EPA STAR Program Final Report (2003-2007)

Date of Report:	July 19, 2007
EPA Agreement Number:	RD-83087901
Title:	Effects of Multiple Stressors on Aquatic Communities in the Prairie Pothole Region
Investigators:	Patrick K. Schoff, Lucinda B. Johnson, Glenn R. Guntenspergen, W. Carter Johnson
Cooperators:	Jennifer H. Olker
Institution:	University of Minnesota Duluth, Natural Resources Research Institute
Research Category:	Ecological Indicators/Assessment/Restoration
Project Period:	04/07/2003 - 04/06/2007

Natural Resources Research Institute, University of Minnesota Duluth
Technical Report NRRI/TR-2007/13

Effects of Multiple Stressors on Aquatic Communities in the Prairie Pothole Region

Executive Summary

The prairie potholes wetlands of the Great Plains comprise some of the most ecologically valuable freshwater resources of the nation, but they are also exceptionally vulnerable to anthropogenic stressors, particularly those associated with agricultural land use practices. They are also considered likely to be severely impacted by climate change. In this study we have quantified relationships among stressors associated with climate, agricultural land use and amphibian communities throughout much of the prairie pothole region.

The specific objectives of this project were to:

1. Quantify the relationships among factors directly affected by climate change (e.g., hydroperiod), differing land use, and amphibian community structure and composition in the prairie pothole region (PPR) of the U.S.
2. Quantify the relationships among physical and chemical wetland attributes (e.g., hydroperiod, thermal regime, pH, and DOC), UV-B radiation, and land use (including associated pesticide use) on amphibian organismal and community responses.
3. Quantify the effects of multiple stressors (shortened hydroperiod, increased UV-B radiation, and atrazine exposure) on the health and organismal responses of *Rana pipiens* (northern leopard frog).
4. Use regional climate scenarios and hydrologic models in conjunction with empirical data gathered through field and mesocosm studies to predict potential effects of multiple stressors on prairie pothole wetlands and their associated amphibian communities.

To achieve our objectives we gathered and integrated data from three hierarchical levels: landscape (referred to in the text as Extensive scale studies), wetland (Intensive scale), and mesocosms. At the landscape scale we established relationships among amphibian community structure, composition and land use, and wetland permanence types across the majority of the U.S. portion of the PPR east of Montana. Wetland scale studies focused on examining relationships among individual wetlands (including hydroperiod and other physical and chemical wetland characteristics), land use (including pesticide use), UV-B, amphibian abundance and community structure, and indices of amphibian health. In mesocosm experiments we focused on quantifying the effects of multiple stressors (hydroperiod and pesticide exposure) on amphibian development and health in artificial ponds.

Summaries of major methods development and results of the studies are reported in the form of extended abstracts in the body of the report that follows. In addition, a final summarization of the various results is included. A digest of results from each of the major portions of the project completes this executive summary.

Methods Development and Project Results

1. We developed a novel stratified random sampling design incorporating GIS and field verification components that enabled us to select study wetlands across the prairie pothole region (PPR) landscape very efficiently and objectively, while obtaining the desired proportion of wetlands in each ecoregion and approximate equal treatment sizes (land cover and hydrology). The selected wetlands represented a variety of exposures to local- (90 m buffer) and landscape-scale (up to 3200 m buffer) stressors, gradients of crop and grassland cover types, water depths, seasonal and event-driven hydrological movements, and anthropogenic stressors, such as the herbicide, atrazine.

2. Our research indicates that *Rana pipiens* (northern leopard frogs) select breeding sites based on both local- and landscape-scale conditions. *Rana pipiens* are more likely to breed in wetlands that are surrounded by grassland (within 90 m), maintain average water levels greater than 30 cm and temperatures below 28°C, and that are embedded in a landscape with adequate wetlands within traveling distance for an individual. However, breeding was virtually absent if the distance between wetlands was greater than 1500 m. Understanding these breeding requirements will help guide conservation planning for maintaining stable amphibian populations in environments faced with climate change and development pressures.

3. Atrazine appears to be a ubiquitous contaminant in PPR wetlands, but very few wetlands contained concentrations high enough to elicit concern under conventional toxicological standards. The US EPA's Interim Reregistration Eligibility Decision (2003) documents state that atrazine presents "…the potential for community-level and population-level risk to aquatic ecosystems at prolonged concentration … from 10 to 20 ppb." Of 149 unique wetland sites that were sampled at least once during the current study, only one (33.8 ppb) equaled or exceeded this atrazine concentration, and only three exceeded 1.0 ppb atrazine. However, atrazine at or above 0.1 ppb, which has been reported to cause endocrine disruption in male *R. pipiens* and *Xenopus*, was found during at least one survey in the majority of PPR wetlands in both 2004 and 2005.

4. We collected *R. pipiens* metamorphs from wetlands located in crop and grassland landscapes that contained a wide range of atrazine concentrations. The sex ratio was virtually 1:1 and 97% of all gonads had normal morphology; however, testicular oocytes were observed in 56% of male metamorphs. The incidence of testicular oocytes ranged from one per pair of gonads to 1856 per pair, with a mean of 76 per affected individual. Neither the prevalence nor the numbers of testicular oocytes per gonad were correlated with wetland atrazine, which has been hypothesized to cause this phenomenon.

5. *Rana pipiens* embryos and larvae growing in mesocosm habitats were exposed to atrazine at 0.1, 20, and 200 ppb, and each of these groups were subjected to two hydrologic regimes in which water was withdrawn throughout the developmental period at "normal" and "accelerated" rates. No significant differences were detected in survival, weight at metamorphosis, or developmental time to completion of metamorphosis among

the treatments. The only treatment effect was seen in the proportion of larvae completing metamorphosis, which was significantly lowered in the 200 ppb atrazine treatments.

6. A stratified random site selection process combined with consistent collection and assessment procedures give us confidence that the 2.5% malformation prevalence found in these studies represents a reasonable estimate of occurrence in the Midwest. In addition, malformed specimens were found in over 75% of all sites in which 50 or more metamorphs were collected. These figures are comparable to those reported in studies in other regions for wetlands where the trematode parasite *Ribeiroia* sp. was not a factor (Johnson et al., 2001). Thus, when compared to the 0.3% prevalence found in archived specimens by Hoppe (2001), it appears that idiopathic malformation prevalence in Midwest anurans may have increased eight-fold in the last several decades.

7. Ultraviolet radiation (UVR) was rapidly attenuated through the wetland water column; 99% of UV-B attenuated within the top 75 cm in prairie pothole wetlands. However amphibian larvae, which commonly occupy the upper 10 cm of the water column, could be exposed to damaging UVR. The Z65% levels (depth that 65% irradiance reaches), which induced malformations in *R. pipiens* laboratory studies (Ankley et al. 2002), ranged from 0.9 to 8.2 cm in the prairie pothole wetlands. Attenuation was not driven solely by DOC content, and models of true color, chlorophyll-a, and direct attenuation value were significant, but weak predictors of extinction coefficient of PPR wetlands

8. Our studies suggest that *R. pipiens* prefers to breed in seasonal, as opposed to semi-permanent wetlands, and that successful breeding is influenced by the abundance and density of wetlands, as well as by the presence of row crops or grassland in the immediate proximity. Natural history data indicates that the species needs 80 - 120 days to complete development. Modeling of prairie pothole region seasonal wetlands using WETLANDSCAPE indicates that a 3°C atmospheric temperature increase may be accompanied by a 28% to 47% reduction in the number of years in which wetland hydroperiods equal or exceed 90 days after spring inundation. Areas with higher evaporative demand during the summer (to the north and west in the PPR) will likely have even more dramatic declines in the number of wetlands attaining the minimum hydroperiod needed for *R. pipiens* metamorphosis.

Semi-permanent wetland hydroperiods were not as markedly affected by a uniform 3°C increase in air temperature, with only a 2% reduction in years with hydroperiods of 90 days or more. Earlier model results suggested that increasing atmospheric temperatures would result in semi-permanent wetlands drying more often and that semi-permanent wetlands located in the south and east of the PPR would come to resemble seasonal wetlands, which breeding *R. pipiens* prefer. However, semi-permanent wetland density is low in this area, which is dominated by row crop agriculture. According to our logistic model, these characteristics are not optimal for northern leopard frog breeding. Thus amphibian metapopulation survival will depend on both the landscape context in which the remaining wetlands are embedded (e.g., inter-wetland distance and surrounding land use), and on local conditions within the wetland.

Effects of Multiple Stressors on Aquatic Communities in the Prairie Pothole Region

Project Design Summary

Introduction

The prairie potholes of the Great Plains comprise some of the most ecologically valuable freshwater resources of the nation (Hubbard 1988, van der Valk 1989). The diversity of wetland hydrologic regimes and the abundance of macroinvertebrates and plant life in these wetlands provide critical habitat for breeding waterfowl, stopover areas for millions of migrating birds, and habitat for amphibian species. Because of their high productivity and importance for flood storage and habitat, these wetlands represent a critical aquatic resource that should be managed on a regional basis to ensure the persistence of the ecological services they support.

Increasing temperatures and/or reduced precipitation in the Prairie Pothole Region (PPR) can be expected to change the hydrologic regime of PPR wetlands, eliminating some and modifying functions in others. In addition, existing stressors associated with agriculture (e.g., draining and impoundment), may make the PPR wetlands especially susceptible to the effects of climate change. Finally, pesticides could interact with climate change effects in as yet unknown ways, resulting in negative consequences for wetland biota.

Hydroperiod may be the best indicator of climate change impacts on wetlands, given its controlling effect on vegetation composition, habitat, and other ecosystem services. Larval amphibians are extremely sensitive to changes in hydroperiod, which may directly affect the diversity and abundance of metamorphosing juveniles. Furthermore, the interactions of stressors associated with climate change (e.g., hydroperiod) have been implicated in global amphibian declines. Because amphibians may integrate effects of climate change and pesticide exposure on wetlands, they are considered excellent indicators of the potential effects of these stressors on aquatic ecosystems.

Objectives

The overall objective of this project was to quantify the relationships among factors directly affected by climate change (e.g., hydroperiod), differing land use, and amphibian community structure and composition in the prairie pothole region (PPR) of the U.S. Specific objectives were to:

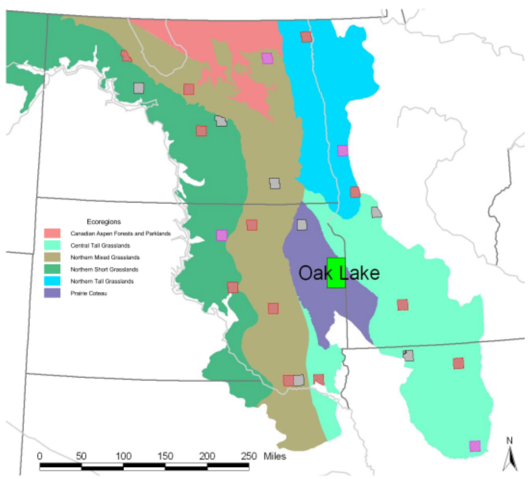
1. Quantify the relationships among factors directly affected by climate change (e.g., hydroperiod), differing land use, and amphibian community structure and composition in the prairie pothole region (PPR) of the U.S.
2. Quantify the relationships among physical and chemical wetland attributes (e.g., hydroperiod, thermal regime, pH, and DOC), UV-B radiation, and land use (including associated pesticide use) on amphibian organismal and community responses.
3. Quantify the effects of multiple stressors (shortened hydroperiod, increased UV-B radiation, and atrazine exposure) on the health and organismal responses of *Rana pipiens*.

4. Use regional climate scenarios and hydrologic models in conjunction with empirical data gathered through field and mesocosm studies to predict potential effects of multiple stressors on prairie pothole wetlands and their associated amphibian communities.

Experimental Design and Site Selection

To quantify the effects of multiple stressors on amphibians we conducted investigations at three spatial scales: landscape (Extensive study), wetland (Intensive study), and mesocosm study. Extensive studies sites were distributed across the U.S. portion of the Prairie Pothole Region (PPR; Figure 1); Intensive study sites were concentrated in a three county region in east-central South Dakota.

Figure 1. US Prairie Pothole Region



Study areas within ecoregions of the Prairie Pothole Region. Thirty-five sites comprise the intensive study area (Oak Lake, SD environs). Gray cells were sampled only in 2004; red only in 2005; pink cells were sampled both years.

All sites were stratified based on hydrologic regime (seasonal v. semi-permanent) and land use (grassland v. cropland). Semi-permanent wetlands are relatively deep, discharge wetlands with surface water persisting through the ice-free season in most years. Seasonal wetlands are shallower, and were normally inundated from spring to mid- or late summer. Hydrologic regimes were determined using the USGS Wetland BASINS data for wetlands with seasonal or semi-permanent hydrologic regimes. Isolated wetlands between 0.5 hectares and 5.0 hectares were selected. Land cover was summarized in 90 meter buffers surrounding these selected wetlands to classify wetlands as "cropland" or "grass"; basins with 70 percent or greater row-crop coverage in the 90 m buffer were classified as "cropland" and basins with 70 percent or greater pasture/hay or grassland were classified as "grass."

Intensive sites were selected across stressors gradient from the crop-rich southern PPR to the grassland-dominated northern PPR. We used National Land Cover Data (NLCD) classes for site selection; sites that met land cover, size, and hydrology criteria were randomly selected for field verification and sampling. A total of 35 sites were included in the Intensive study.

For the Extensive portion of the study we selected 114 wetland study sites based on a random design that incorporated ecoregion, land use and wetland hydrologic regime as stratification levels. GAP land cover data was used for Extensive site selection. Sites were distributed across the 5 ecoregions found in the PPR, with the number of sites weighted by the ecoregion's proportional area in the PPR.

Extended Abstracts

1. Environmentally stratified sampling of amphibian communities in the prairie pothole region

Introduction

Environmental assessment of lakes and streams in the U.S. over the past decade has focused on the application of a probabilistic design that selects sites within regions that represent some degree of climatic and landform homogeneity, such as ecoregions. Such designs require that the population of sample sites is well known. The outcome of this process is a set of sites that are examined individually to determine whether they meet the selection criterion. Due to inaccuracies in the wetland inventory data (e.g., National Wetland Inventory), climate-related drying, and difficulties associated with obtaining landowner permission, this method requires that a large number of potential sites be identified, relative to the number that will ultimately be sampled (known as an "overdraw"). We sought to reduce the expense and time associated with probabilistic site selection by developing a procedure that is less dependent on the accuracy of the wetland inventory.

Methods

We used GIS to characterize environmental conditions across the region to select 149 wetlands representing a stressors gradient from the row-crop dominated southern PPR to the grassland-dominated northern PPR and two hydrologic regimes (semipermanent and seasonal). Sites were distributed across 5 ecoregions in the PPR by a stratified random process, with the number of sites per ecoregion weighted by the ecoregion's proportional area in the PPR. Cells (~20 X 20 km) were randomly selected from all cells in the PPR that met the sampling criteria of wetland density and land cover/land use. Field crews used aerial photographs and NWI data to visit wetlands within each randomly selected cell. Between 4 and 10 sites were selected and sampled in each cell.

Results and Discussion

The stratified random sampling design enabled us to select study wetlands across a large landscape very efficiently and objectively, while obtaining the desired proportion of wetlands in each ecoregion and approximate equal treatment sizes (land cover and hydrology) (Tables 1 and 2). When visiting the predetermined randomly selected cells, field crews were able to select sites one day and sample the next, except for rare instances where there were few wetlands in a cell or where most wetlands were dry. This timing was essential, as there was a limited period in which to sample early breeding amphibians and site selection outside of the breeding season was challenging. By the end of summer, wetlands became harder to find and predictions of their ability to hold water in the next breeding season were unreliable.

Table 1: Number of wetlands sampled in the Prairie Pothole Region by treatment

		Land Use/Land Cover in 90 m buffer of wetlands [total (Intensive)]		
		Crop	**Grass**	**Total**
Hydrology	**Semipermanent**	28 (4)	45 (15)	73 (19)
	Seasonal	35 (7)	41 (9)	76 (16)
	Total	63 (11)	86 (24)	149 (35)

Table 2: Number of wetlands sampled in the Prairie Pothole Region by year and ecoregion for each level of study, including proportion total and original goals based on stratified sampling design.

Ecoregion	2003	2004	Resample in 2005	New in 2005	Total # Sites	prop of TOTAL	GOAL (prop TOTAL)	GOAL #
Central Tall Grassland	0	17	4	6	**23**	**0.20**	**0.274**	**30**
Northern Mixed Grassland	0	22	10	17	**39**	**0.34**	**0.279**	**31**
Northern Short Grassland	0	14	5	17	**31**	**0.27**	**0.228**	**25**
Northern Tall Grassland	0	7	7	7	**14**	**0.12**	**0.142**	**16**
Prairie Coteau [Extensive (Intensive)]	0 (27)	7 (35)	0 (35)	0 (0)	**7 (35)**	**0.06 (N/A*)**	**0.077 (N/A*)**	**8 (N/A*)**

*N/A = not applicable

The selected study wetlands were exposed to a range of stressors at both local- (90 m buffer around the wetland) and landscape-scales (up to 3200 m buffer around the wetland; Table 3). Study sites encompassed the full gradient of percent cover of both grassland and agriculture (and row crop) in the surrounding landscape, with a fairly normal distribution at 500 m or greater. Wetland density in the surrounding landscape varied greatly from 3 to 439 seasonal and semi-permanent wetlands within 2 km.

Local stressors also varied widely across the study sites (Table 3). Wetland water depth ranged from very shallow (<20 cm) throughout the year, to very deep (>150 cm) throughout the year, and included some wetlands in which water depth varied considerably in response to seasonal and event-based rainfall.

The Intensive study wetlands had more restricted ranges for most of these variables, as expected given that they were in close proximity and were contained within one ecoregion. However, the potential range of most stressors was realized in this set of wetlands.

Table 3: Mean, range and standard deviation for landscape scale stressors/conditions (n = 149 wetlands)

	Mean	Minimum	Maximum	Std.Dev.
Stream density (km/km^2) within 10 km	0.455	0.0000	1.218	0.3095
Population density ($people/km^2$) within 10 km	5.765	0.4280	104.024	12.2431
Road density (km/km^2) within 10 km	1.204	0.8031	2.058	0.2229
prop. Ag within 90 m (NLCD)	0.406	0.0000	1.000	0.3392
prop. Ag within 1 km (NLCD)	0.482	0.0003	0.927	0.2480
prop. Ag within 3.2 km m (NLCD)	0.491	0.0848	0.908	0.2090
prop. Row Crop within 90 m (NLCD)	0.347	0.0000	1.000	0.3340
prop. Row Crop within 1 km (NLCD)	0.412	0.0000	0.924	0.2660
prop. Row crop within 3.2 km (NLCD)	0.421	0.0000	0.908	0.2366
prop. Grass within 90 m (NLCD)	0.465	0.0000	1.000	0.3258
prop. Grass within 1 km (NLCD)	0.399	0.0358	0.919	0.2407
prop. Grass within 3.2 km (NLCD)	0.385	0.0561	0.833	0.1995
Number of SP & SS wetlands within 100 m[*]	2.047	0.0000	13.000	1.9533
Number of SP & SS wetlands within 1 km[*]	40.584	3.0000	137.000	26.9838
Number of SP & SS wetlands within 2 km[*]	133.141	3.0000	439.000	91.8353
Average distance to SP & SS wetlands within 100 m[*]	54.033	5.8604	98.814	21.2703
Average distance to SP & SS wetlands within 1 km[*]	582.494	305.8232	818.568	78.8459
Average distance to SP & SS wetlands within 2 km[*]	1232.554	633.0269	1593.829	136.2166
Minimum water depth	59.018	0.0000	228.500	50.7367
Maximum water depth	92.536	0.0000	264.500	54.2624
Average Water Depth	77.095	0.0000	241.000	50.8308
Maximum Atrazine (ppb)	0.151	0.0000	0.480	0.1047
Average Atrazine (ppb) - surveys 2 & 3	0.121	0.0000	0.480	0.0900

[*]SS = seasonal, SP = semipermanent

2. Effects of local and landscape conditions on northern leopard frog (*Rana pipiens*) breeding in the Prairie Pothole Region

Introduction

The ability of amphibian species to maintain their populations can be influenced by the presence and quality of water during crucial breeding and developmental periods, physiologically acceptable temperatures, and tolerable levels of predators, competitors, and diseases. Because of these requirements and the fact that many amphibian taxa exploit both aquatic and terrestrial habitats, they are considered ideal models for integrating the impacts of climate change (e.g., changes in hydrology) and other anthropogenic stressors, such as elevated UV-B irradiation and pesticides on vertebrates. Additionally, their trophic status and unique physiological adaptations make amphibians excellent indicators of stressor effects on aquatic ecosystems.

The US portion of the Prairie Pothole Region (PPR) encompasses an enormous diversity of wetlands embedded in land use types and structure that range from the densely populated southeastern portion in Iowa, to the sparser, less impacted northwestern portion in North Dakota. The northern leopard frog (*Rana pipiens*) was selected as a focal species for this study because it is found throughout the region and is most often associated with emergent wetlands and grasslands, which are common in the PPR.

Landscape characteristics such as broad-scale land use and road density, local impacts (e.g. immediate surrounding upland, modifications, cattle), and within-wetland characteristics (e.g. hydrology, vegetation type, and density) all affect wetland quality and suitability for amphibian breeding. Given their peripatetic nature, many PPR anurans also contend with stressors on the landscape-scale (roads, crops, etc) as they move towards and disperse from breeding and natal sites, as well wetland-scale stressors, such as desiccation, elevated water temperature, predators, and pesticides. We examined the relationships between *R. pipiens* breeding to wetland conditions, local stressors, and land use at varying spatial scales (up to 2 km from study wetlands).

Methods

Amphibian community assessments included observations of breeding adults, larvae, and egg masses, using a combination of calling surveys, larval sampling and visual encounter surveys (VES) conducted three times annually. Nighttime calling surveys were conducted during April, May-June, and July, in accordance with North American Amphibian Monitoring Program (NAAMP) protocol. Time-constrained daytime VES identified amphibian adults, larvae, and eggs present as well as potential predators. Amphibians were identified, measured, staged, and examined for malformations prior to release. For these analyses, breeding evidence includes any observation of eggs, larvae, or metamorphic amphibians during the surveys.

Local conditions, including water depth, water quality (pH, salinity, temperature, and conductivity), and habitat measurements (wetland size, wetland type, dominant cover), were measured at Extensive sites in conjunction with amphibian surveys and weekly at Intensive sites.

Landscape characteristics that could potentially impact wetland quality and amphibian populations were acquired and summarized for buffers surrounding each study site. National Land Cover Data (NLCD) was acquired for the entire region and summarized for 9 buffer widths (90-3200 m) surrounding each study wetland. In addition, population density, stream density, elevation change, and count of and distance to neighboring wetlands were quantified at varying buffer distances surrounding each study site.

Results and Discussion

We found significant relationships between conditions/stressors and the probability of *R. pipiens* breeding at both the local and landscape scale. The strongest predictors of breeding were land cover treatment (crop v. grass), water depth (average, minimum, or maximum), and average distance to wetlands within 2 km (semi-permanent and seasonal combined, and seasonal alone). For all three groups of explanatory data, one-variable models were as good as or better than two- and three-variable models.

Evidence of *R. pipiens* breeding was found in a variety of wetland types in both agricultural and non-agricultural landscapes. However, odds of breeding were >5 times higher in a grassland-surrounded wetland (in 90 m buffer) than in one surrounded by crop. The probability of *R. pipiens* breeding had a strong negative response to increased average distance between wetlands within 2 km (Figure 1). The model with only seasonal wetlands was very similar to that with both seasonal and semi-permanent wetlands. The odds of breeding increased by 4 times (seasonal wetlands) to >6 times (seasonal and semi-permanent wetlands) for each 100 m decrease in the average distance to neighboring wetlands. There appeared to be a "threshold" at ~1500 m, beyond which the probability of breeding dropped to below ~ 5% in both models (Figure 1). The average distance to seasonal wetlands within 1 km had a very similar strongly negative relationship with the probability of breeding, and was also fairly good at predicting breeding in subsequent years and across the region (Table 1).

Local conditions were also good predictors of breeding. The probability of *R. pipiens* breeding showed a strong relationship to increased average, minimum and maximum water depth. In general, if wetlands contained water, breeding was observed. Even in wetlands that eventually dried, the chance of *R. pipiens* breeding was 35%. This relationship reached an asymptote at 50-70 cm deep, with > 90% chance of breeding if the average depth was greater than 52 cm, and > 99% if it was greater than 77 cm.

The probability of *R. pipiens* breeding was also strongly negatively correlated to increased daytime water temperature, which probably reflected *R. pipiens* temperature preferences and tolerances. There appeared to be a temperature "threshold" which limited *R. pipiens* breeding. At daytime water temperatures of 28° C or greater, the chance of breeding was reduced to < 5%. Daytime water temperature had a lower correct classification rate than other local and landscape predictors of breeding.

Our research indicates that *R. pipiens* select breeding sites based on both local- and landscape-scale conditions. *Rana pipiens* are more likely to breed in wetlands that are immediately surrounded by grassland (within 90 m), maintain water levels and temperatures amenable to developing eggs and larvae, and that are embedded in a landscape with adequate wetlands within traveling distance for an individual. Understanding these breeding requirements help guide conservation planning for maintaining stable amphibian populations in environments faced with climate change and development pressures.

Geographic Transferability (to rest of PPR)

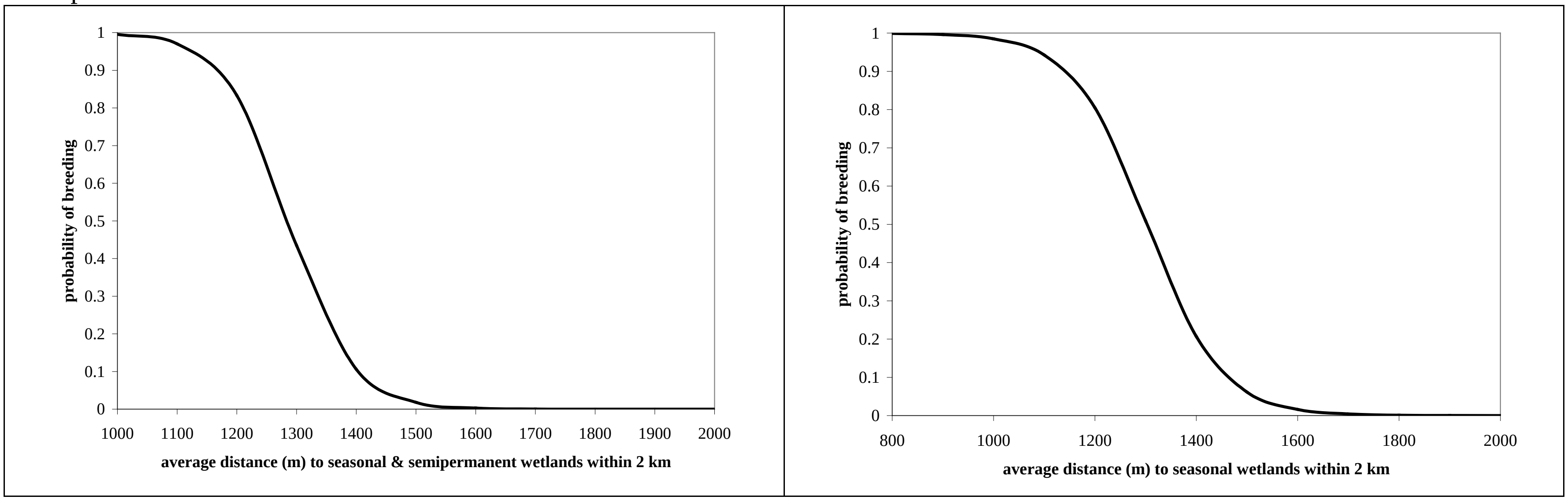
The models predicting *R. pipiens* breeding in the Prairie Coteau region can be used to conservatively predict where breeding was likely to occur in the rest of the PPR, with more wetlands predicting to contain breeding that were observed. There were, however, differences in amphibian abundances, land use practices, and weather conditions that must be considered when applying the intensive model to the rest of the region.

Table 1. Logistic models of evidence of breeding for *Rana pipiens*, based on weekly data in 2004 from 35 Intensive sites.

Scale	Variables In Model	Relation-ship	p-value	AIC[c]	%CC[d,e]	%CC[d] Intensive 2003	%CC[d] Intensive 2005	%CC[d] Extensive 2004	%CC[d] Intensive 2005
Local (treatment)	*Land Cover (Categorical)*	+ grass	0.029	45.41	71.4	68	74	43	53
Local	*Extinction Coefficient (K_dB)*	-	0.001	35.78	72.7	63	-	-	-
Local	*Avg Depth*	+	<.0001	32.43	68.6	82	85	30	38
Local	*Min Depth*	+	<.0001	34.54	71.4	78	83	25	39
Local	*Max Depth*	+	<.0001	34.58	68.6	82	89	39	40
Local	*Dissolved Organic Carbon (DOC)*	-	0.043	46.08	65.7	74	-	-	-
Local	*Daytime Water Temperature*	-	0.013	43.95	68.6	33	71	27	38
Landscape (NLCD[a])	*prop. Wetland within 3200 m*	-	0.043	46.08	68.2	64	66	27	30
Landscape	*Average distance to SP & SS wetlands within 2 km*[b]	-	0.005	42.44	68.6	71	69	42	48
Landscape	*Average distance to SS wetlands within 2 km*[b]	-	0.017	44.44	62.9	68	66	48	48
Landscape	*Average distance to SS wetlands within 1 km*[b]	-	0.039	45.94	65.7	57	80	37	41

[a]NLCD = National Land Cover Data; [b]SS = seasonal, SP = semipermanent; [c]Akaike's Information Criterion; [d]CC = correctly classified; [e]Intensive 2004 cross-validation of model.

Figure 1. Logistic curves for models predicting *Rana pipiens* breeding from the average distance to surrounding seasonal and semipermanent wetlands within a 2 km radius.



3. Mesocosm scale empirical models of amphibian developmental responses to atrazine and hydroperiod manipulation.

Introduction

Like most anurans, developmental timing in *Rana pipiens* is somewhat plastic; depending on environmental cues, normal completion of metamorphosis can be accomplished in as few as 80 or as many as 120 days. Accelerated development may be stimulated by decreasing water levels, perhaps suggesting to the organism that drying is imminent. However, the apparent cost of rapid metamorphosis has been reported to be smaller size and weight as juveniles, which could translate into poorer winter survival and reduced future reproductive capacity. Additional stressors, such as exposure to pesticides, may also affect the timing and progression of development. This experiment was designed to subject *R. pipiens* to sets of stressors that they might be exposed to in the PPR under current and anticipated future climate conditions. Embryos were exposed to biologically relevant atrazine concentrations; specifically, atrazine at 0.1 ppb has been reported to cause endocrine disruption in male *R. pipiens*, 20 ppb atrazine has commonly been found in surface water, and 200 ppb concentrations are occasionally measured in runoff or in surface waters after precipitation events.

Methods

Rana pipiens were raised from embryos through metamorphosis in 100 L mesocosms in a four by two experimental treatment design. The organisms were exposed to atrazine at 0, 0.1, 20, and 200 ppb (static), and two water-removal regimes, designed to replicate the average rate of water loss experienced in PPR wetlands or an accelerated rate (2-times higher than the standard rate), as might be experienced in a warmer climate. Metamorphs were removed from the mesocosms, euthanized, weighed and measured and fixed for future histological examination.

Results and Discussion

Of 225 embryos placed in mesocosms, 103 reached metamorphosis by the end of the experiment (173 days). The earliest metamorph emerged (i.e. reached Gosner stage 45) at day 83, and the median age of emergence was 128 days. The last metamorphs were collected on day 159, although five dead stage 45 metamorphs were found when the experiment was stopped on day 173. As expected, the earliest metamorphosing animals were small, and sizes increased with later metamorphosing dates. However, size peaked at around the median emergence time, and progressively decreased, so that the latest metamorphosing animals weighing about the same as the earliest emerging animals. The largest metamorphs, which completed metamorphosis in about 120 days, were 1.75-fold larger than the earliest and latest emerging metamorphs.

Two-way ANOVA showed no significant relationships between hydrologic regime and survival, number of organisms metamorphosing, length of metamorphosis, weight or size. There was also no relationship between the presence of atrazine at 0.1 and 20 ppb with any of the above variables, as was the case with 200 ppb atrazine, with the exception of an apparent delay or inhibition of metamorphosis, which was significantly reduced in comparison with the control treatment animals, but not from the frogs in the other atrazine exposures.

4. Relationship between ambient atrazine concentration and testicular oocytes in *Rana pipiens* metamorphs.

Introduction

Recent interpretations of selected laboratory and field studies have suggested that low, environmentally relevant concentrations of atrazine may be associated with the development of testicular oocytes (TO) in metamorphic *Rana pipiens* (northern leopard frogs). In contrast, similar studies on *R. pipiens* and other anurans have shown no such effects. The widespread use of atrazine combined with its persistence and ability to mobilize into surface waters create compelling reasons for investigating the possible endocrine disrupting characteristics of this pesticide. However, our understanding of gonadal and germ cell development in anurans is also incomplete, and transient hermaphroditism has been reported in some developing amphibians. In the current study, we have assessed gonadal and germ cell development in male *R. pipiens* metamorphs collected from wetlands contained in a gradient of atrazine concentrations.

Methods

Collection sites were chosen from the randomly selected wetlands in the Intensive and Extensive studies that showed evidence of metamorphosing *R. pipiens* during the final yearly survey. Metamorphic *R. pipiens* were collected by dip netting, euthanized, preserved in the field with Bouin's fixative and 70% ethanol, then returned to the lab, where the gonads were dissected (with kidneys attached), processed, and stained using standard techniques. Oocyte size and configuration were determined in ovaries of female *R. pipiens* metamorphs to be fairly uniform spheres averaging 40 µm. Five µm-thick longitudinal sections were cut through both gonads, with one section mounted every 45 µm. Each gonad was sectioned in its entirety.

Results and Discussion

In 2005 514 *Rana pipiens* at various metamorphic stages were collected from ten PPR wetland sites ranging from northern Iowa to southern North Dakota. Examination of gross gonadal morphology identified 255 males and 259 females in the collection, resulting in a virtual 1:1 sex ratio. In addition, gross tissue assessment indicated normal gonadal appearance in about 97% of all metamorphs. Gonadal abnormalities, including discontinuous or constricted testes, poorly developed testes, and poorly lobed ovaries were identified in sixteen individuals.

Gonads from the 168 male *R. pipiens* that had completed metamorphosis (Gosner stage 46) were sectioned, stained and analyzed for the presence of testicular oocytes. Overall, testicular oocytes were detected in 56.0% (94) of all stage 46 males, and they were found in at least one individual from each collection site. The prevalence of metamorphs with testicular oocytes ranged from 19.2% (n = 26 males) to 100% (n = 3 males) throughout all sites; at the three sites where more than 25 males were collected (n = 26, 42, and 51), prevalences were, respectively, 19.2%, 92.9%, and 21.6%.

Testicular oocytes counted within affected individuals ranged from one to 1856, with a mean of 74.0 TOs per individual. Metamorph testicular oocytes appeared indistinguishable from metamorph ovarian oocytes.

Metamorph collections were attempted from all sites where evidence of *R. pipiens* development was noted during surveys. All wetland study sites had been categorized according to hydrology (seasonal or semipermanent) and landuse (crop or grassland), and the metamorph collection sites were distributed among these different groups. No significant correlations were noted between TO prevalence of the number of TOs within an individual and any site classification.

Mean atrazine concentrations in the sites containing metamorphs ranged from below the method limit of detection (LOD = 0.011 ppb) to 0.096 ppb in Survey 1, 0.025 to 0.399 ppb in Survey 2, and <LOD to 0.805 ppb in Survey 3. There were no significant correlations between TO prevalence or the number of TOs with any of the following site parameters and characteristics: atrazine concentration (high, low, average of Surveys 1 and 2, average of Surveys 2 and 3, and overall average), survey number, and latitude.

Our study design resulted in a sample of *R. pipiens* metamorphs from both crop and grassland wetland sites, which contained a wide variation in atrazine content. Over half of all male metamorphs contained testicular oocytes, but neither the prevalence nor severity (number of TOs/male) correlated with atrazine, which has been hypothesized as a causal factor in this phenomenon.

5. Atrazine concentrations in Prairie Pothole wetlands

Introduction

Atrazine is one of the most commonly used pesticides in the U.S., particularly in areas of corn agriculture. Its resistance to degradation and tendency to partition into water often results in ground- and surface-water contamination, at times in areas far removed from its application. Although atrazine at approved application rates has little conventional toxicological effect on amphibians, recent studies have associated low, environmentally relevant concentrations with endocrine-mediated effects, including hermaphroditic gonads, testicular oocytes, and demasculanized larynges in male frogs. Given its ubiquity in PPR surface waters (US EPA 2003), and its potential for endocrine disruption, atrazine could be an important stressor for amphibians and other organisms in these wetlands.

Methods

Water sampling. Water samples were taken from wetlands situated in a roughly south-to-north transect of the PPR during three repeated surveys in the spring and summer of 2004 and 2005, during which the first samples taken during each survey were collected from the southernmost sites in Iowa and the final water samples were from the northernmost sites in North Dakota. The sampling efforts were timed such that the initial samples would be taken prior to the first seasonal application of atrazine, and prior to the breeding period for the majority of PPR anurans. The second survey corresponded to both pre-emergence atrazine application and to the normal breeding period of common PPR species, including *Rana pipiens* (northern leopard frog) and various toads. The final survey coincided with post-emergent atrazine applications and to metamorphosis in *R. pipiens*.

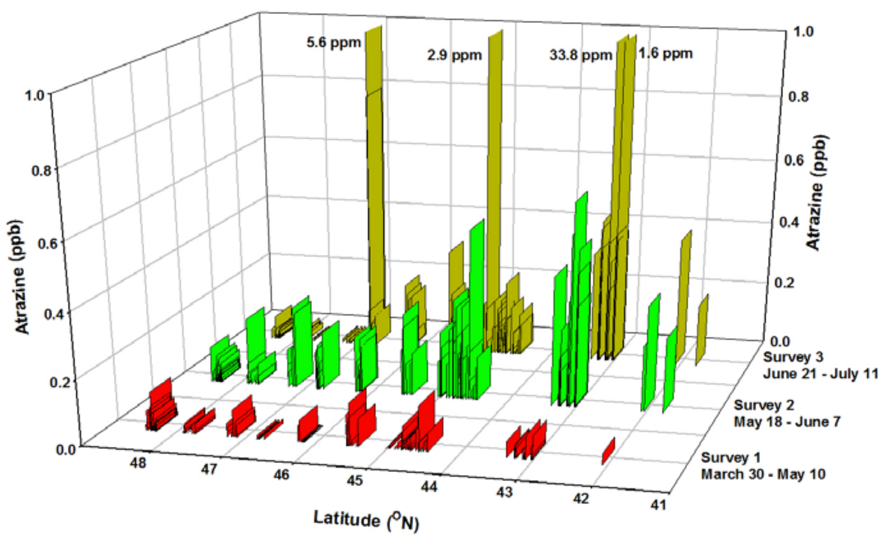
Atrazine assay. Water samples were hand-collected from the approximate center of each wetland, filtered through a 0.2 µm membrane, placed on ice during transport, and held at or below 4º C until analysis. Atrazine concentration was quantified using an enzyme-linked immunosorbent assay with a nominal detection limit of 0.011 ppb (Atrazine HS, Abraxis LLC, Warminster, PA).

Results and Discussion

Range of atrazine in PPR wetlands. Atrazine levels above the method limit of detection (0.011 ppb) were measured in the majority of wetlands sampled in each of the three surveys in 2004 and 2005 (Figure 1). In both years samples from about a third of the wetlands from Survey 1 (April/May) were below nominal detection limits, but in the second and third surveys almost all sites contained measurable atrazine. Excluding elevated concentrations determined to be outliers, wetland atrazine averaged 0.019 - 0.027 ppb during the first surveys (2004 and 2005, respectively), 0.084 - 0.128 ppb during the second surveys, and 0.133 - 0.122 during the third surveys. It is notable that over the two years of study, only two wetlands averaged atrazine concentrations below the limit of detection over the entire season.

In 2004 the three surveys produced 19 wetlands containing elevated atrazine concentrations, ranging from 0.116 - 7.1 ppb, while in the 2005 surveys 12 wetlands contained outlier atrazine concentrations ranging from 0.119 - 33.8 ppb atrazine.

Figure 1. 2005 PPR Wetlands Atrazine Concentration



General patterns of wetland atrazine. Atrazine concentrations in PPR wetlands during the amphibian breeding and developmental period could be characterized as being fairly uniformly distributed, with 90 – 95% of the wetlands containing atrazine at 0.030 – 0.130 ppb, and the remaining 5 – 10% containing concentrations 2 to 260 times the mean (Figure 1). Generally, atrazine concentrations measured in the first survey were below 0.030 ppb, then rose to greater than 0.100 ppb after pesticide application and remained at that concentration through at least mid-summer. The highest wetland atrazine concentrations were detected in the second and third surveys of each year, with the notable exception of two wetlands in 2004 Survey 1, which registered 0.456 ppb and 0.641 ppb atrazine, respectively (Figure 1).

Atrazine concentration generally corresponded positively with reported corn agriculture and atrazine sales, both of which are higher in the southern and eastern portions of the study area, and considerably lower in the northern regions. However, atrazine concentrations in wetlands determined to be crop sites did not differ from atrazine concentrations in grassland sites during either year. Furthermore, outliers were as likely to occur in either treatment group and the highest measured site (33.8 ppb) was in a grassland site.

Atrazine appeared in the overwhelming majority of the PPR wetland sites, but very few wetlands contained concentrations high enough to elicit concern under conventional toxicological standards. The US EPA's Interim Reregistration Eligibility Decision (2003) documents state that atrazine presents "…the potential for community-level and population-level risk to aquatic ecosystems at prolonged concentration … from 10 to 20 ppb." Of 149 unique wetland sites that were sampled at least once during the current study, only one (33.8 ppb) equaled or exceeded this atrazine concentration, and only three exceeded 1.0 ppb atrazine. However, atrazine at or above the 0.1 ppb concentration reported to cause endocrine disruption in male *R. pipiens* and *Xenopus* was found during at least one survey in the majority of PPR wetlands in both 2004 and 2005.

6. Malformed anurans in the central Midwest US

Introduction

In the mid-1990s large numbers of malformed frogs, toads, and salamanders were collected in surveys from a wide variety of wetland habitats and in widely separated locations. The appearance of malformations in populations of familiar wild vertebrates triggered responses from academics and regulatory agencies with dual goals of discovering the cause(s) of amphibian malformations and determining whether similar harm could occur in other vertebrates, including humans. Various etiologies were proposed and their plausibilities tested in laboratory or in field studies; these included trematode parasites, trauma, predation, and environmental retinoid mimics (teratogens). At present, only infection by *Ribeiroia* sp. trematode cercaria has been established as a probable and plausible cause of amphibian malformations. Notably however, amphibian malformations also occur in the absence of *Ribeiroia*, indicating other etiologies exist.

One challenge to determining the parameters of the current amphibian malformation phenomenon has been to determine the "historical" prevalence of malformation within a given population or species. Hoppe (2001) addressed this problem in an analysis of *Rana pipiens* (northern leopard frogs) collected from central Minnesota during the 1960s, calculating a 0.3% prevalence of skeletal malformations. Numerous current studies, which have focused primarily on causation, report anuran malformation prevalence up to 79% (Gardiner and Hoppe 1999). Typically, these studies were performed in ponds or wetland areas where large numbers of malformed individuals had been reported. Also typically, matching "control" or "reference" collection sites have been designated *a posteriori*, based on proximity and similarity of such criteria as physical description and association with agricultural or developed landscapes. An alternative approach is to select survey sites randomly using investigator-determined criteria, such as conditions known to support anuran development, wetland size and location, and defined treatment categories, including row crop agriculture versus grassland, to establish an unbiased framework for establishing malformation prevalence.

Methods

Surveys. Survey timing was based on temporal estimates of northern leopard frog (*Rana pipiens*) metamorphosis, although data on all anurans collected was included in the analysis. Only metamorphic individuals were included in the data set to ensure that the malformation prevalence determined for a particular wetland would represent only the population that originated in that wetland, and not migrants or organisms of multiple age classes.

<u>*Collection protocol*</u>. Metamorphic anurans (including Gosner stage 42 and older) were collected by dip netting in a time- and effort-constrained protocol, which specified that collection continued until 100 - 150 metamorphs were obtained or four person/hours had elapsed. If fewer than ten metamorphs were observed after 2 person/hours, the survey was abandoned.

The current study expands on earlier work (see Schoff et al. 2003), which reported anuran malformation prevalence in northern Illinois, central Wisconsin, and central Minnesota (see Table IA, Indicator and EPA STAR Final Technical Report GAD #R825867).

Results and Discussion

A total of 254 independent surveys were conducted at 115 unique sites over seven years (Table IA). Metamorphic frogs were found in 185 (72.8%) of the surveys, and malformed metamorphs were collected in 73 of these; thus 39.5% of all surveys yielded malformed frogs. However, the vast majority (84.2%) of all metamorphs were collected in the 69 surveys in which greater than 50 frogs were collected (Table IB). Of these surveys, 52 (75.4%) produced malformed frogs. A similar analysis of the 36 sites that yielded 100 or more metamorphs resulted in 88.9% (32) that contained malformed frogs (not shown).

In all surveys 212 out of 8648 individual metamorphs were identified as having skeletal, cutaneous, or eye malformations (Table IA), resulting in a malformation prevalence of 2.45%. The malformation prevalence was virtually unchanged in the subset of surveys yielding 50 or more metamorphs (Table IA & B) and was similar in surveys with 100 or more specimens (2.81%; not shown).

Table I. Skeletal, eye, and integumentary malformations in metamorphosing amphibians

A: All surveys

Project	Total surveys	Surveys w/metas[a]	Surveys with malfs[b]	Survey malf prev[c] (%)	Total N[d]	Total malfs[e]	Malf prev[f] (%)
Indicator	192	142	53	37.3	5975	108	1.81
MS	62	43	20	46.5	2679	105	3.92
Total	254	185	73	39.2	8654	213	2.46

B: Surveys in which ≥ 50 metamorphs were collected

Project	Total surveys	Surveys N[a] ≥50	Surveys with malfs[b]	Survey malf prev[c] (%)	Total N[d]	Total malfs[e]	Malf prev[f] (%)
Indicator	192	48	34	70.8	4730	83	1.75
MS	62	21	18	85.7	2555	102	3.99
Total	254	69	52	75.4	7285	185	2.54

[a] Metamorphosing amphibians
[b] number of surveys that contained malformed metamorphosing amphibians
[c] percentage of sites with any malformed metamorphosing amphibians
[d] metamorphosing amphibians assessed
[e] malformed individuals
[f] percentage of metamorphosing amphibians with malformations

Malformation classification. Most specimens had one readily identifiable abnormality, but a substantial minority had multiple abnormalities that could be categorized independently. For analytical and comparative purposes in metamorphs with multiple abnormalities, we arbitrarily assigned one (the most apparently severe) as the "primary" abnormality and other(s) as subordinate abnormalities. Thus, 297 abnormalities were identified in the 213 abnormal frogs, resulting in an incidence of 1.39 abnormalities per abnormal metamorph.

Of the primary malformations, 71.4% occurred in the hindlimbs, 1.4% in forelimbs, 16% involved the eyes, and the remaining 8.5% occurred in the jaws and other skeletal structures. The vast majority of all limb malformations involved either shortened or missing limbs or portions of limbs. Notably, only three specimens displayed multiple limbs or toes.

Random site selection combined with consistent collection and assessment procedures give us confidence that the 2.5% malformation prevalence found in these studies represents a reasonable estimate of occurrence in the Midwest. These figures are comparable to those reported in other studies, for areas where the trematode parasite *Ribeiroia* sp. was not present (Johnson et al. 2003). Thus, when compared to the 0.3% prevalence found in archived specimens by Hoppe (2001), it appears that idiopathic malformation prevalence in Midwest anurans may have increased eight-fold in the last several decades.

7. UV Radiation and attenuation in Midwestern wetlands

Introduction

Amphibian populations worldwide have suffered from diseases caused by new pathogens, such as the chytrid fungus and ranaviruses, which have caused local extinctions and extirpations, as well as trematode parasites, which have been associated with severe malformations. In addition, long-term data has suggested a pattern of broad declines in many amphibian populations. Researchers have concluded that no single factor is responsible for the widespread amphibian declines, and interactions among stressors such as pathogens, global climate change, and habitat degradation and loss are suspected to be involved. However, investigation of possible integrating factors, such as environmentally induced immune suppression, might help explain the breadth of the phenomenon.

Ultraviolet radiation (UVR) has been shown to cause malformations and be fatal as well as have such an immune-suppressive effect in amphibians, and ambient UVR exposure has increased globally in the preceding decades. Amphibian egg, embryonic, and larval exposure to UVR is influenced by weather, incident radiation at the pond surface, shading by vegetation, and attenuating properties of the water column, which occurs when light is absorbed and scattered by dissolved and particulate material. In lakes and wetlands, UVR attenuation is strongly influenced by dissolved organic carbon (DOC) and color.

Increased UV-B penetration into the water column has been identified as an indirect effect of climate change, resulting from reduced DOC loading from upland and wetland sources. The effects of decreased DOC output may be exacerbated during droughts, when surface water DOC is exposed to photo-bleaching, and negative effects will be most apparent in shallow wetlands, because harmful UV-B doses are restricted to the top 10 cm in most Midwest wetlands. Considering that many amphibians lay their eggs and undergo much of their early development in the upper regions of the water column, changes in UVR exposure may be critical.

Methods

Sites. Solar radiation attenuation was measured in two types of wetlands in the Midwest: ephemeral ponds in northern Minnesota (2002) and prairie pothole wetlands in eastern South Dakota (2003, 2004). All wetlands were less than five hectares, with many less than one hectare. Both wetland types supported amphibian communities dominated by early breeding species (*Rana sylvatica* in MN and *R. pipiens* in SD).

Radiation Measurements. Incident and *in situ* attenuation measurements were taken during the period of larval development of *R. sylvatica* and *R. pipiens* in three open water locations per pond on cloudless (or nearly cloudless), calm days. Measurements were taken directly above the water surface and approximately 1 cm below the water surface to minimize the effects of surface chop. Subsequent measurements were taken at 5 cm increments until reaching pond bottom or the instrument's limit of detection. Vertical attenuation coefficients (K_d) were calculated for UV-B (K_d B) using the averaged K_d from three different locations in each pond. Bulk attenuation coefficients (K_a) were determined in water samples from each pond in the laboratory.

Water Chemistry. Water chemistry was assessed using sub-surface grab samples (~ 0-0.5 m) obtained coincident with vertical attenuation measurements. Analyses were performed for DOC, true color, total suspended solids (TSS), chlorophyll-a (chl-a), and phaeopigment-a.

Statistical Analysis. Models predicting attenuation coefficients and UV-B radiation from water column properties (DOC, true color, TSS, chl-a and K_a B) were constructed. To build diffuse (K_d) attenuation models, a multiple linear regression analysis was conducted using best subsets and Mallows' C_p variable selection methods with all sites where attenuation slope had $R^2 \geq 0.85$. Standard techniques were used to evaluate model fit and relative importance of individual variables.

Results and Discussion

UVR was rapidly attenuated through the water columns of both ephemeral ponds and prairie pothole wetlands. The UV-B extinction coefficients (K_d B) ranged from 12.01 to 109.98 (m^{-1}) for the ephemeral ponds and 6.23 to 58.93 (m^{-1}) for the prairie pothole wetlands, with 1% of the surface radiation (Z1%) reaching depths of less than 34 cm (ephemeral ponds) and 74 cm (prairie pothole wetlands).

In contrast to lakes and some wetlands, water chemistry characteristics were not very strong predictors of K_d B for either type of wetland, combined or separate. The best models (based on AIC) explain less than 36% of the variation in K_d B (Table 2). True color, chl-A, and direct attenuation values (K_a B) were significant, but weak, predictors in several of the models (Table 2).

Table 1: Extinction coefficient for UV-B (K_d B) (mean, min, max, SD) for two wetland types in the Midwest.

			K_d B			
Wetland Type	**Year**	**n**	**Mean**	**Min**	**Max**	**SD**
Ephemeral Ponds	2002	25	47.54	12.01	109.98	32.46
Prairie Pothole Wetlands	2003	19	22.91	6.24	58.93	14.70
	2004	44	22.08	7.74	54.66	11.42
	Total	63	22.33	6.24	58.93	12.38
All wetlands		***88***	***29.49***	***6.24***	***109.98***	***23.03***
Where extinction coefficient slope had $R^2 \geq 0.85$						
Ephemeral Ponds	2002	16	62.26	15.46	109.98	31.92
Prairie Pothole Wetlands	2003	15	25.73	6.24	58.93	15.33
	2004	25	27.02	9.02	54.66	12.39
	Total	40	26.54	6.24	58.93	13.39
All wetlands		***56***	***36.74***	***6.24***	***109.98***	***25.89***

Table 2: Best models (based on AIC) for predicting extinction coefficient for UV-B (K_d B) from water chemistry characteristics for two wetland types in the Midwest. Grey = sig. at α = 0.05.

	Variables	R^2	Adjusted R^2	Mallow's C(p)	AIC*	p
All wetlands (n = 55)	ln(chl-a)	0.32	0.31	5.95	339.75	<0.001
	ln(color) ln(chl-a)	0.38	0.36	2.4514	336.16	<0.001
Ephemeral Ponds (n = 16)	ln(color)	0.24	0.18	-1.4169	109.44	0.055
	ln(K_a)	0.23	0.17	-1.2726	109.65	0.062
	ln(color) ln(TSS)	0.26	0.14	0.3463	111.08	0.147
Prairie Pothole Wetlands 2003 (n = 15)	ln(K_a)	0.28	0.23	-0.7988	79.85	0.041
	ln(color)	0.27	0.22	-0.6344	80.09	0.046
	ln(color) ln(K_a)	0.36	0.26	0.0738	80.10	0.067
Prairie Pothole Wetlands 2004 (n = 24)	DOC	0.05	0.003	0.9654	121.77	0.275
	ln(K_a)	0.03	-0.02	1.3634	122.22	0.386
	ln(color) ln(K_a)	0.12	0.04	1.3278	121.82	0.306
Prairie Pothole Wetlands 2003 & 2004 (n = 39)	ln(K_a)	0.11	0.08	0.5859	200.41	0.038
	ln(color)	0.09	0.06	1.4011	201.29	0.060
	DOC	0.07	0.05	2.0333	201.96	0.093
	ln(TSS) ln(K_a)	0.1303	0.082	1.702	201.43	0.058

*Akaike's Information Criterion

UVR was rapidly attenuated through the water column; 99% of UV-B attenuated within the top 35 cm of ephemeral ponds and within the top 75 cm of prairie pothole wetlands. However amphibian larvae could be exposed to damaging UVR levels in the upper 10 cm of the water column of many of the study wetlands. The Z65% levels (depth that 65% of surface radiation reaches), which represented the ED50 for sunlight-induced malformations in *R. pipiens* (Ankley et al. 2002), ranged from 0.5 to 3.6 cm in the ephemeral ponds and 0.9 to 8.2 cm in the prairie pothole wetlands. Developing amphibians commonly occupy this region of the water column and thus could be exposed to biologically significant UVR.

8. Climate change and amphibians.

Introduction

Previous studies using the simulation model WETSIM have demonstrated that prairie wetlands are highly sensitive to climate variability and climate change. WETSIM models the temporal and spatial dynamics of the hydrology and vegetation of a single semi-permanent wetland basin. Semi-permanent wetlands are relatively deep, discharge wetlands with surface water persisting through the ice-free season in most years. Initial experiments with WETSIM showed that a 3-4° C increase in air temperature greatly decreased wetland hydroperiod (the length of time a temporary pond retains water), slowed the speed of the wetland cover cycle, and increased drought frequency. Overall, the model wetland in a warmer climate was less productive, less dynamic, and supported lower biodiversity.

Results and Discussion

Recent studies applied an updated version of WETSIM to 18 long-term weather stations across the PPR to yield a broad geographic perspective of space and time variability in wetland characteristics during the 20th century. These simulations also served as a baseline against which future climate scenarios could be compared. The most striking result was that habitat for breeding water birds would shift under an effectively drier climate (3° temperature scenario) from the center of the PPR to the wetter eastern fringes, areas that are currently less productive or where most wetlands have been drained. Simulations also suggest that changing farming practices in wetland watersheds, by shifting from high water-use row crops to managed grassland, could offset some portion of the negative effects of climate warming on wetland water budgets, depending on how much the climate warms (Johnson et al. 2005).

Simulations just conducted using a new model, WETLANDSCAPE, provide additional insight into the potential effects of climate change on prairie wetlands. WETLANDSCAPE models a wetland complex, including the three PPR wetland permanence types: temporary, seasonal, and semi-permanent. Initial simulations indicate that wetlands in the northwestern portion of the PPR (Saskatchewan and Alberta, Canada) have already begun to dry from climatic warming during the 20th century. Quite surprisingly, simulations suggest greater vulnerability of the more permanent wetland types to climate warming if the magnitude of warming is uniform across seasons. For example, a 3° C warming could shorten annual hydroperiods of seasonal wetlands by an average of 2 months. WETLANDSCAPE promises to provide more complete analyses of wetland vulnerability to climate change than are currently possible.

Amphibians are ectotherms with life histories that are exceedingly sensitive to temperature and precipitation, and there is good evidence that recent climate change has already resulted in a shift to breeding earlier in the year for some species. Reduced snowfall and increased summer evaporation, outcomes predicted under recent IPCC emission scenario models, could have dramatic effects on the duration or occurrence of seasonal wetlands, which are primary habitat for many species of amphibians. Changes in temperature and precipitation regimes could result in changes in the distribution and abundance of amphibian populations.

The northern leopard frog (*Rana pipiens*) is an early breeding amphibian, which initiates calling soon after ice-out (typically April or early May in the PPR). Development from egg to metamorphic *R. pipiens* occurs in 80-120 days, with metamorphs usually egressing from natal wetlands in July and August. As adults, northern leopard frogs utilize a wide variety of wetlands and are found in many different landscape types; however this species prefers to breed in seasonal wetlands (shallower and more vegetated) immediately surrounded by grassland and in areas of high wetland density and low proportion of agricultural land use. These seasonal wetlands provide adequate protection (vegetation) while minimizing the probability of exposure to predators (fish, invertebrates, etc) and pathogens due to periodic drying. Using these habitat preferences and knowledge of *R. pipiens* reproductive behavior, we can investigate the potential impacts of climate change (increased temperatures, decreased rainfall) on this species' future distribution. We hypothesized that the effects of changes in temperature and precipitation (under different IPCC emission scenarios) on hydrology and hydroperiod may have large effects on amphibians. Early drying of wetlands may result in less time available to complete metamorphosis.

We used WETLANDSCAPE to model the impacts of a warmer climate on the hydroperiod of seasonal wetlands at different locations in the PPR. In all cases, an increase of 3°C uniformly applied across the year resulted in an approximately 25% decrease in the hydroperiod of seasonal wetlands experiencing the climate in Minot, ND and Academy, SD. Results from the literature suggest that *R. pipiens* requires 80-120 days for development from egg to metamorph. Our model results suggest that there is a 28% reduction in the number of years with hydroperiods greater or equal to 90 days after spring inundation under a 3°C increase in temperature at Academy, SD and a 65% reduction in the number of years with hydroperiod greater or equal to 90 days after spring inundation compared to historic means with a 6°C uniform increase in temperature. However, the model results changed with changes in geography. At Brookings, SD the changes in seasonal wetland hydrology were greater at 3°C (47% reduction compared to historic) than at Academy, SD. Areas with higher evaporative demand during the summer (to the north and west in the PPR) will likely have even more dramatic declines in the length of the minimum hydroperiod needed for *R. pipiens* metamorphosis.

Semi-permanent wetland hydroperiods are not as markedly affected by increases in atmospheric temperature (2% change in years with hydroperiods of greater than 90 days at 3°C elevated atmospheric temperatures). Earlier model results suggest that increasing atmospheric temperatures would result in semi-permanent wetlands drying more often and that semi-permanent wetlands located in the south and east of the PPR would most likely resemble seasonal wetlands which are favored by northern leopard frogs. The southern and eastern portions of the PPR are areas where semi-permanent wetland densities are quite low and the average distance between these wetlands is considerably less than seasonal wetlands. In addition, this is an area dominated by row crops in the upland and not grassland cover. These are all landscape factors that our logistic regression models suggest are not optimal for northern leopard frog breeding. Furthermore, many semi-permanent wetlands support fish populations, which potentially prey on amphibian larvae. Thus amphibian metapopulation survival will depend on both the landscape context in which remaining wetlands are embedded (e.g., inter-wetland distance and surrounding land use), as well as local conditions within the wetland.

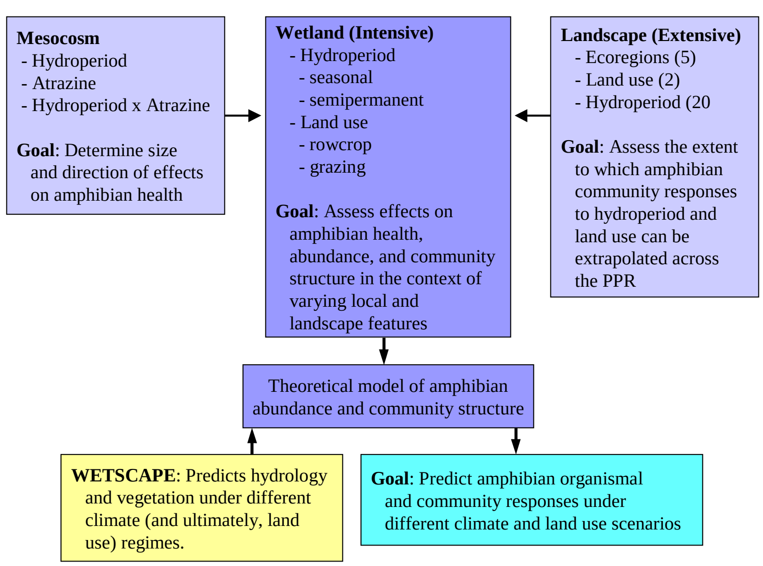
<u>**Effects of Multiple Stressors on Aquatic Communities in the Prairie Pothole Region**</u>

Summary of Project Results

Amphibians in the PPR have adapted to stressors such as variable water levels, predators, pathogens, and various types of competitors. As the landscape has shifted from undisturbed prairie to rowcrop and grazing agriculture, anthropogenic stressors, such as pesticides, nutrients, habitat degradation and fragmentation, roads, and livestock have been added, as well as increased exposure to UV radiation. And finally, we anticipate that the overarching effects of global climate change will begin to be realized within the next several decades, and may exacerbate or intensify the impact of existing stressors. Various climate-change scenarios have been proposed, with the majority focusing on increasing air temperatures of 3 – 5 degrees Celsius throughout the region over the next century.

We examined the effects of several existing and anticipated stressors on a model amphibian, the northern leopard frog (*Rana pipiens*), which is widely distributed over the PPR, as well as other parts of the US, and has been the subject of current ecotoxicological research addressing pathogenesis and epidemiology, malformation, and population declines. Our research design (Figure 1) used a series of hierarchically integrated studies to determine the current conditions for amphibians in the PPR and to predict how changing stressors could affect them in the future. We conducted field studies at both wetland and landscape scales with the overall goal of predicting effects over larger area using data from smaller-scale studies. Mesocosm-based experiments tested some specific effects of combining two stressors, atrazine and accelerated hydroperiod, on *R. pipiens* survival and growth. Finally, combining models of PPR wetland response to climate change with our field-derived data will allow us to predict conditions that will be necessary for sustaining *R. pipiens* populations.

Figure 1. Multiple Stressors Project Organization



Our data indicated that *R. pipiens* prefers to breed in seasonal wetlands; those that tend to dry at least once through the year. This appears to be adaptive, since the periodic drying reduces pathogen loads and eliminates most fish populations, which can be major predators of tadpoles. Logistic modeling, based on an extensive survey of seasonal and semipermanent wetlands throughout the US PPR, suggests that physiologically-driven constraints for *R. pipiens* breeding include a requirement that the water not exceed 28°C, and that they appear to prefer depths of at least 30 cm, although breeding can take place in shallower water. Modeling also indicates that the probability of breeding depends on the distribution of suitable wetland habitats in the proximity; *R. pipiens* are peripatetic, and will travel considerable distances to breed. However, our data indicates that breeding *R. pipiens* were not found in wetlands separated by 1500 m or more.

Natural history data for *R. pipiens* indicates that full development requires 80-120 days. In addition, work on these and other anuran species has shown that accelerated development (i.e. complete development at the shorter end of that range) produced smaller metamorphs with reduced fat stores; conditions that are negatively correlated with winter survival and fecundity. Other stressors, such as pesticides, have also been shown to change developmental rates. However, our mesocosm experiments did not show a correlation between accelerated hydroperiod (i.e. simulated drying) and survival, successful completion of metamorphosis, or size at metamorphosis. In addition, atrazine exposure did not significantly affect any of these parameters, except at the 200 ppb concentration, in which metamorphosis was delayed or inhibited.

Atrazine was detected in virtually of our test sites, and wetland concentrations corresponded broadly to presumed pesticide use in the regional uplands. Although wetland atrazine concentrations rarely approached established levels of concern for aquatic organisms, they often exceeded concentrations reported to act as endocrine disruptors in frogs. Notably, testicular oocytes were commonly found in male metamorphs throughout the PPR; however, there appeared to be no correlation between atrazine presence or concentration and the prevalence or intensity of the abnormality. One possible explanation could be that *Rana pipiens* males may routinely undergo a transitory hermaphroditic stage prior to complete development, as occurs in some other anurans. Further histological analysis of the specimens cultured under various atrazine exposure regimes in the mesocosm experiments may help further clarify this phenomenon.

Hydrology models have indicated that the PPR wetlands are extremely responsive to changing climatic conditions. The WETSCAPE and WETLANDSCAPE models used in the current study predict that with a temperature increase of 3°C, seasonal ponds will dry earlier in the season and are not likely to have the minimum hydroperiod necessary for *R. pipiens* metamorphosis, and that semipermanent wetlands will begin to act more like seasonal wetlands. Under this climate scenario, *R. pipiens* breeding success and survival will depend on the distribution of semipermanent wetlands in the landscape and characteristics of the surviving wetlands.

References

Ankley GT, Diamond SA, Tietge JE, Holcombe GW, Jensen KM, Defoe DL, and Peterson R. 2002. Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (*Rana pipiens*). Environmental Science and Technology 36:2853-2858

US Environmental Protection Agency. 2003. Interim Reregistration Eligibility Decision documents. http://www.epa.gov/oppsrrd1/REDs/atrazine_combined_docs.pdf

Gardiner DM, Hoppe DM. 1999. Environmentally induced limb malformations in mink frogs (*Rana septentionalis*). J Exp. Zool. 284:207-16

Hoppe DM. 2001. Linking malformations to amphibian declines: history of malformed anurans in Minnesota and interspecific differences in their occurrence, in MJ Lannoo, ed., Status and Conservation of United States Amphibians Volume 1: Conservation Essays: Berkeley, Calif. University of California Press.

Hubbard DE. 1988. Glaciated prairie wetland functions and values: a synthesis of the literature. U.S. Fish and Wildlife Service Biological Report 88(43). Fort Collins, CO.

Johnson PTJ, Lunde KB, Haight RW, Bowerman J, and Blaustein AR. 2001. *Ribeiroia ondatrae* (*Trematoda: Digenea*) infection induces severe limb malformations in western toads (*Bufo boreas*): Canadian Journal of Zoology 79:370-379

Johnson PTJ, Lunde KB, Zelmer DA, Werner JK. 2003. Limb deformities as an emerging parasitic disease in amphibians: Evidence from museum specimens and resurvey data. Conservation Biology 17:1724-1737

Johnson WC, Mellett BV, Gilanov R, Voldseth RA, Guntenspergen GR, Naugle DE. 2005. Vulnerability of Northern Prairie wetlands to climate change. BioScience 55:863-872

Schoff PK, Johnson CM, Schotthoefer AM, Murphy JE, Lieske C, Cole RA, Johnson LB, Beasley VR. 2003. Prevalence of skeletal and eye malformations in frogs from north-central United States: estimations based on collections from randomly selected sites. Journal of Wildlife Diseases 39:510-521.

van der Valk AG, ed. 1989. Northern Prairie Wetlands. Iowa State University Press, Ames.

Graduate Student

Angela K. Rohweder, M.S. (anticipated Fall 2007)
Thesis title: Correlation between atrazine and testicular oocytes in the northern leopard frog (*Rana pipiens*).

Presentations

Rohweder, A.K., Olker, J.H., Johnson, L.B., Schoff, P.K. Relationships between land use, atrazine and amphibian malformations in the Prairie Pothole Region. (presentation; Society for Environmental Toxicology and Chemistry, Midwest Regional Meeting, Madison, WI, April 2005)

Rohweder, A.K., Olker, J.H., Schoff, P.K., Johnson, L.B., Guntenspergen, G.R. A survey of atrazine in prairie pothole wetlands and preliminary gonadal examination of *Rana pipiens*. (presentation; Society for Environmental Toxicology and Chemistry North America annual meeting, Montreal, QC, Canada, November 2006)

Presentations

Schoff, P.K., Johnson, L.B., Guntenspergen, G.R., Johnson, W.C. Multiple Stressors and Amphibian Malformations: Cause, Correlation, Coincidence? (presentation; USGS Upper Midwest Environmental Science Center, La Crosse, WI, February, 2004)

Olker, J.H., Rohweder, A.K., Schoff, P.K., Johnson, L.B., Guntenspergen, G.R. Effects of multiple stressors on amphibian communities in the prairie pothole region. (presentation; Minnesota Chapters of the Society of Conservation Biology and American Fisheries Society joint annual meeting, Grand Rapids, MN, March 2005)

Rohweder, A.K., Olker, J.H., Johnson, L.B., Schoff, P.K. Relationships between land use, atrazine and amphibian malformations in the Prairie Pothole Region. (presentation; Society for Environmental Toxicology and Chemistry, Midwest Regional Meeting, Madison, WI, April 2005)

Stricker, C.A., Guntenspergen, G.R., Rye, R.O. Stable isotope evidence linking prairie wetland food webs to biogeochemical processes (presentation, American Geophysical Union Annual Meeting, May, 2005)

Olker, J.H., Hollenhorst, T.P., Johnson, L.B., Schoff, P.K., Rohweder, A.K., Guntenspergen, G.R. Effects of Hydrology and Land Use on Amphibian Breeding Potential and Community Structure in the Prairie Pothole Region. (presentation; Minnesota Chapters of the Society of Conservation Biology, American Fisheries Society, and Wildlife Society joint annual meeting, Brainerd, MN, March 2006)

Hollenhorst, T.P., Olker, J.H., Johnson, L.B., Schoff, P.K., Guntenspergen, G.R. A probabilistic sampling design for aquatic communities in the prairie pothole region. (presentation; Minnesota Chapters of the Society of Conservation Biology, American Fisheries Society, and Wildlife Society joint annual meeting, Brainerd, MN, March 2006)

Rohweder, A.K., Olker, J.H., Schoff, P.K., Johnson, L.B., Guntenspergen, G.R. A survey of atrazine in Prairie Pothole wetlands and preliminary gonadal examination of *Rana pipiens*. (presentation; Society for Environmental Toxicology and Chemistry, Midwest Regional Meeting, St. Cloud, MN, March 2006)

Olker, J.H., Hollenhorst, T.P., Johnson, L.B., Schoff, P.K., Rohweder, A.K., Guntenspergen, G.R. Effects of Hydrology and Land Use on Amphibian Breeding Potential and Community Structure in the Prairie Pothole Region. (presentation; Society of Conservation Biology Annual Meeting, San Jose, CA, June 2006)

Rohweder, A.K., Olker, J.H., Schoff, P.K., Johnson, L.B., Guntenspergen, G.R. A survey of atrazine in prairie pothole wetlands and preliminary gonadal examination of *Rana pipiens*. (presentation; Society for Environmental Toxicology and Chemistry North America annual meeting, Montreal, QC, Canada, November 2006)

Johnson, L.B., Guntenspergen, G.R, Olker, J.H., Schoff, P.K., Johnson, W.C., Hollenhorst, T.P., Rohweder, A.K., Johnson, C.M. Predicting effects of climate change on amphibian communities from prairie pothole wetlands. (presentation; American Society of Limnology and Oceanography Aquatic Sciences Meeting, Santa Fe, NM, February 2007)

Olker, J.H., Hollenhorst, T.P., Johnson, L.B., Schoff, P.K., Johnson, C.M., Guntenspergen, G.R., Johnson, W.C., Beasley, V. Broadscale land use impacts on northern leopard frog (*Rana pipiens*) in the upper Midwest. (presentation; Amphibian Research and Monitoring Initiative workshop “Understanding agriculture’s effects on amphibians and reptiles in a changing world,” St. Louis, MO, February 2007)

Schoff, P.K., Rohweder, A.K., Olker, J.H., Hollenhorst, T.P., Johnson, L.B., Guntenspergen, G.R. A survey of atrazine in prairie pothole wetlands and preliminary gonadal examination of *Rana pipiens* (presentation; Amphibian Research and Monitoring Initiative workshop “Understanding agriculture’s effects on amphibians and reptiles in a changing world,” St. Louis, MO, February 2007)

Manuscripts in preparation

Potential effects of climate change on amphibians in the Prairie Pothole Region. (Science)

UV Radiation and attenuation in Midwestern wetlands. (Wetland Ecology)

Atrazine concentrations in Prairie Pothole wetlands. (Environmental Toxicology and Chemistry)

Relationship between ambient atrazine concentration and testicular oocytes in *Rana pipiens* metamorphs. (Environmental Toxicology and Chemistry)

Malformed anurans in the central Midwest US. (Journal of Wildlife Diseases)

Acknowledgments and disclaimers

This research has been supported by a grant from the U.S. Environmental Protection Agency’s Science to Achieve Results (STAR) program. Award number RD-83087901.

Although the research described in this report has been funded wholly or in part by the U.S. Environmental Protection Agency’s STAR program through grant (number), it has not been subjected to any EPA review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.