutes in Benz-all solution prior to each surgery.

C. On-site preparations to be completed before surgery
1. Fill 5 gallon pail with 2-3 gallons of pond water.
2. Capture frogs suitable for implantation (transmitters should not exceed 5% (38g) of the frogs weight) and place the frogs into holding buckets until needed for surgery. If holding time will exceed 0.5 hours, place the frogs into a 2 m x 2 m holding pen until needed for surgery.
3. Prepare anesthesia in the 1-gallon glass jar (Green 2001). Place 1 liter of filtered pond water (well water) into the jar and add 0.2 g of Finquel. Mix the solution thoroughly and then add sodium bicarbonate until the pH of the solution is about 7 as determined by the pH paper.
4. Place anesthesia jar in a second 5 gallon pail with 2-3 gallons of pond water. **Note: This allows the anesthesia solution to maintain the same temperature as the frog's holding water avoiding any shock to the frog when exposed to the anesthesia. Ensure the temperature is not above 25°C**
5. Place the surgery tray onto the operating table.
6. Fill the 50-ml squeeze bottle with the anesthesia and place syringe onto the operating table.
7. Place the instrument and transmitter containers onto the operating table readily accessible to the assistant.
8. Place one absorbable suture package (Polysorb 6/0), two packages of gauze and one sterile drape near the surgery tray.
9. Place the sponge into the surgery tray. Dampen the sponge with sterile saline solution.

D. Surgical Procedures

Note: The surgeon should maintain sterile, gloved hands throughout the surgery. If the surgeon's gloves are contaminated at anytime during surgery, they should be discarded and replaced with a new sterile pair)

1. Assistant: Put on nitrile gloves

2. Assistant: Place the frog into the anesthetizing jar and close the lid. Do not expose the frog for more than 2 minutes. Generally, 90 seconds sedates the frog. **Precaution: Watch the frog closely to ensure the frog's head does not submerge in the anesthesia for any length of time.**
3. Assistant: Remove the frog from the anesthetizing jar and place into the recovery container while the drug continues to take effect, usually 2-3 minutes.
4. Surgeon: Put on sterile gloves.
5. Assistant: Partially open suture packages, gauze and drapes. Minimize contamination by only touching the packaging. Fold packaging back exposing the sterile contents for the surgeon.
6. Surgeon: Remove sterile drape from package and place over the operating sponge. Remove sterile gauze from package and place on drape. Care must be taken to avoid touching the packaging material.
7. Assistant: Once the frog is anesthetized, weigh the frog to the nearest gram and measure the frog's snout-vent length to the nearest millimeter. Record the weight and length on the appropriate study data sheet.
8. Assistant: Place the frog, ventral side up, onto the sponge located in the surgery tray. **Note: When touched, the frog should still have a slight response. If the frog does not respond at all when touched, rinse the frog with fresh water until a slight response to touch is noted. If during surgery, the frog appears to be over responding to the procedure, rinse the frog's skin with the anesthetic from the syringe.**
9. Assistant: Place moist sterile gauze pads across the head and hind legs of the frog.
10. Assistant: Remove basket of needed surgical instruments from instrument container and rinse them with sterile saline solution. Place the rinsed instruments onto the sterile drape on the operating table.
11. Assistant: Spray the area to be cut with Bactine antiseptic spray.
12. Surgeon: With a scalpel, scissors, and tissue forceps, make an incision along the left side of the frog's abdominal region through the skin and muscle exposing the body cavity. The incision should be just long enough to permit insertion of the transmitter. **Note: Extreme care should be taken not to cut the blood vessels or the internal organs in the vicinity of the incision.**
13. Surgeon: Take a transmitter from the transmitter container and rinse it with sterile saline solution. Verbally indicate the transmitter identification number to the Assistant.
14. Assistant: Ensure the transmitter is working properly by turning the radio receiver on and tuning it to the appropriate frequency. Record the transmitter's identification number onto the appropriate data sheet.
15. Surgeon: Hold the incision open with the tissue forceps and/or the tissue spreader as appropriate, and insert the transmitter into the frog's body cavity. Note: In some cases, it may be necessary for the Assistant to help hold the incision open while the surgeon inserts the transmitter.

16. Surgeon: Close the peritoneum with one continuous suture with the Polysorb 6/0 suture. Use the single-instrument tie knot at the beginning and end of the continuous suture.
17. Surgeon: Close the skin with 4 or 5 simple interrupted sutures using the remaining Polysorb 6/0 suture material. Use the single-instrument tie knot for each of the interrupted sutures.
18. Surgeon: Spray sutures with Bactine antiseptic spray.
19. Assistant: Scrub instruments with the toothbrush, rinse them with saline solution and place them back into the instrument container with Benz-all solution. Note: Instruments should soak in Benz-all solution for a minimum of 15 minutes before being used again.

E. Recovery and release of the surgically implanted frog

1. Assistant: Place the frog into the recovery container. Place a piece of cut sponge (1"x ½"x ½") under the frog's chin. Add fresh pond water (well water) to the container until the water level is just under the frog's mouth. **Do not allow water to enter the mouth.** Monitor the frog; if the frog is not alert in 5 to 10 minutes completely exchange the water in the recovery container.
2. Assistant: Once the frog is fully alert, place it into the holding enclosure. Holding enclosure and recovery containers will be protected from direct sunlight using tarps, canopies, boards or tables.
3. After a minimum of 4 hours, observe the frog to determine if it is behaving "normally." If the frog is behaving "normally," release the frog near the capture site. If the frog is not behaving "normally," maintain the frog overnight in an enclosure and re-evaluate its condition the following morning.

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Appendix B. Standard Operating Procedure for Amphibian Care, Maintenance, and Disposal.

Upper Midwest Environmental Sciences Center	SOP No. 417.0
2630 Fanta Reed Road, P.O. Box 818	Date: 1/26/01
La Crosse, Wisconsin 54603	Page 1 of

STANDARD OPERATING PROCEDURE

PROCEEDURE TITLE: Care, Maintenance and Disposal of Amphibians

APPLICABILITY: Branch of Terrestrial Sciences, Upper Midwest Environmental Sciences Center.

PURPOSE: To provide a set of standard procedures for the maintenance, handling and disposal of laboratory amphibians at the Upper Midwest Environmental Sciences Center.

PROCEEDURE:

A. Receiving study animals

1. Animals will be received and handled at the Center according to General 133 and Green (2001).

B. Animal housing

1. In each area where amphibians are held, an inventory of holding tanks will be maintained. Aquariums at least 10 gallons in size provide adequate space for most amphibians. Aquariums will be set up according to the species it will hold. Totally aquatic amphibians, like the clawed frog and various newts will be kept in an aquarium half full of dechlorinated water with some type of floating material to provide a rest area. Green frogs, bullfrogs, salamanders and other highly aquatic amphibians will be housed in an aquarium divided in two sections: one for land and one for water. Ranids, tree frogs and other species who do not spend a great deal of time in the water will be set up with substrate and a shallow dish (8"x 8"x 2") of water. The dish should be a non-porus material that is easily cleaned. Aquariums should have a lid that will not injure frogs that brush against it. Clear plexi-glass with air exchange holes will help hold humidity and temperature levels while not injuring the amphibians. Plexi-glass can be cut to fit the tank and is easily cleaned.
2. Several substrate materials can be used depending on the species of amphibian to be housed. Astroturf, out door carpet, sand, aquarium gravel and soil can be used. These substrates will need to be cleaned every two weeks and recorded on daily log sheets. Carpet and gravel are easier to

clean and maintain. Most amphibians require hiding places to feel secure and reduce stress. Well placed rocks, over turned bowls (6"x6"x6"), branches or artificial plants provide cover and security and are easily cleaned.

3. Temperature regulation is critical and species dependent. Tropical species require warmer air temperatures ranging from 75°F to 80°F. North American species prefer slightly cooler air temperatures ranging from 60°F to 75°F. Care must be taken to avoid air temperatures above 80°F. High air temperatures create stress and the animal is more susceptible to illness. Heat lamps can be used during the day to maintain target temperatures but should not be left on at night. Do not place the lamps too close to the tank; excessive heat and bright concentrated light may injure the animal. Water temperatures will be held at room air temperatures.

C. Animal densities

1. Overcrowding creates stress that inhibits the animals' immune system, places the animals into closer contact with waste materials and exposes the animals to potentially harmful pathogens carried by other animals sharing a common tank. No more than one or two small amphibians (<50g) or one large amphibian may be housed in a 10- gallon tank. Never place different species in the same tank. If more than one animal is in the tank make sure they are at the same life cycle stage. One animal per tank is the best set up.

D. Food

1. Food items include crickets, grubs and earthworms offered at last three times per week. Eight to ten crickets every other day works well for *R. pipiens*. Food will be purchased from commercial vendors, pet stores or propagated in the lab. Bait stores should be avoided. Maintain records recording the food type and source. The food items should be sprinkled with a calcium/mineral supplement available from pet supply stores. Salamanders and newts require a varied diet and should be feed a mix of foods. Frogs and toads do not require the mix of foods. Aquatic species can be fed worms, fish, pieces of meat or dried pelleted food found at local pet stores. Dead or spoiled food items will be removed each day. Feeding records will be kept on daily log forms.

E. Cleaning

1. Water dishes should be cleaned daily and the tanks wiped down with a clean towel. The frogs do not need to be removed for daily cleaning. Do not use the same cloth for more than one tank. Gloves will be washed with antibacterial soap and rinsed with clean water between tank cleanings. Aquatic species with water filtration devices do not need to be changed daily. All food items from the previous feeding and waste materials should be removed daily. Substrate and

any artificial items should be cleaned twice a month in a Benz-all solution (1.35oz bottle of Benz-all mixed in 1 gallon of water makes a solution of 1:750 dilution of Benzalkonium Chloride), rinsed with clean water and air or towel dried. Tanks will be cleaned by spraying the inside with a Benz-all solution, thoroughly scrubbing the glass, rinsing with clean water and wiping dry before new animals are placed in them. Each animal will be placed in a small plastic holding container (8"x8"x4") while the tank is being cleaned. Approximately 2-3 cm of clean water at room temperature will added to the holding container and a lid fastened on top. The animals will be returned to the same tanks after the tank, media and water bowl have been cleaned. Cleaning records will be kept on daily log sheets.

F. Disposal of Animals

1. Dead animals will be removed immediately and the tank cleaned. Animals reaching the end of the study will be euthanized by placement in a .02% solution of MS-222 for 10 minutes. Before disposal, prick the leg or lightly press on the eye to ensure the animal is dead. Larger amphibians may require more exposure time to the MS-222. Dead animals will be frozen and incinerated according to GEN 132.7.

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Agricultural Land Uses are not Associated with Genetic Damage or Malformations in Frogs in Southeastern Minnesota

by

Bart L. Bly, Mark B. Sandheinrich, Dean A. Jobe, Melinda G. Knutson, and Brian R. Gray

Farm Ponds as Critical Habitats for Native Amphibians: Final Report

Submitted to

Legislative Commission on Minnesota Resources
100 Constitution Avenue, Room 65
St. Paul, Minnesota 55155-1201

June 2002

U.S. Geological Survey
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603

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Agricultural Land Uses are not Associated with Genetic Damage or Malformations in Frogs in Southeastern Minnesota

by

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Abstract:
Flow cytometry (FC) is a laboratory method that can be used to indicate genetic damage in amphibians. It is useful for evaluating sites with high rates of amphibian malformations or sites exposed to contaminants or other stressors. We used FC to compare the effects on amphibians of different types of agricultural land uses surrounding breeding ponds in southeastern Minnesota. Exposed ponds were surrounded by grazed grassland or row crop agriculture and received more fertilizers, pesticides, and animal wastes than the reference ponds, represented by natural wetlands and nongrazed grasslands. Amphibian metamorphs from reference and exposed ponds were examined for malformations and blood samples were analyzed with FC. We found no significant differences in amphibian genetic integrity or malformations between the reference and exposed ponds. Malformations were rare, but were observed in both the reference and exposed ponds.

Key words: amphibian, agriculture, farm pond, flow eytometry, genetic damage, malformation

Introduction

Concerns over high rates of amphibian malformations in some locations (Reaser and Johnson 1999) and global amphibian population declines (Wake 1991; Alford et al. 2001) have led to increased interest in monitoring amphibian populations. Population declines have been documented for some amphibian species in the midwestern United States (Christiansen 1981; Hay

1998; Lannoo 1998). Amphibian populations with known exposures to toxicants are a high priority for monitoring and assessment.

Amphibians are vulnerable to waterborne contaminants because of their biphasic life cycle (larva and adult) and semi-permeable skin (Harfenist et al. 1989; Mahaney 1994). Some studies suggest that high concentrations of pesticides and fertilizers from field run-off (Berrill et al. 1997; Berrill et al. 1998) or retinoids (Gardiner and Hoppe 1999; Sessions et al. 1999) may be responsible for inducing malformations. Many known toxicants have mutagenic effects on amphibians in laboratory studies (Harfenist et al. 1989). Toxicants can induce clastogenic formation of micronuclei, leading to abnormal DNA content (Fernandez et al. 1993; Krauter 1993). Amphibians that breed in farm ponds containing agricultural chemicals may be environmental indicators of contaminants in agricultural landscapes.

Herbicides are applied to 97% of the corn acreage in the 18 top-producing corn states in the United States, including Minnesota (Hunst and Gowse 2001). The herbicide, atrazine is one of the most pervasive agricultural chemicals found in surface waters in the United States. In a study of midwestern reservoirs, 92% were found to be contaminated with atrazine; concentrations were generally <5 ppb (Solomon et al. 1996).

The FC is a laboratory method that can be used to indicate genetic damage in amphibian populations. It is useful in evaluating sites with high rates of amphibian malformations or sites with a history of exposure to contaminants or other stressors. The FC has been used to detect aneugenic and clastogenic effects induced by environmental contaminants and other stressors on the vertebrate genome (Bickham et al. 1988; Lamb et al. 1991; Fernandez et al. 1993). The FC monitors multiple cellular characteristics, estimates cellular DNA content, and detects small changes in DNA caused by exposure to environmental contaminants (Dallas and Evans 1990). The FC can also be used to screen for abnormal DNA profiles, such as aneuploid mosaicism, a chromosomal condition associated with exposure to pesticides. The FC has been used to assess genetic damage in amphibians (Bonin et al. 1997; Lowcock et al. 1997; Murphy et al. 1997), but only in a limited number of locations.

We used FC to compare the effects on amphibians of different types of agricultural land uses surrounding breeding ponds in southeastern Minnesota. Exposed ponds were surrounded by grazed land or row crop agriculture and received more fertilizers, pesticides, and animal wastes than the reference ponds, represented by natural wetlands and nongrazed grasslands. We expected that blood from amphibians developing in exposed ponds would exhibit larger variances in DNA population size and more abnormal DNA profiles than blood from amphibians developing in reference ponds.

Methods

Pond Types

Exposed ponds were constructed ponds surrounded by agricultural row crops or pastures grazed by domestic livestock. The row crops surrounding exposed ponds in our study area were corn and soybeans. Reference ponds were natural wetlands, generally marshes and oxbows of river floodplains, or constructed ponds surrounded by ungrazed grassland. Exposed ponds received higher inputs of fertilizers, pesticides, and animal wastes than the reference ponds; assays of nitrogen and phosphorus in the ponds support this assumption (Knutson et al. 2002). Amphibians from four reference and five exposed ponds were examined in the field for deformities, and blood samples were collected for analysis with FC.

Collection of Specimens and Deformity Assessments

Deformity assessments were conducted at the ponds when amphibians were late-stage metamorphs (May to July 2000). Deformity assessments consisted of examining as many as 100 individual metamorphs for physical malformations. Up to 10 metamorphs, including any deformed individuals, from each pond were collected live for necropsy and blood collection for FC analysis.

Necropsy and Blood Collection

Blood samples for FC analysis were collected in the laboratory from 58 metamorphs representing three species: *Rana clamitans* (*N*=40), *Rana pipiens* (*N*=13), and *Rana palustris* (*N*=5). Specimens were euthanized with methane tricaine sulfonate (MS-222; Argent Laboratories, Redmond, Washington), and two 10-µL samples of auricular blood were collected with 40-µL heparinized capillary tubes. Each sample was resuspended in 1.5-mL cryovials containing 200-µL freezing solution (0.25 M sucrose, 0.04 M trisodium citrate, 5% dimethyl sulfoxide; pH 7.61) and manually agitated. Blood specimens were flash frozen in liquid nitrogen and stored at -70° C until analysis. In addition, metamorphs were necropsied to determine their overall health and examined for *Ribeiroia* and other parasites.

Reference Blood

Accurate estimation of DNA content for the target species requires knowledge of the DNA content of an internal reference (Tiersch et al. 1989). A reference specimen of known DNA content obtained from *Xenopus laevis* (6.3 pg of DNA/haploid nucleus, certified free of potential mutagens) was used as an internal control in every sample (Xenopus Express, Homosassa, Florida). A healthy *X. laevis* was euthanized as previously described and 4 mL of auricular blood were gathered with a 10-mL syringe and transferred to 80 mL of freezing solution. After manual agitation, the solution was allowed to incubate at room temperature for 1 min to allow tissue and clotted blood to settle out of solution. After incubation, 210-µL aliquots of reference blood were transferred into 1.5-mL cryovials and flash frozen in liquid nitrogen. Reference blood was stored at –70° C until needed.

Sample Preparation

Before sample preparation, ribonuclease stock solution (2 mg/mL) was prepared by adding 20 mg of Ribonuclease A (Sigma Chemical, St. Louis, Missouri) to 10 mL of autoclaved deionized water. One hundred mL of stock staining solution (API: 0.01 M Trizma Base, 0.01 M NaCl, 0.1% NP-40; pH 7.62) was combined with

300 µL of ribonuclease solution and 5 mg of propidium iodide (Sigma Chemical, St. Louis, Missouri). The solution was brought to a final volume of 140 mL using distilled water. The API staining solution was covered to prevent exposure to light and was stored at 4° C until needed. Before each run of samples, 300 µL of fresh ribonuclease stock solution was added to the API staining solution.

Samples for FC analysis were thawed and 200 µL of *Xenopus laevis* reference blood was added to each target sample. Samples were gently vortexed and immediately placed in an ice bath. The API staining solution (750 µL) was added to each sample, gently mixed, returned to the ice bath, and reincubated in the dark for 2 hours. After incubation, each sample was transferred to a 12x75 mm culture tube by passage through 53 µm nylon mesh to remove clumped cells or tissue debris.

Flow Cytometry

After incubation and filtration, samples were analyzed for DNA content using a FACScan flow cytometer (Becton-Dickinson Immunocytometry Systems, San Jose, California). Before data acquisition, the linearity and alignment were calibrated with DNA Quality Control Particles (Becton-Dickinson Immunocytometry Systems). For each target sample, 20,000 stained nuclei were collected. Propidium iodide, when excited by the argon laser (488 nm), emits fluorescent light over the range of 550–650 nm that is detected by a photomultiplier tube (FL2) within the flow cytometer. The FL2-Width vs. FL2-Area dot plots were used to detect and differentiate erythrocytes from debris, and FL2-A histograms were used to determine DNA content. Data were analyzed using CellQuest software (Becton-Dickinson Immunocytometry Systems). Triplicate analysis was performed on 10% of the samples to assess intra-assay variation; the relative SD, defined as the SD divided by the mean, of the triplicate sample runs was 1.63%.

The C-value (pg of DNA/haploid nucleus) and an estimated sample coefficient of variation (CV) were calculated for every sample. The C-value was calculated from the following equation (Lowcock et al. 1997):

$$C_t = C_r P_t \ / \ P_r$$

Where C_t is the C-value of the target species, C_r is the C-value of the internal reference, P_t is the peak channel of the target species, and P_r is the peak channel of the internal reference. The estimated sample CV is defined as the SD of the target peak divided by the mean channel of the target peak, multiplied by 100, and was calculated by the analysis software. The C-values and CVs were averaged among replicate FC analyses of the same individual to obtain a mean value for every specimen. Histograms with the target species' DNA profile were analyzed for aneuploid mosaicism.

Statistical Methods

All statistical comparisons were performed using the CV values. The association between CV and pond exposure status was estimated using a general linear mixed model and restricted maximum likelihood [Littell, 1996 #4124]. Ponds, as the experimental units, were treated as random effects. This method was also used to estimate the correlation among CVs measured on frogs obtained from a common pond. Adequate replication for these analyses was available for *R. clamitans* only.

Results and Discussion

Pond-averaged C-values for *R. pipiens* ranged from 6.53 to 7.08 (Table). Mean CVs within the ponds ranged from 1.91 (SE=0.07; *R. palustris*) to 6.31 (SE=0.23; *R. pipiens*). For *R. clamitans,* the association between pond category and CV was small and nonsignificant (β=0.01, SE=0.24, *t* test, df=3, p=0.98), as was the estimated within-pond correlation among CV values (r=0.08, restricted likelihood ratio test, p=0.48). Residuals appeared approximately normal (Anderson-Darling test, $P > 0.25$). For *R. calamitans,* we found high power (≥90%) to detect an increase of ≥0.5 CV units from a reference mean of 3.0 CV using as few as three ponds in each of two pond types (reference and exposed, Thomas and Krebs 1997; Zar 1999). Lack of replication within species and among ponds in our pilot study precluded examination of inter-species or pond-type effects (Table).

Deformities were rarely observed (two individuals) and occurred in the reference and exposed ponds. The CV's of the deformed frogs were not large, 2.15 and 3.85 for the exposed and reference ponds, respectively. During the necropsy, *R. clamitans* specimens from three ponds—two exposed and one reference pond—were found to contain the parasite *Ribeiroia ondatrae* (Table). *R. ondatrae* were found in the limb bud region of the deformed individuals, indicating that the deformities were most likely because of parasite loads instead of DNA damage (Sessions and Ruth 1990; Johnson et al. 1999; Johnson et al. 2002). The *R. pipiens* from an exposed pond had aneuploid peaks and a higher mean CV than the other ponds, but no deformities (Table). Aneuploid mosaicism (Figure) was observed in 10% of the specimens analyzed, the highest number of abnormal profiles occurring in *R. pipiens* (Table).

We did not find evidence that agricultural land uses surrounding breeding ponds were associated with elevated CVs or higher malformation rates. Although we found some aneuploidy, this is not always indicative of genetic damage and may arise through spontaneous variation in amphibian populations (Lowcock and Licht 1990).

Concentrations of atrazine in water from farm ponds in our study area ranged from 0.04 to 0.55 ppb (μg/L, J. Elder, U.S. Geological Survey, Water Resources, Middleton, Wisconsin, unpublished data, June 2001). At these concentrations, we would not expect genetic damage from atrazine in our reference or exposed farm pond populations. These concentrations are orders of magnitude lower than those shown to be toxic (47.6 mg/L, *Rana pipiens* larvae, 96-h LC50) in the laboratory (Howe et al. 1998). Recent laboratory work indicates that atrazine and nitrate concentrations commonly found in the environment do not alter survival or behavior of several native frog species (Allran and Karasov 2000, 2001). However, a recent laboratory study of *Xenopus laevis* found that at concentrations of 0.1 ppb and above, 16% to 20% of the animals developed multiple reproductive organs and, at 25 ppb of atrazine, testosterone levels in males dropped 10-fold (Hayes et al. 2002). These effects may go unobserved in field studies because no mortality or external abnormalities are apparent to the field observer. In a study of *Rana clamitans* in Quebec, Canada, researchers using FC demonstrated that frogs exposed to agricultural contaminants from potato fields had more deformities and DNA alterations than frogs from ponds adjacent to cornfields and control ponds (Bonin et al. 1997). Our C-values for *R. clamitans* ranged from 6.55 to 6.60 pg of DNA/haploid nucleus (Table) and were within the range of values calculated in the Canadian study (Bonin et al. 1997).

Our study was limited by small sample sizes and statistical analysis on only one species. The experimental unit was the pond. However, the low within-pond correlation of CV values among *R. clamitans* individuals observed in our study suggests that studies with an organismal focus may benefit from relatively large sample sizes within ponds. Our findings regarding land use associations should be substantiated using larger sample sizes from more ponds and with other amphibian species. Also, amphibians with known genetic damage should be included in future studies to provide a benchmark for comparisons.

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Table. Amphibian deformity, DNA, and parasite statistics for exposed and reference ponds in southeastern Minnesota, 2000.

					Deformity assessment [c]		DNA analysis and presence of *Ribeiroia*					
Species	Collection date	Minnesota township	Pond type [a]	Exp. group [b]	Def.	N	Mean C-value[d]	Mean CV[e]	SE	Aneuploid peaks [f]	Rib. present [g]	N[h]
Rana clamitans	6-15-2000	Altura	Graze	Exp	1	25	6.55	3.02	0.30	1	1	9
R. clamitans	6-28-2000	Brownsville	Graze	Exp	0	30	6.57	2.87	0.33	0	2	4
R. clamitans	5-25-2000	Utica	Agric	Exp	0	276	6.57	3.28	0.14	0	0	8
R. clamitans	6-15-2000	Utica	Ngraz	Ref	1	102	6.57	2.83	0.15	0	1	10
R. clamitans	5-25-2000	Lewiston	Natur	Ref	0	42	6.60	3.32	0.14	0	0	9
R. palustris	7-19-2000	Eitzen	Ngraz	Ref	0	37	6.74	1.91	0.07	0	0	5
R. pipiens	7-12-2000	Houston	Ngraz	Ref	0	36	7.08	2.88	0.28	2	0	7
R. pipiens	7-06-2000	Sheldon	Agric	Exp	0	107	6.53	2.83	0.37	0	0	3
R. pipiens	7-05-2000	Caledonia	Graze	Exp	0	91	6.91	6.31	0.23	3	0	3

[a] Types of land uses surrounding the ponds: Graze=grazed grassland; Agric=agricultural row crops; Ngraz=nongrazed grassland; Natur=natural wetlands.
[b] Exp=exposed (i.e., grazed and agricultural) and Ref=reference (i.e., nongrazed and natural) for pond categories.
[c] Number of frogs deformed and total examined in the field deformity assessment.
[d] Average DNA weight (pg of DNA/haploid nucleus).
[e] Average coefficient of variation (CV; SD of the target peak, divided by the mean of the target peak channel, multiplied by 100).
[f] Number specimens showing an aneuploid peak (Figure) with flow cytometry (FC) analysis.
[g] Number of specimens with the parasite *Ribeiroia ondatrae*.
[h] Number of specimens used in FC analysis and examined for parasites.

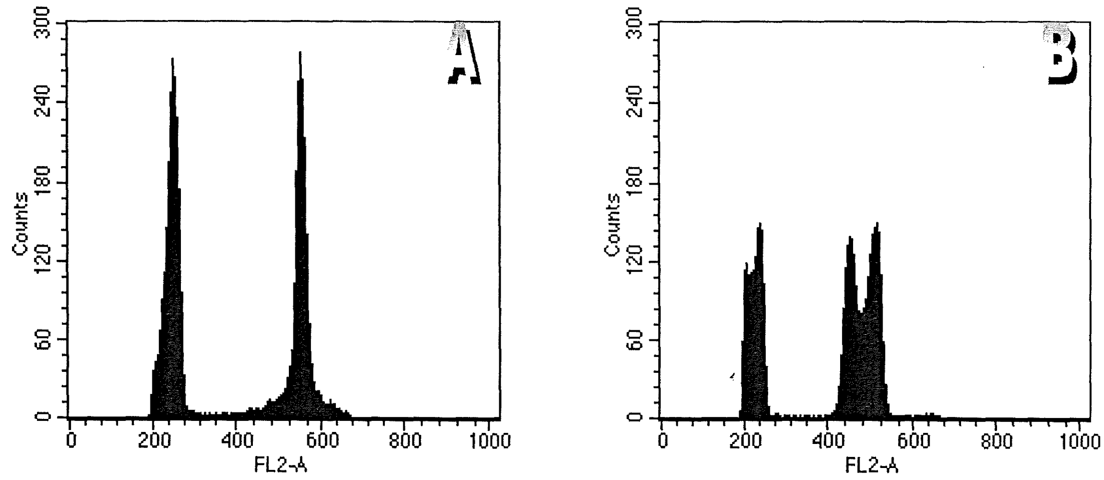

Figure. Histograms comparing normal DNA peaks (*A*) and an aneuploid mosaic peak (*B*). Histogram (*A*) shows a normal DNA peak for the reference specimen (*Xenopus laevis*) on the left and a normal target species (*Rana pipiens*) peak on the right. Histogram (*B*) shows a normal reference peak on the left and an aneuploid mosaic target species (*R. pipiens*) peak on the right. Histogram (*B*) was generated with a specimen collected from a reference pond in southeastern Minnesota, 2000.

Resources for Monitoring Pond-breeding Amphibians in the Northcentral USA

by

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Farm Ponds as Critical Habitats for Native Amphibians: Final Report

Submitted to

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Abstract
Public and private land managers are interested in monitoring amphibian populations to evaluate the risk of population declines. In this report, we describe monitoring methods and resources useful for biologists undertaking monitoring of amphibians breeding in pond environments in the northcentral USA. We include states in the U.S. Geological Survey Amphibian Research and Monitoring Initiative, Upper Mississippi Region (Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin). The monitoring resources are derived from the literature and our experiences with a study of amphibians breeding in small farm ponds in southeastern Minnesota (Driftless Area Ecoregion) conducted from 2000 to 2001. We provide an overview of methods and list resources for conducting anuran calling surveys, egg mass surveys, larval surveys, and amphibian deformity assessments, and we list precautions to prevent the spread of diseases. We also present one method of collecting habitat information associated with a breeding site. The appendixes list equipment and resources useful for conducting amphibian surveys. Examples of data sheets are provided, along with a list of amphibians present in the northcentral USA.

Key words: amphibian, midwestern USA, monitoring, northcentral USA, pond, resources

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Introduction

Declines in amphibian populations around the world, including some in the northcentral USA (Hay 1998; Lannoo 1998; Bury 1999; Alford et al. 2001) and high rates of deformed frogs in some locations (Helgen et al. 1998) have stimulated interest in amphibians as bioindicators of the health of ecosystems. Public and private land managers are interested in monitoring amphibian populations to evaluate the risk of population declines (Mossman et al. 1998).

We describe monitoring methods and resources useful for biologists undertaking monitoring of amphibians breeding in pond environments in the northcentral USA. We included states in the U.S. Geological Survey (USGS) Amphibian Research and Monitoring Initiative (ARMI), Upper Mississippi Region (Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin). The monitoring resources are derived from the literature and our experiences in a study of amphibians breeding in small farm ponds in southeastern Minnesota (Driftless Area Ecoregion) conducted from 2000 to 2001 (Knutson et al. 2002).

As concern about amphibians increases, more agencies and herpetologists are engaged in monitoring activities. Amphibian monitoring methods are rapidly evolving because new research is focusing on improving monitoring methods. The USGS ARMI is monitoring amphibians across the USA and is a resource for monitoring methods (http://www.mp2-pwrc.usgs.gov/armi/index.cfm). The USGS Science Centers with active research on amphibians in the northcentral USA include Upper Midwest Environmental Sciences Center (La Crosse, Wisconsin), Northern Prairie Wildlife Research Center (Jamestown, North Dakota), National Wildlife Health Center (Madison, Wisconsin), and Columbia Environmental Research Center (Columbia, Missouri) (http://biology.usgs.gov/pub_aff/centers.html).

General Considerations

Anyone undertaking amphibian survey work has a responsibility to avoid harming the amphibians or their habitats. Persons planning to sample amphibians should work in cooperation with state or federal wildlife professionals. Lack of knowledge about sensitive habitats or populations could result in the spread of diseases, damage to breeding habitats, or local reproductive failure of amphibian populations. State and federal laws protect amphibians from exploitation. Collection permits are required from the appropriate state and/or federal authorities before collecting or handling amphibians. Consult your state wildlife management agency for guidance. Permission for sampling should also be obtained from the landowner.

Qualifications and Training

Biologists undertaking amphibian surveys should be familiar with the amphibian species in their area. A number of field guides and general herpetology references are available to assist biologists who are unfamiliar with amphibians (Wright and Wright 1949; Conant and Collins 1991; Stebbins and Cohen 1995; Harding 1997; Petranka 1998; Moriarty and Bauer 2000). Surveyors should be able to identify anurans by call and identify amphibian adults, eggs, and larvae in the field by sight or through the use of keys (Altig et al. 1998; Parmelee et al. 2002). In addition, skills in the identification of aquatic vegetation are useful. Training with a professional is strongly encouraged. Some universities offer herpetology courses as part of their academic program and some offer short summer courses at biological field stations. For biologists new to amphibian surveys, we recommend consulting herpetologists in your state to assist you.

Collecting and Handling

While performing amphibian surveys, it may be necessary to handle amphibian eggs, larvae, and adults. The following procedures will minimize the risk of injury to amphibians during collecting and handling (Fellers et al. 1994; Lips et al. 2001). Before handling amphibian eggs, larvae, or adults, wash your hands so they are free of soap, insect repellent, sunscreen lotion, and any other potential toxins. Hands should be moistened with water before handling any amphibians.

Handling of amphibian eggs should be minimized. When possible, identify eggs in place. Larvae should be handled with a dip net and not removed from the water for more than 2 min. During larval surveys, larvae can be held in buckets filled with pond water and placed in a cool place out of direct sunlight. Larvae should be released as soon as they are identified.

Preventing the Spread of Diseases

Disposable gloves should be used for handling animals when disease is suspected. To prevent the spread of potential pathogens or the introduction of novel species to new sites, animals should not be transported among sites. Any animals that are removed from the site for captive rearing or other purposes should not be released back into the environment. They should be euthanized and either preserved as voucher specimens, or disposed of properly (Green 2001).

If sampling will include contact between field gear (footwear, clothing, and equipment) and aquatic habitats, preventing contamination among sites is important. To prevent the spread of diseases from one amphibian population to another, all field gear should be cleaned and sanitized among study sites. The USGS National Wildlife Health Center (Madison, Wisconsin) has developed standard operating procedures for handling amphibians and disinfecting equipment (Green 2001). These guidelines also cover biosecurity precautions and reporting procedures if you suspect amphibian disease at a site. The Fieldwork Code of Practice developed by the Declining Amphibian Populations Task Force (http://www.npwrc.usgs.gov/narcam/techinfo/daptf.htm) also describes accepted safety precautions to take to prevent the spread of disease.

Sampling Design

Sampling design (where and how frequently to sample) may be the most important consideration in a monitoring study and determines what information can be derived from the data. Careful planning is especially important if you have specific management objectives for conducting the survey. If you are unsure about whether your planned design will meet your management objectives, consult references (Thompson et al. 1998; Yoccoz et al. 2001), a statistician, or a research biologist. The USGS Florida Caribbean Science Center (Gainesville, Florida) has investigated statistical design and analysis with respect to amphibian surveys. They describe issues related to sampling design on their Web site (http://www.fcsc.usgs.gov/armi/Framework/framework.html). Before you start, consider the types of habitats you want to include in your project or study, their size and distribution, and what maps are available showing these habitats. Stratified random sampling, aided by computer software, is often used to randomly select sample points from different habitat types.

Sampling and Recording Data

Standard survey techniques for amphibians include anuran calling surveys, egg mass surveys, larval surveys, and visual searches for adults (Heyer et al. 1994; Olsen et al. 1997). For those unfamiliar with amphibians, locating, collecting, and identifying amphibians (adults, eggs, larvae) can be challenging. We present resources for conducting amphibian surveys, including a list of field equipment (Appendix A), examples of field data sheets (Appendix B), resources for amphibian identification (Appendix C), amphibian species found in the northcentral USA (Appendix D), and species of management concern (Appendix E). Species names are based on Crother (2001).

Careful recording of the data collected during sampling is important for the effort to have any long-term value. The examples of data sheets (Appendix B) list the essential information to record. In the past, recording of sampling sites generally involved mapping on USGS quad sheets. Today, global positioning system (GPS) equipment makes it easy to record the spatial coordinates of sampling sites. We recommend recording location information at each site to accurately link your data with digital maps.

Anuran Calling Surveys

Anuran calling surveys are used to identify locations where adult frogs and toads are attempting to breed. Some states have been collecting anuran calling data over the last decade (Hemesath 1998; Mossman et al. 1998). Amphibian habitat associations have been derived from calling survey data (Knutson et al. 1999; Knutson et al. 2000), as well as population trend estimates (Mossman et al. 1998).

Anuran calling surveys are easier to perform than egg or larval surveys and are frequently conducted by volunteers. However, calling surveys do not provide evidence that breeding is successful. Eggs, larvae, and metamorphs are needed to confirm successful reproduction for anurans. Calling surveys are not used to survey salamanders because salamanders do not call. However, salamanders often breed in the same locations as anurans and may be detected by visual search or larval sampling.

Calling anurans can be heard in wetland habitats from early spring through midsummer. Frogs and toads (*Rana* and *Bufo* spp.) often conceal themselves in vegetation—including emergent vegetation, flooded grass, and shrubs—while calling. Treefrogs (*Hyla* spp.) also call from trees adjacent to breeding ponds. Most anuran calling surveys are conducted after dark. Headlamps are useful for keeping your hands free and for walking to breeding sites in the dark. Many anurans will also chorus during the day, especially at the peak of breeding activity.

Anurans make a variety of calls. Release calls are given by males of many species attempting to avoid accidental amplexus with other males. These calls are typically quieter than mating calls. The American Bullfrog and Northern Leopard Frog will sound alarm calls when approached or disturbed. Variations on mating calls are given by males trying to defend their calling territory. Most anuran call recordings will point out these differences. During daylight hours, bird songs may sound like amphibians. Later in the summer, a variety of insect calls must be distinguished from anuran calls.

Protocols for anuran calling surveys have been developed by the USGS North American Amphibian Monitoring Program (NAAMP 2002). Several states have state anuran calling programs that cooperate with North American Amphibian Monitoring Program. We recommend using protocols adopted by your state wildlife management agency so that your data are compatible with other, similar data collected in your state. Numerous resources, including sound recordings, are available to help you learn the calls for frogs in your area (Appendix C). Times and minimum air temperature guidelines are available to plan the timing of calling surveys in each state (NAAMP 2002).

Visual Encounter Surveys

Visual encounter surveys identify amphibian adults and possibly metamorphs at a site. The details of conducting visual searches have been described in several references (Crump and Scott 1994; Olsen et al. 1997).

Egg Mass Surveys

Egg mass surveys provide evidence that mating occurred. The number of egg masses is also an indication of the number of adults that bred at that location (Crouch and Paton 2000). Some amphibian species are most effectively surveyed by egg mass surveys because their egg masses are large and easily found (Crouch and Paton 2000). Searching for egg masses while attempting to locate calling individuals allows one to observe the relation among calling adult anurans, their eggs, and their choice of egg-laying sites. Polarized sunglasses help reduce glare when searching for eggs during the day.

Each species lays its eggs in characteristic ways (Stebbins and Cohen 1995). Most ranids lay their eggs in large masses, either in floating sheets or spherical masses near the water's surface, sometimes attached to vegetation. Toads lay eggs in long strings, typically in shallow water. Treefrogs lay their eggs in small masses or individually, attached to vegetation. Pond-breeding salamanders usually lay their eggs in masses attached to vegetation, at or below the water surface. While not all amphibians attach their eggs to vegetation, vegetation (living and dead) is often used for support by amphibians during the egg-laying process. As a result, pond-breeding amphibian eggs are usually found in association with vegetation. All pond-breeding amphibians in our region have pigmented eggs (Parmelee et al. 2002). Eggs or egg masses that are white or translucent are likely snail eggs that can be quite large.

Larval Surveys

Performing larval surveys is another method of detecting the presence of pond-breeding amphibians. The presence of larvae is good evidence that breeding was successful and that site conditions support larval development. There are a number of methods used to survey amphibian larvae (Heyer et al. 1994; Olsen et al. 1997). We recommend defining a search area for larval surveys. If your pond is small, you may want to search the entire pond. If your pond is large, you can define a search area, such as a 20-m diameter circle. Most amphibian larvae prefer shallower (<1 m depth) water, so shorelines and shallow areas should be your focus.

Dip nets or seines can be used to collect larvae. In our surveys, we attempted to standardize our dip net effort by placing all larvae collected during a 20-min dip net effort in a bucket. We then identified larvae by species and recorded their abundances (Appendix B).

The ability to successfully collect larvae depends on the density of larvae and the habitat characteristics. Small, temporary ponds may have relatively high densities of larval amphibians that can be collected with little effort. Larger, interconnected, permanent wetlands tend to have more dispersed populations of larval amphibians that increases the effort required.

Most amphibian larvae can be found among aquatic vegetation or other sheltering objects, where they seek food and refuge from predators. Toad tadpoles can often be seen in large schools in shallow, open water. Collecting amphibian larvae with a dip net requires walking carefully and slowly through the water, sweeping the net through stands of aquatic vegetation. In shallow, turbid, sparsely vegetated areas, larvae can often be found resting on the bottom. To prevent the escape of larvae, work from deeper water towards shallower areas. Immediately place collected larvae in a bucket containing water from the site. Put 2 to 3 L of water in the bucket and place it out of direct sunlight to prevent the larvae from overheating.

Funnel traps are another tool for collecting larvae (Adams et al. 1997). Funnel traps are useful when it is logistically feasible to deploy and check them regularly and when dense vegetation impedes the use of dip nets or seines. Because of the logistical considerations of sampling many sites, we collected the same species with less time using dip nets.

Identifying larvae in the field can be difficult for novices. Training by a herpetologist in the field is the best way to learn to identify larvae. Keys to amphibian larvae and eggs (Watermolen 1995, 1996; Parmelee et al. 2002) are useful in identifying species or groups of species. Some species can only be differentiated during the larval stage by examination of larval tooth patterns with the aid of a microscope (Altig et al. 1998; McDiarmid and Altig 1999). We recommend this only if you have training in amphibian larval identification. If you are unsure of your identifications, options include consulting a herpetologist or raising the larvae in the laboratory and making an identification from a metamorph or juvenile amphibian.

Amphibian Deformity Assessment

Recent concerns about amphibian deformities (Helgen et al. 1998; Johnson et al. 1999; Souter 2000; Rosenberry 2001; Johnson et al. 2002) have led management agencies to conduct deformity assessments to assess risks on public lands. Deformity assessments are usually performed on metamorphs from mid-June through mid-August. Accurate descriptions of any malformations you find are important for identifying causes (Meteyer 2000). The USGS North American Reporting Center for Amphibian Malformations provides guidance on how to conduct surveys for malformations and report your findings (http://www.npwrc.usgs.gov/narcam/).

Amphibian Disease Assessment

Amphibian disease is an emerging concern among herpetologists. Amphibian declines and species extinctions may be linked to novel and catastrophic diseases (Hero and Gillespie 1997; Daszak et al.

1999; Carey 2000; Green and Sherman 2001; Kiesecker et al. 2001; Young et al. 2001). If you encounter a die-off or disease outbreak of amphibians, you should act quickly to have the problem diagnosed. The USGS National Wildlife Health Center (Madison, Wisconsin) is experienced in identifying amphibian pathogens. The Center has guidelines on handling and shipping specimens for diagnosis (Green 2001). Contact them for assistance before sending specimens.

Collecting Voucher Specimens

To verify the identification of eggs and larvae encountered in the field you will initially need to collect and preserve voucher specimens (McDiarmid 1994); (McDiarmid and Altig 1999; Simmons 2002 (in press)). A set of voucher specimens can be sent to a specialist for positive identification. Once you are confident in your identification skills, collections will not be necessary. Most states require collection permits issued by the state Department of Natural Resources or similar agency. The permits must be carried in the field during sampling and must accompany any preserved specimens. Remember to observe all wildlife laws and only collect where it is legal and where the collection of a few individuals will not affect the population. Species that are classified as endangered, rare, threatened, or of special concern (Appendix E) should be collected only with special permission from appropriate authorities.

Preserving Eggs and Larvae

Larvae should be anesthetized according to procedures recommended by Green (2001). There is no perfect preservative, and the techniques for preserving specimens are still debated (McDiarmid 1994; McDiarmid and Altig 1999). We recommend preserving amphibian eggs and larvae by placing them in a small vial filled with a 10% formalin solution. Alcohol is more pleasant to work with and safer than formaldehyde, but tends to dehydrate specimens. Whatever preservative you use, read the relevant Material Safety Data Sheets to learn how to safely handle and store that chemical.

Larvae can be placed individually, or as a lot of 5 to 20 individuals in screw top vials. Do not place too many individuals in one container. Immediate labeling is a must; use pencil or indelible ink on all submerged tags. Field tags should be linked to corresponding field notes; labels with detailed information must be kept with the specimens. Do not rely on your memory as a record of locality, date, and habitat information. The minimum information includes as follows: date, locality (kilometers from a crossroad or other landmark or GPS coordinates), habitat description, and name of the collector. We recommend maintaining a numbered log that links to tags on the vials. Other important information includes notes on live coloration (specimens quickly lose color in preservative). Specimens should be deposited in a museum or university collection where they can be appropriately cataloged, maintained, and available for researchers worldwide.

Habitat Assessment

Decisions about what habitat data to collect should be made by clarifying the research questions. Measuring habitat variables can be time-consuming. We tried various methods and found that simple habitat assessments were best, unless you have a specific need to be more detailed. The habitat assessment area should correspond to the area sampled for amphibians. Several references describe methods of collecting habitat information (Heyer et al. 1994; Olsen et al. 1997).

We present one example of measuring biotic and abiotic habitat variables at a site (Appendix B). The method is relatively simple and is based primarily on visual estimates of cover. Habitat assessments should be done after surveys for amphibians to avoid disturbing amphibians before the survey. Familiarity with aquatic vegetation is helpful (Fassett 1957; Borman et al. 1997; Chadde 1998), although we present estimates of cover by vegetative growth habit, not species or genera.

Cover information can be collected on the various types of vegetation (Appendix B). Vegetation is broadly defined as determined by plant habit (i.e., submerged, emergent, terrestrial, etc.). Information on substrate characteristics (sediment particle size estimates) can also be collected.

Canopy Cover

Visual estimates can be made of tree cover directly overhead, including overhanging canopy from trees with trunks located outside of the survey area. Canopy cover is estimated for woody vegetation >3 m in height. Because forest canopies often consist of multiple layers, we estimate total canopy cover and canopy cover above a height of 5 m (upper canopy). The estimate of upper canopy coverage may equal, but should not exceed the total canopy coverage.

Aquatic Habitat Cover

We estimated the total amount of aquatic habitat (habitat currently covered with water) contained within the sampling area.

Vegetation Cover

We also estimated vegetation cover for the entire sampling area, including submerged, floating–leaved (both rooted and nonrooted), emergent, woody/shrub (<3 m tall), and terrestrial vegetation (nonwoody vegetation including grasses and forbs). Because water levels may vary and aquatic plants may be found on dry substrates, plant categories can be determined according to growth preferences and not on hydrologic conditions present at the time of the assessment. The coverage of dormant woody vegetation can also be recorded.

Litter, Log, and Rock Cover

We estimated the coverage of dead leaf and plant litter, downed log, and rock cover for the entire sampling area of both aquatic and terrestrial portions of the site combined.

Water Depth

Because water depth usually varies across a sampling area, we suggest estimating water depth at five points randomly placed within the survey area. A measuring pole can be constructed from a PVC pipe. When measuring water depth, avoid resting the bottom of the measuring pole on submerged vegetation or large woody debris. If the water depth is greater than can be measured, record "Greater than" the maximum measurable depth.

Substrate Characterization

Underwater substrates can be characterized by particle size and organic content. Substrate type can be examined by sight and feel at the same five points used to determine average water depth. Only a small quantity (~ 2 cm^3) of substrate is needed for characterization and should be taken to a substrate depth of about 2 cm (Yin et al. 2000).

Landscape Context

The quality of the landscape surrounding your study site (context) is important to the persistence of amphibian populations. Persistence may be less likely if potential breeding sites are isolated or the surrounding landscape is potentially hostile to amphibians (row crops, major roads, industrial zones). If you record your survey site accurately with a GPS receiver, you will be able to evaluate the quality of the landscape surrounding your site using digital land cover maps and GIS software.

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Appendix A. Equipment List

Dip nets: 14 inches x 16 ½ inches aluminum frame with 24 inches aluminum handle. Net bag: 1/16 inches mesh, 18 inches deep. (Duraframe Dipnet, Viola, Wisconsin; 'intermediate wide teardrop')

Thermometer: Pocket alcohol thermometer with protective case, -10 to -110° C. (Fisher Scientific, Cat. No.15-021-5B)

Headlamp (Petzl "Duo").

Global Positioning System (GPS) receiver (Garmin GPS III, Garmin International, Olathe, Kansas).

PVC measuring pole: 2-m PVC pipe marked with centimeter gradations and fitted with 7.6-cm (3 inches) PVC pipe flange to prevent the measuring pole from sinking into soft sediments.

Plastic buckets: 3-5 gallon capacity.

10% buffered formalin (Fisher Scientific)
Directions for preparing:
http://www.jcu.edu.au/school/phtm/PHTM/frogs/pmfrog.htm - S4.

Glass specimen vials with plastic caps (Fisher Scientific).

Meter tape (25 m).

Watch or stop watch.

Sprayer for disinfectant (general duty 12-L capacity sprayer).

Hip and /or chest waders.

Small kayak: May be useful for surveying certain habitat types.

Amphibian call recordings (Appendix C).

Regional amphibian and reptile guides (Appendix C).

Covered clipboard.

Rite-in-the-rain paper.

Data sheets.

Collection permits.

Appendix B. Examples of Field Data Sheets

Study description:

Survey site location: ______________________ UTM coordinates: _______________ E _______________ N
UTM error: _____________________________ Datum:________________ Spheroid:______________________
Habitat type: _________ Date begin: _________ end: _____ Time (e.g., 1600) begin: _________ end: ________ .
Observer initials:__________Recorder's initials:_________ Temperature: Air _____° C Water ______° C
Sky conditions:_______ Wind speed: _______ Water present (Yes/ No) _______ (For road/trail calling surveys)
Data entered in computer (date): _______________ Data proofed (date) : ______________ Point ID # : ______

Check the assessments made:

		Specimens collected: (list species, numbers, and purpose)
Frog chorus survey		
Egg mass survey		
Larval survey		
Water quality		
Vegetation		
Deformity assessment		(Collection requires appropriate state and/or federal permits)

Calling Survey (5 min)

Species code	Species	Call index[a]	Notes

[a] 0 = No frogs of a given species can be heard calling.
1 = Individuals of a species can be heard; calls not overlapping.
2 = Individual frogs can be heard calling; but some overlap, can estimate number of frogs present.
3 = Full chorus; numerous frogs can be heard; chorus is constant and overlapping.

Additional Observations: Fill out for observations of other herpetofauna and for egg mass and larval surveys

Taxa (reptile, amphibian)	Life stage[a]	Species code	Species	Number[b]	Abundance code[c]	Notes[d]

[a] Life stage : egg, larva, metamorph, adult.
[b] Number: Total number of individuals or egg masses encountered.
[c] Abundance code: Larval survey, 0 (0), 1 (1–10), 2 (11–100), 3 (>100) Do not enter species name or code if species ID is not positively known.
[d] Notes: Enter information on sex of individuals, if known (m/f), or any other pertinent data.

Field Data Sheet (Page 2)

Additional Observations (Continued): Fill out for observations of other herpetofauna and for egg mass and larval surveys

Taxa (reptile, amphibian)	**Life stage[a]**	**Species code**	**Species**	**Number[b]**	**Abundance code[c]**	**Notes[d]**

[a] Life stage : egg, larva, metamorph, adult.
[b] Number: Total number of individuals or egg masses encountered.
[c] Abundance code: Larval survey, 0 (0), 1 (1–10), 2 (11–100), 3 (>100) Do not enter species name or code if species ID is not positively known.
[d] Notes: Enter information on sex of individuals, if known (m/f), or any other pertinent data.

Habitat Assessment

Water depth (centimeters):

Depth 1	**Depth 2**	**Depth 3**	**Depth 4**	**Depth 5**	**Avg. Depth**

Substrate characterization (codes 1–7[a]):

Substrate 1	**Substrate 2**	**Substrate 3**	**Substrate 4**	**Substrate 5**

[a] Silt/clay = 1, mostly silt with sand = 2, mostly sand with silt = 3, hard clay = 4, gravel = 5, sand = 6, organic muck = 7.

Canopy, vegetation, and litter cover (assessed for entire survey area):

Cover type	**Cover class[a] (1–5)**	
Trees/shrubs canopy cover	Upper (>5 m)	
	Total (>3 m)	
Aquatic habitat		
Floating-leaved		
Submerged		
Emergent		
Woody/shrubs (Less than 3 m tall)		
Terrestrial (grasses and forbs)		
Leaf and plant litter		
Downed log		
Rock		

[a] Visual estimate of coverage 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, 5 = 81–100%.

Field Data Sheet (Page 3)

Beaufort Scale for determining wind speed:

	Wind speed		
Code	**kph**	**mph**	**Indicators**
0	0–2	0–1	Calm, smoke rises vertically.
1	3–5	2–3	Light air movement, smoke drifts.
2	6–11	4–7	Slight breeze, wind felt on face; leaves rustle.
3	12–19	8–12	Gentle breeze, leaves and small twigs in constant motion.
4	20–30	13–18	Moderate breeze, small branches are moved, raises dust and loose paper.
5	31–39	19–24	Fresh breeze, small trees in leaf begin to sway; crested wavelets form.
6	40–50	25–31	Strong breeze, large branches in motion.

Sky conditions codes (codes 3 and 6 are not used).

Code	Sky condition
0	Few clouds
1	Partly cloudy (scattered) or variable sky
2	Cloudy or overcast
3	
4	Fog or smoke
5	Drizzle or light rain (not affecting hearing ability)
6	
7	Snow
8	Showers (affecting hearing ability)

Codes for estimating vegetative cover:

Cover class	Visual estimate of coverage (%)
1	1–20
2	20–40
3	40–60
4	60–80
5	80–100

Field Data Sheet (Page 4)

Growth habit of representative taxa:

Habit	Representative taxa
Submerged	*Elodea* (water weeds), *Ceratophyllum* (Coontail), *Potamogeton* (pond weeds), Algae
Floating–leaved	Rooted: *Nymphae* and *Nuphar* (water lilies) Nonrooted: *Lemna* and *Spirodela* (Duckweed), Algae
Emergent	*Typha* spp. (Cattail), *Sagittaria* spp. (Arrow heads)
Woody/shrub (<3 m tall)	May include moist soil species such as *Salix* (Willow) or upland species such as *Cornus* (Dogwood). Also includes seedlings of tree species (i.e., *Acer* spp.).
Terrestrial (grasses and forbs)	May include moist soil species such as *Leersia* (cut-grass) or more upland species.

Substrate types and codes:

Substrate code	Substrate type and physical description
1	Silt/clay: Fine particle size, feels smooth when rubbed between fingers.
2	Mostly silt with sand: Material appears fine grained, but has slight gritty feel when rubbed between fingers
3	Mostly sand with silt: Sandy appearance, with finer material present. Feels gritty to the touch
4	Hard clay: Fine material, without gritty feel. Substrate tends not to be flocculent because of cohesiveness.
5	Gravel: Coarse substrate with particles between 3 and 32 mm.
6	Sand: Sandy appearance, gritty feel, no finer material (silt/clay) evident.
7	Organic muck: Dark or black smooth substrate. May contain some identifiable, but darkly stained plant material

Appendix C. Resources for Amphibian Identification

Some of this information is adapted from Moriarty and Bauer (2000).

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Appendix D. List of Amphibian Species Found in the Northcentral USA

Order	Family	Taxonomic order	IT IS number	Letter codes	Common name[a]	Scientific name
Caudata	Sirenidae	1000.9	173736	SIINTE	Lesser Siren	*Siren intermedia*
Caudata	Amphiumidae	1002.0	173612	AMTRID	Three-toed Amphiuma	*Amphiuma tridactylum*
Caudata	Proteidae	1004.0	208249	NEMACU	Mudpuppy	*Necturus maculosus*
Caudata	Cryptobranchidae	1006.0	208176	CRALLE	Hellbender	*Cryptobranchus alleganensis*
Caudata	Salamandridae	1008.0	888117	NOVIRI	Eastern Newt	*Notophthalmus viridescens*
Caudata	Ambystomatidae	1009.0	173594	ABANNU	Ringed Salamander	*Ambystoma annulatum*
Caudata	Ambystomatidae	1010.0	208204	ABBARB	Streamside Salamander	*Ambystoma barbouri*
Caudata	Ambystomatidae	1011.0	173598	ABJEFF	Jefferson Salamander	*Ambystoma jeffersonianum*
Caudata	Ambystomatidae	1012.0	173599	AMLATE	Blue-spotted Salamander	*Ambystoma laterale*
Caudata	Ambystomatidae	1013.0	173590	ABMACU	Spotted Salamander	*Ambystoma maculatum*
Caudata	Ambystomatidae	1014.0	173591	AMOPAC	Marbled Salamander	*Ambystoma opacum*
Caudata	Ambystomatidae	1016.0	173604	AMTALP	Mole Salamander	*Ambystoma talpoideum*
Caudata	Ambystomatidae	1017.0	173605	AMTEXA	Small-mouthed Salamander	*Ambystoma texanum*
Caudata	Ambystomatidae	1018.0	173593	AMTIGR	Tiger Salamander	*Ambystoma tigrinum*
Caudata	Plethodontidae	1021.0	173699	ANAENE	Green Salamander	*Aneides aeneus*
Caudata	Plethodontidae	1022.0	999104	DECONA	Spotted Dusky Salamander	*Desmognathus conanti*
Caudata	Plethodontidae	1023.0	173633	DEFUSC	Northern Dusky Salamander	*Desmognathus fuscus*
Caudata	Plethodontidae	1024.0	173641	DEOCHR	Allegheny Mountain Dusky Salamander	*Desmognathus ochrophaeus*
Caudata	Plethodontidae	1024.1	173634	DEWELT	Black Mountain Salamander	*Desmognathus welteri*
Caudata	Plethodontidae	1024.2	173640	DEMONT	Seal Salamander	*Desmognathus monticola*
Caudata	Plethodontidae	1025.0	173685	EUBISL	Northern Two-lined Salamander	*Eurycea bislineata*
Caudata	Plethodontidae	1026.0	550246	EUCIRR	Southern Two-lined Salamander	*Eurycea cirrigera*
Caudata	Plethodontidae	1027.0	173687	EULONG	Long-tailed Salamander	*Eurycea longicauda*
Caudata	Plethodontidae	1028.1	173687	EUGUTT	Three-lined Salamander	*Eurycea guttolineata*
Caudata	Plethodontidae	1029.0	208311	EULUCI	Cave Salamander	*Eurycea lucifuga*
Caudata	Plethodontidae	1030.0	208314	EUMULT	Many-ribbed salamander	*Eurycea multiplicata*
Caudata	Plethodontidae	1031.0	173697	EUTYNE	Oklahoma Salamander	*Eurycea tynerensis*

Order	Family	Taxonomic order	IT IS number	Letter codes	Common name[a]	Scientific name
Caudata	Plethodontidae	1032.0	208353	GYPORD	Spring Salamander	*Gyrinophilus porphyriticus*
Caudata	Plethodontidae	1034.0	173678	HESCUT	Four-toed Salamander	*Hemidactylium scutatum*
Caudata	Plethodontidae	1035.0	208278	PLALBA	Western Slimy Salamander	*Plethodon albagula*
Caudata	Plethodontidae	1036.0	173649	PLCINE	Eastern Red-backed Salamander	*Plethodon cinereus*
Caudata	Plethodontidae	1037.0	999112	PLDORS	Northern Zigzag Salamander	*Plethodon dorsalis*
Caudata	Plethodontidae	1039.0	173650	PLGLUT	Northern Slimy Salamander	*Plethodon glutinosus*
Caudata	Plethodontidae	1039.1	173661	PLKENT	Cumberland Plateau Salamander	*Plethodon kentucki*
Caudata	Plethodontidae	1039.2	208289	PLMISS	Mississippi Slimy Salamander	*Plethodon mississippi*
Caudata	Plethodontidae	1040.0	173667	PLRICH	Southern Ravine Salamander	*Plethodon richmondi*
Caudata	Plethodontidae	1041.0	173668	PLSERR	Southern Red-backed Salamander	*Plethodon serratus*
Caudata	Plethodontidae	1042.0	173634	PLWEHR	Wehrle's Salamander	*Plethodon wehrlei*
Caudata	Plethodontidae	1043.0	208302	PSMOND	Mud Salamander	*Pseudotriton montanus*
Caudata	Plethodontidae	1044.0	173681	PSRUBE	Red Salamander	*Pseudotriton ruber*
Caudata	Plethodontidae	1045.0	173730	TYSPEL	Grotto Salamander	*Typhlotriton spelaeus*
Anura	Pelobatidae	1046.0	173426	SCHOLB	Eastern Spadefoot	*Scaphiopus holbrookii*
Anura	Pelobatidae	1047.0	206989	SPBOMB	Plains Spadefoot	*Spea bombifrons*
Anura	Microhylidae	1048.0	173467	GACARO	Eastern Narrow-mouthed Toad	*Gastrophryne carolinensis*
Anura	Microhylidae	1049.0	173468	GAOLIV	Great Plains Narrow-mouthed Toad	*Gastrophryne olivacea*
Anura	Bufonidae	1050.0	173473	BUAMER	American Toad	*Bufo americanus*
Anura	Bufonidae	1052.0	173484	BUCOGN	Great Plains Toad	*Bufo cognatus*
Anura	Bufonidae	1053.0	173487	BUHEMI	Canadian Toad	*Bufo hemiophrys*
Anura	Bufonidae	1054.0	173478	BUFOWL	Fowler's Toad	*Bufo fowleri*
Anura	Bufonidae	1055.0	173476	BUWOOD	Woodhouse's toad	*Bufo woodhousii*
Anura	Hylidae	1056.0	173522	ACCREP	Northern Cricket Frog	*Acris crepitans*
Anura	Hylidae	1057.0	173511	HYAVIV	Bird-voiced Treefrog	*Hyla avivoca*
Anura	Hylidae	1058.0	173502	HYCHRY	Cope's Gray Treefrog	*Hyla chrysoscelis*
Anura	Hylidae	1059.0	173505	HYCINE	Green Treefrog	*Hyla cinerea*
Anura	Hylidae	1060.0	173503	HYVERS	Gray Treefrog	*Hyla versicolor*
Anura	Hylidae	1060.1	173508	HYGRAT	Barking Treefrog	*Hyla gratiosa*
Anura	Hylidae	1061.0	173528	PSBRAC	Mountain Chorus Frog	*Pseudacris brachyphona*

Order	Family	Taxonomic order	IT IS number	Letter codes	Common name[a]	Scientific name
Anura	Hylidae	1062.0	207304	PSCRUC	Spring Peeper	*Pseudacris crucifer*
Anura	Hylidae	1063.0	207301	PSSTRE	Strecker's Chorus Frog	*Pseudacris streckeri*
Anura	Hylidae	1064.0	207310	PSFERI	Southeastern Chorus Frog	*Pseudacris feriarum*
Anura	Hylidae	1065.0	207312	PSMACU	Boreal Chorus Frog	*Pseudacris maculata*
Anura	Hylidae	1066.0	173525	PSTRIS	Western Chorus Frog	*Pseudacris triseriata*
Anura	Ranidae	1067.0	207006	RAAREA	Crawfish Frog	*Rana areolata*
Anura	Ranidae	1068.0	173448	RABLAI	Plains Leopard Frog	*Rana blairi*
Anura	Ranidae	1069.0	173441	RACATE	American Bullfrog	*Rana catesbeiana*
Anura	Ranidae	1070.0	207002	RACLAM	Green Frog	*Rana clamitans*
Anura	Ranidae	1072.0	173435	RAPALU	Pickerel Frog	*Rana palustris*
Anura	Ranidae	1073.0	173443	RAPIPI	Northern Leopard Frog	*Rana pipiens*
Anura	Ranidae	1074.0	173460	RASEPT	Mink Frog	*Rana septentrionalis*
Anura	Ranidae	1075.0	173436	RASPHE	Southern Leopard Frog	*Rana sphenocephala*
Anura	Ranidae	1076.0	173440	RASYLV	Wood Frog	*Rana sylvatica*

[a]Adapted from Lannoo (1998), Crother (2000), and the Integrated Taxonomic Information System (ITIS).
All amphibians found in the northcentral USA are included, not only pond-breeders.
Names follow Crother (2000).
States include Iowa, Illinois, Indiana, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin (U.S. Geological Survey Amphibian Research and Monitoring Initiative, Upper Mississippi Region).
The list may not be comprehensive for every state and is subject to revision.

Appendix E. State Conservation Status of Amphibian Species Found in the Northcentral USA

Common name	Scientific name	Status by state[a]												
		MO	IA	IL	IN	OH	KS	KY	MN	WI	MI	ND	SD	NE
Lesser Siren	*Siren intermedia*	P		P	P			P			X			
Three-toed Amphiuma	*Amphiuma tridactylum*	R												
Mudpuppy	*Necturus maculosus*	P	E	P	SPC	P	P	P	P	P	P	P	P	P
Hellbender	*Cryptobranchus alleganensis*	R		E	E	E		P						
Eastern Newt	*Notophthalmus viridescens*	P	E	P	P	P	T	P	P	P	P			
Ringed Salamander	*Ambystoma annulatum*	R												
Streamside Salamander	*Ambystoma barbouri*				P	P		P						
Jefferson Salamander	*Ambystoma jeffersonianum*			P	P	P		P			P			
Blue-spotted Salamander	*Ambystoma laterale*		E	P	SPC	E			P	P	P			
Spotted Salamander	*Ambystoma maculatum*	P		P	P	P		P		P	P			
Marbled Salamander	*Ambystoma opacum*	P		P	P	P		P			T			
Mole Salamander	*Ambystoma talpoideum*	R		P				P						
Small-mouthed Salamander	*Ambystoma texanum*	P	P	P	P	P	P	P			E			
Tiger Salamander	*Ambystoma tigrinum*	P	P	P	P	P		P	P	P	P	P	P	P
Green Salamander	*Aneides aeneus*				E	E								
Spotted Dusky Salamander	*Desmognathus conanti*			E				P						
Northern Dusky Salamander	*Desmognathus fuscus*				P	P		P						
Allegheny Mountain Dusky Salamander	*Desmognathus ochrophaeus*					P		P						
Black Mountain Salamander	*Desmognathus welteri*							P						
Seal Salamander	*Desmognathus monticola*							P						
Northern Two-lined Salamander	*Eurycea bislineata*					P		P						
Southern Two-lined Salamander	*Eurycea cirrigera*			P	P	P		P						
Long-tailed Salamander	*Eurycea longicauda*	P		P	P	P	T	P						
Three-lined Salamander	*Eurycea guttolineata*							P						
Cave Salamander	*Eurycea lucifuga*	P		P	P	E		P						
Many-ribbed salamander	*Eurycea multiplicata*	P					E							

Common name	Scientific name	Status by state[a]												
		MO	IA	IL	IN	OH	KS	KY	MN	WI	MI	ND	SD	NE
Oklahoma Salamander	*Eurycea tynerensis*	P					P							
Spring Salamander	*Gyrinophilus porphyriticus*					P		P						
Four-toed Salamander	*Hemidactylium scutatum*	P		T	E	SPC		P	SPC	SPC	SPC			
Western Slimy Salamander	*Plethodon albagula*	P												
Eastern Red-backed Salamander	*Plethodon cinereus*			P	P	P		P	P	P	P			
Northern Zigzag Salamander	*Plethodon dorsalis*	P		P	P			P						
Northern Slimy Salamander	*Plethodon glutinosus*			P	P	P		P						
Cumberland Plateau Salamander	*Plethodon kentucki*							P						
Mississippi Slimy Salamander	*Plethodon mississippi*							P						
Southern Ravine Salamander	*Plethodon richmondi*				P	P		P						
Southern Red-backed Salamander	*Plethodon serratus*	P												
Wehrle's Salamander	*Plethodon wehrlei*					P		P						
Mud Salamander	*Pseudotriton montanus*					P								
Red Salamander	*Pseudotriton ruber*				E	P		P						
Grotto Salamander	*Typhlotriton spelaeus*	P												
Eastern Spadefoot	*Scaphiopus holbrookii*	R		P	SPC	E	P	P						
Plains Spadefoot	*Spea bombifrons*	P	P									P	P	P
Eastern Narrow-mouthed Toad	*Gastrophryne carolinensis*	P		P			P							
Great Plains Narrow-mouthed Toad	*Gastrophryne olivacea*	P					T							
American Toad	*Bufo americanus*	P	P	P	P	P	P	P	P	P	P	P	P	P
Great Plains Toad	*Bufo cognatus*	P	P						P			P	P	P
Canadian toad	*Bufo hemiophrys*						P		P			P	P	P
Fowler's Toad	*Bufo fowleri*	P	P	P	P	P	P	P			P			
Woodhouse's toad	*Bufo woodhousii*	P	P					P				P	P	P
Northern Cricket Frog	*Acris crepitans*	P	P	P	P	P	P	P	E	E	PRO		P	P
Bird-voiced Treefrog	*Hyla avivoca*			P				P						
Cope's Gray Treefrog	*Hyla chrysoscelis*	P	P	P	P	P	P	P	P	P	P		P	P

Common name	Scientific name	Status by state[a]												
		MO	IA	IL	IN	OH	KS	KY	MN	WI	MI	ND	SD	NE
Green Treefrog	*Hyla cinerea*	P		P			P	P						
Gray Treefrog	*Hyla versicolor*	P	P	P	P	P		P	P	P	P	P	P	P
Barking Treefrog	*Hyla gratiosa*							P						
Mountain Chorus Frog	*Pseudacris brachyphona*					P								
Spring Peeper	*Pseudacris crucifer*	P	P	P	P	P	P	P	P	P	P			
Strecker's Chorus Frog	*Pseudacris streckeri*	R		T										
Southeastern Chorus Frog	*Pseudacris feriarum*	P		P	P	?	P							
Boreal Chorus Frog	*Pseudacris maculata*		P						P	P	SPC	P	P	P
Western Chorus Frog	*Pseudacris triseriata*	P	P	P	P	P	P		P	P	P	P	P	P
Crawfish Frog	*Rana areolata*	R	E	P	E		T	P						
Plains Leopard Frog	*Rana blairi*	P	P	P	SPC		T				P		P	P
American Bullfrog	*Rana catesbeiana*	P	P	P	P	P		P	P	P	P		P	P
Green Frog	*Rana clamitans*	P	P	P	P	P	P	P	P	P	P			
Pickerel Frog	*Rana palustris*	P	P	P	P	P	P	P	P	P	P			
Northern Leopard Frog	*Rana pipiens*	R	P	P	SPC	P	P	P	P	P	P	P	P	P
Mink Frog	*Rana septentrionalis*						P		P	P	P			
Southern Leopard Frog	*Rana sphenocephala*	P	P	P	P	?	P	P						
Wood Frog	*Rana sylvatica*	R		P	P	P		P	P	P	P	P	P	

[a]Status: P = Present, E = Endangered, R = Rare, T = Threatened, PRO = Protected, SPC = Special concern, X = Presumed extirpated, ? = Status unknown.
The list is adapted from field guides and state Web sites and is subject to revision. All amphibians found in the northcentral USA are included, not only pond-breeders.

Role of *Ribeiroia ondatrae* (Platyhelminthes: Trematoda) Metacercariae in the Development of Malformed Frogs in Minnesota and Wisconsin

by

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Farm Ponds as Critical Habitats for Native Amphibians: Final Report

Submitted to

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Abstract

Metacercariae of *Ribeiroia ondatrae* have been shown in laboratory and field studies to elicit significant limb malformations in metamorphs of several amphibian species. During a five-year study of trematode metacercariae from Minnesota and Wisconsin anurans, *Ribeiroia* was distributed throughout eastern Minnesota and western Wisconsin. *Ribeiroia* was most abundant (100% prevalence, mean intensity >110 worms per host) at two Minnesota malformation hotspots during the same years that extremely high (>50%) malformation rates occurred at the sites. Subsequent declines in malformation rates at these two sites have been correlated with reduced *Ribeiroia* abundance in metamorphs. *Ribeiroia* has not been found at malformation hotspots in western Minnesota, indicating that causes other than *Ribeiroia* are responsible. Preliminary studies on malformed anurans from eastern U.S. wildlife refuges indicated that, though infrequent, *Ribeiroia* is present at some sites. We suggest that in order for high rates of malformations to occur at a site, *Ribeiroia* infection rates must exceed a species-specific threshold. Environmental conditions that support increased intermediate snail host populations will, in turn, provide more *Ribeiroia* cercariae to penetrate tadpoles at critical times during limb formation. High *Ribeiroia* infection rates may elicit more frequent and severe amphibian malformations.

Key Words: Anura, malformations, parasites, *Ribeiroia*, Trematoda, Minnesota, Wisconsin, amphibian, deformity.

Introduction

Metacercariae of *Ribeiroia ondatrae* are known to cause development of malformed limbs in amphibians. Severe limb malformations were induced in *Hyla regilla* exposed in the laboratory to "biologically relevant" numbers of *R. ondatrae* cercariae (Johnson et al 1999). The malformations were similar to those observed in field studies conducted at malformation hotspots in northern California. Increases in cercarial exposure were associated with an increase in the malformation rate and a decline in tadpole survivorship. Experimentally-induced malformations included ectromelia (missing limbs), ectrodactyly (missing digits), cutaneous fusions (skin webbings), taumely (bony triangles), polydactyly (extra digits), polymelia (extra limbs) and femoral projections. Less abundant malformations included brachymelia (shortened limbs), permanent extension of limbs, brachydactyly (shortened digits) and syndactyly (fusion of digits).

Similarly, laboratory-induced *R. ondatrae* infections resulted in high rates (40-80%) of severe limb malformations in surviving *Bufo boreas* (Johnson et al. 2001a). Survivorship declined with increasing parasite exposure (42% in the heaviest treatment). In contrast to the previously mentioned study with *H. regilla*, cutaneous fusion was the predominant malformation among infected toads in all exposures. Infection also caused polymelia (fore and hind), ectromelia, polydactyly and various other limb malformations. Johnson et al. (1999, 2001a) have, therefore, demonstrated that (i) teratogenic effects of *R. ondatrae* are not limited to treefrogs, (ii) *R. ondatrae*-induced malformations are not confined to hind limbs and (iii) rates and types of malformations resulting from infections vary among amphibian species.

Laboratory studies conducted on *Rana pipiens* by R. Cole (pers. comm., USGS National Wildlife Health Center, Madison, Wisconsin) have also supported the hypothesis that *Ribeiroia* can induce developmental malformations and decrease survivorship of infected tadpoles and metamorphs.

Field studies also implicate *Ribeiroia* as a cause of amphibian malformations. Over a two-year period, Johnson et al (2001b) monitored rate and severity of malformations in *H. regilla*, *B. boreas*, *Rana catesbeiana* and *Taricha torosa* from two northern California ponds. Both ponds were highly eutrophic spring-fed stock ponds. Rates of malformations differed according to species, life-history stage, pond, and season. Larval stages were more likely to be malformed and at greater severity than emerging and adult amphibians. Larval *T. torosa* exhibited the highest rate of malformations, ranging from 15-50%, followed by larvae and metamorphs of *H. regilla* (10-25%), and finally by metamorphs of *B. boreas* and *R. catesbeiana*, both of which had rates of <5%. The most severe malformations were observed in *H. regilla*. More than 60% of malformations in treefrogs involved extra hind limbs, femoral projections and cutaneous fusions. Similarly, severe malformations of *R. catesbeiana* were dominated by extra and missing hind- and forelimbs. In *B. boreas* and *T. torosa*, the most common malformations were missing limbs and digits, which accounted for about 75% and 95%, respectively, of the total malformations.

During 1998, Johnson and Lunde collected frogs, toads and salamanders from 103 ponds in six northwestern states (CA, OR, WA, ID, MT, CO). At 42 ponds malformations were found in six amphibian species at rates ranging from 5-90%, and *Ribeiroia* was found at 40 of the 42 ponds and was almost never found at ponds with low deformity rates (Johnson et al. 2002).

Both laboratory and field studies support the hypothesis of Sessions and Ruth (1990) that amphibian malformations may be caused by trematode metacercariae. However, *Ribeiroia* has not been identified at all amphibian malformation hotspots. For example, Ouellet et al. (1997) observed a high rate of malformations in *Rana clamitans*, *R. pipiens*, *Bufo americanus* and *R. catesbeiana* from agricultural sites exposed to pesticide runoff in the St. Lawrence River Valley of Quebec, Canada in 1992 and 1993. According to Ouellet, parasites "were not encountered in relation to limb structures."

The status of malformed frog investigations in Minnesota was summarized by Rosenberry (2001). Suspected causes for malformed frogs in Minnesota include parasites, chemicals (including pesticides and endocrine disrupters) and ultraviolet light. It was concluded that one or more combinations of chemicals, biological and physical factors are likely responsible for malformations in Minnesota frogs.

We are investigating the frog-parasite communities of various water bodies in western Wisconsin (1997-present) and Minnesota (1999-present). Our objective is to determine if parasites are associated with malformed frogs in Minnesota and Wisconsin. This report summarizes our findings from 1997-2001.

Materials and Methods

Field Collections

We collected frogs and examined them for parasites from 1997-2001. Specimens were collected from western Wisconsin, malformation hotspots and farm ponds in Minnesota, USFWS national wildlife refuges in the eastern USA, and from northwestern Iowa (Table 1). We surveyed 44 sites throughout Minnesota, western Wisconsin and northwestern Iowa. We collected amphibians from eight sites in three Wisconsin counties (La Crosse, Trempealeau and Bayfield). Amphibians from 17 farm ponds and 12 malformation hotspots and five reference sites in Minnesota were examined for parasites. We collected from two sites in Dickinson Co., Iowa in 2001. Frogs from seven eastern USFWS national wildlife refuges were examined in 2000 and 2001.

Frogs were submitted for examination by the USGS (farm pond research study), the Minnesota Pollution Control Agency (MPCA), D. Hoppe (University of Minnesota-Morris, malformation hotspots), and M. Lannoo. Sites are described in Lannoo et al. (in press, 2003). Frogs from Minnesota malformation hotspots (>5% of animals with malformations) were paired with frogs from corresponding reference sites (<5% of animals with malformations). The two northwestern Iowa sites are located 150 km SW from the nearest known hotspot and represent control sites distant from the region where hotspots are prevalent.

Deformity assessments at each site consisted of species-specific collections of metamorphosing amphibians. Each specimen was inspected for morphological malformations and the snout-vent length was measured. All malformed specimens (up to ten total) and a sample of normal individuals were collected for parasite necropsies. The remaining individuals were released at the site. Amphibians collected for parasite examination were captured alive and maintained in coolers with ice or blue ice for transport to the University of Wisconsin-La Crosse. Amphibians were kept in a walk-in cooler (4°C) until necropsy and were euthanized in MS-222 (Green 2001). With the aid of a stereo-dissecting microscope, each specimen was examined first for deformities and then for parasites.

Necropsy Procedures

The skin was removed and the exposed surfaces of the skinned frog and the inside surfaces of skin were examined for parasites. In addition, muscle and connective tissues were teased apart to expose the presence of deeply embedded parasites. Locations of parasites were recorded as: tail resorption site, legs, back, abdomen, forelimbs, head, mandible, coelom and viscera. The viscera were further subdivided into lungs, liver, urinary bladder, kidneys, gonads, stomach, intestine, rectum, mesenteries and Eustachian tube/pharynx.

Live metacercariae were counted or the minimum number estimated. Representative metacercariae were carefully excysted manually and photographed. *Ribeiroia* metacercariae were identified by their characteristic esophageal cecae and other features described by Beaver (1939) and Basch and Sturrock (1969). Other metacercariae were identified to genus and type using primary literature cited in Yamaguti (1975) and Schell (1985).

The skeletal system was maintained intact and following examination for parasites, carcasses were preserved in 10% neutral buffered formalin in a standardized position and sent to M. Lannoo for radiographic analyses and complete description of malformations.

Statistical Analyses

We calculated the rate of morphological abnormalities as the percentage of malformed individuals relative to the total number examined for each site and amphibian species. Obvious traumatic injuries were not included as malformations. Parasite data were recorded as prevalence (percentage of individual hosts in a species infected with a parasite species) and mean intensity (mean number of worms per infected host).

Results and Discussion

We examined 904 amphibians for parasites from 1997-2001 in the eastern USA (Table 1); twelve species were presented (Table 2). *Ribeiroia* was found at 22 sites (two eastern USFWS refuges, two sites in La Crosse County, Wisconsin and 18 sites in Minnesota). *Ribeiroia* metacercariae were recovered from *R. pipiens*, *R. clamitans*, *R. septentrionalis*, *R. sylvatica* and *B. americana*.

Frog malformation hotspots are rare in southeastern Minnesota and western Wisconsin. Of the 40 farm ponds monitored by USGS during 2000 and 2001, no malformation rates exceeded 5% for any species. Among sites adjacent to the Mississippi River, malformation rates were <3%. We observed high rates of malformations (>5% at a site) in 1997, 1998, and 2000 (but not in 1999) for *R. clamitans* metamorphs at one Trempealeau County farm pond.

Ribeiroia were not found in any frogs from Wisconsin during 1997 and 1998. At this time, we assumed that *Manodistomum* was the metacercaria responsible for causing limb malformations in frogs (Sessions and Ruth 1990). Frogs from the Upper Mississippi River Valley (UMRV) harbor a diverse fauna of metacercariae that can occur in large numbers (hundreds to thousands of trematodes per individual frog). *Alaria*, *Fibricola*, *Manodistomum*, *Euryhelmis*, *Clinostomum*, *Apharyngostrigea pipientis*, Meta A, Meta B, other "globbies" (including *Aurodistomum chelydrae*, *Glypthelmins quieta*, an unknown progenetic digene, and several other small unknown digenes) and echinostomes are all frequent and often abundant parasites infecting frogs from the UMRV. We may have overlooked the presence of *Ribeiroia* in frogs from the UMRV during 1997 and 1998.

Ribeiroia was initially identified in frogs from the Upper Midwest in 1999. We initially found *Ribeiroia* in *R. pipiens* and *R. clamitans* from La Crosse County, Wisconsin in 1999 and 2000, respectively.

During 2000, *Ribeiroia* was found at 3 of 16 southeastern Minnesota farm ponds examined in Houston and Winona counties. Prevalence ranged from 30-47% and mean intensity of infection ranged from 2-33.2 worms per infected host. Only two malformed *R. clamitans* were observed at these sites; both malformations were associated with *Ribeiroia* metacercaria. Two malformed frogs were also found at *Ribeiroia*-negative sites.

In Minnesota during 2001, *Ribeiroia* was found at 6 of 13 sites examined. Again, prevalence and mean intensities were relatively low, except for one site where all 10 *R. pipiens* were infected with *Ribeiroia*; mean intensity of infection was 33.6 worms (range 19-68). One other site contained a normal *R. clamitans* with 68 metacercariae. Other sites had prevalences of *Ribeiroia* ranging from 10-40% and mean intensities of 2-4 worms per infected host. Only seven of 111 amphibians from farm ponds in southeastern Minnesota were malformed and of those seven only four harbored *Ribeiroia*. In addition to *Ribeiroia*, farm ponds in Houston and Winona counties also harbored *Fibricola*, "globbies," *Manodistomum* and echinostome metacercariae in 2001 and *Fibricola*, "globbies," an unknown neascus, echinostome and *Clinostomum* metacercariae in 2000.

In 2000, 17 out of 18 frogs from USFWS wildlife refuges in the eastern USA were malformed and only one harbored *Ribeiroia* metacercariae (*R. pipiens*, 37 *Ribeiroia)*. Other metacercariae found from the 18 frogs included *Fibricola*, *Alaria*, globbies, *Manodistomum*, neascus, an unknown metacercaria ("Meta E"), a progenetic metacercaria ("Meta P"), echinostome metacercariae, and an unknown (possibly gorgoderid) immature digene.

In 2001 frogs, nine *R. clamitans* from one USFWS refuge were infected with *Ribeiroia* (mean intensity = 18.8) and two of these (with five and nine *Ribeiroia*) had deformities in a hind foot. Two frogs from another refuge were malformed and one of these harbored a single *Ribeiroia*. In addition to *Ribeiroia*, the frogs also harbored globbies, *Manodistomum*, Meta E and echinostome metacercariae, *Fibricola*, and *Alaria*. One particularly interesting structure found in the frogs was a large thick-walled cyst that contained amorphous material. These "masses" were located most frequently in the tail resorption site but also occurred in the gill resorption site and occasionally elsewhere. The masses ranged in size up to five times the diameter of a typical metacercarial cyst. Several of the smaller masses appeared to contain remains of dead metacercariae that were apparently being resorbed. These were unlike the melanized cysts previously seen in frogs from many North American sites.

During 1999, we examined four severely malformed *Rana septentrionalis* from a site in north central Minnesota. This 12-ha lake is well known as a frog malformation hotspot, characterized by extremely high malformation and mortality rates for virtually all amphibian species (Gardiner and Hoppe 1999). Amphibian populations have crashed over the past few years along with most fish and several free-living invertebrate populations. All four of the malformed frogs had various degrees of satellite hind limb formation ranging from severe to minor. Satellite limbs originate in the region of the pelvis, but do not articulate with the pelvis; rather, these limbs arise superficially (i.e., just under the skin) and occur in the presence of a normal right and left hind limb. The four frogs harbored an average of 110 *Ribeiroia* (range=96-125). Metacercariae occurred primarily in the tail resorption site and in subdermal fascia surrounding the anus. *Ribeiroia* were so numerous that they fell away in packets of 25-70 when the skin was removed. In addition, each mink frog harbored high numbers (mean=11) of metacercariae encysted along the mandibular margin.

During 1999, *Ribeiroia* metacercariae were also found in south central Minnesota. The site was a constructed farm pond located near Henderson, Minnesota, where in 1995 middle school students found many malformed frogs for the first time in Minnesota (Souder 2000). When we visited the site in mid-August, malformation rates were low. Two of three malformed *R. pipiens* we were examined were infected with *Ribeiroia* (1 and 2 metacercariae) and one of three normal *R. pipiens* was infected with *Ribeiroia*. *Ribeiroia* was not found in six frogs (3 normal, 3 malformed) from four other sites that are being monitored because they have been malformation hotspots in previous years.

We did not find *Ribeiroia* in 1999 at another Traverse County, Minnesota site that had malformation rates of metamorphosing *R. pipiens* exceeding 60% (pers. comm., D. Hoppe, University of Minnesota-Morris). None of six severely malformed and three normal leopard frogs that we examined harbored *Ribeiroia*. Malformation rates were again high in 2000 at this site, but we did not examine any frogs. In 2001, malformation rates were low. We visited in early August and collected only one malformed *R. pipiens* out of 14 frogs collected. None harbored *Ribeiroia*. *Manodistomum* and *Fibricola* were common parasites at this site in both 1999 and 2001.

We did recover *Ribeiroia* from a malformation hotspot near Hibbing, Minnesota in 2000. This small oval pond was constructed in 1996 by pushing soil toward the middle of the depression to create a donut shaped pond with a central island. Malformation rates approached 96% in 2000. All twelve *R. pipiens* sent to us from this site were infected with *Ribeiroia* (mean intensity of 155.5 worms; range 51-266). Ten of the 12 frogs were malformed. Malformations included severe cutaneous fusions, satellite limb formation, brachydactyly, polydactyly and unusual soft tissue growths--some of which project externally and some internally from the skin. Many growths had encysted *Ribeiroia* metacercariae located at their proximal ends. *Ribeiroia* were found primarily in the tail resorption site (76.7%) or attached to the inside of the skin (18%). *Ribeiroia* were also found along the margin of the mandible (2.4%). A few *Ribeiroia* were found extending down to the knee and even out onto the foot in several of the more heavily infected frogs. Five frogs had *Ribeiroia* metarcercariae (2.5% of total) located within the coelomic cavity near the urinary bladder.

By 2001, malformation rates at many of the Minnesota hotspots were on the decline (pers. comm., P. Jones, USGS Water Resources). We found *R. pipiens*, *R. sylvatica* and *B. americanus* individuals infected with *Ribeiroia* metacercariae. At the Henderson, Minnesota hotspot, malformations were still relatively high; six of 10 *R. septentrionalis* collected were malformed. All of these frogs were infected with *Ribeioria*, mean intensity 35.4 worms per infected host. A normal *R. clamitans* harbored 12 *Ribeiroia* metacercariae. Likewise, four of 11 *R. pipiens* necropsied from another Minnesota site in 2001 harbored *Ribeiroia* (mean intensity of 2.8).

In August 2001 a new malformation hotspot was identified near Cottage Grove, Minnesota (23% malformation rate). This site is a storm water retention reservoir located in a city park. All malformed and normal toads necropsied harbored heavy infections of *Ribeiroia* (18.8 and 13 worms per infected malformed and normal host, respectively).

We found high prevalence and mean intensities of *Ribeiroia* at some sites, but *Ribeiroia* has never been found at several sites with high malformation rates. Malformation hotspots are brought to the attention of authorities when there is a congruence between amphibian malformations and humans interested in the outdoors. This occurs with school or scouting group trips and in areas where people enjoy wildlife (Souder 2000). We found one previously unreported hotspot simply because we were unable to locate a designated reference site. None of the 12 *R. pipiens* examined (including five malformed) had *Ribeiroia*.

Malformed frog hotspots tend to occur within a broad band running northwest to southeast across Minnesota; few reported hotspots occur in the southwestern grassland and northeastern boreal forested portions of the state. Hotspots are more associated with North Central Hardwoods and Driftless Area ecoregions, less associated with Lake

Agassiz Plain, Northern Glaciated Plain and Western Corn Belt Plain ecoregions. There may be some tendency for hotspots to occur at the junctions of recognized ecoregions. Our data indicate that *Ribeiroia* positive sites are located in the eastern half of Minnesota and *Ribeiroia* negative hotspots are located in the western half of Minnesota.

We observed that *Ribeiroia* metacercariae encyst subdermally in fascia connecting skin to the underlying muscle. The majority of metacercariae are located in tissues surrounding the anus (including tail resorption site, rump and inguinal region, 72.5%) or attached to the underside of skin in the region surrounding the anus (21.3%). Fewer *Ribeiroia* metacercariae were found along the margin of the mandible (3.5%), gill resorption site (0.8%) or attached to the parietal peritoneum of the coelom near the urinary bladder (1.2%).

Frogs with heavy worm burdens of *Ribeiroia* were more likely to have *Ribeiroia* metacercariae occurring at locations away from the anus. *Ribeiroia* were found encysted distal to the knees and down onto phalanges only in anurans that were heavily infected with *Ribeiroia*. Two frogs had *Ribeiroia* encysted between the orbits of the eyes. According to P. Johnson (pers. comm., University of Wisconsin-Madison), *Ribeiroia* cercariae will contact tadpoles at almost any spot on the body, but unlike most other species of cercariae, do not necessarily penetrate and encyst at the point of contact. Rather *Ribeiroia* cercariae will move toward a major body opening (such as anus or nostrils), enter the opening, penetrate the epithelium or mucosa and then encyst. Penetration of the anus and encystment of metacercariae in surrounding tissues brings *Ribeiroia* metacercariae in close contact with the hind limb bud.

Numerous other species of trematode metacercariae encyst in the region of the anus and must be distinguished from *Ribeiroia*. Some researchers advocate clearing and staining of anuran specimens as the standard for diagnosing *Ribeiroia* infections. In our opinion, clearing and staining does not allow for discrimination of *Ribeirioa* from other metacercarial species. While molecular assays would allow such discrimination, a faster and cheaper method involves manual excision of metacercariae with a sharp probe or syringe needle and examination of live metacercariae as a wet mount. Preserved metacercariae can be dissected from anuran hosts, but their definitive identification is often compromised.

There appears to be little correlation regarding the location of *Ribeiroia* metacercariae cyst and the side of the frog on which a limb is malformed. We noted several cases in which *Ribeiroia* occurred on the side of the frog opposite to the malformed hind limb. Because both hind limb buds originate together near the anus, it is plausible that a metacercaria located on one side of the limb bud might negatively impact the formation of a limb on the opposite side of the tadpole, if the trigger for abnormal development is a substance secreted by the parasite. While the mechanism by which *Ribeiroia* elicits abnormal limb formation is not known, as few as one *Ribeiroia* metacercaria may result in one or more significant limb abnormalities in an individual anuran (Johnson et al. 1999).

In conclusion, high burdens of *Ribeiroia* have now been found at several frog malformation hotspots in Minnesota. We found the highest mean intensities (>100 worms) of *Ribeiroia* during the same years that sites had extremely high malformation rates.

The finding of *Ribeiroia* at reference sites and at USGS farm pond sites during the past several years, all of which have been documented to have low rates of malformations in metamorphosing amphibians, indicates that *Ribeiroia* is more widely dispersed in Minnesota than was previously thought. In 1999, MPCA stated that parasites could not be a cause of amphibian malformations in Minnesota because the causative parasite had never been reported from the state. Our data indicate that *Ribeiroia* is widely distributed in Minnesota. At some ponds, *Ribeiroia* occurs at low levels (both prevalence and mean intensity of infection) and may not be present in sufficient numbers to infect tadpoles when limb buds are most susceptible to malformations. Likewise, there may be insufficient *Ribeiroia* cercariae penetrating tadpoles and encysting at critical locations where deformities can be induced.

However, there are malformation hot spots in Minnesota and elsewhere where we have not been able to find *Ribeiroia*. In 1999, a site where *H. regilla* had high rates of missing limbs and eyes was identified near Irvine, California (pers. comm., D. Gardiner, University of California-Irvine). We found no *Ribeiroia* in 25 malformed frogs from the Irvine site. Similarly, we have not found *Ribeiroia* or any other trematode metacercariae at a farm pond in Trempealeau County, Wisconsin, where high rates of ectromelia occurred in metamorphs of *R. clamitans* during 1997, 1998 and 2000. This pond is surrounded by corn and soybean fields that receive manure from a large poultry-rearing facility.

While it may be argued that we could have missed identifying all rare parasites at some sites, we feel that the small size of many of the ponds examined and the uniformity of parasite loads among frogs from a given site, reduces the probability that rare *Ribeiroia* infections were missed.

Adult *R. pipiens*, *R. clamitans*, and *R. catesbeiana* are able to melanize metacercarial cysts of most trematode species. Cyst melanization can make metacercarial species identification difficult or impossible. However, melanized metacercarial cysts often contain live and active metacercariae. We have been able to successfully infect ducklings, chicks and pigeons with *Ribeiroia* metacercariae obtained from melanized cysts and to obtain gravid *Ribeiroia* as a result of these experimental infections. The most advanced melanization responses occur in adult anurans. Therefore, we do not believe the absence of *Ribeiroia* indicates that the frog host has immunologically eliminated all traces of parasites prior to our examining frogs for parasites. Our experience is that while frogs do react to parasites (melanization being a primary mechanism for elimination) and eventually kill, digest and absorb dead parasites, it is unlikely that they eliminate all traces of parasites within weeks of metamorphosis.

Therefore, we conclude that *Ribeiroia* is one cause of malformed amphibians in Minnesota, especially at malformation hotspots where extremely high burdens of *Ribeiroia* were found in anuran hosts. High malformation rates at other ponds where *Ribeiroia* have not been found must result from other causes. Increases in malformation rates may be anticipated at *Ribeiroia*-positive ponds currently exhibiting low malformation rates if *Helisoma* snail (the first intermediate host for *Ribeiroia*) populations increase, in turn, producing more *Ribeiroia* cercariae, or when an infected definitive host (bird or mammal) deposits a large number of eggs into a pond harboring large populations of *Helisoma*.

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Table 1. Numbers of specimens and collection locations for frogs examined for parasites from 1997-2001 (Number of sites=44).

Year	# Specimens	Location
1997	80	Western Wisconsin
1998	65	Western Wisconsin
1999	68	Malformation sites and controls (42), Minnesota
2000	245	Southeastern Minnesota (farm ponds, USGS, 149), Malformation sites and controls, Minnesota, and USFWS refuges, eastern USA (18)
2001	446	Southeastern Minnesota (farm ponds, USGS, 111), Malformation sites and controls, Minnesota, (260), northwest Iowa (40), USFWS refuges, eastern USA (35)

Table 2. Species of amphibians examined for parasites.

Rana pipiens (Northern Leopard Frog)
Rana sphenocephala (Southern Leopard Frog)
Rana clamitans (Green Frog)
Rana septentrionalis (Mink Frog)
Rana sylvatica (Wood Frog)
Rana palustris (Pickerel Frog)
Rana catesbeiana (American Bullfrog)
Bufo americana (American Toad)
Hyla versicolor (Gray Treefrog)
Pseudacris crucifer (Spring Peeper)
Pseudacris triseriata (Western Chorus Frog)
Ambystoma tigrinum (Tiger Salamander)