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# Land use and anuran biodiversity in southeast Kansas, USA

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***Abstract*.—The relationship of anuran breeding site biodiversity to land use was examined in southeast Kansas, USA. Eight breeding pools or temporary ponds were sampled from March to July 1995. Each site has some adjacent woodland, but varied in the remaining adjacent land use. Two sites were relatively unimpacted reference or "natural" sites, two were impacted by abandoned coal or lead/zinc mines, and four were impacted by cropland. Adult density was determined with visual and audio censuses. Tadpoles were examined for malformations and density was estimated. Eggs were collected from the sites, hatched in the laboratory, and examined for malformations. Total audio anuran density was statistically higher (ANOVA, P<0.05) in natural area breeding pools (1,048.7/ha) compared to pools in agricultural (519.0/ha) and mined areas (164.8/ha). Visual densities followed the same pattern (459.9/ha natural > 315.1/ha agricultural > 262.0/ha mined) but were not statistically different. Tadpole densities were significantly (P<0.05) higher in natural area breeding pools (137.6/m$^2$) compared to agricultural (59.4/m$^2$) and mined areas (28.5/m$^2$). The percentage of tadpoles with malformations was significantly lower (P<0.05) in natural areas (0.4%) compared to agricultural (4.6%) and mining (8.3%). Malformations found in the field included spinal cord, optic, edemas, and tumors. Eggs incubated from natural sites had significantly (P<0.05) higher percentages of eggs hatching successfully (98.8%) and lower percentages of tadpoles with malformations (17.5%) than did eggs from agricultural (88.2% and 51.0%, respectively) and mined areas (40.4% and 76.1%, respectively). Eggs incubated from natural sites also had the lowest malformation rate (17.5%) compared to eggs from agricultural sites (51.0%) and mined sites (76.1%), but these differences were not statistically different. These data provide evidence for the link between land use and the individual and population characteristics of anurans in breeding pools.**

**Key words.** *Amphibian, anuran, land use, tadpole, watershed, biodiversity, malformations*

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## Introduction

The reasons for declines in amphibian populations (Blaustein and Wake 1990; Wake 1991) are complex, and include diseases, ultraviolet radiation, pollutants, and habitat modifications (Alford and Richards 1999). In spite of many changes to the natural landscape and land use in southeast Kansas, the region supports a greater diversity of anurans — 17 species — than the rest of the state (Conant and Collins 1993). Hecnar and M'Closkey (1996) showed regional differences in amphibian species richness related to land use history. Southeast Kansas is also an ecotone between eastern deciduous forests and prairie, and is at the edge of distribution for many anuran species. Anuran populations that reside in peripheral areas such as this are of considerable interest to many biologists and geneticists who study divergence and speciation (Ptacek 1984).

The conversion of the rural landscape in southeast Kansas, as elsewhere, from pre-settlement conditions may adversely affect breeding and nonbreeding habitats and water quality in anuran breeding pools. Small wetlands are important to juvenile recruitment and their loss and increased isolation can have a negative effect on rescue efforts (Semlitsch and Bodie 1998). Such isolation influences the probability of dispersal among wetlands and is one of the critical factors in managing aquatic-breeding amphibians (Semlitsch 2000). Development of road networks, such as the many rural roads constructed on section lines (Public Land Survey System) in Kansas, can be responsible for fragmentation as well as direct mortality (Fahrig et al. 1995; Vos and Chardon 1998). But direct water quality impacts on breeding pools can occur as well. Agricultural runoff can carry fertilizers, pesticides, and sediments. Underground and strip-mining of coal, lead, and zinc earlier this century in southeast Kansas has left piles of mine tailings. Leachate from mined areas can be acidic and contain elevated concentrations of metals.

The goal of this project was to assess the relationship between adjacent land use and the biodiversity of anurans inhabiting breeding pools. The specific objectives were to (1) identify breeding pools with different surrounding land uses, (2) estimate adult anuran density, (3) estimate the density of and malformation rates in tadpoles, and (4) estimate the hatch-

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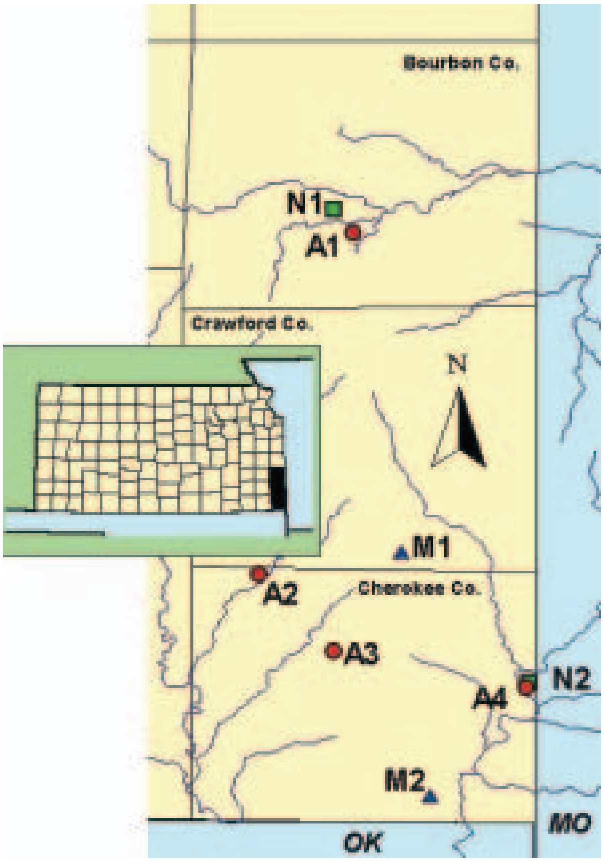


**Figure 1.** Locations of study sites in southeast Kansas.
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**Plate 1.** View of site N1 in Bourbon County, Kansas. A shallow artificial pond chiefly fed by a small ephemeral stream that originates in and runs through a meadow and a wooded area.
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ing success of eggs and the malformation rates of their hatched tadpoles incubated in the laboratory.

## Methods

### *Study area and sites*

The study area is located in the Osage Cuestas physiographic region in three counties in southeast Kansas USA

**Plate 2.** View of site N2 in Cherokee County, Kansas. A shallow artificial pond fed by runoff from a mature woodland area.
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**Plate 3.** View of site A1 in Bourbon County, Kansas. A shallow man-made pond adjacent to a plowed crop field.
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**Plate 4.** View of site A2 in Cherokee County, Kansas. A natural wetland surrounded on three sides by a plowed crop field.
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(Figure 1). Site selection focused on finding anuran breeding pools located in "micro-watersheds" with different immediate surrounding land uses. Three land uses were considered: agricultural, mined, and natural. Agricultural areas were plowed fields and row crops. Areas with live-

**Plate 5.** View of site A3 in Cherokee County, Kansas. A natural pool on the edge of a plowed crop field.
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**Plate 6.** View of site A4 in Cherokee County, Kansas. An artificial wetland in the middle of a plowed crop field.
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**Plate 7.** View of site M1 in Crawford County, Kansas. A shallow pool chiefly fed by a small ephemeral stream that originates in and runs through land that was previously mined for coal.
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stock were not used. Mined areas were on land that had clear signs of previous mining activity, including mine tailings. Natural areas were defined as lands with no cultivation, grazing, or mining activities occurring within the micro-watershed now or in the recent past, and approximating an undisturbed vegetative cover.

Over 70 potential sites were evaluated according to water depth, vegetation, percentage of the land use category in the micro-watershed, and availability of adjacent non-breeding habitats. The final sites chosen included eight sites in three land use categories: four agricultural sites, two mined land sites, and two natural sites. All sites, except one (A1), are on private property.

The first natural site (N1) is a shallow artificial pond (Plate 1). The pond is fed by a small ephemeral stream that originates in and runs through a meadow and a wooded area before entering the pond. Runoff from the surrounding wooded area also feeds the pond. The area around the pond is undergoing succession and consists of dense cedars, brush, small and medium sized trees, and grasses. Mature forest lies just to the south and the area has never been cultivated. N2 is a shallow artificial pond (Plate 2). The pond is fed by runoff from a surrounding mature woodland area. An area of grasses mixed with small trees and dense brush is located just north of the pond and a grassed area is located to the south.

The first agricultural site, A1, is a shallow man-made pond adjacent to a plowed crop field on the Hollister Wildlife Area owned by the State of Kansas (Plate 3). The pond is fed by runoff from the field where sunflowers were grown. Adjacent to the other sides of the pond are a prairie, a densely wooded area, and an ephemeral stream. This site is probably the least intensively cultivated site of all the agricultural sites. Agricultural site A2 is a natural wetland (Plate 4) fed by runoff from a crop field where soybeans were grown. The field surrounds the wetland on three sides while small to medium sized trees, aquatic and wet soil plants, and grasses are adjacent to the wetland on the fourth side. Woodlands are nearby to the east and west. Agricultural site A3 is a natural pool at the edge of a plowed crop field (Plate 5). The pool is fed by runoff from the field where soybeans were grown. On the opposite side of the pool from the crop field is a grassed area with dense brush and small trees. Adjacent to the north end are mature woodlands next to a creek. The last agricultural site, A4, is an artificial wetland in the middle of a plowed crop field (Plate 6). The wetland is fed by runoff from the field where soybeans were grown in 1995. A dense growth of small trees is present on both sides in the wetland. A grassed area is adjacent to the wetland, and woodlands lie adjacent to the south, the east, and the west.

The first mined site, M1, is a shallow pool fed by a small ephemeral stream (Plate 7) located on a partially reclaimed coal mine now managed by Pittsburg State University as part of the Monahan Outdoor Education Center. The stream originates in and runs through land that was previously mined for coal and by smaller amounts of runoff from the immediate wooded area. The area adjacent to the pool is undergoing succession with trees of varying sizes and dense brushy vegetation. Native grass areas are adjacent. The last mined site, M2, is a man-made pool at the edge of a pile of mining spoils (chat) left over from lead and zinc mining activities that occurred earlier in this century (Plate 8). The pool is fed by runoff from the chat piles. Adjacent to the pool are cattails, dense brush, grasses, and trees of varying sizes. A wooded area next to a creek is just west of the pool.

### Sample collection

Sampling started on 13 March 1995 with each site sampled at two-week intervals for a total of seven samples per site ending on 30 June 1995. Sampling took place in the evenings starting approximately one hour after sunset. All tasks were performed in the same order by the same person during each sample event at each site. A miner's head light was used for illumination.

At each site, the pool was approached quietly with the light dimmed in order to tape record the calls of adult anuran males and count them. If the number of calling anurans was low, it was possible to stand in one or a few spots and count the different individuals. If the number of calling anurans was high, multiple parallel transects were slowly walked with the light dimmed as much as possible (modified from Heyer et al. 1994). Every attempt was made to avoid counting the same individual twice. Anurans were recorded by species and number of individuals in order to estimate density.

Visual census of adults consisted of walking along multiple parallel transects and counting the anurans encountered on both sides of the transect (modified from Heyer et al. 1994). Transects were approximately two meters apart. Anurans were recorded by species and number of individuals in order to estimate density.

During sample period three, the tadpoles in the pools had reached a size large enough to be handled briefly and were sampled. Tadpole sampling was performed throughout the remainder of night sampling periods and in addition, each site was visited and tadpoles sampled during the daylight twice in July approximately two weeks apart.

Tadpole sampling was performed along the edges of two adjacent sides of each sample site. The other two sides of each sample site were sampled during alternate sampling events. Tadpole sampling was performed every five meters (m) at smaller sites and every 10 m at larger sites. Five m is the minimum distance recommended to avoid sampling the same tadpoles more than once (Heyer et al. 1994). Tadpoles were trapped with a plastic storage container with its bottom cut out (24.5 by 38 centimeters, cm). They were removed with a dipnet, counted, examined for malformations, and released. Numbers of individual tadpoles and numbers and types of malformations were recorded. Malformations were classified based on the system in the Frog Embryo Teratogenesis Assay Xenopus (FETAX, Bantle et al. 1991).

When found, the eggs of non-threatened and non-endangered species were collected, brought back to the laboratory, and incubated at 18°C. The eggs were kept in glass beakers with water collected from the site. Water was changed daily using site water kept in a refrigerator (Bantle 1995). Necrotic eggs were removed at the same time.

After the eggs hatched, any remaining necrotic eggs were removed and counted, followed by fixation in 10% formalin for 24 hours (Hull 1995) and preservation in 70% ethanol to preserve them. Newly hatched tadpoles were killed using tricaine methanesulfonate (MS-222) added directly to the beaker to relax the tadpoles and avoid unnatural contortions. Tadpoles were fixed and preserved as for the necrotic eggs.

Preserved tadpoles were then counted and examined for malformations at 15X. Malformation types and number of individuals were recorded in order to calculate the percent of tadpoles hatching successfully and the percent with malformations. Fixation, examination methods, malformation types, and malformation data sheets were modeled after Bantle et al. (1991).

### Data analysis

Adult density and field tadpole data were analyzed as a two-way ANOVA with interaction using land use group (natural, agricultural, mined) and sampling period (seven biweekly samples) as main effects. Audio and visual counts (the total count or by individual species) were expressed as density (numbers per hectare, ha) using average breeding pool area.

**Table 1.** Species present (X) with species richness at each site and land use group. Site codes are mapped in Figure 1.

| | Mined | | Agricultural | | | | Natural | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **M1** | **M2** | **A1** | **A2** | **A3** | **A4** | **N1** | **N2** |
| *Acris crepitans* | X | X | X | X | X | X | X | X |
| *Bufo americanus* | X | X | X | | X | X | X | X |
| *Gastrophryne carolinensis* | | | | | | X | | X |
| *Hyla versicolor/chrysoscelis* | | | X | | X | X | X | X |
| *Pseudacris crucifer* | | | X | | | | X | X |
| *Pseudacris triseriata* | X | | X | X | X | | X | |
| *Rana areolata* | | | X | | | | X | |
| *Rana catesbeiana* | X | X | X | | X | | | X |
| *Rana sphenocephala* | X | X | X | X | X | X | X | X |
| **TOTAL** | **5** | **4** | **8** | **3** | **6** | **5** | **7** | **7** |
| **LAND USE** | **5** | | | | **9** | | **9** | |

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Field tadpole data included tadpole density (numbers per square meter, $m^2$) and the percentage of field tadpoles with malformations.

Laboratory tadpole data included both percent eggs hatched and percentage tadpoles malformed. These data were analyzed with a one-way ANOVA using land use group as the main effect. Laboratory tadpole data from different sample periods were pooled by site because eggs were not consistently found at all sites in all sample periods. The percentages of types of specific malformations were pooled by land use and so were not analyzed statistically.

All percentage data were arcsine transformed before analysis (Zar 1996). As a result, standard errors from percentage data are asymmetrical and both lower and upper standard errors are presented in data tables. The general linear models procedure in SAS was used for all analyses (SAS 1988) and a significance level of 0.05 was used, except as noted.

## Results

### *Species found*

Nine species of anurans were found during visual and audio sampling of adults (Table 1). Overall, agricultural and natural sites both yielded the same nine species but the species present varied at each site. At agricultural sites, species richness varied from three to eight, and while species richness was

**Table 2.** Adult density by land use group. Data are numbers per hectare. C is census type: A = audio, V = visual. Within each line, means with different lower case letters are significantly different (P<0.05, except for northern spring peeper where P=0.06).

| **Species** | **C** | **Mined Mean (SE)** | **Agricultural Mean (SE)** | **Natural Mean (SE)** |
|---|---|---|---|---|
| *Rana sphenocephala* | A | 23.2 (29.8) a | 77.2 (21.1) a | 59.7 (29.8) a |
| | V | 47.0 (39.8) a | 102.7 (28.2) a | 65.9 (39.8) a |
| *Pseudacris triseriata* | A | 3.4 (12.6) a | 38.5 (8.9) b | 0.0 (12.6) a |
| | V | 3.4 (7.1) a | 17.7 (5.0) a | 1.9 (7.1) a |
| *Bufo americanus* | A | 8.4 (24.0) a | 29.0 (17.0) a | 43.4 (24.0) a |
| | V | 42.1 (19.1) a | 28.8 (13.5) a | 33.9 (19.1) a |
| *Acris crepitans* | A | 129.8 (148.0) a | 250.9 (104.7) a | 546.4 (148.0) a |
| | V | 126.8 (48.6) a | 91.0 (34.4) a | 186.8 (48.6) a |
| *Pseudacris crucifer* | A | 0.0 (62.9) b | 4.2 (44.5) b | 177.1 (62.9) a |
| | V | 0.0 (9.6) b | 1.4 (6.8) b | 50.5 (9.6) a |
| *Hyla versicolor/chrysoscelis* | A | 0.0 (41.0) b | 106.6 (29.0) a | 202.0 (41.0) a |
| | V | 0.0 (13.1) c | 38.6 (9.3) b | 100.3 (13.1) a |
| *Rana catesbeiana* | A | 0.0 (7.5) a | 7.7 (5.3) a | 9.1 (7.5) a |
| | V | 42.7 (13.9) a | 28.1 (9.9) a | 12.4 (13.9) a |
| *Rana areolata* | A | 0.0 (5.1) a | 4.2 (3.6) a | 9.5 (5.1) a |
| | V | 0.0 (4.9) a | 6.3 (3.4) a | 5.7 (4.9) a |
| *Gastrophryne carolinensis* | A | 0.0 (0.8) a | 0.5 (0.5) a | 1.7 (0.8) a |
| | V | 0.0 (0.8) a | 0.5 (0.5) a | 1.7 (0.8) a |
| **Total density** | **A** | **164.8 (198.3) b** | **519.0 (140.2) b** | **1048.7 (198.3) a** |
| | **V** | **262.0 (86.0) a** | **315.1 (60.8) a** | **459.0 (86.0) a** |

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**Plate 8.** View of site M2 in Cherokee County, Kansas. A man-made pool at the edge of a pile of mining spoils (chat) left over from lead and zinc mining.
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**All landscape photos by Lewis Anderson (Plates 1-8).**

seven at both natural sites. The mined sites had only five species, four at one site and five at the second. Only one agricultural site (A1) had more than six species.

Based on overall occurrences, there may be three clusters of species. Cluster one included species generally found at all three groups of sites: the southern leopard frog (*Rana sphenocephala*), western chorus frog (*Pseudacris triseriata*), American toad (*Bufo americanus*), Blanchard's cricket frog (*Acris crepitans*), and the bullfrog (*Rana catesbeiana*) [Table 1]. Cluster two included species not found at the mined sites, less common at the agricultural sites, and common at the natural sites: northern spring peeper (*Pseudacris crucifer*) and gray treefrog (*Hyla versicolor / chrysoscelis*). Cluster three included species uncommon at all sites: northern crawfish frog (*Rana areolata*) and eastern narrowmouth toad (*Gastrophryne carolinensis*).

### *Adult density*

Total audio density was significantly greater in natural sites (1048.7 / ha) than in the agricultural and mined sites (Table 2), but there was no significant difference between the agricultural (519.0 / ha) and mined (164.8 / ha) sites. Total visual density did not vary significantly among the groups of sites, but the rank order was the same as for audio density. Overall, estimates of audio density were about twice the visual densities at agricultural and natural sites, but just over half at the mined sites.

The densities of some species varied with land use group. *P. triseriata* audio density was significantly higher in agricultural sites than in mined and natural sites (Table 2). Natural sites had no *P. triseriata* in the audio census. Visual density was also higher at the agricultural sites, but not significantly so, and *P. triseriata* were found during the visual census at one natural site. The density of *R. sphenocephala* was also higher at agricultural sites in both audio and visual census (Table 2), although the differences were not significant.

Visual density of the *H. versicolor*/*chrysoscelis* complex was significantly higher in natural sites compared to agricultural sites (Table 2). The same pattern existed for audio density, but was not significant. Audio and visual density of

**Plate 9.** Eastern Narrowmouth Toad, *Gastrophryne carolinensis.* DOI: 10.1514/journal.arc.0040014g010

**Table 3.** Field tadpole density (per m2) and percent malformations by land use category. Standard errors for percentage data are asymmetrical upper and lower standard errors. Within each line, means with different lower case letters are significantly different (P<0.05).

| | Mined Mean (SE) | Agricultural Mean (SE) | Natural Mean (SE) |
|---|---|---|---|
| **Density (per m2)** | 28.5 (11.1) c | 59.4 (7.7) b | 137.6 (11.8) a |
| **% Malformed** | 8.3 (2.2/1.9) b | 4.6 (0.8/0.8) b | 0.4 (0.5/0.1) a |

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**Table 4.** Percentages of types of malformations in field tadpoles from each land use category.

| Category | Spinal Cord | Optic | Edema | Tumor |
|---|---|---|---|---|
| **Mined** | 78.9 | 5.3 | 10.5 | 5.3 |
| **Agricultural** | 67.5 | 12.5 | 13.8 | 6.3 |
| **Natural** | 92.8 | 7.2 | 0.0 | 0.0 |

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*P. crucifer* approached being significantly higher in natural sites (P = 0.06) compared to agricultural sites (Table 2). Mined sites had no *P. crucifer* or *H. versicolor* / *chrysoscelis* complex individuals in either audio or visual census.

### *Field-collected tadpoles*

Tadpole density was significantly higher in natural sites (137.6/$m^2$) compared to agricultural (59.4/$m^2$) and mined sites (28.5/$m^2$, Table 3). Natural sites also had significantly lower percentage of tadpoles with malformations in the field (0.4%) compared to agricultural (4.6%) and mined sites (8.3%, Table 3). Four different types of malformations were found on tadpoles in the field (Table 4). Spinal cord malformations were the most prevalent in all land use categories varying from 67.5% of malformations found on tadpoles in agricultural sites to 92.8% at natural sites. Optic malformations varied from 5.3% at mined sites to 12.5% at agricultural sites. No tadpoles with edema were found at natural sites compared to 10.5% of tadpoles at mined sites and 13.8% at agricultural sites. No tumors were found on tadpoles at natural sites, but tumors were found on 5.3% of tadpoles with malformations at mined sites and on 6.3% at agricultural sites.

### *Laboratory-hatched tadpoles*

The percentage of eggs hatching successfully in the laboratory was significantly higher in natural and agricultural sites (98.8% and 88.2%, respectively), compared to mined sites (40.4%, Table 5). Natural sites also had the lowest percentage of tadpoles with malformations from eggs incubated in the laboratory (17.5%), compared to agricultural sites (51.0%) and mined sites (76.1%, Table 5); however, these differences were not statistically significant.

Five different types of malformations were found on tadpoles hatched from eggs incubated in the laboratory: notochord / spinal cord, head / face, edema, stunted, and severe (having three or more different malformations) [Table 6]. Notochord / spinal cord and stunted malformations were most prevalent on tadpoles from eggs collected at mined and agricultural sites, and edema was the most prevalent malformation from natural sites.

## Discussion

The characteristics of these anuran populations were related to land use. Differences were found with regard to adult anuran total visual and audio density, field tadpole density, tadpoles percent with malformations (field and laboratory), and laboratory eggs percent hatching successfully. In four of these six measurements, differences due to land use were statistically significant, and in all cases the ranks of values were best in natural sites and worst in mined sites, with agricultural sites intermediate.

In addition, although five of the nine species were common to all three land use groups, two other species (*P. crucifer* and *H. versicolor* / *chrysoscelis* complex) were more common at the natural sites. This difference was not related to geographic distribution, since the natural sites spanned from the region from north to south. Perhaps unassessed habitat requirements or water quality sensitivities were a factor. Two species tended to be denser at the agricultural sites: *P. triseriata* and *R. sphenocephala*. The presence of more grassed areas as non-breeding habitat at the agricultural sites may be a factor in this trend. Although the data were collected from only one season, the replication of sites, the independence of variables, and the consistency of patterns provide evidence of an overall link between land use and characteristics of amphibians and their populations.

Mensing et al. (1998) found relationships between some of six land use types and the species richness or diversity of several biotic groups, including amphibians. Knutson et al. (1999) examined landscape level habitat associations for frogs and toads, and found a consistent negative association with urban land use. Relationships of these anuran populations to agriculture varied, being negative in Iowa, but positive in Wisconsin. The presence of isolated remnant forest patches in Wisconsin may have been responsible for the positive agricultural effect (Knutson et al. 1999).

What factors are associated with this overall relationship of land use to amphibian population diversity? Variability in physical habitat, food, predation, and the availability or quality of nonbreeding and breeding habitats could be specifically influential. However, the impact of land use on water quality can be equally important. For example, while habitat loss has been blamed in general for amphibian population declines, it can not explain differences in tadpole malformation rates or hatching success related to land use among the sites studied here.

Anurans have been shown to bioconcentrate or bioaccumulate pollutants. Tritiated water has been shown to appear

**Plate 10.** Southern Leopard Frog, *Rana sphenocephala.* DOI: 10.1514/journal.arc.0040014g011

**Plate 11.** Western Chorus Frog, *Pseudacris triseriata.* DOI: 10.1514/journal.arc.0040014g012

**Table 5.** Percentage of lab-reared eggs hatching and with malformations as tadpoles by land use category. Standard errors for percentage data are asymmetrical upper and lower standard errors. Within each line, means with different lower case letters are significantly different (P<0.05).

| | Mined Mean (SE) | Agricultural Mean (SE) | Natural Mean (SE) |
|---|---|---|---|
| **% Hatched** | 40.4 (10.0/9.7) b | 88.2 (4.2/5.0) a | 98.8 (1.2/3.2) a |
| **% Malformed** | 76.1 (17.3/23.6) a | 51.0 (17.4/17.5) a | 17.5 (22.4/14.3) a |

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**Table 6.** Percentages of types of malformations from lab-reared tadpoles in each land use category.

| Category | Notochord Spinal Cord | Head/ Face | Edema | Stunted | Severe |
|---|---|---|---|---|---|
| **Mined** | 48.8 | 2.4 | 4.9 | 41.5 | 2.4 |
| **Agricultural** | 46.8 | 1.1 | 9.6 | 33.5 | 9.0 |
| **Natural** | 26.9 | 8.5 | 36.2 | 19.2 | 9.2 |

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within 2.5 minutes in the lymphatic system and the plasma of a chronically catheterized *Bufo marinus* (Wentzell 1993). Wentzell (1993) also noted the kidney is the first organ exposed to the incoming ambient water. Atrazine has been shown to bioconcentrate in northern leopard frogs (*Rana pipiens*), but no impacts were observed (Allran and Karasov 2000). Russell et al. (1997) surveyed the distribution of the accumulation of PCBs and DDT metabolites in green frogs (*Rana clamitans*) and found variation in the amounts and kinds of contaminants accumulated. Bishop and Gendron (1998) reviewed literature on contaminant levels and effects in amphibians for the Great Lakes basin and reported some population declines could be influenced by exposures to environmental contaminants.

In agricultural areas, pesticides may be present in breeding pools. Several organophosphates caused abnormal pigmentation, abnormal gut development, notochord defects, and reduced growth to embryos of the African clawed frog *Xenopus laevis* (Snawder and Chambers 1989). Organochlorine pesticides impaired the peripheral or central nervous system by the likely inhibition of enzymes or metabolites by substances normally destroyed during nerve action (Livingston 1977). Fenitrothion and carbaryl showed effects such as microcephaly, edema, altered external morphology, heart abnormalities, and notochord / spinal cord abnormalities in *X. laevis* (Elliot-Feeley 1982).

*R. pipiens* larvae were exposed to atrazine and nitrates in the lab and no significant growth or developmental effects were noted except for slowed larval growth due to nitrates (Allran and Karasov 2000). Bridges and Semlitsch (2000) found significant variation in the time to death among tadpoles of nine species of *Rana* and ten subpopulations of *R. sphenocephala* exposed to carbaryl. Significant variation in the tolerance to carbaryl was found within a population of *H. versicolor* tadpoles (Semlitsch et al. 2000).

At Pelee Island in Canada, hybrid toads (*B. woodhousii* X *B. americanus*) have disappeared from agricultural areas with heavy chemical use (Green 1989). Cooke (1981) caged tadpoles in a potato field sprayed with oxamyl, a carbamate nematicide, and found a very high incidence of deformities of the tail and hind limbs, and high mortality among the deformed tadpoles.

*R. pipiens* embryos exposed to paraquat in a laboratory situation showed abnormal tail development, reduced muscular response, abnormal swimming behavior, and stunted growth (Dial and Bauer 1984). The types of malformations found in these studies are consistent with those found in agricultural areas in this study including notochord / spinal cord malformations (sometimes also referred to as tail abnormalities or malformations), microcephaly (head/face), and stunted growth (Tables 4 and 6).

In mined areas, problems can arise from the leaching of metals such as lead and zinc, and from low pH. Smelters also have been active in southeast Kansas in the past, sometimes close to mines. Lead concentrations found in tadpoles living in water subject to deposition from smelters and lead mine effluent are much higher than in tadpoles found residing in roadside ditches (Birdsall et al. 1986). Because of differences in the feeding habits of larval and adult amphibians, tadpoles living in roadside drainage accumulate more lead than the adults (Birdsall et al. 1986). Also, *B. americanus* tadpoles have shown no avoidance of water containing lead in octogonal fluvarium tests (Vial 1992).

Niethhammer et al. (1986) showed that *R. catesbeiana* had much higher levels of lead, zinc, and cadmium in their tissues than did reptiles, birds, or mammals collected from a river impacted by metal pollution from abandoned mine tailing piles. Khangarot and Ray (1987) showed that amphibians exposed to heavy metals display a variety of adverse effects such as erratic body movements, slower growth and development rates, morphological deformities and death.

Rowe et al. (1996) found oral deformities in tadpoles from a basin contaminated by coal ash. Loumbourdis et al. (1999) noted a tendency for retarded growth in tadpoles of *R. ridibunda* exposed to cadmium. The metamorphosis of *R. luteiventrus* tadpoles was delayed when they were exposed to soils contaminated with heavy metals (Lefcort et al. 1998). Freda (1991) pointed out the influential roles of pH,

**Plate 12.** Gray Treefrog, Hyla versicolor/chrysoscelis. DOI: 10.1514/journal.arc.0040014g013

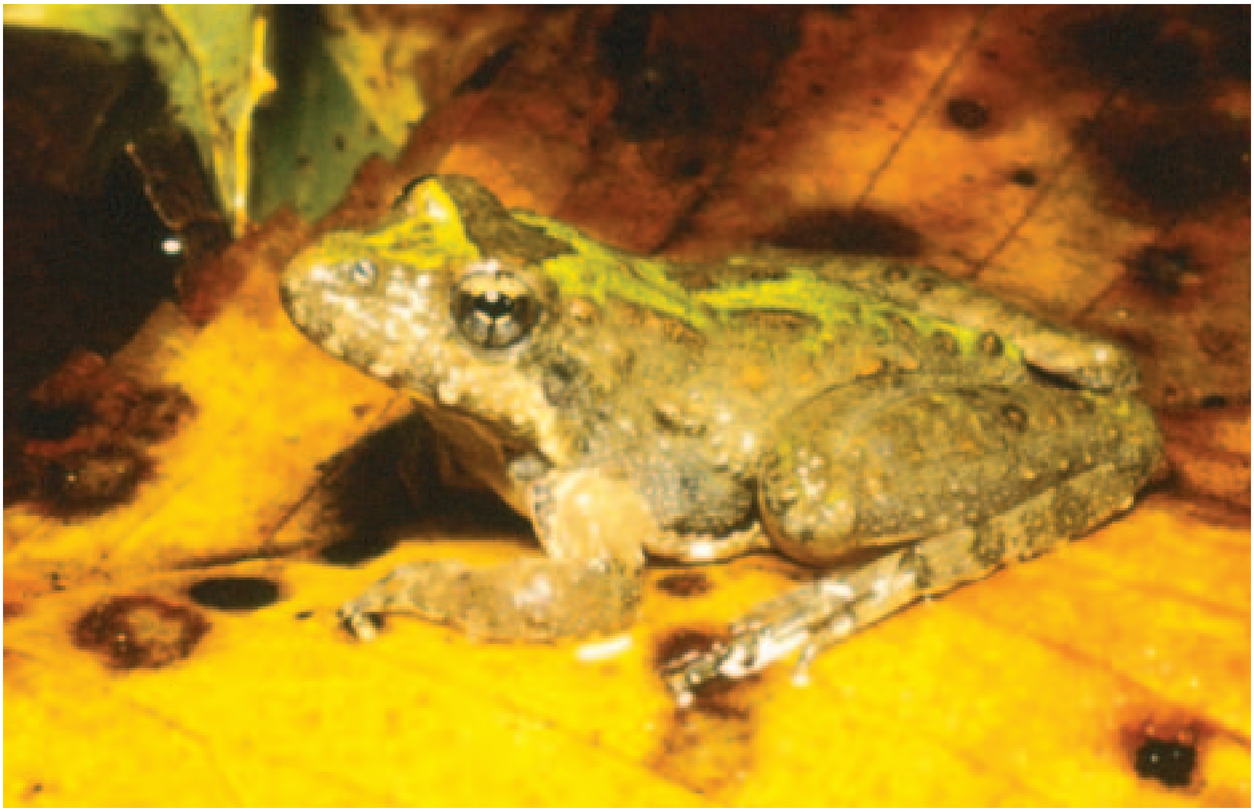

**Plate 13.** Northern Cricket Frog, *Acris crepitans.* DOI: 10.1514/journal.arc.0040014g014

hardness, dissolved organic carbon, developmental stage, and species on the impact of aluminum toxicity. Additionally, Freda (1991) noted the endpoints of toxic effects may vary from early egg mortality to sperm motility or to the success of fertilization.

Tests on anuran embryos under acidic conditions indicate a high survival rate to a certain threshold, and then the survival rate drops drastically. Embryos that do hatch often display developmental abnormalities and more abnormalities at lower pH (Pierce et al. 1984). Larvae of *R. sylvatica* showed greater tolerance to acidic conditions but toxicity of the acidic water does have adverse effects (Pierce et al. 1984). Preest (1993) showed that low pH disrupts osmoregulation in *Ambystoma maculatum* larvae causing slower growth and ultimately reducing fecundity and increasing exposure to predation.

At M2, the lead/zinc mined site, no tadpoles were found during any sample event nor were any tadpoles found during two intensive searches of the entire pool. Tadpoles with stunted growth comprised 41.5% of the total malformations found from eggs originating from mined sites in this study compared to 33.5% at agricultural sites and 19.2% at natural sites (Table 6).

Since land use likely affects water quality, water quality provides a possible explanation for the higher rate of tadpoles with malformations, the lower percentage of eggs hatching successfully, and potentially, the lower density of tadpoles in agricultural and mined land breeding pools. It is less clear what the relative role of water quality is in producing the lower adult anuran densities and species richness in agricultural and mined land breeding pools compared to physical habitat availability or quality.

The application of metapopulation dynamics (Gilpin and Hanski 1991, Fiedler and Jain 1992) and the concepts of sources and sinks to amphibians has been recognized by Hecnar and M'Closkey (1996) and Alford and Richards (1999). Considering the relationship of spatial scale to the species status of green frogs (*R. clamitans*), Hecnar and M'Closkey (1997) detected differences in occupancy and abundance at various spatial scales and concluded that the status of the green frog was dependent on the scale used.

Overall, the higher diversity of amphibians seen in the region of southeast Kansas may be maintained by a heterogeneous landscape, where losses in impacted areas are balanced by survival and recruitment from unimpacted areas. The analysis of Alford and Richards (1999) that habitat patch isolation may be of greater significance on populations of amphibians, compared to other animals is relevant to the consideration of the status of amphibians in southeast Kansas. Alford and Richards (1999) concluded that the dynamics of pond use was primarily affected by breeding pond isolation. The amphibian landscape of southeast Kansas contains many isolated patches of breeding pools, pools isolated not only by physical distance, but by the distance generated by patches of poor water quality. These pools have varied breeding and nonbreeding habitats as well, cast against a background of dispersal pathways and environments.

Even when adult anurans from neighboring populations migrate into an impacted breeding patch, low juvenile recruitment due to poor tadpole survivorship can result in reduced adult density and diversity in the patch. Freda (1991), Bishop and Gendron (1998) and Loumbourdis et al. (1999) show the key role of pollutants on the tadpole stage. If water quality is sufficiently poor over a larger regional area, there may be no immigration at all into central breeding pools and overall biodiversity may decrease as observed by Hecnar and M'Closkey (1996) for the effect of habitat loss. Species having shorter life spans may be the first to disappear from an area and any differential tolerances of the adults, larvae, and eggs from different species (Bridges and Semlitsch 2000 and Semlitsch et al. 2000) may also play a role. The limited travel range of anurans and the consideration that some tend to be patrophilic may explain localized extirpations of anuran populations such as observed here.

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**Plate 14.** Crawfish Frog, *Rana areolata.* DOI: 10.1514/journal.arc.0040014g015

**Plate 15.** Spring Peeper, *Pseudacris crucifer.* DOI: 10.1514/journal.arc.0040014g016

**Plate 16.** Bullfrog, *Rana catesbeiana.*

DOI: 10.1514/journal.arc.0040014g017

**Plate 17.** American Toad, *Bufo americanus.*

DOI: 10.1514/journal.arc.0040014g018

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