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USE OF FETAX TO EXAMINE ACUTE SURVIVAL OF *XENOPUS LAEVIS* LARVAE IN WATER FROM NATURAL AND CONSTRUCTED PONDS IN THE UPPER MIDWEST

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***Abstract.*—Agricultural ponds are exposed to many contaminants that can negatively affect breeding amphibians. Despite the risks, such ponds are important amphibian breeding habitat if natural wetlands are scarce. We compared the survival of *Xenopus laevis* larvae reared in water from natural and constructed ponds. Grazed ponds had higher nutrient levels (total nitrogen and total phosphorus) than natural ponds in 2000, but we observed no difference in nutrients between agricultural and natural ponds in 2001. All treatments had high survival rates (74-91%) in both years and we detected no significant differences in survival among treatments. These results support previous research on the same test ponds; thus we conclude that certain constructed agricultural ponds in the Driftless Area ecoregion of the Upper Midwest may be suitable for anuran larval survival. However, it is difficult to determine the biological significance of our findings, as indirect factors that could not be controlled for during this research, may influence the water chemistry of ponds in this region.**

Key Words.*—**agriculture; anurans; effects; FETAX; pond; type; ***Xenopus laevis

INTRODUCTION

Amphibian population declines are a phenomenon with global significance (Houlahan et al. 2000; Blaustein and Kiesecker 2002; Blaustein and Johnson 2003; Lannoo 2005). Although no single explanation is known, several possible natural (e.g., parasitic infections or disease) and anthropogenic (e.g., toxicants and habitat destruction) exist (Johnson et al. 2002; Johnson and Sutherland 2004; Stuart et al. 2004; Green 2005; Pounds et al. 2006). Exposure to environmental contaminants during critical life stages, such as the embryo and metamorph may be critical to amphibian survival (Fort et al. 1999; Relyea 2005).

Agriculture may negatively affect amphibian populations by generating contaminants, such as fertilizers and pesticides (e.g., Kroening and Andrews 1997; Marco et al. 1999; Rouse et al. 1999; Wijer et al. 2003; Hayes et al. 2006). Previous researchers reported fewer species of anurans near agricultural sites than in upstream or downstream areas (Bishop et al. 1997). Despite the risks, agricultural ponds may be important breeding sites for amphibians in certain situations (Knutson et al. 2004).

The Frog Embryo Teratogenesis Assay *Xenopus* (FETAX; American Society for Testing and Materials 1998) is a standardized assay designed to assess the effects of water-based toxicants on amphibians (Burkhart et al. 1998; Fort et al. 1999; but see Tietge et al. 2000). We used the FETAX bioassay to test the hypothesis that acute survival of *Xenopus laevis* larvae would differ among water samples from agricultural and natural ponds in the upper Midwest. If this is true, survivorship in water exposures from agricultural habitats (i.e., row crop or within grazed fields) will be lower than from other locations.

METHODS

We conducted standardized FETAX bioassays to evaluate the effect of pond water on the survival of *Xenopus laevis* larvae at the University of Wisconsin-La Crosse (American Society for Testing and Materials 1998). We purchased adult *X. laevis* from a biological supplier (Xenopus I, Inc., Dexter, Michigan, USA) and quarantined them for two months prior to water collection and FETAX analyses. All *X. laevis* were kept in a flow-through well water system and cared for according to accepted methods (ASTM 1998). Well water had an alkalinity of 300 ± 2 mg/l (CaCO3), pH of 8.06 ± 0.01 and a hardness of 348 ± mg/l (CaCO3).

***Study area and pond selection.*—**We conducted our study in the Driftless Area ecoregion in southeastern Minnesota (Houston and Winona Counties). We chose this state because it has many “hot spots” for amphibian malformations and mortality (Souder 2000; Rosenberry 2001; Lannoo et al. 2003). The ecoregion represents an area approximately 41,986 km^2 that was not glaciated during the recent Wisconsin glacial period that ended

FIGURE 1. The driftless area ecoregion (outlined in yellow) of the upper Midwestern United States.

roughly 10,000 years ago (Fig. 1; McNab and Avers 1994; Albert 1995). This ecoregion possesses large relief relative to the surrounding landscape. The bedrock is composed mostly of limestone, sandstone and dolomite (Albert 1995). Existing natural ponds are often associated with river or stream floodplains, while agricultural ponds used for livestock and erosion control account for most other lentic habitats in the area (Knutson et al. 2004). The land surrounding most of these agricultural ponds consists largely of row crop, pasture, or grasslands (Albert 1995; Knutson et al. 2004).

We randomly selected 40 test ponds using Geographical Information Systems (GIS) as in Knutson et al (2004). We initially classified ponds as natural or constructed. Natural ponds (n = 10) were designated based on their natural origin and/or association with rivers, streams, oxbow sloughs, etc. We designated constructed ponds as those existing high in the watershed and likely created for the purposes of erosion control or as a water source for livestock (*see* Knutson et al. 2004). We evenly distributed the remaining 30 constructed ponds among three treatment groups: agricultural ponds, grazed ponds, and ungrazed ponds. We defined these groups based on the width of the grass buffer surrounding them and the adjacent land use determined on-site and via GIS analysis of aerial photos. Agricultural ponds were adjacent to row-cropped lands and surrounded by a grass buffer of < 30 m. Grazed ponds were often used by domestic livestock (primarily beef and dairy cattle), and ungrazed ponds were surrounded by a grass buffer of ≥ 30 m. We derived buffer strip widths from riparian buffer standards recommended by the United States Department of Agriculture (United States Department of Agriculture, Natural Resources Conservation Service 1999). We did not identify many natural ponds so were unable to control for land uses surrounding them. Land use around natural ponds occasionally included a small amount of agriculture (≤ 25%) that existed beyond the 30 m buffer.

Water collection.—Pond water samples were composites, comprised of water collected at mid-depth, 1 m from shoreline at four equidistant locations around pond perimeter and mixed. All glassware used for collection and storage of samples were washed with soap and tap water, and rinsed with reverse osmosis deionized water and pesticide grade acetone (Fisher Scientific, Inc., Waltham, MA, USA). All water samples were labeled and immediately placed on ice or refrigerated until needed for analyses.

Due to limited resources, we were only able to collect and analyze water from a randomly selected sub-sample of the 40 available study ponds. In 2000, we collected water samples from 25 study ponds (five natural, 10 row crop, five grazed, and five ungrazed). We eliminated the five grazed and five ungrazed ponds and added five additional natural ponds to our analysis in 2001. The agricultural and natural ponds sampled in 2000 were also sampled in 2001. We collected water samples for FETAX and nutrient analyses every two weeks from April 15-July 20 (n = 6 sampling periods/yr).

The time frame of the selected sampling regime corresponded with the egg and larval developmental period of several anuran species present in test ponds (e.g., *Bufo americanus*, *Pseudacris crucifer*, *Pseudacris triseriata*, *Rana pipiens*, *Rana sylvatica*; Vogt 1981). This also helped ensure that test subjects were exposed to potential changes in the water quality of test ponds occurring over time. Due to travel time required to begin a single sampling regime (~ 1 h) and to ensure use of the resources necessary to collect water, sampling trips were pre-determined and we could not control for the effects that rain events had on water quality in test ponds.

Laboratory bioassay.—On days that water samples were collected, two breeding pairs of *X. laevis* were placed in aquaria with FETAX solution. At this time, breeding was induced with hormone injections as described in ASTM (1998). Within 24 h of water collection, we harvested embryos and initiated FETAX assays via standard methods (ASTM 1998; *but see more efficient methods recently published in* McCallum and Rayburn 2006).

During 2000 and 2001, FETAX bioassays (96 h acute bioassays) were conducted on each pond a total of six times per year. Test ponds were of four different "treatment" types in 2000 and two different "treatment" types in 2001. Bioassays consisted of two 60 mm diameter petri dishes (Fisher Scientific, Inc., Pittsburgh, PA, USA) per pond, each containing 25 embryos cultured in 10 ml of test solution (i.e., pond water) for 96 h. Each bioassay also contained a double control, with embryos housed in 10 ml of sham solution (6-aminonicotinamide; Fisher Scientific, Inc., Pittsburgh, PA, USA) and embryos housed in 10 ml of negative control solution (FETAX solution) as described in ASTM (1998). During assays, petri dishes were held in an environmental chamber at 25-28 C, with a light regimen of 12 h light: 12 h dark for a total of 96 h. We recorded embryo survival at the end of each assay.

Nutrient analyses.—We conducted nutrient analyses on water samples within 30 d of collection at the Upper Midwest Environmental Sciences Center (UMESC) Water Quality Laboratory, La Crosse, Wisconsin, as described in Knutson et al. (2004). We analyzed unfiltered samples from 2000 for total nitrogen and total phosphorus, and analyzed unfiltered water samples from 2001 for total nitrogen, total phosphorus, ammonia and nitrate following standard methods after digestion (persulfate method; APHA, 1998).

Statistical analysis.—We used a repeated-measures analysis of variance to determine differences in survival of *Xenopus laevis* larvae cultured in water from different pond types (ungrazed, grazed, agricultural and natural in 2000; agricultural and natural in 2001) (ANOVA; Zar 1984). A power analysis estimated an 85% probability of detecting a 20% difference in the survival of *X. laevis* between agricultural and natural ponds with a Type I error (α) of 0.05 for the FETAX bioassays (Zar 1984). We used the statistical software SPSS 11.5 to conduct all statistical analyses (Chicago, IL, USA)

We rank transformed data prior to analysis and multivariate analysis of variance (MANOVA) was used to determine if there was a significant difference in water quality between natural and constructed ponds in both years. We chose MANOVA because it simultaneously considers the effects of independent variables (e.g., pond type) on several dependent variables (e.g., phosphorus, total nitrogen, etc.; Zar 1984). We performed a MANOVA on each set of data because we measured different ponds and parameters in 2000 and 2001. If statistically significant, we followed the MANOVA with a univariate analysis of variance to determine which of the water quality parameters differed among pond types. We performed a Bonferroni test for post hoc analysis of the water quality data to assess differences in nutrient concentrations among the four types of ponds (Zar 1984). We used Spearman's rank correlation to determine if acute *Xenopus* survival during FETAX was correlated with the rank of each of the nutrient values in 2000 and 2001 (Zar 1984).

TABLE 1. Characteristics of ponds where water was collected for FETAX assays, Houston and Winona Counties, Minnesota, USA in 2000.

Pond name/type	Dominant land uses within 2500 m	Pond area (ha)	Mean max depth (m)	Mean total nitrogen (mg/l)	Mean total phosphorus (mg/l)
Alt/ungrazed	Forest	0.4	2.7	0.57	1.10
Uti/ungrazed	Grassland	0.2	0.7	0.19	1.29
StCh/ungrazed	Corn/soybean	0.4	2.5	0.07	3.99
She/ungrazed	Corn/soybean	0.0	1.3	0.22	1.14
Eit/ungrazed	Forest/grassland	0.1	1.5	0.13	1.28
Alt/grazed	Corn/soybean	0.3	2.8	0.78	2.60
Uti/grazed	Grassland	0.0	0.9	0.20	1.54
StCh/grazed	Grassland	0.1	1.1	3.56	5.62
She/grazed	Forest	0.2	1.8	0.43	3.26
Eit/grazed	Grassland	0.2	0.6	1.66	4.95
Alt/agriculture	Corn	0.5	1.4	0.82	2.85
Uti/agriculture	Corn/soybean	0.1	1.7	0.25	1.46
Lew/agriculture	Corn/soybean	0.3	1.1	0.44	2.07
StCh/agriculture	Corn	0.4	0.7	0.36	1.38
Hou/agriculture	Corn	0.5	1.6	0.30	1.07
Mou/agriculture	Corn/soybean	0.1	1.1	0.15	2.77
She/agriculture	Corn/soybean	0.1	0.7	0.21	1.07
Cal/agriculture	Corn/soybean	0.1	0.6	14.76	16.69
Bro/agriculture	Corn	0.1	1.3	0.08	2.42
Eit/agriculture	Corn/soybean	0.2	0.7	0.18	1.22
Alt/natural	Forest	5.6	0.7	0.15	0.76
Uti/natural	Forest	0.3	0.8	0.28	1.01
StCh/natural	Grassland	1.6	1.1	0.04	4.80
Cal/natural	Forest	0.3	0.4	0.16	0.64
Eit/natural	Forest	0.8	0.8	0.06	0.74

RESULTS

Water quality varied among the pond types (Table 1). In 2000, we found significant differences in water quality among the four ponds (F = 2.367, df = 40, P = 0.047, Wilks' lambda; Table 1) with grazed ponds having higher total nitrogen (P = 0.045) and total phosphorus (P = 0.023) than natural ponds. In 2001, we detected no significant difference in water quality between agricultural and natural ponds (F = 2.260, df = 14, P = 0.115, Wilks' lambda; Table 2).

In 2000, spearman's rank correlation determined that *Xenopus* survival was negatively correlated with total nitrogen (R = -0.53, P = 0.049) and also negatively correlated with total phosphorus (R = -0.57, P = 0.030). In 2001, *Xenopus* survival was negatively correlated with total phosphorus (R = -0.552, P = 0.014). In 2000, there was no detectable difference between the mean acute survival of *Xenopus laevis* larvae cultured in water from ponds in grazed pastures (78%), agricultural fields (78%), ungrazed fields (91%) and natural settings (91%; $F_{3,22}$ = 2.52, P = 0.08). There was also no difference in survival of *X. laevis* in water from artificially constructed ponds only (i.e., ponds in ungrazed fields, grazed fields and agricultural fields; $F_{2,17}$ = 3.28, P = 0.06). In 2001, mean acute survival of *Xenopus laevis* cultured in water from agricultural fields (74%) did not differ from that of natural wetlands (81%; $F_{1,17}$ = 0.46, P = 0.51). Response of *X. laevis* to positive and negative control solutions in all bioassays was within acceptable limits (ASTM 1998).

TABLE 2. Characteristics of ponds where water was collected for FETAX assays, Houston and Winona Counties, Minnesota, U.S.A. in 2001, Houston and Winona Counties, Minnesota, USA (modified from Kapfer et al. *In Press*).

Pond name/type	Dominant land uses within 2500 m	Pond area (ha)	Mean max depth (m)	Mean total nitrogen (mg/l)	Mean ammonia (mg/l)	Mean nitrate (mg/l)	Mean total phosphorus (mg/l)
Alt/agricultural	Corn	0.5	1.0	3.33	0.89	0.01	0.73
Uti/agricultural	Corn or Soybean	0.1	2.0	2.75	0.06	1.17	0.21
Lew/agricultural	Corn	0.3	0.5	2.02	0.17	0.11	0.20
Hou/agricultural	Corn	0.5	1.4	1.79	0.07	0.12	0.64
Mou/agricultural	Corn or Soybean	0.1	1.0	1.89	0.05	0.17	0.11
She/agricultural	Corn or Soybean	0.1	0.9	1.58	0.03	0.01	0.29
Cal/agricultural	Corn or Soybean	0.1	0.2	24.30	24.27	0.04	25.53
Bro/agricultural	Corn	0.1	1.5	1.90	0.06	1.06	0.09
Eit/agricultural	Corn or Soybean	0.2	0.6	2.72	0.16	0.27	0.47
Alt/natural	Forest	5.6	0.4	0.90	0.04	0.02	0.21
Uti/natural	Forest	0.3	0.8	1.19	0.11	0.00	0.23
Lew/natural	Grassland	0.5	0.3	0.85	0.07	0.13	0.08
Stch/natural	Grassland	1.6	1.4	5.43	0.03	4.75	0.03
Hou/natural	Forest	0.3	0.2	1.63	0.03	-0.01	0.42
Mou/natural	Grassland	0.1	0.3	2.45	0.05	0.00	0.52
She/natural	Grassland	0.3	0.2	1.62	0.05	0.02	0.48
Cal/natural	Forest	0.3	0.4	1.10	0.03	0.04	0.20
Bro/natural	Forest	0.4	0.5	2.28	0.52	0.01	0.40
Eit/natural	Forest	0.8	2.5	0.79	0.05	0.25	0.05

DISCUSSION

Grazed and ungrazed study ponds in 2000 had similar survival as agricultural and natural ponds, respectively, which resulted in their elimination in 2001. Our expectation that larval survival would be lower in constructed or agricultural ponds was incorrect; we reject the hypothesis that larval survival differs based on pond type. Our belief that nutrient levels would be lower in artificial ponds was correct in 2000, but incorrect in 2001.

Based on past research (e.g., Jofre and Karasov 1999) the ammonia levels we detected were high enough to affect acute survival. Conversely, most nitrate concentrations were too low to have an effect (Rouse et al. 1999). de Wijer et al. (2003) report that ammonium nitrate reduced survival of *Rana temporaria* tadpoles, but increased nitrate levels only indirectly enhanced mass at metamorphosis in *R. temporaria* and *Bufo bufo*. The low intensity of agricultural practices in our study region may partially explain the comparably low levels of nutrients detected in most test ponds and why acute larval survival was not significantly affected (Stamer et al. 1998; Hunst and Howse 2001; Sands and Parker 2001).

Several factors must be considered in regards to this research project: (1) *Xenopus laevis* is morphologically and physiologically different from native amphibians, although our results provide some insight into the pond types that may be at risk and require future research; (2) research on the same ponds found that those surrounded by grazed habitat and subject to direct disturbance by livestock contain fewer amphibians (Knutson et al. 2004); and (3) our study focused mostly on the direct effects that agricultural ponds may have on acute larval survival only. Although not analyzed during this research, indirect effects (such as those determined by Hayes et al. 2002 and Tavera-Mendoza et al. 2002, or the frequency and intensity of livestock grazing, frequency of pesticide application to land surrounding agricultural ponds, pesticide products used by land-owners) associated with agricultural ponds should not be ignored. In addition, chronic and behavioral studies using FETAX on water from these ponds is also worth pursuing, should time and resources allow.

Despite the potential toxicological risks present in agricultural settings, mean survival of larvae at 96 h was high for all pond types. The complex topography and erosive soils typical of the Driftless Area Ecoregion, which may result in less intensive agriculture associated with test ponds, must be considered. Agriculture could have a lesser effect on developing anuran larvae in this region compared to other areas where agricultural practices may be more intense. Further comparative research on this topic is necessary. The biological significance of these results, when taken alone, is difficult to determine. It is when they are considered concurrently with other research conducted on the same ponds (Knutson et al. 2004; Kapfer et al. in press), that a slightly clearer picture presents itself: should natural ponds be unavailable, certain agricultural ponds in this region are used by native amphibians for breeding and may be suitable for amphibian adult and larval survival.

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LITERATURE CITED

Albert, D.A. 1995. Regional landscape ecosystems of Michigan, Minnesota, and Wisconsin: a working map and classification. Gen. Tech. Rep. NC-178. St. Paul, MN: U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station. Jamestown, ND: Northern Prairie Wildlife Research Center Online. http://www.npwrc.usgs.gov/resource/habitat/rlandscp/index.htm [Version 03JUN98]

American Society for Testing and Materials. 1998. Standard guide for conducting the Frog Embryo Teratogenesis Assay-Xenopus (FETAX). E 1439-91. Pp. 826-836 *In* Annual Book of ASTM Standards, Vol. 11.05, Philadelphia, Pennsylvania, USA.

APHA. 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. American Public Health Association, Washington, D.C., USA.

Bishop, C.A., N.A. Mahony, J. Struger, P. NG, and K.E. Pettit. 1997. Anuran development, density and diversity in relation to agricultural activity in the Holland River watershed, Ontario, Canada (1990-1992). Environmental Monitoring and Assessment 57:21-43.

Blaustein, A.R., and P.T.J. Johnson. 2003. The complexity of deformed amphibians. Frontiers in Ecology and the Environment 1:87-94.

Blaustein, A.R., and J.M. Kiesecker. 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters 5:597-608.

Burkhart, J.G., J.C. Helgen, D.J. Fort, K. Gallagher, D. Bowers, T.L. Propst, M. Gernes, J. Magner, M.D. Shelby and G. Lucier. 1998. Induction of mortality and malformation in *Xenopus laevis* embryos by water sources associated with field frog deformities. Environmental Health Perspective 106:841-848.

Carey, C., and C.J. Bryant. 1995. Possible interactions among environmental toxicants, amphibian development, and decline of amphibian populations. Environmental Health Perspectives 103:13-17.

de Wijer, P., P.J. Watt, and R.S. Oldham. 2003. Amphibian decline and aquatic pollution: effects of nitrogenous fertilizer on survival and development of larvae of the frog *Rana temporaria*. Applied Herpetology 1:3-12.

Duellman, W.E., and L. Trueb. 1994. Biology of Amphibians. McGraw-Hill, New York, New York, USA.

Fort, D.J., T.L. Propst, E.L. Stover, J.C. Helgen, R.B. Levey, K. Gallagher and J.G. Burkhart. 1999. Effects of pond water, sediment and sediment extracts from Minnesota and Vermont, USA, on early development and metamorphosis of *Xenopus*. Environmental Toxicology and Chemistry 18:2305-2315.

Green, D.M. 2005. Biology of amphibian declines. Pp. 28-33 *In* Amphibian Declines: the conservation status of United States species. M.J. Lannoo (Ed.). University of California Press, Berkeley, California, USA.

Hayes, T.B., A. Collins, M. Lee, M. Mendoza, N. Noriega, A.A. Stuart and A. Vonk. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences 99:5476-5480.

Hayes, T.B., P. Case, S. Chui, D. Chung, C. Haeffele, K. Haston, M. Lee, V. P. Mai, Y. Marjuoa, J. Parker, and M. Tsui. 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we understanding the impact? Environmental Health Perspectives 114:40-50.

Houlahan, J.E., C.S. Findlay, B.R. Schmidt, A.H. Myer, and S.L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature 404:752-755.

Hunst, M., and G. Howse. 2001. Minnesota agricultural statistics. United States Department of Agriculture, National Statistics Service. Minnesota Agricultural Statistics Service, St. Paul, Minnesota, USA.

Jofre, M.B., and W.H. Karasov. 1999. Direct effect of ammonia on three species of North American anuran amphibians. Environmental Toxicology and Chemistry 18:1806-1812.

Johnson, P.T.J., and D.R. Sutherland. 2004. Amphibian deformities and *Ribeiroia* infection: an emerging helminthiasis. Trends in Parasitology 19:332-335.

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., M.B. Sandheinrich, and M.G. Knutson. In press. Use of artificial enclosures to determine the survival of *Rana pipiens* larvae in upper Midwestern agricultural ponds. Journal of the Iowa Academy of Sciences.

Knutson, M.G., W.B. Richardson, D.M. Reineke, B.R. Gray, J.R. Parmelee and S. E. Weick. 2004. Agricultural ponds support amphibian populations. Ecological Applications 14:669-684.

Kroening, S.E., and W.J. Andrews. 1997. Water-quality assessment of part of the Upper Mississippi River Basin, Minnesota and Wisconsin-nitrogen and phosphorus in streams, streambed sediment, and ground water, 1971-1994. USGS Water-Resources Investigations Report, 97-4107.

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. Pp. 233-262 *In* Multiple Stressor Effects in Relation to Declining

Amphibian Populations. Linder, G., S. Krest, D. Sparling and E. Little (Eds). American Society for Testing and Materials, West Conshohocken, Pennsylvania, USA.

Lannoo, M.J.. (Ed.) 2005. Amphibian Declines: the Conservation Status of United States Species. University of California Press, Berkley, California, USA.

Marco, A., C. Quilachano and A.R. Blaustein. 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. Environmental Toxicology and Chemistry 18:2836-2839.

McCallum, M.L., and J. Rayburn. 2006. A simple method for housing *Xenopus* during oviposition and obtaining eggs for use in FETAX. Herpetological Review 37:331-332.

McNab, W.H., and P. Avers. 1994. Ecological Subregions of the United States. U.S. Forest Service. WO-WSA-5, Washington D. C., USA.

Pough, F.H., R.M. Andrews, M.L. Crump, A.H. Savitsky, and K.D. Wells. 1998. Herpetology. 2nd Edition. Prentice-Hall, Inc., Upper Saddle River, New Jersey, USA.

Pounds, J.A., M.R. Bustamante, L.A. Coloma, J.A. Consuegra, M.P.L. Fogden, P.N. Foster, E. La Marca, K.L. Masters, A. Merino-Viteri, R. Puschendorf, S.R. Ron, A. Sanchez-Azofeifa, C.J. Still, and B.E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161-167.

Relyea, R.A. 2005. The lethal impact of Roundup on aquatic and terrestrial amphibians. Ecological Applications 15:1118-1124.

Rosenberry, D.O. 2001. Malformed frogs in Minnesota: an update. U.S. Department of the Interior, United States Geological Survey. USGS Fact Sheet 043-01.

Rouse, J.D., C.A. Bishop, and J. Struger. 1999. Nitrogen pollution: an assessment of its threat to amphibian survival. Environmental Health Perspectives 107:799-803.

Sands, J.K., and B.D. Parks. 2001. Iowa Agricultural Statistics. United States Department of Agriculture, National Agricultural Statistics Service. Iowa Agricultural Statistics, Des Moines, Iowa, USA.

Souder, W. 2000. A Plague of Frogs: the Horrifying True Story. Hyperion Press, New York, New York, USA.

Stamer, J.K., W.A. Battaglin and D.A. Goolsby. 1998. Herbicides in Midwestern reservoir outflows, 1992-93. USGS Fact Sheet, FS-134-98.

Stebbins, R.C., and N.W. Cohen. 1995. A Natural History of Amphibians. Princeton University Press, Princeton, New Jersey, USA.

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783-1786.

Tavera-Mendoza, L., S. Ruby, P. Brousseau, M. Fournier, D. Cyr and D. Marcogliese. 2002. Response of the amphibian tadpole (*Xenopus laevis*) to atrazine during sexual differentiation of the testis. Environmental Toxicology and Chemistry 21:527-531.

Tietge, J.E., G.T. Ankley, D.L. Defoe, G.W. Holocombe and K.M. Jensen. 2000. Effects of water quality on development of *Xenopus laevis*: a Frog Embryo Teratogenesis Assay-*Xenopus* assessment of surface water associated with malformations in native anurans. Environmental Toxicology and Chemistry 19:2114-2121.

United States Department of Agriculture, Natural Resources Conservation Service. 1999. National Handbook of Conservation Practices, Conservation Practice Standard: filter strip, Code 393. U.S. Department of Agriculture, Natural Resources Conservation Service, Washington, D.C., USA.

Vogt, R.C. 1981. Natural History of Amphibians and Reptiles of Wisconsin. The Milwaukee Public Museum, Milwaukee, Wisconsin, USA.

Zar, J.H. 1984. Biostatistical Analysis, 2nd Edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA.

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JOSHUA M. KAPFER (left) recently earned his Ph.D. from the University of Wisconsin-Milwaukee. His doctoral research focused on the spatial ecology and habitat selection of Bullsnakes (*Pituophis catenifer sayi*) in the upper Midwest. He is pictured here at his study site in southwestern Wisconsin holding a radio-tagged Bullsnake. Although broadly interested in vertebrate ecology, his research to-date has focused on "herptile" conservation and, recently, the behavioral ecology of African cichlid fishes from Lake Malawi. He currently teaches Zoology at the University of Wisconsin-Milwaukee, and works as a conservation biologist for the Wisconsin Department of Natural Resources, Bureau of Endangered Resources

MARK SANDHEINRICH, Ph.D. (right) is Chair of the Department of Biology and Director of the River Studies Center at the University of Wisconsin-La Crosse. He has 25 years of experience and more than 25 publications on aquatic ecology and on the effects of contaminants in aquatic systems. He and his students currently conduct research on: (1) the bioaccumulation, maternal transfer, and reproductive effects of methylmercury exposure in fish; (2) bioaccumulation of methylmercury in aquatic food webs; and (3) identification of controls on ecosystem sensitivity to mercury in atmospheric deposition. He recently served as an Expert Panelist on effects of methylmercury on fish, birds, and wildlife for the Eighth International Conference on Mercury as a Global Pollutant, August 6-11, 2006

MELINDA KNUTSON received her Ph.D. in Ecology and Evolutionary Biology (1995) from Iowa State University. Beginning in 1991, her federal research focused on the ecology of the Driftless Area Ecoregion of the midwestern USA, including the bird communities of upland and large floodplain forests along the Upper Mississippi River and amphibians living in agricultural landscapes. She also led the development of models to predict and map bird abundances across large regions and management tools to support regional conservation planning. She was a scientist for the USGS Upper Midwest Environmental Sciences Center for 10 years and she currently works for the U.S. Fish and Wildlife Service (FWS). Her current work involves helping FWS refuges in Regions 3 and 5 improve their biological monitoring programs and archive biological data collected from refuges.