Wetland Atrazine Concentrations and Occurrence of Testicular Oocytes in *Rana pipiens* from Randomly-Selected Sites in the Prairie Pothole Region

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Abstract

The Prairie Pothole Region (PPR) of the United States supports a high density of wetlands interspersed in an agricultural matrix. Row-crops, primarily corn and soybeans, dominate the southern PPR, while small grains and grasslands typify the north. Recent laboratory and field studies report that the corn herbicide atrazine disrupts normal sexual development in amphibians. In 2005, we measured atrazine concentrations during three surveys in 10 wetlands distributed from the southern (intensive corn production) to the northern (sparse or no corn production) PPR and collected *Rana pipiens* (northern leopard frog) metamorphs. Atrazine was detected in at least one water sample from every wetland, with concentrations ranging from < 0.011 (i.e. < method nominal detection limit) to 0.805 µg/L. The overall sex ratio did not diverge from 1:1; however there was a strong negative correlation between average atrazine concentrations at each site where more than 25 total specimens were collected and the female to male sex ratio. Gross gonadal abnormalities were noted in 3% of all metamorphic *R. pipiens.* The gonads of 55 % of all fully metamorphosed (Gosner stage 46) males contained testicular oocytes (TO's). Atrazine concentrations were not significantly correlated with gross gonadal anomalies or TO's. The lack of consistent, significant correlations between atrazine concentrations and gonadal anomalies in *R. pipiens* does not support the hypothesis linking atrazine with endocrine disruption in developing amphibians.

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Introduction

Atrazine has been one of the most frequently used herbicides worldwide since its registration in the United States in 1959 [1]. Nearly 35,000 metric tons of active ingredient were applied on crops in the United States in 2002 alone [2]. Although primarily used to control annual broadleaf and grassy weeds in corn, atrazine is also used in the production of other crops, including sorghum, Christmas trees, sugarcane, as well as on rangeland. The herbicide is usually applied in early spring and summer by soil-directed ground spraying, but aerial spraying is also used, depending on weather and field conditions. While primarily considered a pre-emergent herbicide, applications are also made prior to planting and post emergence [3].

Because of its widespread application, relatively slow degradation, and transport through water and air, atrazine often migrates great distances from where it is originally applied [4]. Atrazine is commonly detected in surface [1] and ground water [5], as well as in air [6] and rain [7]. Nations and Halberg [8] reported concentrations as high as 40 µg/L in rainfall in the Midwestern US, and approximately 0.6% of atrazine applied yearly to crops in the Midwestern and Northeastern United States was deposited via rainfall [7]. Dissolved atrazine can also be transported via runoff [9,1,10]. Scribner et al. [11] reported median atrazine concentrations ranging from 4.27-10.9 µg/L in Midwestern streams during post-application runoff, while a single maximum concentration of 172.2 µg/L was measured in another study of Midwestern streams [12]. Although volatilization of atrazine is minimal [1], some pesticides enter the atmosphere via wind erosion [6],

which can be transported great distances and return to the surface via wet or dry deposition [7].

In 2005, approximately 88% of atrazine used in the US was applied to corn [13], which is grown primarily in Iowa and Illinois and areas of Minnesota, South Dakota, Nebraska, Kansas, Missouri, Indiana and Ohio. Portions of this corn production area are contained within the Prairie Pothole Region (PPR) of the North-Central United States (Figure 1), a region characterized by high densities of wetlands of various sizes and hydrologic regimes, interspersed in a matrix of row crop agriculture, pasture, and grassland.

Prairie Pothole Region wetlands support multiple amphibian species such as salamanders, toads and frogs, including *Rana pipiens* (northern leopard frog), which has been a focal species for numerous studies investigating possible endocrine disrupting effects of atrazine [14,15]. Recent studies have reported conflicting data regarding the effects of atrazine on amphibian sexual development. Some laboratory studies suggest that exposure to low atrazine concentrations during development caused hermaphroditism (occurrence of testicular and ovarian tissue within the same gonad) [14,16] in *Xenopus laevis* and *R. pipiens* [17], while other researchers found no significant gross morphological or histological gonadal effects in similar exposures studies using *Rana clamitans* [18] or *X. laevis* [19].

Field studies have generated similarly disparate data. Hayes et al., [17] reported that *R. pipiens* collected from atrazine-contaminated sites across the United States exhibited hermaphroditism and Reeder et al. [20] reported a strong but non-significant

relationship between atrazine and intersex (animal with both male and female gametes) cricket frogs (*Acris crepitans*). However, in other field studies atrazine was not significantly correlated with hermaphroditism [21] or with the presence of testicular oocytes (TO's; egg cells embedded in testicular tissue and an indicator of hermaphroditism) (Figure 2) [15] in various ranids, nor with TO presence in *X. laevis* exposed in outdoor mesocosms [22]. The primary objectives of the current study were to 1) determine the prevalence of testicular oocytes (TO's) in metamorphic *R. pipiens* harvested from randomly selected wetlands distributed across the PPR, and 2) explore possible correlations between the range of atrazine concentrations in these wetlands and the presence or prevalence of testicular anomalies in metamorphic *R. pipiens*.

Materials and Methods

Site Selection

Our objectives included measurement of atrazine concentration throughout the *R. pipiens* breeding and developmental period and collection of metamorphic (Gosner stage 44-46; [23] frogs as they emerged from the wetlands. We used a stratified, random process to select 109 PPR wetlands (sites) representing a land-use gradient from predominantly row-crop agriculture in the south to small grain and grassland agriculture in the north. Ten of these wetlands produced *R. pipiens* metamorphs and constitute the study sites (Figure 1).

Site Surveys and Atrazine Analysis

Each site was surveyed three times during the spring and summer to collect water and conduct biotic surveys. Survey timing was based on local knowledge of agricultural practices and amphibian phenology, and was planned to coincide with anticipated herbicide applications or significant stages in *R. pipiens* life history. The first survey and water sampling timed to take place prior to annual atrazine application and expected *R. pipiens* egg-laying, the second survey was intended to take place after atrazine application and during *R. pipiens* embryo to early larval period, and the third in conjunction with the final stages of *R. pipiens* metamorphosis, but prior to their emergence and emigration from the wetland. In each set of surveys, site visits progressed from south to north, which was designed to correlate with the presumed timing of corn planting (and associated herbicide application) and *R. pipiens* breeding and development. Collection dates were as follows: Survey 1 (S1), March 30 - May 10; Survey 2 (S2), May

18 - June 7; Survey 3 (S3), June 21 – July 11. Water samples were hand-collected from the approximate center of each wetland using a 50 ml plastic syringe, filtered through a 0.2 µm membrane, placed on ice during transport, and held at or below 4º C until analysis. Atrazine was quantified using an enzyme-linked immunosorbent assay (ELISA) with a nominal detection limit of 0.011 µg/L (Atrazine HS; Abraxis LLC, Warminster, PA.). Two aliquots from each water sample were analyzed and averaged to obtain a single atrazine concentration for each wetland.

Metamorphic Rana pipiens Collection

Visual searches for eggs, larvae and adults, site perimeter searches and random dip net sweeps were used to assess *R. pipiens* breeding and development during the three surveys, and specimen collections were planned to coincide with metamorphosis. Collections were made using dip nets and continued until either 100 metamorphic *R. pipiens* were collected or a total of 240 person-minutes had elapsed. Specimens were euthanized in 3-amino benzoic acid ethyl ester (MS-222), 2 g/L. Immediately following euthanization, a 1cm longitudinal cut was made through the abdominal wall and specimens were fixed in Bouin's solution (Sigma, St. Louis, MO) for 48 hours, then transferred to 70% ethanol, which was replaced every 48 hours until the yellow pigment was substantially absent.

Gross Gonadal Morphology

Each metamorph was assigned a randomized unique identifier, which was used throughout all analyses so that specimen origin was unknown to the examiner until after all analyses were completed. Metamorphs were measured for snout-vent length (SVL) and morphological sex (i.e. male, female or ambiguous, based on gonadal anatomy) was determined. All gonads were digitally photographed, then removed from the body cavity with kidneys attached and placed in 70% ethanol in individual vials until histological analysis.

Histological Analysis

Gonads with attached kidney tissues were prepared for histological analysis using a Tissue Tek VIP Processor (Tissue Tek; Analytical Instruments, Minneapolis, MN.) in 40-minute stages. Tissues were dehydrated in a series of seven ethyl-alcohol stages (70%, 80%, 95%, 95%, 100%, 100%, 100%), followed by three clearing stages using Clear-Rite (Richard Allen Scientific, Kalamazoo, MI), and four infiltration stages with Paraplast tissue embedding media, (Electron Microscopy Sciences, Hatfield, PA). Tissues were then embedded using a Tissue Tek embedding console, and 5µm thick longitudinal sections were cut throughout both gonads using a Reichert-Jung rotary microtome. Each set of gonads was sectioned in its entirety with every fifth section mounted and stained with Harris's hematoxylin and eosin using standard methods.

Statistical Analyses

Potential relationships between atrazine concentrations and metamorph size (SVL), gross gonadal abnormalities, TO prevalence (number of specimens per site having TO's) and mean number of TO's per specimen were tested using Pearson's correlation test ($\alpha = 0.05$). Atrazine concentrations from individual surveys (S1, S2 and S3 concentrations) and multiple surveys (S1+S2+S3, S2+S3 concentrations) as well as maximum observed concentration were used in these analyses. When testing correlations between atrazine and TO prevalence or mean number of TO's per specimen, all 10 sites were initially included in the analysis, then analyses were repeated using data from only the five sites containing more than 10 stage 46 males. Statistica 9.7 was used for all statistical analyses (Statistica 9.7, StatSoft, Tulsa, OK).

Results

Atrazine

Atrazine was detected in water samples from all 10 study wetlands (sites) during at least one of three surveys (S1, S2 and S3) in 2005; concentrations ranged from < 0.011 (method nominal detection limit) to 0.805 µg/L and were generally lowest during S1, increased in S2, and remained elevated in S3 (Figure 3). Wetland atrazine concentrations showed a significant negative correlation with latitude ($r = -0.64$, $\alpha = 0.05$, $p = < 0.05$), with southernmost wetlands containing higher mean atrazine concentrations than northernmost sites (Figure 3). Atrazine concentrations in samples from the subset were not significantly different from concentrations in the entire set of 109 study sites (< 0.011 to 33.80 µg/L) (2-sample t-test, $p = > 0.3$).

Rana pipiens Growth, Sex Ratio, and Gonadal Gross Morphology

A total of 514 Gosner stage 44-46 [23] *R. pipiens* were collected from the 10 study wetland sites (Table 1). Mean snout-vent length (SVL) for all specimens was 43.1 mm. Considerably lower mean SVL's of 34 and 32.1 mm were measured at sites 3 and 4, respectively, however no significant differences in SVL were noted at sites with > 25 total specimens. Gross morphological analysis of gonads revealed an overall female to male sex ratio of 1.02:1 (Table 1). Notably, in sites with more than 25 specimens, there was a strong negative correlation between average atrazine concentration and female to male sex ratio. ($r = - 0.92$, $p < 0.05$; $n = 6$; Figure 4).

Abnormal gonads were detected in 3% (16/514) of the metamorphs representing three of 10 sites. The most frequent anomaly was discontinuous or constricted testes (n = 10 specimens), followed by unidentifiable tissue masses located on or near the testes (n = 3 specimens) and shallow-lobed ovaries (n = 3 specimens). There was no significant correlation between atrazine concentration and gross gonadal abnormalities.

Gonadal Histology

Testes from 164 Gosner stage 46 male *R. pipiens* were examined histologically for the presence of testicular oocytes (Figure 2, Table 1). Fifty-five percent (90/164) of the specimens contained one or more TO, while at least one specimen from every site exhibited this anomaly (Table 1; Figure 5). The median number of TO's per affected specimen was 18.5; however, several specimens had comparatively high numbers of TOs (maximum 1856), which produced an overall mean of 78.9 TO's per affected specimen (Table 1). The total number of TOs in the left versus right testis differed significantly in 24% (22/90) of the affected specimens when tested individually; there was no preference for the left or right testis in these 22 specimens. However, across all affected specimens there was no significant difference in TO abundance between right and left gonads (two-sided t-test, $\alpha = 0.05$).

There was no significant difference between TO prevalence (number of specimens per site with TO's) or mean number of TO's per specimen and any of our atrazine measures, including concentrations from individual surveys (S1, S2, S3), combinations of surveys (mean of S1, S2, and S3 (Figure 6), mean of S2 and S3), or

maximum concentrations for all sites (Figure 5). In addition, there was no correlation between TO prevalence or quantity and gross gonadal abnormalities.

Discussion

Because we utilized a stratified random sampling design to systematically and uniformly sample wetlands across five ecoregions, we are able to conclude with confidence that atrazine is present throughout the PPR, as it was detected in wetlands from northern Iowa to northern North Dakota. Prairie pothole region land use varies from heavy row-crop agriculture (primarily corn and soybeans) in the south, to grasslands, pasture and small grains (wheat, oats and barley) in the north. In 2005, 12.8 million acres of field corn were planted in Iowa, 7.3 million acres in Minnesota, 4.45 million acres in South Dakota and approximately 1.4 million acres in North Dakota. Because corn production is much more widespread in the southern portion of the PPR (northern Iowa, southern South Dakota and western Minnesota) than in the north (northern South Dakota and North Dakota) [24; http://www.nass.usda.gov], we expected to measure regional differences in atrazine concentrations in PPR wetlands. Also, based on limited corn production and low reported atrazine use in North Dakota compared to other states within the PPR [24; http://www.nass.usda.gov], we anticipated that the northernmost wetlands would be atrazine-free and could be used as reference sites.

While we observed the expected latitudinal concentration trend (Figure 3), we were unable to find sites that had no atrazine sometime during the spring or summer. This suggests that either atrazine was used more widely in northern regions than expected, or that residues were transported to non-target locations [4] via wind [25] and wet [26] or dry [27; http://www.epa.gov/glnpo/lmmb/results/atra_datarpt.html] deposition.

Atrazine concentrations throughout the PPR were typically lowest in early spring (S1) and peaked mid-May through July (S2 or S3). The low concentrations detected during survey 1 suggest that the first survey was conducted prior to herbicide application and that early spring atrazine may have been carried over from the previous year or deposited from remote areas. The substantially higher atrazine concentrations detected during surveys 2 and 3 indicate that the majority of atrazine found in PPR wetlands during the summer entered following local or remote application. The trend of increasing atrazine concentration throughout the spring and summer, as well as the range of atrazine concentrations (< 0.011 - 0.805 µg/L) reported in the 10 study sites was also observed in the total 109 sites the larger study, which suggests that reported concentration trends and means accurately reflect the PPR landscape means. Similar concentrations and seasonal patterns were detected in agricultural regions of Canada [15] and Michigan [21]. Atrazine concentrations in the majority of our samples from these 10 sites were < 0.1 µg/L, however, two samples with high concentrations (0.700 and 0.805 µg/L) were collected from Sites 1 and 8 respectively (Figure 3; Figure 5). Wetland atrazine concentrations in this range are not uncommon in areas of heavy corn production. Concentrations of 0.700 µg/L were measured in both Michigan [21], and Canada [15]. Furthermore, much higher atrazine concentrations have been encountered in field sites across the PPR, including > 6 µg/L measured in water from a ditch adjacent to a corn field in central Iowa [17] and concentrations of 5.6 and 33.8 µg/L in PPR wetland water samples (Schoff et al., unpublished).

A portion of the data retrieved from each of our sites depended on the presence of metamorphic *R. pipiens* at the end of the summer, and initial site selection occurred without prior knowledge of amphibian presence or breeding. Because of this, we acquired a relatively small number of sites with substantial numbers of metamorphic *R. pipiens.* However, atrazine concentrations measured at these 10 sites are characteristic of PPR atrazine concentrations measured in 109 stratified, randomly selected wetlands during the same year.

Many other field studies have used atrazine sales [17] and agricultural land-use data [21,15] or the presence of substantial amphibian populations [21] to drive site selection. While using these *a priori* methods may increase the likelihood of selecting wetlands with the desired high or low atrazine concentrations and/or sizeable amphibian populations, they may also introduce non-random sampling bias. Results generated from studies incorporating non-random sampling of amphibian populations must be interpreted cautiously because studied populations may not be truly representative of the regional amphibian population and the value of statistical tests inherently decrease as a result. This is because most statistical tests only consider random error, and if populations are not randomly selected, these tests may exaggerate the actual level of significance.

There was a strong negative correlation between average atrazine concentrations at each site where more than 25 total specimens were collected and the female to male sex ratio ($r = -0.92$, $p < 0.05$, $n = 6$, Figure 4), however the overall sex ratio did not significantly differ from an expected 1:1 ratio (Table1) ($X^2 = 1.69$, $p = 0.89$, d.f. = 5). The small sample size of this study precludes the conclusion that atrazine causes lower

female to male sex ratios; however Coady et al. [19] also reported male-skewed sex ratios in frogs. Although they did not find consistent (experiment-wide) deviations from an expected 1:1 sex ratio, they reported that laboratory-reared *X. laevis* exposed to 0.1 µg/L atrazine produced significantly male-skewed sex ratios.

Three percent of all *R. pipiens* metamorphs in the current study had grossly abnormal gonads. The most common anomaly was discontinuous testicular tissue, which was also the most common gross gonadal anomaly in a laboratory study of metamorphic *X. laevis* [19] and was exhibited in four percent of all *X. laevis* serving as reference specimens in an outdoor mesocosm study in South Africa [22]. We found no correlation between discontinuous testes and atrazine concentration, or the presence or prevalence of TO's in Gosner stage 46 *R. pipiens*, which was also consistent with results reported by Jooste et al. [22] in *X. laevis*.

Notably, TO's were detected in specimens from all 10 of our PPR study sites, which is comparable to results published by Murphy et al. [21], in which TO's were found in specimens from seven of eight agricultural and non-agricultural sites sampled in south-central Michigan in 2002 [21]. In addition, TO's were found in 55% of all fully metamorphosed *R. pipiens* individuals collected during our study (Table 1), which is similar to TO prevalence in *R. pipiens* collected from an agricultural region of Canada [15]. Testicular oocytes have also been identified in other species, including *R. clamitans* [18,21] and *R. catesbeiana* [21]. However, TO prevalence may differ among species, because Murphy et al.[21] reported that juvenile *R. pipiens* displayed a 4-20-fold greater incidence of TO's than other ranids. Jooste et al. reported a TO prevalence of 57% in *X.*

laevis reference specimens and 57% in those exposed to 1µg/L atrazine in an outdoor microcosm experiment [22]. In recent years, additional field and laboratory studies have reported TO occurrence in atrazine-exposed frogs. Testicular oocytes were noted in ranids (*R. pipiens, R. clamitans* and *R. catesbeiana*) from Michigan agricultural areas [21] and in *R. pipiens* and *R. clamitans* from agricultural regions of Canada [15]. Hayes et al. [17] reported that > 60% of developing *R. pipiens* exposed to 0.1 µg/L atrazine expressed TO's. Similar atrazine concentrations of ≥ 0.1 µg/L were common in PPR wetlands; eight of our ten sites contained ≥ 0.1 µg/L atrazine at least once during the study, and half of all water samples met or exceeded this concentration. Consequently, developing anurans could have been exposed to critical concentrations of atrazine during sensitive developmental periods.

Although TO's were present in over half of our specimens, we found no correlation between TO prevalence or quantity in Gosner stage 46 *R. pipiens* and mean atrazine (Figure 6), S1 or S2 atrazine concentrations. Other studies also report a lack of positive correlation between TO prevalence in *R. pipiens* [15] or *X. laevis* [19] and atrazine. However, McDaniel et al. [15] reported a correlation between the proportions of frogs with TO's and the number of different pesticides at each site, suggesting that TO's may have resulted from exposure to multiple chemical stressors. Orton et al. [28] reported a female-skewed sex ratio in *R. pipiens* exposed to a combination of nitrate and atrazine, further supporting the hypothesis that multiple stressors may result in partial or complete feminization in frogs. Although atrazine was the only chemical measured in wetlands as part of our study, numerous pesticides and other chemicals are commonly

used in the PPR [29]. Besides direct agricultural applications, chemicals can be deposited via rainfall. Goolsby et al. [7] detected atrazine and other herbicides in rainfall on Isle Royale, in Lake Superior. Thus, *R. pipiens* could have been exposed to multiple chemicals throughout their breeding and developmental periods. Although we didn't observe a shift in expected sex ratios or increased prevalence of TO's in atrazine-exposed populations of *R. pipiens*, exposure to multiple anthropogenic stressors has recently been associated with female-skewed sex ratios [28], testicular oocyte prevalence [15] and potential amphibian decline [30].

While it has been speculated that atrazine may cause a disruption of normal developmental processes in *R. pipiens* [17], testicular oocytes were identified in ranid species decades before atrazine was registered for use in the United States [31,32]. These early data suggest TO expression may be part of a normal, transient phase for *R. pipiens*, rather than a result of atrazine exposure. Further laboratory, mesocosm and field studies are necessary to assess the combined effects of multiple stressors on amphibian development.

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Table 1. Site characteristics, field-collected R. pipiens by site, and testicular oocyte (TO) prevalence and quantity in fully metamorphosed males.

Site Characteristics and Atrazine Concentration				All Specimens[c]			Gosner Stage 46 Males		
Site	Lat N	LC[a]	Mean Atrazine (µg/L)[b]	n	M	Sex Ratio[d] (F:M)	n	TO Prevalence	Average # TO/Affected Specimen (SE)
1	43.090	Crop	0.700	31	18	0.72:1	16	0.94	54.5 (20.9)
2	43.357	Grass	0.264	26	15	0.73:1	11	0.73	13.5 (4.2)
3	44.462	Grass	0.088	10	5	1:1	3	1.00	19 (16.0)
4	44.658	Crop	0.080	129	61	1.11:1	25	0.16	64 (37.4)
5	45.561	Crop	0.187	98	51	0.92:1	50	0.20	8.3 (3.3)
6	46.223	Grass	0.113	2	2		2	0.50	335 (NA)
7	46.731	Crop	0.096	9	7	0.29:1	7	0.57	14 (7.0)
8	46.818	Grass	0.291	2	1	1:1	1	1.00	1 (NA)
9	47.258	Grass	0.028	121	55	1.2:1	41	0.93	140.3 (49.3)
10	47.767	Grass	0.017	86	40	1.15:1	8	0.75	9.8 (2.7)
Overall				514	255	1.02:1	164	0.55	78.9 (22.1)

Site numbers ordered by latitude.

[a]Land cover classification based on > 70% crop or grassland within a 90 m buffer around wetland (USGS , GAP Analysis Program).

[b]Average atrazine concentration by site. Water samples collected during three surveys (S1, S2, S3) between March-July 2005.

[c]Gosner stage 44-46 specimens.

[d]Sex determination based on gross morphological analysis of gonads.

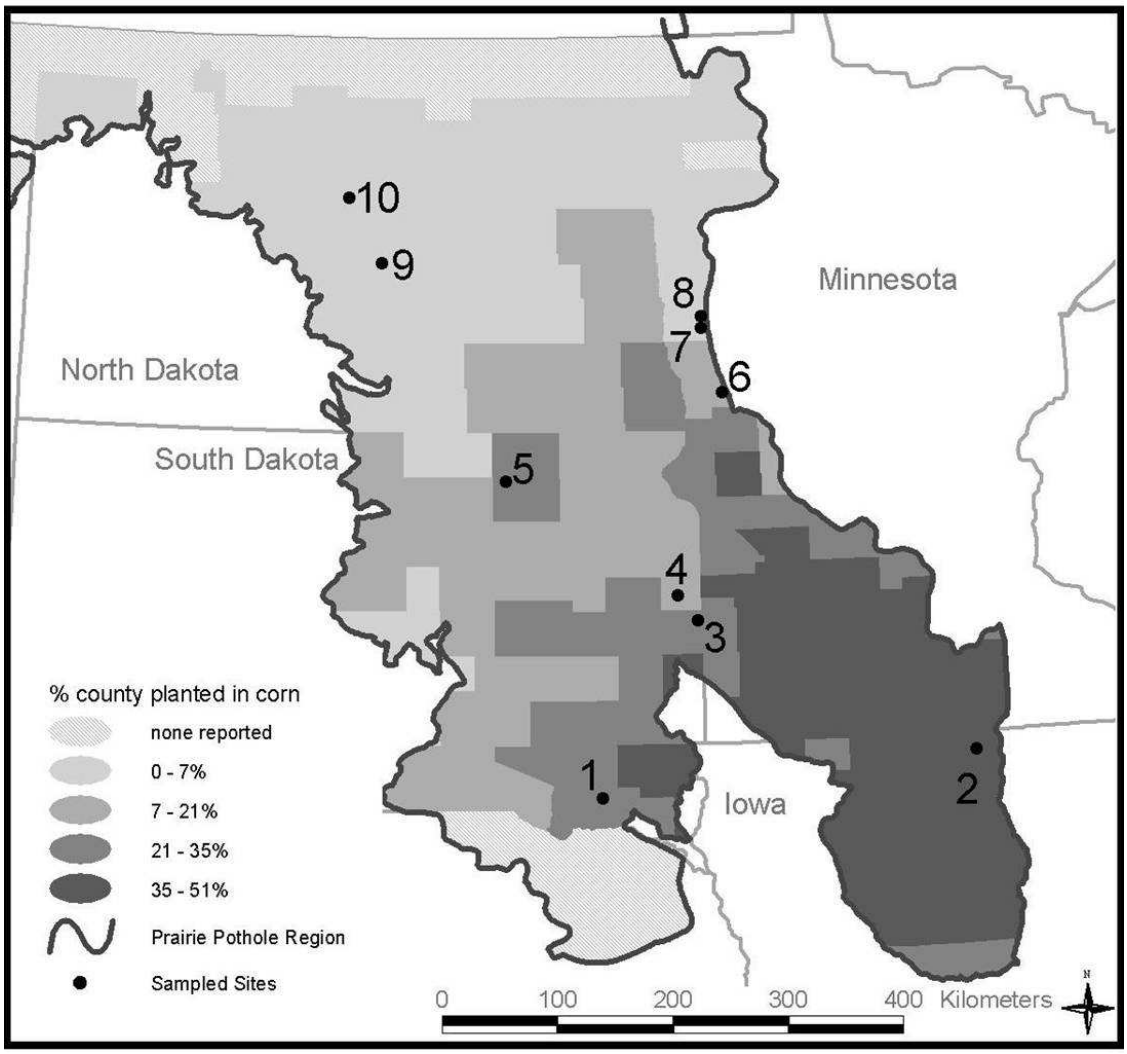

Figure 1. Location of study wetlands (sites) where water samples and metamorphic *R. pipiens* were collected within the Prairie Pothole Region of the North Central United States. Site numbers have been ordered by latitude (S to N). Crop data obtained from the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) and represent all corn planted in Iowa, Minnesota, Nebraska, North Dakota and South Dakota in 2005.

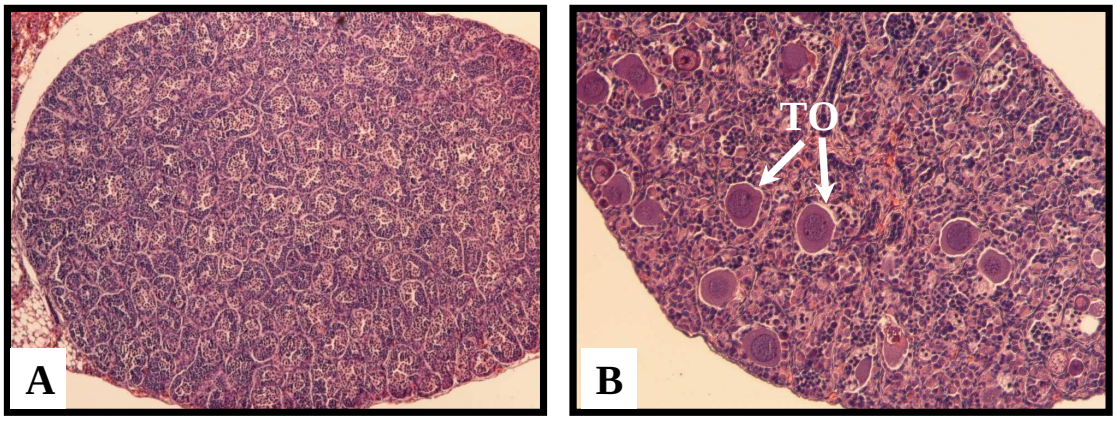

Figure 2. Photomicrographs of longitudinal cross sections of testicular tissue from field-collected Gosner stage 46 *R. pipiens*. (A) Normal-appearing testicular tissue. (B) Section containing multiple testicular oocytes (TO).

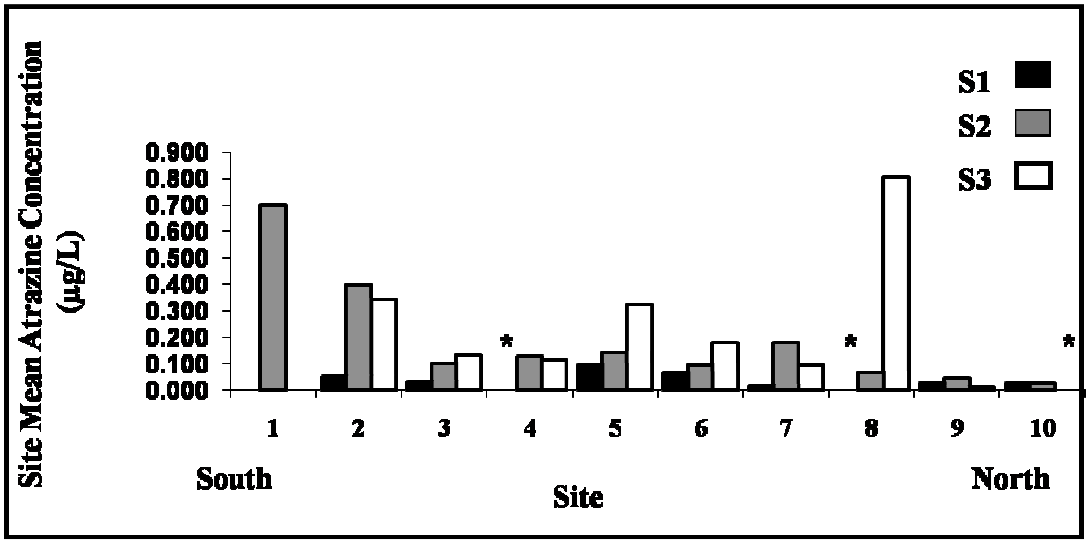

Figure 3. Atrazine concentrations (µg/L) measured in PPR wetlands (sites) during three surveys (S2, S2 and S3) March-July, 2005. Sites ordered by latitude (S to N). Concentrations < 0.011 µg/L (LOD) (*) were set to 0.5 x LOD. Site 1 was not one of our routinely sampled sites therefore only one water sample was collected.

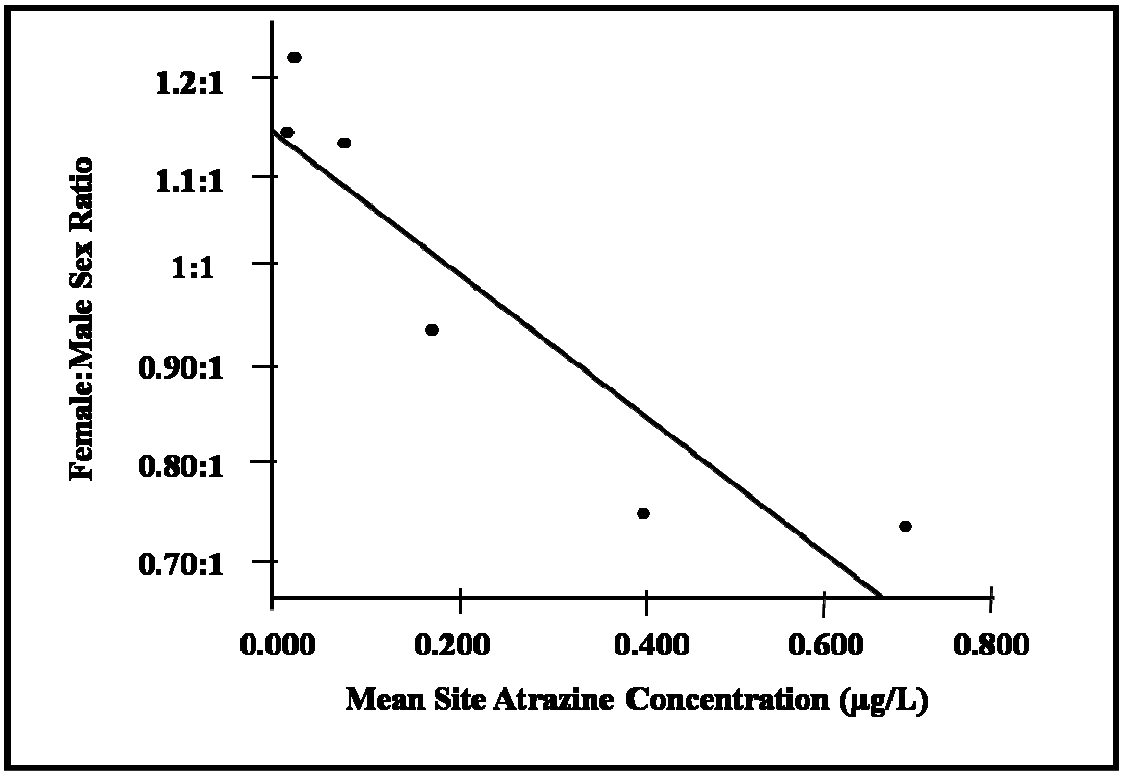

Figure 4. Relationship between atrazine concentration and sex ratio of metamorphic *R. pipiens* in Prairie Pothole Region wetlands. Mean atrazine concentrations from three surveys or sites with > 25 total specimens were included in the analysis. Sex ratios were based on gonadal sex. ($r = -0.92$, $p < 0.05$; $X^2 = 1.69$, $df = 5$, $p < 0.89$).

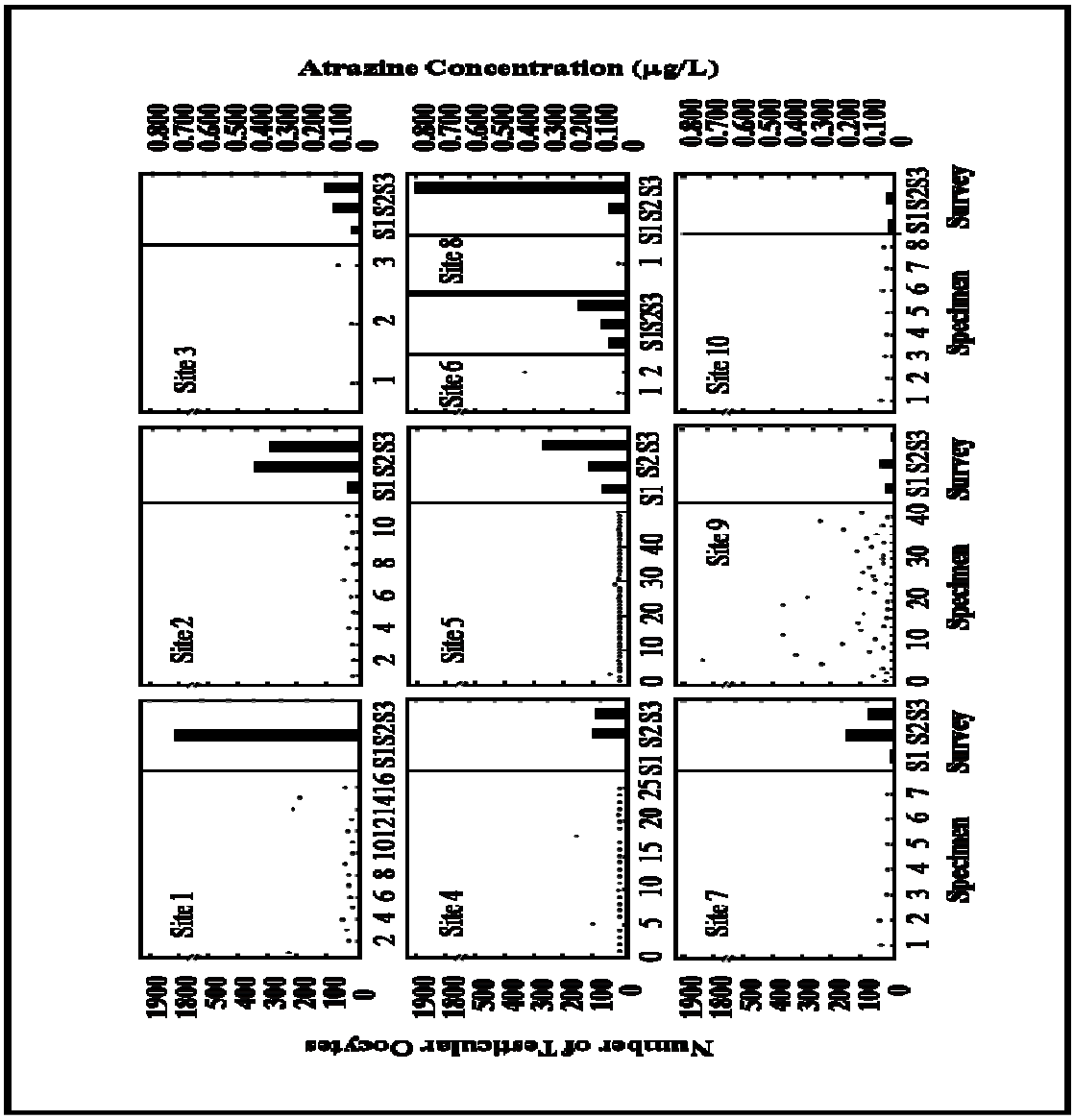

Figure 5. Testicular oocyte number in field-collected *R. pipiens* metamorphs, and atrazine concentrations by site. Sites 6 and 8 were joined due to small sample sizes. Atrazine concentrations measured in water samples collected from Prairie Pothole Region wetlands (sites) during three surveys (S1, S2 and S3) March-July, 2005. Concentrations < 0.011 µg/L (LOD) were set to 0.5 x LOD. Site 1 was selected based on the presence of metamorphs and was not one of our routinely sampled sites, therefore only one water sample was collected and analyzed.

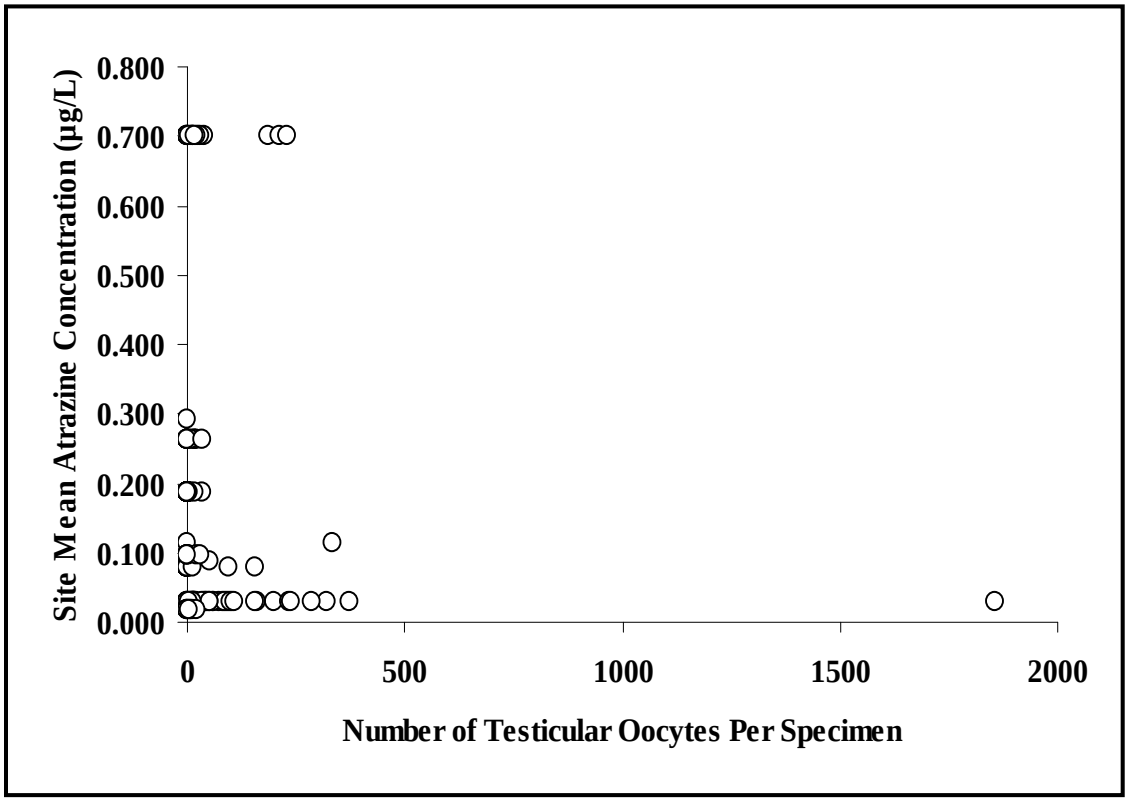

Figure 6. Total number of testicular oocytes in individual Gosner Stage 46 *R. pipiens* and mean atrazine concentrations (µg/L) as measured in water samples from their respective natal wetlands. Atrazine concentrations were measured during three surveys (S2, S2 and S3) March-July, 2005.