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FARM PONDS AS CRITICAL HABITATS FOR NATIVE AMPHIBIANS: FIELD SEASON 2000 REPORT

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Abstract:

We studied constructed farm ponds in the Driftless Area Ecoregion of southeastern Minnesota, western Wisconsin, and northeastern Iowa. These ponds represent potentially significant breeding, rearing, and over-wintering habitat for amphibians in a landscape where natural wetlands are scarce. This region contains thousands of small farm ponds constructed with cost-sharing dollars from the US Department of Agriculture and the state, yet no studies have been conducted to determine how the ponds benefit wildlife. We collected amphibian, wildlife, invertebrate, and water quality data from 40 randomly-selected farm ponds in southeastern Minnesota beginning March 2000. We selected 10 ponds in each of 4 surrounding land use classes: row crop agriculture, grazed grassland, ungrazed grassland, and natural wetlands. Data collection protocols included measuring amphibian presence/absence at all life cycle stages, water samples collected for water quality analysis, a FETAX study of amphibian sensitivity to pond water quality, and a Microtox study (a rapid, biotic water quality assessment technique). In addition, we conducted 22 amphibian deformity assessments among 6 amphibian species and screened for the parasite *Riberoiria* at 18 ponds, collected amphibian blood for flow cytometry analyses, and measured bird, reptile, and mammal presence near the ponds. Detailed vegetation and land use/cover measurements were obtained for each pond. Hundreds of photographs were taken documenting field techniques, vegetation changes, and the amphibians and wildlife present.

Dry weather conditions prevailed in May 2000, followed by heavy rainfall in late May and June and continued wet weather through July. Heavy rainfall corresponded with the planting of crops. Extensive soil erosion was observed surrounding many of the ponds. The ponds attenuated soil erosion by slowing water movement and stopping sediment high on the watersheds. This process can cause the ponds to rapidly fill up with sediment. 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),* and pickerel frog (*Rana palustris).* The blue-spotted salamander (*Ambystoma laterale)* was identified from a single larval specimen. We also identified 4 snake species, including the fox snake (*Elaphe vulpine),* brown snake *(Storeria dekayi),* redbelly snake *(Storeria occipitomaculata),* and

garter snake *(Thamnophis sirtalis)*. Approximately 73 species of birds, 14 mammal species, 4 fish species, and 28 invertebrate taxa were also identified. We observed high abundances of American toad, eastern gray treefrog, and green frog at many ponds. Lower abundances of the other species were observed. Despite full choruses of spring peepers at many ponds, we observed low abundances of tadpoles for this species, possibly indicating a sensitivity to habitat conditions at the ponds. Deformities represented < 4% of the individuals examined at any pond. Dan Sutherland, University of Wisconsin La Crosse, identified *Riberoiria* (the parasite responsible for amphibian deformities elsewhere) at a few locations, but in low numbers. Water quality in the ponds ranged from very clear, stream-fed ponds (natural wetlands) to very nutrient-rich, stagnant waters. There were some significant differences in water quality, nutrients, and the FETAX test between the natural and nongrazed land use classes (less disturbed) and the grazed and agricultural land uses (more disturbed). There were no significant differences in amphibian species richness among the 4 classes of surrounding land use.

We obtained additional funding from the USGS Amphibian Research and Monitoring Initiative (ARMI) ($98K) to expand the post-breeding habitat use component of the study. This work, “Effects of agricultural and urban land use on movement and habitat selection by northern leopard frogs (*Rana pipiens*)”, will involve radio-telemetry of leopard frogs during the post-breeding stage of the life cycle around 2 farm ponds and an urban-edge marsh within the USFWS Upper Mississippi River National Wildlife and Fish Refuge. The objective of the study is to determine how adult leopard frogs use the landscape after leaving the breeding pond and how they respond to potential hazards such as roads, agricultural fields, and urban development.

Two University of Wisconsin graduate students, Josh Kapfer (amphibian toxicology) and Brian Pember (amphibian post-breeding habitat use) are undertaking various aspects of the project. We are sharing information with other amphibian researchers in the Midwest, including Perry Jones (USGS Water Resources Division, Mounds View, MN), David Green (USGS Wildlife Health Lab in Madison), Lucinda Johnson (NRRI, Duluth), Nancy Shappell (USDA Fargo, ND), and Jeff Canfield (MN Pollution Control Agency).

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

Website: http://www.umesc.er.usgs.gov/terrestrial/amphibians.html

INTRODUCTION

Constructed farm ponds represent significant breeding, rearing, and over-wintering habitat for amphibians in the Driftless Area Ecoregion of southeastern Minnesota, western Wisconsin, and northeastern Iowa, a landscape where natural wetlands are scarce (Fig. 1). Despite intensive agricultural use adjacent to the ponds, these ponds harbor an abundance of frogs and toads. 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 and create wildlife habitat, yet no studies have been conducted to determine how the ponds benefit wildlife. Habitat and water quality in these ponds directly affects livestock health where grazing is practiced. The karst geology of the area promotes rapid water movement from surface waters into ground water used for human consumption. Minnesota has been plagued by the discovery of frog deformities in some agricultural areas; farm ponds have not been examined to determine if amphibian deformities are a problem in the Driftless Area. Our study addresses the following questions:(1) How are measures of amphibian individual, population and community health associated with land uses surrounding the pond, such as row crops, grassland, and grazed grassland? (2) Are measures of amphibian individual, population, and community health more associated with surrounding landscape features or within-pond characteristics? (Fig. 2) (3) What design features associated with a pond (size, depth, vegetation) will maximize wildlife benefits?

The study will evaluate current farm management practices surrounding farm ponds and identify those that lead to sustainable amphibian populations, high diversity, and low incidence of deformities. Based on our experience and study results, we will recommend screening/monitoring methods suitable for assessing the health of amphibian populations in farm ponds. We will also develop a guide to the design, construction, and management of farm ponds for use by contractors, private landowners, and state and federal agencies.

STUDY AREA

Our study plots were located in Houston and Winona counties in Minnesota. These counties are within the Driftless Area ecoregion of the midwestern USA. The ecoregion is distinct from those surrounding it, in that it was not covered by ice during the last

(Wisconsin) glaciation (Mickelson et al. 1982). The ecoregion covers portions of the states of Minnesota, Wisconsin, Iowa, and Illinois (Fig. 1). The landforms are characterized by maturely dissected, upland plateaus with steep (150 m) bedrock ridges descending to river drainages that ultimately flow to the Mississippi River, which bisects the ecoregion (McNab and Avers 1994). Complex topography and erosive soils support a less intensive agriculture than in many parts of the Midwest, with agriculture occupying 30–40% of the landscape (McNab and Avers 1994). Forests interspersed with pastures, temporary grasslands, and small towns are also present. The forests form a connected, dendritic pattern, confined to steep slopes adjacent to streams and rivers. 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.

METHODS

We randomly selected 40 farm ponds, 10 ponds in each of 4 surrounding land use classes: row crop agriculture, grazed grassland, ungrazed grassland, and natural wetlands (Fig. 1) in Houston and Winona counties, Minnesota. The ponds were selected using USGS 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's but not on the NWI's (constructed after 1988) were added to the set of ponds from which the study ponds were selected. We assumed that the 4 types of surrounding land uses represented the range of amphibian habitat conditions found in farm ponds in this region. Grassed buffers surrounding the ponds were <30 m wide for the row crop class. Domestic livestock (cattle or horses) had access to the water in the ponds in the grazed grassland class. Natural wetlands proved scarce and 'pristine' sites were impossible to find; therefore, some were lightly grazed or adjacent to croplands. We did not control for distance to roads and we excluded ephemeral wetlands and ponds within 80 m of barnyards or livestock confinement areas as study ponds. Most of the ponds were privately owned and written permissions to conduct the research 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).

Amphibians

We surveyed amphibians using chorus surveys, egg mass surveys, larval surveys, and visual encounters. We surveyed calling anurans 6 times at each pond, starting late March and ending in late July. We used standardized 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 (15-30 April; 20 May – 5 June; and 1-15 July). All adult amphibians caught were identified, weighed, checked for deformities, and snout-vent length measured.

Reproductive effort was assessed through egg mass counts. The littoral zone of each pond was searched for egg masses at each pond visit (approximately every 2 weeks). The density 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*), while others lay clusters of 2-3 eggs on vegetation (e.g. *Pseudacris crucifer*).

Reproductive success was assessed through larval surveys conducting by dipnetting at each pond once every 2-3 weeks. Larval surveys allowed identification of larval salamanders, which are not identified by calling surveys. We also experimented with funnel traps (Ranger Products, Detroit, MI), both unbaited and baited with glow sticks. Three traps were rotated among ponds for a 3-week period in May and June.

We experimented with drift fences and pitfall traps for capturing salamanders. Three drift fences (15 m long) were constructed parallel to the littoral zone at 2 ponds, with pit traps at each end. Traps were operated for 18–hour periods during April and May, excluding mid-day to avoid heat stress to captured animals. All amphibians captured in the pit traps were identified, weighed, and snout-vent length measured.

Deformity assessment.--Twenty-two deformity assessments among 6 species of anurans (metamorphs) were conducted at 18 ponds using U.S. Fish and Wildlife Service protocols (North American Reporting Center for Amphibian Malformations 1998, http://www.npwrc.usgs.gov/narcam/techinfo/ protocol.htm). Metamorphs were identified to species, counted, and examined for deformities. Ten individuals were collected of each species during the deformity assessment. If deformed individuals were found, up to 5 were included in the collection; the balance were apparently healthy individuals. The 10 collected animals were taken to the lab and examined for parasites.

Flow cytometry.--Blood was collected from the specimens examined for deformities. This blood was frozen for the flow cytometry analysis.

Procedures for handling, transporting, anesthetizing, and housing amphibians were approved by the USGS Upper Midwest Environmental Sciences Center's Animal Care and Use Committee. We followed the Declining Amphibians Task Force Fieldwork Code of Practice (http://www.npwrc.usgs.gov/ narcam/techinfo/daptf.htm), including cleaning all boots and equipment between site visits with a bleach solution.

Amphibian voucher specimens were collected to aid 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. We examined eggs and larvae under a dissecting microscope to make accurate identifications and build a key to amphibian eggs and larvae of the Driftless Area to aid in field identification. We took >1000 photographs of field activities, amphibians, other vertebrates, and vegetation surrounding the ponds. We are maintaining a digital database of the photographs with attributes for each image.

Sampling frames for detecting amphibian species.-- We approached the problem of optimal allocation of field effort for standardized field surveys of amphibians through a probability-based statistical analysis of dipnet data. For a given species of anuran, let p represent the true probability of observing at least one tadpole or metamorph for a single visit when dipnetting is the means of detection. Let $\hat{p} = \frac{\#\ \text{successes}}{\#\ \text{visits}}$ be an estimator of p, where a success is defined as observing at least one tadpole or metamorph of a given species during the visit.

In order to develop a sampling plan for presence/absence data, it is useful to consider a geometric probability distribution as a model of the number of visits needed for detection of tadpoles or metamorphs of a given species. 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" will be defined as a visit in which the presence of tadpoles or metamorphs for a given species in observed.

Genetics

We collected blood in the laboratory from 220 animals obtained from 22 deformity assessments. Blood samples were placed into a freezing solution and flash-frozen in liquid nitrogen (Murphy et al. 1997). Frozen samples were maintained in a freezer at -80°C until analysis. Blood has also been obtained from a reference specimen of *Xenopus laevis*. We are in the process of running the DNA profiles. Use of the cytometer is courtesy of Gundersen Lutheran Medical Center, La Crosse, WI, Lyme Disease Research Group, Dr. Steven Callister, senior scientist.

Post-breeding habitat use

We worked to refine methods of attaching radio-transmitters to leopard frogs (*Rana pipiens*) for purposes of describing post-breeding habitat use. Beginning 1 August 2000, frogs were captured in a wet meadow adjacent to a natural pond, one of the farm pond reference sites, and outfitted with radio transmitters. We could not obtain radio prior to August, due to a backlog of orders at the manufacturer. The frogs were outfitted with radio transmitters (2 grams, Holohill, Inc., model BD-2G, Ontario, Canada) attached to each frog's back with a harness. The frogs experienced problems with skin erosion and they slipped out of the transmitters with all combinations of materials and strapping methods we tried. We decided to hold the frogs in enclosures while we worked on refining our methods. The frogs were confined for observation in 1.8 X 1.8 m cages on the periphery of the pond, beginning 29 August 2000 and released from the enclosures on 12 October 2000. We tried nickel bead chain, aluminum bead chain, plastic cable ties, and sewing elastic as harness materials. Frogs were located and their health status evaluated every 2-4 days from 1 August – 26 October 2000. We removed the harness and transmitter and released frogs that developed skin lesions. All transmitters were removed and tracking ceased by 26 October 2000. Additional funding for this component of the study was obtained from the USGS Biological Resources Division, the Milwaukee Zoological Society, and the University of Wisconsin, La Crosse. University of Wisconsin, La Crosse graduate student, Brian Pember, is undertaking this study as part of his Master's thesis.

Other Vertebrates

Mammal presence was monitored once at each pond (excluding grazed ponds) using 3 scent stations placed equidistant around the riparian zone perimeter of each pond during August 2000, using protocols modified from the Minnesota DNR Predator - Furbearer Scent Post Station Survey (Bill Berg, Minnesota DNR, pers. comm., Sargeant et al. 1998). 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 boards (0.6 m X 2 m strips of corrugated sheet metal) to estimate reptile and small mammal presence (Parmelee and Fitch 1995). Cover boards were initially placed in the grass buffers, equidistant around the riparian zone perimeter in March. The boards were checked at each pond visit. 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 (Ralph et al. 1995). Incidental observations of birds (ducks, herons, swallows, shorebirds, nocturnal birds) at each visit were also recorded.

Aquatic Predators

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. 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 and Cummins 1984; Thorpe and Covich 1991). We sampled each pond 3 times, twice in June and once in July.

Water Chemistry and Toxicity

We collected water for chemical analysis and toxicity testing from 26 ponds during the weeks of 24 April, 8 May, 22 May, 12 June, 26 June, 10 July, and 24 July 2000. In addition, the remaining 14 ponds were sampled for chemical analysis once during the week of 22 May. 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. Sample numbers and codes were assigned to each sample to ensure blind testing of each sample by laboratory staff. Water for chemical analysis (100 ml) was frozen until analysis. Water samples for the FETAX and MICROTOX analyses (200 ml, described below) were collected in chemically clean amber bottles with Teflon caps and reduced head space (to minimize volatilization), transported on ice to the laboratory, and stored in the dark at 4 C until use.

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 1989) and UMSC standard operating procedures. Chlorophyll-*a*, nitrogen (nitrate+nitrite), and total phosphorus concentration were measured in the laboratory according to standard methods (APHA 1989).

We conducted the FETAX assay as described in ASTM E 1439-98 (American Society for Testing and -Materials 1998). Twenty normally-developing blastula-stage embryos were placed in 60-mm covered plastic petri dishes containing negative control, pond, or positive control solutions. Water samples from each pond were tested in duplicate dishes. Four dishes of 20 embryos were exposed to FETAX solution alone and served as negative controls. The FETAX solution consisted of 625 mg NaCl, 96 mg $NaHCO_3$, 30 mg KCl, 15 mg $CaCl_2$, 60 mg $CaSO_4$´$2H_2O$, and 75 mg $MgSO_4$ per liter of deionized water. Four dishes of 20 embryos were exposed to 6-aminonicotinamide, 2 dishes at 5.5 mg L^{-1} FETAX solution (approximate 96-h EC50, malformation) and 2 dishes at 2,5000 mg L^{-1} FETAX solution (approximate 96-h LC50) as specified in ASTM E 1449-98 and served as positive controls. Each dish contained 8 ml of solution. Embryos were cultured at 23.0 ± 1.0 C in a computer-controlled environmental chamber on a 12 h light:12 h dark photoperiod. All solutions were changed every 24 h during the 4-d test and dead embryos were removed and recorded. After 4 d (stage 46 embryos), living embryos were fixed in 3% buffered formalin (pH = 7.0) for later examination of malformation and determination of growth.

The head-tail length (index of growth) of surviving larvae were measured with a dissecting microscope integrated with a video camera, an IBM-compatible computer, and digitizing software. The type and rate of malformations were determined among the control and exposed larvae with a dissecting microscope. The "Atlas of Abnormalities" (Bantle et al. 1991) was used in scoring malformations. Statistical analysis of mortality, growth, and malformation data were determined with ANOVA and Dunnett's test ($p < 0.05$). University of Wisconsin, La Crosse graduate student, Josh Kapfer, is undertaking this work as part of his Master's thesis.

In conjunction with the FETAX assay, we determined the toxicity of water samples to *Vibrio fischeri* with the Microtox (r) Chronic Toxicity Test. Luminescence of bacteria incubated in pond water were compared to that of bacteria incubated in control water. Statistically significant differences were indicative of an effect. Those samples of pond water causing toxicity to *Vibrio fischeri* were compared with those samples having deleterious effects on the early embryo-larval development of frogs (FETAX).

Vegetation

We measured vegetation using a modification of aquatic plant sampling developed by Yin et al. (Yin et al. 1998). 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. We recorded 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. The landscape visual estimates will be used to ground truth the quantitative landscape data derived from GIS maps described below.

Landscape

We are in the process of generating the landscape data. We are using 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 are using National Wetland Inventory 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). The radiotelemetry component of the study will provide us with better estimates of home range sizes for leopard frogs.

RESULTS

Amphibians

Presence/absence.-- We developed a key to amphibian eggs and larvae of the to aid in field identification (Appendix A). Using all methods of capture and identification, we identified eastern gray treefrogs, American toads, green frogs, spring peepers, chorus frogs and leopard frogs at ≥32 ponds, making them the most common species identified (Fig. 3). Common and scientific names for all species are based on the Integrated Taxonomic Information System (www.its.usda.gov, Appendix B). Pickerel frogs, tiger salamanders, and wood frogs were less common; these species were found at 13 or fewer ponds (Fig. 3). Calling surveys had the best correlation with the total number of ponds where each species was identified, followed by dipnetting and visual search for adults (Table 1). Egg mass surveys were least successful in identifying species presence (Figs. 4 and 5). However, egg mass surveys were useful for wood frogs and American toads because their egg masses are easily observed and identified. Calling surveys were not as useful for leopard frogs and pickerel frogs, missing about ½ of the ponds where these species were ultimately found. For leopard frogs and pickerel frogs, adult visual search was the most successful survey method. We recommend calling surveys plus dipnetting and egg mass surveys for most species. In addition, adult visual search should be used where leopard or pickerel frogs are suspected. For tiger salamanders, dipnetting and funnel traps are the best methods of identification.

We found drift fences with pit traps to be too time-intensive for our purposes. Humane treatment of animals requires that traps be set and checked on a strict timetable to avoid mortality. Drift fences will be most useful when the number of sites is very low and travel distances to check them short. Salamanders were the only non-calling species in our ponds, and they were easily identified in the larval stage by dipnetting.

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

Sampling frames for detecting amphibian species by dipnetting.-- We dipnetted for amphibian larvae at the 40 farm ponds from 27 March – 7 August 2000, for a total of 202 visits. The dates for first and last observation vary by species (Table 2). (Note that leopard frog and pickerel frog larvae are difficult to distinguish in the field prior to the metamorph stage. These larvae were recorded as leopard frogs until they could be distinguished. This accounts for the late appearance of pickerel frogs in dipnets and the low probabilities of detecting pickerel frogs.)

Table 2. Summary of dates and number of observed successes (*i.e.* species present), *n*, for each amphibian species 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

Common Name	n	Date of First Observance	Date of Last Observance
Green frog	43	April 4	August 2
Leopard frog	43	May 10	August 7
Pickerel frog	3	June 19	July 27
Spring peeper	31	May 24	July 12
Tiger salamander	9	May 2	July 26

The sum of the number of observations from Table 2 is greater than 202, the total number of visits, because more than one species may be observed in a single visit. The estimated probability of observing tadpole or metamorph presence for a single visit on a randomly selected day between 27 March and 7 August 2000 varied from 0.01 (pickerel frog) to 0.37 (American toad) (Table 3).

Table 3. Summary of sampling results: estimated probability of observing the presence of tadpoles or metamorphs in a single visit between 27 March and 7 August 2000 for farm ponds in Houston and Winona Counties, Minnesota.

Common Name	$\hat{p}$	95% CI for p	Estimated mean number of days until first observed presence
American toad	0.3713	(0.3047, 0.4379)	2.6933
Chorus frog	0.1238	(0.0783, 0.1692)	8.0800
Eastern gray tree frog	0.2178	(0.1609, 0.2747)	4.5909
Green frog	0.2574	(0.1971, 0.3177)	3.8846
Leopard frog	0.2129	(0.1564, 0.2693)	4.6977
Pickerel frog	0.0149	(-0.0018, 0.0315)	67.3333
Spring peeper	0.1535	(0.1038, 0.2032)	6.5161
Tiger salamander	0.0446	(0.0161, 0.0730)	22.4444

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 ($\hat{p}$) given in Table 3 can be used to estimate the probabilities of observing the presence of tadpoles or metamorphs 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 (Table 4).

Table 4. Probability of observing the presence of tadpoles or metamorphs for each species within a given number of visits: 27 March and 7 August 2000 for farm ponds in Houston and Winona Counties, Minnesota.

# Visits	American toad	Chorus frog	E. gray tree frog	Green frog	Leopard frog	Pickerel frog	Spring peeper	Tiger salamander
1	0.3713	0.1238	0.2178	0.2574	0.2129	0.0149	0.1535	0.0446
2	0.6047	0.2322	0.3882	0.4486	0.3804	0.0295	0.2834	0.0871
3	0.7515	0.3272	0.5215	0.5905	0.5123	0.0439	0.3934	0.1278
4	0.8438	0.4105	0.6257	0.6959	0.6161	0.0581	0.4865	0.1667
5	0.9018	0.4835	0.7072	0.7742	0.6978	0.0721	0.5653	0.2038
6	0.9382	0.5474	0.7710	0.8323	0.7622	0.0859	0.6320	0.2393
7	0.9612	0.6034	0.8209	0.8755	0.8128	0.0994	0.6885	0.2732
8	0.9756	0.6525	0.8599	0.9075	0.8526	0.1128	0.7363	0.3055
9	0.9847	0.6955	0.8904	0.9313	0.8840	0.1260	0.7767	0.3365
10	0.9904	0.7332	0.9143	0.9490	0.9087	0.1390	0.8110	0.3660

Table 4 may be used to make statements such as, “If 5 visits are made at randomly selected farm ponds from the 40 ponds in the study on randomly selected dates between 27 March and 7 August 2000, then the estimated probability of getting at least one visit in which green frog tadpoles or metamorphs are present is 0. 7742.”

During the time frame 24 May through 6 July 2000, five of the observed species were observed to share common tadpole and/or metamorph presence, including the American toad, chorus frog, green frog, leopard frog, and spring peeper (Table 5). 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 0.66 (American toad) (Table 5).

Table 5. Summary of sampling results: estimated probability of observing the presence of tadpoles or metamorphs in a single visit between 24 May and 6 July 2000 for farm ponds in Houston and Winona Counties, Minnesota.

Common Name	$\hat{p}$	95% CI for p	Estimated mean number of days until first observed presence
American toad	0.6696	(0.5825, 0.7568)	1.4933
Chorus frog	0.1786	(0.1076, 0.2495)	5.6000
Green frog	0.3036	(0.2184, 0.3887)	3.2941
Leopard frog	0.2054	(0.1305, 0.2802)	4.8696
Spring peeper	0.2589	(0.1778, 0.3401)	3.8621

The estimated success probabilities for the American toad and the spring peeper are noticeably higher in the 24 May to 6 July window than the 27 March – 7 August window, while those for the other species is only slightly higher. 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 6) can be used to estimate the probabilities of observing the presence of tadpoles or metamorphs within a certain number of visits for each species.

Table 6. Probability of observing the presence of tadpoles or metamorphs for each species within a given number of visit visits: 24 May to 6 July 2000 for farm ponds in Houston and Winona Counties, Minnesota.

# visits	American toad	Chorus frog	Green frog	Leopard frog	Spring peeper
1	0.6696	0.1786	0.3036	0.2054	0.2589
2	0.8909	0.3253	0.5150	0.3685	0.4508
3	0.9639	0.4457	0.6622	0.4982	0.5930

4	0.9881	0.5447	0.7648	0.6013	0.6984
5	0.9961	0.6260	0.8362	0.6831	0.7765
6	0.9987	0.6928	0.8859	0.7482	0.8344
7	0.9996	0.7477	0.9205	0.7999	0.8773
8	0.9999	0.7927	0.9447	0.8410	0.9090
9	1.0000	0.8297	0.9615	0.8737	0.9326
10	1.0000	0.8601	0.9732	0.8996	0.9500

The common window for the observance of at least one tadpole or metamorph by dipnetting shared by the eastern gray tree frog and the pickerel frog was 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 tadpole or metamorph of the species listed for a single visit on a randomly selected day between 29 June and 27 July 2000 was considerably higher for the eastern gray tree frog than for the pickerel frog (Table 7).

Table 7. Summary of sampling results: estimated probability of observing at least one tadpole or metamorph in a single visit between 29 June to 27 July 2000 for farm ponds in Houston and Winona Counties, Minnesota.

Common Name	$\hat{p}$	95% CI for p	Estimated mean number of days until first observed presence
Eastern gray tree frog	0. 7813	(0. 6380, 0. 9245)	1.2800
Pickerel frog	0. 0938	(-0.0072, 0.1947)	10.6667

Using the same assumptions needed for the geometric probability distributions discussed above, binomial distributions with the estimated success probabilities ($\hat{p}$) given in Table 7 can be used to estimate the probabilities of observing the presence of tadpoles or metamorphs within a certain number of visits for each species. Eight visits are needed to have a 50% probability of detecting the pickerel frog, while only 1 visit carries a 78% probability of detecting the eastern gray treefrog (Table 8).

Table 8. Probability of observing at least one tadpole or metamorph for each species within a given number of visits: 29 June and 27 July 2000 for farm ponds in Houston and Winona Counties, Minnesota.

# visits	E. gray tree frog	Pickerel frog
1	0.7813	0.0938
2	0.9521	0.1787
3	0.9895	0.2557
4	0.9977	0.3255
5	0.9995	0.3887
6	0.9999	0.4460
7	1.0000	0.4980
8	1.0000	0.5450
9	1.0000	0.5877
10	1.0000	0.6263

Species richness.-- Species richness did not differ among land use classes, when presence was determined by a combination of all survey methods (least square [LS] means = 5.7-6.5 species; SE = ±0.4; P > 0.35), tads or metamorphs (LS means = 3.0-4.1 species; SE = ±0.6; P > 0.65), adults (LS means = 3.1-3.4 species; SE = ±0.4; P > 0.97), or egg masses (LS means = 0.9-1.5 species; SE 0.= ±0.3; P > 0.62). When we used only choral survey data, natural ponds and agricultural ponds had higher species richness (5.8, 5.1 [SE = ± 0.40] species, respectively) than grazed or non-grazed ponds (4.0 [SE = ± 0.40] species, both) (P > 0.006). Square-root transformation of the species counts did not change the above results, so we report the untransformed results.

Abundance.-- Our data are inappropriate for amphibian abundance estimates, except for the choral survey data, which represents an index of abundance. We will be incorporating measures of abundance in future analyses.

Multivariate predictors of amphibian species presence/absence.-- Over the next year, we will be working on a multivariate model of species presence/absence using the habitat predictor variables collected in the study. These include, but are not limited to pond area, variance in water depth, vegetation variables, invertebrate biotic index, mean total nitrogen, mean total phosphorus, mean dissolved oxygen, mean conductivity, land use classes within varying distances of the focal pond. The amphibian response variables are presence/absence at different life stages, therefore, logistic regression will be used to evaluate the habitat variables.

Deformities and Riberoiria.--Deformity assessments were conducted at 16 ponds. Deformity rates for all ponds were < 4%, most had no deformities. All deformities found were minor, i.e. missing digits, limb truncations, and an eye deformity. Some were determined to be the result of trauma. The *Riberoiria* parasite was found at only 3 ponds in green frogs, at low rates of infestation (Table 9).

Table 9. Ponds and species where deformity assessments were conducted during field season 2000, with the maximum number of *Riberoiria* parasites detected per animal, for farm ponds in Houston and Winona Counties, Minnesota.

Species	Pond	Maximum # *Riberoiria*
American toad	Hou-Graze	-
	Stc-Agric	-
Gray treefrog	Bro-Graze	-
	Cal-Ngraz	-
	Mou-Agric	-
Chorus frog	She-Natur	-
	Stc-Agric	-
Green frog	Alt- Graze	4
	Bro-Graze	27
	Lew-Agric	-
	Uti-Agric	-
	Uti-Ngraz	4
Pickerel frog	Eit-Ngraz	-

Species	Pond	Maximum # *Riberoiria*
Leopard frog	Cal-Graze	-
	Eit-Natur	-
	Hou-Ngraz	-
	She-Agric	-
	Stc-Natur	-

Genetics

Laboratory analysis using flow cytometry is underway.

Post-breeding habitat use

A total of 26 frogs were captured and outfitted with radio transmitters via a harness during 2000. Eleven frogs were observed in the enclosures from 29 August to 12 October 2000. Four these frogs were subsequently released with transmitters on 12 October. After release the frogs followed a small feeder stream. Three of the frogs remained within 2 m of the stream, often located in or near the stream buried under silt and debris or in a crayfish burrow.

The most common post-breeding habitat utilized by leopard frogs was wet meadow, followed by aquatic and natural grasslands. Vegetation height was > 74 cm. Frogs were frequently found occupying ‘tunnels’ between the soil and the litter layer. The frogs moved within these tunnels as we approached, making them very difficult to relocate, even with radios attached. The maximum total distance moved by an individual frog was 207 m, the minimum was < 5 m.

Skin erosion and transmitter loss was a problem with all of the harness methods (Table 10). Alternate methods of affixing the chain around the frogs were not effective. Lesions formed where the harness contacted the skin, most notably on the belly and above the legs on the sides of the frogs. One known mortality attributed to skin erosion occurred after a frog was released, however another frog found 21 days after being released due to skin lesions had begun to heal. The open sores were closed and the frog appeared to be in good health. The longest length of time that any individual frog carried a transmitter was 56 days (44 days in the enclosure). That frog had an aluminum bead chain harness and experienced no skin erosion. However, this frog also shed the aluminum bead chain 5 times during the 56-day period.

Table 10. Harness attachment types and mean number of days (SD) until we observed the first evidence of transmitter loss or skin erosion; we also report total days (SD) tracked for leopard frogs on a farm pond in Houston and Winona Counties, Minnesota, 2000.

Attachment type	# Frogs	Transmitter loss	Skin erosion, initial	Total days tracked
Nickel bead chain	15	3.2 (1.2)	8.5 (2.2)	7.3 (2.3)
Aluminum bead chain	4*	11.5 (3.2)	9**	27 (3.9)
Elastic harness	4*	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)

* One frog was switched from aluminum bead chain to elastic harness after 13 days.

**Only 1 frog developed skin erosion, mean value is misleading.

Nickel bead chain.-- Frogs with nickel bead chains (our first harness type) frequently developed skin erosion and lost their transmitters. 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 refitted with tubes and the harness material was passed through the tube, eliminating the need to glue the harness material directly to the transmitter. The nickel harnesses also rusted and discolored the skin, usually within 2-7 days. One rusty chain broke after 7 days during handling. When transmitter loss occurred, the loss took place within the first three days in all of the frogs carrying the nickel bead chain except 2. These frogs carried the transmitter for 12 days each.

Aluminum bead chain.-- Frogs with aluminum bead chains had the least problems with skin erosion, but also experienced transmitter loss. One frog carried a transmitter 13 days and then switched to an elastic harness. Two frogs lost transmitters, one of them 5 times. One frog experienced skin erosion after 9 days and was released 4 days later. Two frogs carried transmitters without developing skin erosions until they shed their transmitter.

Elastic harness.-- Elastic harness attachments caused skin erosion, held moisture, and required 2 people to fit and sew the harness. All 4 frogs experienced transmitter loss. One frog lost the harness twice. Three frogs developed lesions. One frog carried a transmitter 13 days with no signs of erosion until the transmitter slipped off.

Plastic cable tie.-- Frogs with plastic cable tie harnesses developed the first signs of skin erosion from 7-20 days after attachment. Four frogs shed the transmitters. The fifth frog was not recovered, but appears to have gone into an underground burrow. Four frogs experienced lesions. The remaining frog was absent after 2 days.

Other Vertebrates

Reptiles.-- We observed 6 reptile species and 2 turtle species at the farm ponds (Fig. 6). The common garter snake was the most frequently observed reptile.

Birds.-- We observed 73 species of birds at the ponds based on morning point counts (Table 11, Fig. 7A, 7B, and 7C). The most common species at the ponds were the song sparrow, red-winged blackbird, American robin, American goldfinch, common yellowthroat, and savannah sparrow. In addition, we observed the whip-poor-will, woodcock, and common nighthawk during night and evening hours.

Table 11. Bird species identified by morning point counts conducted at each pond from 20 May – 20 June 2000 for farm ponds in Houston and Winona Counties, Minnesota, 2000.

Common name	AGRIC	GRAZE	NATUR	NGRAZ	Grand Total
Mallard	2		2	1	5
Wood Duck			6	1	7
Canada Goose			2		2
Green Heron			1		1
Common Moorhen	1				1
Pectoral Sandpiper	1				1
Killdeer	4	1			5
Ruffed Grouse		1		1	2
Ring-necked Pheasant	2	2	1	1	6
Wild Turkey		1	3		4
Domestic chicken	1				1

Common name	AGRIC	GRAZE	NATUR	NGRAZ	Grand Total
Mourning Dove	1		1		2
Turkey Vulture			1		1
Hairy Woodpecker	1	1			2
Downy Woodpecker			1		1
Yellow-bellied Sapsucker	1	1	1		3
Red-headed Woodpecker	1				1
Red-bellied Woodpecker		1	4	1	6
Northern Flicker	1	1	2	1	5
Chimney Swift			1		1
Ruby-throated Hummingbird	1				1
Eastern Kingbird		3		2	5
Great Crested Flycatcher			1	1	2
Eastern Phoebe	1	1			2
Eastern Wood-Pewee		1	1	2	4
Acadian Flycatcher			1		1
Willow Flycatcher			1		1
Horned Lark	1				1
Blue Jay		3	1		4
American Crow	3	6	2	2	13
European Starling		2	1		3
Bobolink	2	6	1	4	13
Brown-headed Cowbird	1	5	2	1	9
Red-winged Blackbird	8	7	8	9	32
Eastern Meadowlark	2	2	1	2	7
Baltimore Oriole	2	2	5	2	11
Common Grackle	4	1	1		6
American Goldfinch	7	3	4	6	20
Savannah Sparrow	3	7	3	6	19
Chipping Sparrow		1	1		2
Field Sparrow	1	2	2	3	8

Common name	AGRIC	GRAZE	NATUR	NGRAZ	Grand Total
Song Sparrow	9	8	10	9	36
Swamp Sparrow			1		1
Eastern Towhee	1	2		2	5
Northern Cardinal		2	2		4
Rose-breasted Grosbeak	3	2	4	3	12
Blue Grosbeak		1			1
Indigo Bunting	5	2	3	4	14
Dickcissel		1	2	2	5
Scarlet Tanager		2		1	3
Barn Swallow	4	6	3	1	14
Tree Swallow	1	4	2	2	9
Northern Rough-winged Swallow	1	3		2	6
Cedar Waxwing				2	2
Red-eyed Vireo	1	1	1	1	4
Warbling Vireo	1	4	3	1	9
Yellow-throated Vireo			1		1
Blue-winged Warbler	1		1	3	5
Yellow Warbler	3	1	6	3	13
Common Yellowthroat	6	2	7	5	20
Hooded Warbler			1		1
American Redstart		1	4	4	9
Gray Catbird	1	5	6	5	17
Brown Thrasher		1			1
House Wren	2	6	1		9
Sedge Wren		1	1	1	3
White-breasted Nuthatch		3		2	5
Black-capped Chickadee		2	2	1	5
Blue-gray Gnatcatcher		1	1		2
Wood Thrush			1		1
American Robin	4	8	5	4	21

Common name	AGRIC	GRAZE	NATUR	NGRAZ	Grand Total
Eastern Bluebird		1	1		2

Mammals.-- We observed 14 mammal species at the ponds (Fig. 8). The raccoon and white-tailed deer were the most common mammals observed.

Aquatic Predators

Four species of fish (Fig. 9) and 28 aquatic invertebrate taxa (Fig. 10) were identified during sampling. Most of these taxa are potential predators on amphibian larvae and some are indicators of water quality. Snail species were identified because of their role as definitive hosts for the *Riberoria* parasite, which is linked to amphibian deformities. Fish were found primarily at natural and nongrazed ponds. No fish were observed at ponds surrounded by row crops (agricultural). Water boatmen, leeches, and midge larvae were observed at $\geq$30 ponds.

Water Chemistry and Toxicity

Water quality.-- At each pond we measured water clarity (turbidity, NTU), dissolved oxygen (DO, mg L^{-1}), conductivity (μmhos cm^{-1}), and water temperature (C) (Table 12). DO showed no significant variation across the range of ponds sampled. Means (SE) vary from 11.3 ($\pm$1.4) mg L^{-1} in ponds from grazed areas to 9.3 ($\pm$ 0.7) mg L^{-1} in the natural area ponds. These estimates, made during daylight hours, suggest pond waters contain sufficient concentrations of oxygen that pond life is not limited by lack of oxygen. DO and water temperature change throughout the day, depending on the amount of sunlight reaching the ponds and the amount of plant and algal growth in the ponds. Oxygen concentration depends mainly on the daily rate of plant and algal photosynthesis and respiration occurring in the pond. Where many plants exist, oxygen concentrations may be extremely high during midday because of photosynthesis, then extremely low early in the morning before sunrise because of plant/algal respiration. Because of this diel variation in DO and water temperature only general remarks can be made about the importance of these measurements. Certainly, if the DO readings were consistently < 2-5 mg L^{-1} then we might conclude problems exist. This was not the case. Fish grow poorly in water with < 2 mg L^{-1}, and can not survive long in water without oxygen.

Conductivity ranged from an average of 429 (±40.3) µmhos cm^{-1} in the natural ponds to 159.8 (±36.2) µmhos cm^{-1} in the nongrazed ponds. There were no significant differences among land use types. Conductivity is a measure of the dissolved ions in water (such as calcium, magnesium, chloride, etc.) and primarily reflects underlying geology and hardness of water. High conductivity may reflect high contributions of ground water to the water in a pond, or a recent runoff event from a storm - carrying dissolved material in overland flows. Typically, extremes in conductivity are not indicative of water quality problems.

Turbidity (SE) ranged from an average of 10.44 (±1.58) NTU in the natural ponds to 44.75 (±9.8) NTU in the grazed ponds. Here, the two more intensively disturbed ponds (grazed and row crop) were significantly ($P<0.009$) more turbid than the less disturbed ponds (natural and nongrazed). Turbidity is a measure of the amount of fine materials suspended in water; it does not tend to vary dielly but reflects levels of resuspension of fine particles from the bottom sediments (e.g., disturbance by animals or through overland runoff after rains). Typically, ponds in agricultural settings, surrounded by plowed fields or in pastures where cattle water directly in the pond, tend to have high levels of turbidity. Also, ponds containing dense populations of bottom-feeding fish (e.g., carp or catfish) will have high levels of turbidity (e.g., > 20 NTU).

Water temperatures ranged from 21.8 (±0.9) C in the grazed ponds to 22.1 C in the natural and nongrazed ponds. This slight variation in temperature is not surprising given the similar size and geographic location of the ponds. What little variation measured here may reflect the slight shading effect of higher turbidity in the grazed ponds relative to the natural and nongrazed ponds.

Nutrients.-- Estimates of total nitrogen (TN) and total phosphorus (TP) were made from samples taken at each pond on at least 7 dates through the summer of 2000 (Table 12). When averaged across all dates concentrations of TN were lowest in the nongrazed ponds (mean ±1 standard error: 1.39 mg L^{-1} ±0.3) and highest in the grazed ponds (4.37 mg L^{-1} ± 1.26). Although there is a trend for the grazed and row crop ponds to contain more nitrogen, this trend is not statistically significant due to the high levels of variation among ponds. Only TN concentrations in ponds from grazed landscapes were significantly higher ($P<0.05$) than those in natural, nongrazed or row crop landscapes. There were no differences in average TN concentrations between ponds in grazed and row crop landscapes, or between those in natural and nongrazed ponds. This is contrary to what was expected at the outset of the study; we expected increasing concentrations from least to most agriculturally disturbed landscapes. However, because analysis of total nitrogen involves digestion of all organic matter in the samples, these estimates may reflect the amount of organic matter suspended in the samples. A better evaluation of the more labile nitrogen fraction (nitrate) would focus on the dissolved, more reactive nitrogen components. Such an analysis will be performed on samples taken during the second summer (2001) of this study.

When averaged across all dates, TP was lowest in the ponds from natural landscapes (0.27 mg L^{-1} $\pm$0.07) and highest in the ponds from row crop landscapes (1.76 mg L^{-1} $\pm$0.07). While these concentrations more closely followed the expected pattern, they were not exceptionally high. Statistically, the grazed and row crop ponds contained greater concentrations of TP than the natural and nongrazed ponds; there were no differences between grazed and row crop, or between natural and nongrazed ponds.

Table 12. Mean (1 SE of mean) of total phosphorus, total nitrogen, water temperature, conductivity, dissolved oxygen, and turbidity for farm ponds in Houston and Winona Counties, Minnesota, 2000.

Parameter	Pond Type			
	Natural	Nongrazed	Grazed	Row Crop
Total Phosphorus (mg L^{-1})	0.27 (0.07)	0.29 (0.11)	1.50 (0.7)	1.76 (1.45)
Total Nitrogen (mg L^{-1})	1.55 (0.42)	1.39 (0.30)	4.37 (1.26)	3.32 (1.50)
Dissolved Oxygen (mg L^{-1})	9.30 (0.70)	11.02 (1.42)	11.3 (1.4)	9.4 (0.84)
Turbidity (NTU)	10.44 (1.58)	18.0 (6.3)	44.75 (9.8)	25.7 (6.4)
Conductivity (µmhos cm^{-1})	429.4 (40.3)	159.8 (36.2)	317.4 (89.9)	238.9 (100.1)
Water Temperature (C)	22.1 (0.89)	22.1 (0.58)	21.8 (0.9)	22.4 (0.5)

Effects of Pond Water on African Clawed Frog Larvae.-- The 4-d mortality of embryo *Xenopus laevis* exposed to water from the 4 types of ponds ranged 6.2% - 46%. In general, embryo mortality in water from ponds in grazed pastures and row crops was greater than that in water from ponds in non-grazed pastures and natural wetlands (Fig. 11). However, with the exception of water samples collected during the week of May 8, 2000, there was not a statistically significant difference in mortality of embryos exposed to water from the different pond types during any week. For samples collected during the week of May 8, 2000, mortality was greater in water from ponds located in row crops (46%) and grazed pasture (28%) than in water from ponds located in non-grazed pasture (6.8%) and natural wetlands (9.6%). We are currently measuring growth and the type and rate of malformations of embryos surviving to day 4 of each of the assays.

Effects of Pond Water on Luminescence of Vibrio fischeri.-- Assays conducted on water collected during weeks of April 24, 2000 and May 8, 2000 were inconclusive because all pond samples bioluminesced greater than the Microtox control samples; bioluminescence of samples exceeded the measurement range, which is calibrated by the Microtox control sample. In subsequent assays, the Microtox instrument was calibrated with control samples prepared in the same manner as the pond water samples. This resulted in quantifiable measures of light output from the bacteria. On all dates, bacterial bioluminescence in 20% - 100% of pond water samples exceeded that in the control; the pond water was not toxic to the bacteria in these samples (Fig. 12). In addition, there was no significant difference among pond types in the toxicity of the water to the bacteria.

Vegetation

The analysis of pond vegetation data is underway.

Landscape

The analysis of the landscape data shows that the dominant land use surrounding the ponds is agricultural, followed by forest and grassland (Table 13). County and township roads are most common in the landscape surrounding the ponds (Table 14). Intermittent and permanent streams also are found in the landscape surrounding the ponds (Table 15). More in-depth landscape analyses are underway.

Table 13. Summary of the area (ha) of different types of land uses within 2,500 m of the centroid of each farm pond, by *a priori* land use classes for farm ponds in Houston and Winona Counties, Minnesota, 2000.

Sum of Hectares		Land use				
Class	Sub Class	Agriculture	Grazed	Natural Wetland	Non-grazed	Grand Total
Agricultural		9744.22	9715.04	5930.88	8130.72	33520.86
Farmstead		333.05	332.91	226.58	284.11	1176.66
Forest		6372.44	6446.28	9008.33	7945.82	29772.87
Grassland		2936.63	2875.30	2930.59	3031.33	11773.86
Other		25.34	6.20	20.08	7.69	59.31
Shrub		16.73	5.56	31.25	14.15	67.69
Urban		7.03	16.39	52.04	0.29	75.75
Wetland	Permanent	49.15	48.10	421.66	45.48	564.39
	Temporary	45.07	83.87	909.24	70.07	1108.24
Wetland Total		94.22	131.97	1330.90	115.54	1672.63

Table 14. Summary of the length (km) of different types of roads within 2,500 m of the centroid of each farm pond, by *a priori* land use classes for farm ponds in Houston and Winona Counties, Minnesota, 2000.

Sum of Lkilometers		Land use				
Road Class	Road Description	Agriculture	Grazed	Natural Wetland	Non-grazed	Grand Total
1	Interstate Highw		5.48	3.48	9.1	18.06
2	US Trunk Highway		4.2			4.2
3	Minnesota Trunk	16.35	18.96	32	8.97	76.28
4	County State Aid	81.73	93.34	66.39	82.85	324.31
5	County Roads	3.58	18.84	11.36	15.76	49.54
6	Township Roads	164.15	145.76	112.21	139.56	561.68
7	City Streets		1.08	7.04		8.12

Table 15. Summary of the length (km) of different types of roads within 2,500 m of the centroid of each farm pond, by *a priori* land use classes for farm ponds in Houston and Winona Counties, Minnesota, 2000.

Sum of Lkilometers		Land use				
Flow Class	Flow Description	Agriculture	Grazed	Natural Wetland	Non-grazed	Grand Total
0	No flow associated	31.66	24.9	29.18	31.35	117.09
1	Perennial	19.61	23.98	47.34	22.47	113.4
2	Intermittent	107.17	72.38	74.45	70.81	324.81
4	Unknown Stream	52.41	92.78	97.53	77.84	320.56

DISCUSSION

We had a successful field season in 2000. The weather initially was dry, but by late May the ponds had filled up (or overflowed) and we had adequate water for the rest of the season. During field season 2001, we plan to refine our methods for both toxicology and the radio telemetry study of post-breeding habitat use. An additional grant from USGS ($98K) was obtained to enhance our assessment of post-breeding habitat use.

Amphibians

We plan to refine our methods of larval amphibian sampling using dipnets. We will develop a standardized dipnet method that will provide us better estimates of larval abundance classes. Abundance information, even classes of abundance, will help us refine our population parameters for amphibians breeding in farm ponds. We will also use the probability analysis of larval amphibian sampling from 2000 to refine our field schedule and reduce the number of pond visits needed to detect amphibian species.

Genetics

We will collect blood samples for flow cytometry analysis in 2001 using the same methods as the 2000 season. DNA profiles from propidium-iodide-stained erythrocytes will be obtained for 10,000-20,000 nuclei from each sample (individual) and plotted in the form of a frequency distribution using a flow cytometer. This information will be used to measure the DNA content of erythrocyte nuclei as well as to reveal the presence of abnormal profiles (aneuploids, polyploids, etc.). For normal profiles, c-values (in pg DNA/haploid nucleus) and coefficients of variation (as an index of intra-individual genome size variability) will be calculated.

Post-breeding habitat use

We encountered more logistical and technical problems than we initially anticipated, based on communication with other researchers using this method (M. Lannoo and N. Scott). Two to three hours and a crew of 3-4 people were sometimes required to locate and catch one frog in the tall, dense vegetation surrounding the pond. We missed the breeding season due to transmitter delays, so could not catch frogs at the pond, where they would be easier to locate. The enclosures saved us time re-locating the frogs and helped us compare attachment methods, but did not provide adequate information on habitat use.

The aluminum bead chain harness was the best attachment method of those we tried (reduced skin erosion and ease of application), however, the frogs still slipped out of these harnesses.

Plan for 2001.— We will continue to identify a method of attaching radio transmitters to northern leopard frogs that will allow us to obtain the best information on post-breeding habitat use. We will work on methods development for surgical abdominal implants of radios, based on literature review and communication with other scientists engaged in similar work. Experimental animals will be closely observed for complications associated with surgery and post-surgical health and behavioral changes. If this method works, all frogs will carry implanted radios. If not, we will select among the various types of harness attachments, based on data from last summer. Animals with radios will be closely observed to assess adverse effects of radio attachment, including skin erosion, radio loss, radio entanglement in vegetation, and behavioral changes.

We will examine post-breeding habitat use by applying radio transmitters to northern leopard frog populations living in (a) constructed farm ponds in southeastern Minnesota and (b) natural wetlands of the UMRWFR that adjoin urban areas. We will select 2 farm ponds and one UMRWFR marsh adjoining urban development in southeastern Minnesota. Farm ponds will be adjacent to row crops and secondary roads. Marshes on the UMRWFR will be adjacent to transportation corridors, industrial sites, or home sites. Frog populations at each site will be estimated three ways - chorusing surveys, tadpole surveys and visual encounter searches. At each site, all adult northern leopard frogs found at the breeding pond during breeding season (late May, early June) will be tagged with an elastomer tag (injection of flourescent dye). Of these, 15 randomly-selected, healthy adults with a snout-vent length > 60 mm will be fitted with a Holohil BD-2G transmitters. Radio-tagged frogs will be tracked 2-3 times per week from May until hibernation. Transmitter batteries have ~ 20–week life span; frogs with transmitters reaching this time limit will be recaptured and fitted with new transmitters as needed. If skin erosion occurs, transmitters may be moved to other individuals from the same breeding pond, as determined by the presence of an elastomer tag. Each time a frog is located, its position will be determined using a portable GPS unit. The frog's position, general vegetation types within 5 m and specific land cover type at the point of contact will be recorded. A hygrometer will be used to measure the microclimate occupied by the frog (air temperature, relative humidity and surface temperature). Monitors will be placed at each site to record hourly ambient and ground level air temperatures and ambient humidity levels. Rain gauge stations will record daily precipitation totals. G.I.S. maps will be used to map frog locations and calculate home range size and habitat preferences.

Home range sizes will be calculated using the harmonic mean, minimum convex polygon and bivariate normal home range models (Samuel and Fuller 1994). Compositional analysis will be used to determine habitat preferences or ranking (Samuel and Fuller 1994). Mr. Pember will be attending a workshop on “Wildlife Radiotelemetry: Design and Analysis” at the University of Idaho, Moscow, ID in March 2001. Methods taught in this course will be incorporated into the data analysis.

Other Vertebrates

Data on reptiles and mammals will be collected using the same methods as 2000. We will conduct point counts for birds at the ponds only if time permits. We will continue to record bird observations at each pond visit.

Aquatic Predators

Data on aquatic predators will be collected using the same methods as employed in 2000.

Water Chemistry and Toxicity

Plan for 2001.-- During the second year of the study (2001), effects of pond water from the 4 habitat types on the African clawed frog, *Xenopus laevis*, will again be assessed. Because of the apparent insensitivity of the bioluminescent bacteria *Vibrio fischeri* to differences in pond water, the Microtox assay will not be conducted. In addition to the laboratory assays with *Xenopus*, embryo and larval survival of native frogs will be evaluated through the use of mesocosms within ponds. Mortality will be assessed at three critical stages in amphibian larval development: (1) hatching success of the embryos, (2) number of larvae that survive beyond absorption of the yolk sac, and (3) survival rate of amphibians post-metamorphosis.

Selection of ponds for the mesocosm study.-- For the assessment of survival of native amphibians, only ponds in agricultural fields, natural wetlands, and non-grazed pastures will be used. Ponds in grazed pastures will not be used due to the likelihood of mesocosm disturbance by grazing animals.

Three other factors, based on data collected during 2000, will be considered when selecting ponds for the mesocosm study: (1) FETAX results, (2) average water depth and water-level fluctuation, and (3) presence and abundance of the northern leopard frog.

Mesocosms.-- Mesocosms (12’ long X 3’ wide X 4’ high) will be constructed of a welded aluminum framework and sides of plastic mesh (approximately ½ “). Behind this mesh, on the inner wall of the aluminum framework, a layer of window screen will be attached. This double screening will exclude aquatic predators but allow water to flow in and out of the mescocoms. One mesocosm will be placed in 12 of the 40 ponds studied during 2000 (4 ponds from each of 3 habitat types).

To assess embryo mortality, 100 leopard frog embryos will be placed in each mesocosm. These embryos will be contained within 8 small, floating enclosures constructed from plastic fish traps (25 embryos/enclosure). Small enclosures have been used in previous studies to hold developing tadpoles (Bishop et al. 2000, Nancy Shappell USDA Fargo, ND., pers. comm.) and allow specific numbers of embryos to be easily maintained. These chambers will also reduce the risk of embryos being lost or consumed by predators. After all embryos have hatched and absorbed their yolk sac, mortality will be recorded.

Tadpole mortality will be also assessed. One hundred leopard frog tadpoles will be collected (stage 26, Gosner 1960) and placed into each mesocosm. Mortality will be determined after metamophosis of the tadpoles. Information on embryo and tadpole mortality will compared to information obtained from the FETAX assay.

Vegetation

Pond vegetation will be assessed using the same methods as in 2000 only if time permits. Vegetation in the ponds is not expected to change much from one year to the next. Surrounding land uses will be recorded, as in 2000. Crops can change from alfalfa to row crops from one year to the next.

Landscape

The landscape variables will be incorporated into the multivariate analysis during 2001.

Management brochure (Result 2)

We will begin to draft the management brochure this year, based on the analysis of data from 2000. We will begin to assemble the artwork (photos, drawings) needed to effectively communicate with the public.

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Figure 11. Mortality of embryo *Xenopus laevis* after 4-d exposure to water from ponds located in 4 habitats, for farm ponds in Houston and Winona Counties, Minnesota, 2000. Because of the unavailability of adequate numbers of embryos, *Xenopus laevis* were not exposed to water collected 6/12/00 from ponds located in grazed pasture and non-grazed pasture.

Figure 12. Bioluminescence of *Vibrio fischeri* after 22 h of exposure to water from ponds in 4 habitat types, for farm ponds in Houston and Winona Counties, Minnesota, 2000.

Appendix B. List of common and scientific names for species identified on farm ponds in Houston and Winona Counties, Minnesota, 2000.

Taxa	Common name	Scientific name	Order	Family
Birds	Mallard	*Anas platyrhynchos*	ANSERIFORMES	ANATIDAE
	Wood duck	*Aix sponsa*	ANSERIFORMES	ANATIDAE
	Canada goose	*Branta canadensis*	ANSERIFORMES	ANATIDAE
	Green heron	*Butorides virescens*	CICONIIFORMES	ARDEIDAE
	Common moorhen	*Gallinula chloropus*	GRUIFORMES	RALLIDAE
	Pectoral sandpiper	*Calidris melanotos*	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
	Mourning dove	*Zenaida macroura*	COLUMBIFORMES	COLUMBIDAE
	Turkey vulture	*Cathartes aura*	CICONIIFORMES	CATHARTIDAE
	Hairy woodpecker	*Picoides villosus*	PICIFORMES	PICIDAE
	Downy woodpecker	*Picoides pubescens*	PICIFORMES	PICIDAE
	Yellow-bellied sapsucker	*Sphyrapicus varius*	PICIFORMES	PICIDAE
	Red-headed woodpecker	*Melanerpes erythrocephalus*	PICIFORMES	PICIDAE
	Red-bellied woodpecker	*Melanerpes carolinus*	PICIFORMES	PICIDAE
	Northern flicker	*Colaptes auratus*	PICIFORMES	PICIDAE
	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

Taxa	Common name	Scientific name	Order	Family
	Acadian flycatcher	*Empidonax virescens*	PASSERIFORMES	TYRANNIDAE
	Willow flycatcher	*Empidonax traillii*	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
	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
	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
	Scarlet tanager	*Piranga olivacea*	PASSERIFORMES	THRAUPIDAE
	Barn swallow	*Hirundo rustica*	PASSERIFORMES	HIRUNDINIDAE
	Tree swallow	*Tachycineta bicolor*	PASSERIFORMES	HIRUNDINIDAE

Taxa	Common name	Scientific name	Order	Family
	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
	Common yellowthroat	*Geothlypis trichas*	PASSERIFORMES	PARULIDAE
	Hooded warbler	*Wilsonia citrina*	PASSERIFORMES	PARULIDAE
	American redstart	*Setophaga ruticilla*	PASSERIFORMES	PARULIDAE
	Gray catbird	*Dumetella carolinensis*	PASSERIFORMES	MIMIDAE
	Brown thrasher	*Toxostoma rufum*	PASSERIFORMES	MIMIDAE
	House wren	*Troglodytes aedon*	PASSERIFORMES	TROGLODYTIDAE
	Sedge wren	*Cistothorus platensis*	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
	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

Taxa	Common name	Scientific name	Order	Family
	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
	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)

Taxa	Common name	Scientific name	Order	Family
	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
	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

Taxa	Common name	Scientific name	Order	Family
	White-tailed deer	*Odocoileus virginianus*	ARTIODACTYLA	CERVIDAE
	Domestic cow	*Bos taurus*	ARTIODACTYLA	BOVIDAE