ASSESSING THE DISTRIBUTION AND ENVIRONMENTAL ASSOCIATIONS OF BURROWING CRAFYISH IN A RESTORED MISSOURI PRAIRIE

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SUMMARY TO MANAGERS

Crayfish are a keystone species in terrestrial and aquatic environments, but little is known on the environmental associations to the distributions for most species. The results of this report establish baseline information on the assemblage of crayfish species at Prairie Fork Conservation Area, including distributional and soil associations. Three species of crayfish were observed at PFCA: *Procambarus gracilis* (grassland crayfish), *Orconectes virilis* (virile crayfish), and *Procambarus acutus* (white river crayfish). One of these species, *P. gracilis,* is known as a burrowing species that only occurs in prairie and grassland habitats. The other two are stream-dwelling species, though alarmingly, *P. acutus* is not native to Callaway County and likely an invasive species. Communication with Pat Jones about the history of the property illuminated on the translocation of *P. acutus* and that it was introduced as an aquaculture species sometime in the past.

As the only burrowing crayfish found at PFCA, *P. gracilis* occurred at densities (as high as 0.09 burrows/m^2). Crayfish burrows were found in all soil types sampled, but burrow density did not associate with soil type, hydrological classification, or soil compaction. Sizes of crayfish burrows were significantly affected by soil type, as larger burrows were found in Calwoods soil types, and the smallest in Armster. Burrow size was also significantly affected by hydrological classification, and the largest burrows were found in semi-aquatic soil types, followed by hydric and non-hydric (upland) soils. The size of captured crayfish was significantly larger in Calwoods soils.

The application of inverse distance weighting procedures by ArcGIS provided insight into the expected abundance of crayfish at PFCA. Attached in this document is a map providing estimated abundances of crayfish in the area, and local hot spots of crayfish abundance. Crayfish burrows appear to have an affinity to hydric Keswick and Calwoods soils that are in close proximity to water bodies, and dissimilitude toward stream banks along Prairie Fork.

Information in this report can be used as a guide in the reintroduction of crayfish dependent species, e.g. crawfish frogs. The recommendation made by the author at the conclusion of this study is that reintroduction of frogs be focused at transect locations 1061 and 524. The sites contain the highest abundance of crayfish burrows found at PFCA, and also have sizable water bodies or high water tables with, to the author's knowledge, no fish predators.

INTRODUCTION

Crayfish are the third most imperiled floral or faunal group in North America following mussels and snails (NatureServ 2012), and are proportionately the most imperiled faunal group in Missouri, with 56% (20) of 36 species listed as species of conservation concern (Missouri Natural Heritage Program 2013). Information on the ecology and conservation of crayfish species is lacking, with published research on crayfish life histories accounting for only 12% of 347 United States (U.S.) and Canadian species, and the majority of these focusing only on stream dwelling species (Welch and Eversole 2006, Taylor et al. 2007, Loughman 2010, Moore et al. 2013). The sparse ecological work that has been conducted on burrowing crayfish species indicates that they and their burrows provide several ecosystem services including soil mixing, respiration, nutrient cycling, and refugia for a suite of animals such as snakes, frogs, salamanders, non-crayfish crustaceans, nematodes, arachnids, mammals, and insects (Creaser 1931, Carpenter 1953, Williams et al. 1974, Richardson 1983, Norrocky 1991, Stone 1993, Pintor and Soluk 2006, Welch et al. 2008, Loughman 2010).

The fossorial nature of burrowing crayfish species has limited the amount of research conducted, resulting in life history or habitat information on only a small proportion of these species, even though burrowing crayfish account for 32% of all imperiled crayfish species (Schuster 1976, Hamr and Sinclair 1985, Correia and Ferreira 1995, Johnston and Figiel 1997, Welch and Eversole 2006, Welch et al. 2008, Loughman 2010, Taylor et al. 2011, Helms et al. 2013a, 2013b). Burrowing crayfishes are particularly vulnerable to land-use disturbance and can have very limited distributions, in the case of one species, only a few acres (Helms et al. 2013b). In Missouri, prairie landscapes were once a dominate habitat (approximately 27% of the state), and species adapted to these environments after the retreat of glaciation (Schroeder 1981). Of these species, are a diverse array of the genus *Orconectes sp.*, and a few known burrowing crayfish. One species in particular, *Procambarus gracilis* (grassland crayfish) is only associated with grasslands and prairies (Pflieger 1996). Prairie landscapes are now in great decline in the state, mostly by effect of agriculture (grazing, cultivation), and information on the use and distribution of crayfish in remnant or restored prairies are lacking (Schoeder 1981). A survey of the burrowing crayfishes on regional and local scales explicates on the biodiversity of the area, and often provides baseline data on their distribution. Further, understanding the environmental associations (e.g., soil types, soil compaction, proximity to water, etc.) of burrowing crayfish communities facilitates future management decisions on restoration, reintroduction, and establishment of crayfish dependent species (especially prairie specialists e.g. endangered crawfish frogs) through having more extensive knowledge of burrowing crayfish habitat. The goal of this study was establish baseline information on the distribution of burrowing crayfish in a restored Missouri prairie. This goal was addressed with a series of field surveys that would 1) the potential habitats suitable for burrowing crayfish species, 2) their relative densities in these habitats, 3) what habitat/soil characteristics influence their distribution, and 4) the species composition and their respective distributions.

METHODS

Sample sites: Field surveys were conducted in a restored prairie in central Missouri through April-June 2014. Prairie Fork Conservation Area (PFCA) consists of 711 acres in Callaway County, Missouri and was donated to and managed by the Missouri Department of Conservation since 1997. A total of 32 sites were sampled over the course of the study, comprised of 24 terrestrial and 8 lentic sites. Welch and Eversole (2006) emphasize the bias that can occur when burrowing crayfish sampling focuses only on aquatic and semi-aquatic habitats, so a mixture of terrestrial environments were sampled systematically. Of the 24 terrestrial sites, 8 were composed of pond banks and shores (semi-aquatic), 8 in floodplains and hydric soils, and 8 in non-hydric soil, upland habitats (Welch and Eversole 2006) (Table 1, Figure 1). Hydric soils are defined by USDA Natural Resource Conservation Service (NRCS) Soil Survey standards, as poorly drained soils with a water table <1 m in depth (2013). A map delineating the surface water bodies and soil types of PFCA was created for site selection, and a grid of 250 m^2 blocks were created over the map and each assigned a unique value. Values that correspond to the grid map were then picked from a random number generator until 32 sites were selected that fall within each sample environment.

Field sampling: Transects have been effectively used in sampling burrowing crayfish population size, abundance, and density in a variety of terrestrial habitats (Correia and Ferreira 1995, Johnston and Figiel 1997, March and Robson 2006, Helms et al. 2013a, 2013b). A transect 50 m long x 5 m wide (250 m^2) was established at each of the 24 terrestrial sampling sites for a total sampled area of 6000 m^2. The 8 semi-aquatic site transects were established 50 m along the shoreline and 5 m wide, away from the water's edge. Transects at the other 16 terrestrial sites were positioned in a similar manner to the semi-aquatic sites, but the 5 m width was split evenly into 2.5 m sections on either side of the 50 m long transect line. GPS points were recorded at all crayfish burrow openings and counted to estimate the burrow densities for each transect (burrows/m^2). Burrowing crayfish can create multiple burrows and entrances, and may not always occupy the same burrow at any one point in time (March and Robson 2006). To estimate the number of crayfish present at a site, all burrows were examined for recent activity, and active burrows were counted, mapped (GPS point), and flagged. All burrows, active or inactive, were measured by calipers at the opening of the burrow for burrow width. Active burrows are defined as having disturbed soil and cleared plant material around the entrance, and/or by having a plugged chimney (Loughman 2010, Engelbert 2013, Helms et al. 2013a). To assess active burrows, the opening of the burrow was disturbed by removing any mud or chimney that was present and by partially excavating the opening (March and Robson 2006). Active burrows were then inspected 24 hrs after disturbance and observed for any new signs of activity (March and Robson 2006). If new activity was observed, those burrows were then excavated to remove the occupant, and the crayfish carapace length (mm), sex, and species were noted.

Eight aquatic samplings were also conducted at the 8 semi-aquatic sites by the use of seines and dipnets. Lentic sampling was conducted for two 15 minute sessions (30 minutes total), as this method has been shown to effectively catch burrowing species in Missouri (Englebert 2013). Crayfish species and relative abundances were recorded.

Environmental associations: To determine if burrowing crayfish distribution were associated with environmental variables, soil characteristic measurements were taken at each of the 24 terrestrial sites. Soil compaction and type have been shown to influence the behavior and distribution of burrowing crayfish species (Thoma and Armitage 2008, Helms et al. 2013a). Soil compaction was sampled 5 times at haphazard intervals at each transect by using a pocket penetrometer. Crayfish burrow density (active and inactive) was compared against soil type and compaction. Soil types were mapped using NRCS Soil Survey data of PFCA and ArcGIS software (Environmental System Research Institute, Redlands, CA), and crayfish burrow density (active and inactive) was compared among soil types found on PFCA. Densities of crayfish burrows will also be mapped over PFCA using inverse distance weighting (IDW) for visual exploration of crayfish distribution and future management. Burrow physical characteristics (width) were compared against soil type and compaction.

Survey of burrowing crayfish species: Vouchers were taken of all species found on PFCA for taxonomic identification. Specimens were shipped to Dr. Chris Taylor of the Illinois Natural History Survey (INHS) for verification and deposition in the INHS museum. Dr. Taylor is a leading crayfish taxonomist in North America, and currently houses the Missouri crayfish collection. Reference numbers of vouchers were provided for future inquiry. Incidental capture of other aquatic crayfish species were also noted, as well as, their relative abundances. GPS locations of all crayfish burrows and species found on PFCA were plotted on an ArcGIS map for future use by managers.

Analytical procedures: Crayfish burrow densities and species in terrestrial sample sites were compared among site habitat types (e.g., terrestrial vs. aquatic/semi-aquatic), soil types, and soil compaction categories using one-way ANOVAs. Correlative analyses of burrow width, crayfish length, and soil compaction will be compared by using the General Linear Model approach. All statistical analyses will be conducted with SAS 9.2 statistical software. Mapping of crayfish burrow density and IDW were conducted with ESRI ArcGIS 9 software package.

RESULTS:

Field sampling: A total of 275 burrows were found in the 6000m^2 study area at PFCA, with a resulting density of 0.045 burrows/m^2. Burrows were observed in all but 7 transects, with two of these having crayfish captured by additional lentic sampling, for a total of 5 transects not containing burrows or crayfish. Of the 275 burrows found, 34% were active (n=94) and crayfish density was determined to be 0.015 crayfish/m^2, with activity usually represented by fresh mud deposited at the opening of the burrow. Excavation of active burrows resulted in the capture of 37 crayfish, though some crayfish were observed but evaded capture (39% capture rate). During additional lentic sampling, 174 crayfish were captured (0.73 crayfish/min) at 6 (out of 8) sites.

Environmental associations: Soil types delineated by NRCS data were comprised of 6 soil series: (percent of total area at PFCA in parentheses) Keswick (47%), Calwoods (27%), Landes (2%), Gorin (9%), Armster (8%), Armstrong (7%) and Landes (2%). The area represented by hydric, non-hydric, and semi-aquatic hydrologic classifications were 40%, 59%, and 11% of the total area, respectively.

There were no significant differences in crayfish burrow density between transect type (hydric, non-hydric, and semi-aquatic), or by soil type. Burrow density is presented in Table 2 for transect and soil type. Total burrow density did not correlate significantly with soil compaction. Active burrow data was not normal, and a Kruskal-Wallis test was used to determine that there were no significant differences in active burrow density by transect type or soil type.

Burrow width ranged from 16 – 79.5 mm in diameter and averaged 23.96 mm (SD = 19.6 mm). There were no significant correlations between width and soil compaction. Burrow width was significantly different ($F_{1,184} = 34.53$, $p < 0.0001$) between transect types and soil type ($F_{1,184} = 30.81$, $p < 0.0001$). Non-hydric soils had the widest burrows, followed by hydric and semi-aquatic soils (values presented in Table 3, Figure 2). Soil type values and significant differences are presented in Table 4 (Figure 3). Burrow width was significantly correlated with crayfish carapace length ($R^2 = 0.414$, $p < 0.0001$), as burrow width increased so did carapace length (Figure 4).

Carapace length of captured burrowing crayfish ranged from 22.5 – 40.8 mm and averaged 31.5 mm (SD = 4.6mm). There were no significant differences in carapace length between transect type, but did significantly differ between soil type ($F_{1,31} = 4.21$, $p < 0.014$) (Figure 5). Carapace lengths at Calwoods (average 34.2mm, SD = 3.7mm) sites were significantly different from Armster (average 26.2mm, SD = 0.35mm) soil types (Figure 5).

Exploratory analysis of crayfish burrows using IDW indicated that burrowing crayfish prefer hydric Keswick and Calwoods soils that are in close proximity to water bodies (Figure 6).

Survey of burrowing crayfish species: Three crayfish species were observed at PFCA; *Procambarus gracilis* (grassland crayfish), *P. acutus* (white river crayfish), and *Orconectes virilis* (virile crayfish). The only burrowing crayfish captured was *P. gracilis*, with the two other species captured only during additional lentic sampling. The species composition of crayfish at PFCA was primarily comprised of *P. gracilis* (70% of total, n = 147), followed by *O. virilis* (18%, n = 39), and *P. acutus* (12%, n = 25). The male to female ratio of *P. gracilis* captured was 1:1. All species accounts and locations were entered into the Missouri Department of Conservation's crayfish database. A map of where crayfish species occurred is presented in Figure 7.

DISCUSSION

Through the use of field surveys, soil analysis, and mapping software this study was able to determine the distribution, environmental variables, and species composition of burrowing crayfish (and other crayfish species) at a local scale. A relatively small number of studies have examined burrowing crayfish distribution and environmental controls due to the their fossorial life cycles, elusive behavior, and often localized distributions contributing to a lack of information on these individuals (Grow and Merchant 1980, Norrocky 1991, Johnston and Figiel 1997, Welch et al. 2008, Loughman 2010, Loughman et al. 2012, Helms et al 2013a, 2013b). The lack of knowledge, previous to this study, contributed to the disparity of information on the distribution and environmental associations of burrowing crayfish in Missouri prairies.

Environmental associations: Crayfish (or crayfish activity) were observed in 27 out of 32 sites at PFCA, with no preference observed in soil type, NRCS soil hydrologic type, or soil compaction. The lack of association of crayfish burrows and their presence in non-hydric upland soils may be explained by the seasonality of the study, and that, observationally, all transects containing crayfish had a water table that was visible after excavation of < 1 m in depth. The presence of a relatively higher water table alone does not qualify a soil for hydric soil delineation by NRCS, and further elucidation on the distributional effect of seasonally high water tables by burrowing crayfish at PFCA is warranted (2013).

Though no evidence was present that burrow density was a factor of hydrologic or soil type, the size of the crayfish burrow (and the size of the individual) was affected (Tables 2,3; Figures 2,3). Not surprisingly, the positive correlation between burrow width and carapace length demonstrates that larger individuals create wider burrows (Figure 4). Interestingly, the burrow width was a factor of soil type and hydrological delineation, and the size of the individual. The data demonstrates that smaller individuals were found in semi-aquatic environments, and that the largest individuals were observed furthest from water. A recent paper by Helms et al. (2013a) found similar results in the burrowing crayfish, *Cambarus diogenes*, as smaller burrows were observed closest to streams with increasing size of burrows as distance increased from the water body. Helms et al. suggest that smaller individuals reduce the risk for desiccation by burrowing quickly after leaving a water source (2013a). Larger burrows and individuals (carapace length) were found in Calwoods soil types, with the smallest found in Armster. Calwoods soils contain the least amount of sand and most silt according to NRCS data, with sand/silt/clay content remaining similar (though in different composition) in all other soil types found (2013). The presence of larger individuals in Calwoods may be a factor of soil type in relation to the distance from a water body, but this variable was not taken account of in this study and elicits further inquiry. However, other studies have documented the effect of soil texture on the size of the individual and their affinities to sand are species dependent (Dorn and Volin 2009, Helms et al. 2013a). Juveniles of the species *C. diogenes* preferred sandy soils along near-stream habitats, and this may explain the negative incidence of smaller individuals in silty soils (Helms et al. 2013a).

The use of IDW as an exploratory technique is a useful tool to elucidate on the distribution of species in response to environmental variables, especially those with patchy distributions (Merrill et al. 2009, Bonter et al. 2010). Though simplified and limited by the amount of variables and samples taken in this study, IDW's can be used by managers to predict the suitable habitat and abundance of a species in a given area. Plotting of burrow abundance in this case, provided an estimate of the range and abundance of burrows along transect gradients, and presented a physical map that can be easily interpreted by managers (Figure 6). Burrow abundance was observationally highest in Calwoods and Keswick soils that were in close proximity to a water body. The ranges of contours presented in this map can also be used to provide insight into the reintroduction of burrowing crayfish dependent species (e.g. crawfish frogs).

Survey of burrowing crayfish species: Only one burrowing crayfish species was observed over the course of this study, with two other species found only in water sampling (Figure 7). *Procambarus gracilis* is commonly associated with remnant prairie landscapes, and has been observed in neighboring counties of PFCA (W. L. Pflieger, Missouri Department of Conservation [MDC], unpublished data). The occurrence of this species at PFCA may indicate that the restorative actions in place are maintaining a natural hydrologic regime suitable for these individuals. *Orconectes virilis* is the most dispersed crayfish to occur in Missouri, and is not characterized as a burrowing crayfish (Plieger 1996). Though observed in relatively low abundance, the presence of *P. acutus* in Callaway County is a cause for concern. This crayfish was not previously known to occur in the county or surrounding areas, and is known in other areas of Missouri for its potential to displace native crayfish species (Pflieger 1996, DiStefano et al. 2009). The occurrence of *P. acutus* at PFCA is thought to be related to aquacultural stocking of this species for human consumption (Pat Jones, personal comm.). Further investigation into the distribution of *P. acutus* in the watershed, and removal is warranted to evade potential negative effects on native species.

Conclusion: Crayfish distributional information and the influence of environmental mechanisms on such are needed for future conservation efforts, especially for a group that so highly imperiled (Taylor et al. 2007). In this study, associations were found between the locations of individuals based on size. Soil and water proximity may explain the location of younger to juvenile individuals, as well as, larger individuals that are of reproductively mature size (~53 mm total length) (Pflieger 1996). This study also stresses the importance of basic species surveys on local and regional scales, as not only were multiple species accounted for, but also the appearance of a potentially invasive crayfish. The occurrence of a non-native crayfish at PFCA suggests that not only should surveys be completed, but that surveys should be repeated temporally to ascertain distributional changes and introductions.

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Transect #	Transect type	Soil type	Latitude	Longitude
4	Hydric	Keswick	38.87843	-91.73463
330	Hydric	Keswick	38.88827	-91.72812
436	Hydric	Keswick	38.89016	-91.73558
504	Hydric	Keswick	38.89107	-91.73671
619	Hydric	Landes	38.89266	-91.73093
877	Hydric	Keswick	38.89556	-91.7349
947	Hydric	Keswick	38.89653	-91.74181
1006	Hydric	Keswick	38.89787	-91.74005
167	Non-hydric	Armster	38.88382	-91.73281
185	Non-hydric	Armster	38.88425	-91.73049
473	Non-hydric	Gorin	38.8906	-91.73442
645	Non-hydric	Gorin	38.89287	-91.73611
873	Non-hydric	Keswick	38.89558	-91.73721
1061	Non-hydric	Calwoods	38.89919	-91.73772
1189	Non-hydric	Calwoods	38.90282	-91.73997
1340	Non-hydric	Calwoods	38.90683	-91.73586
27	Semi-aquatic	Keswick	38.87931	-91.73231
36	Semi-aquatic	Keswick	38.87978	-91.73403
216	Semi-aquatic	Keswick	38.88568	-91.73796
524	Semi-aquatic	Armstrong	38.89095	-91.72519
687	Semi-aquatic	Landes	38.89328	-91.73264
721	Semi-aquatic	Keswick	38.89375	-91.73436
1040	Semi-aquatic	Keswick	38.89877	-91.74004
1320	Semi-aquatic	Calwoods	38.90641	-91.73817

Table 1. List of transects selected randomly for study with location information.

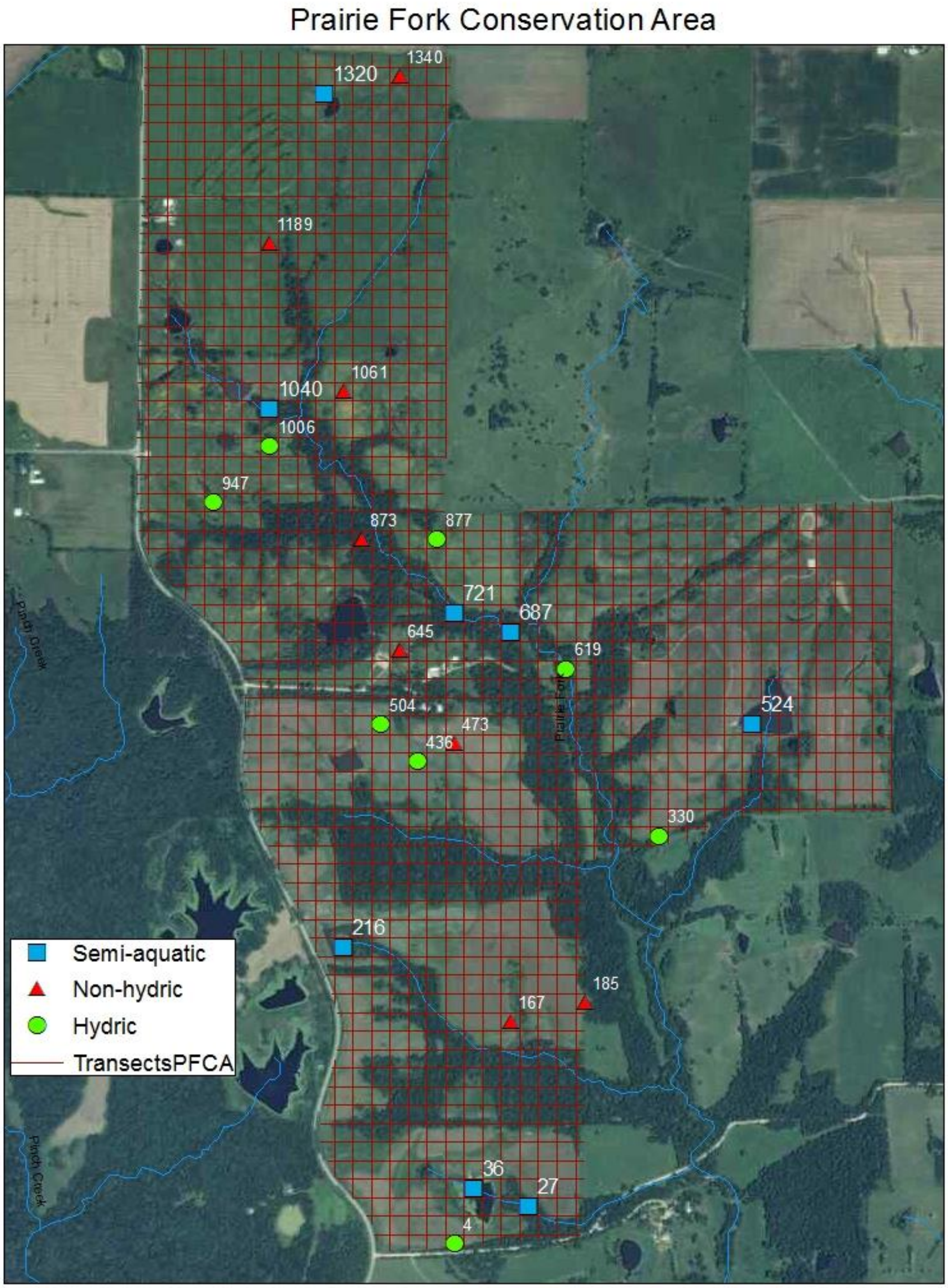

Figure 1. Map of Prairie Fork Conservation Area displaying the 250 m^2 grid system, selected transects, and their respective transect type. Lentic sites are denoted by blue squares, non-hydric by red triangles, and hydric by green circles.

Transect Type	Soil Type	N	Mean (burrows/m^2)	Std Dev	Min	Max
Hydric	Keswick	7	0.029	0.03	0	0.088
	Landes	1	0	.	0	0
Non-Hydric	Armster	2	0.062	0.03	0.044	0.08
	Calwoods	3	0.093	0.12	0.016	0.236
	Gorin	2	0.004	0.01	0	0.008
	Keswick	1	0	.	0	0
Semi-aquatic	Armstrong	1	0.22	.	0.22	0.22
	Calwoods	1	0.032	.	0.032	0.032
	Keswick	5	0.045	0.08	0	0.18
	Landes	1	0.004	.	0.004	0.004

Table 2. Total burrow density (burrows/m2) for transect type and soil type.

Transect type	Obs	N	Mean (mm)	Std Dev	Min	Max
Hydric	37	37	36.62973	8.739148	20	59.2
Non-hydric	75	75	42.84933	11.81247	20.6	79.5
Semi-aquatic	73	73	29.64658	7.384144	16	51.1

Table 3. Summary statistics for burrow widths (mm) for each transect type.

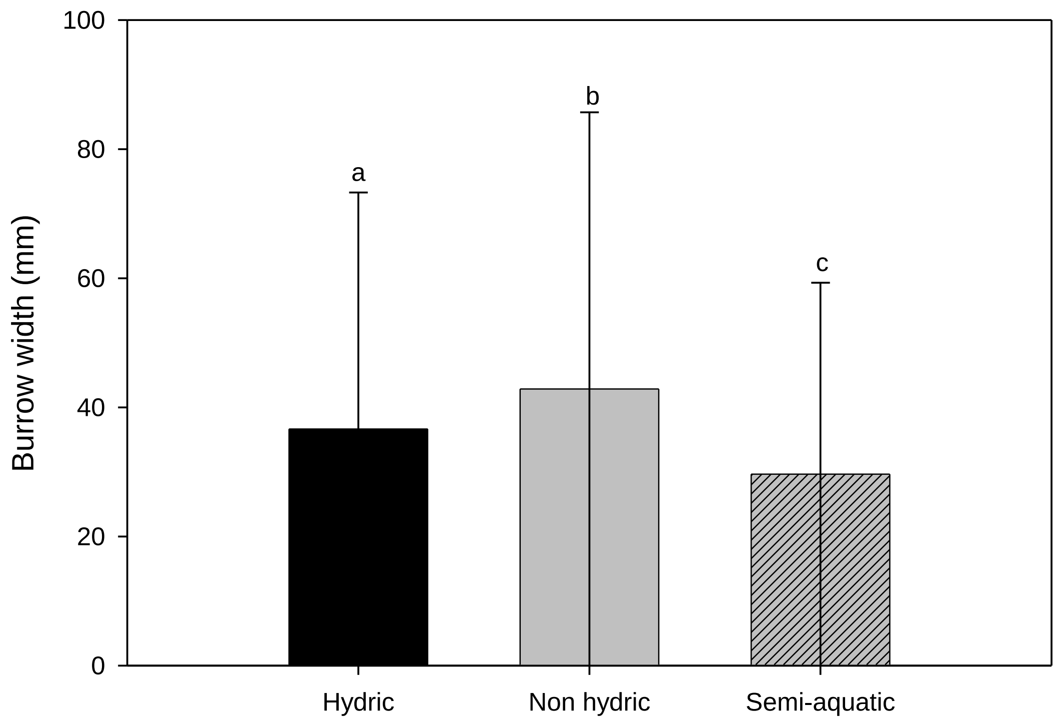

Figure 2. Burrow widths (mm) between transect type. Letters denote significant differences ($p <$ 0.0001).

Soil type	Obs	N	Mean (mm)	Std Dev	Min	Max
Armster	8	8	23.7	2.1	20.6	27.1
Armstrong	49	49	27.9	6.5	16	41.5
Calwoods	70	70	44.5	10.5	23	79.5
Keswick	57	57	35.7	8.6	20	59.2
Landes	1	1	26.5	.	26.5	26.5

Table 4. Summary statistics for burrow widths (mm) by soil type.

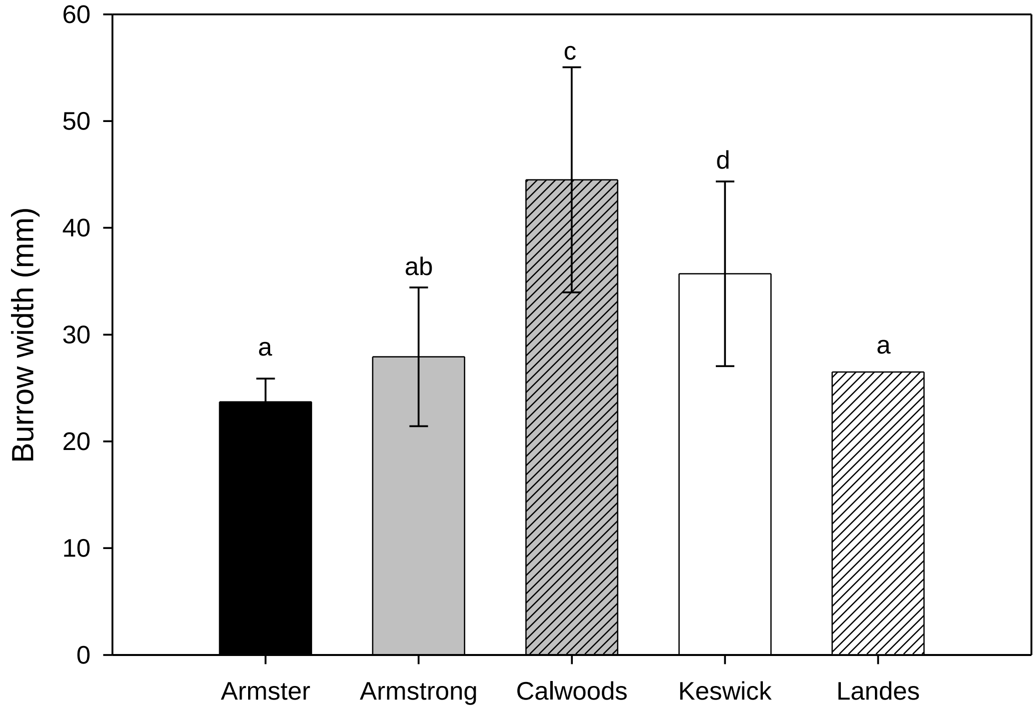

Figure 3. Burrow widths (mm) between soil type. Letters denote significant differences ($p <$ 0.0001).

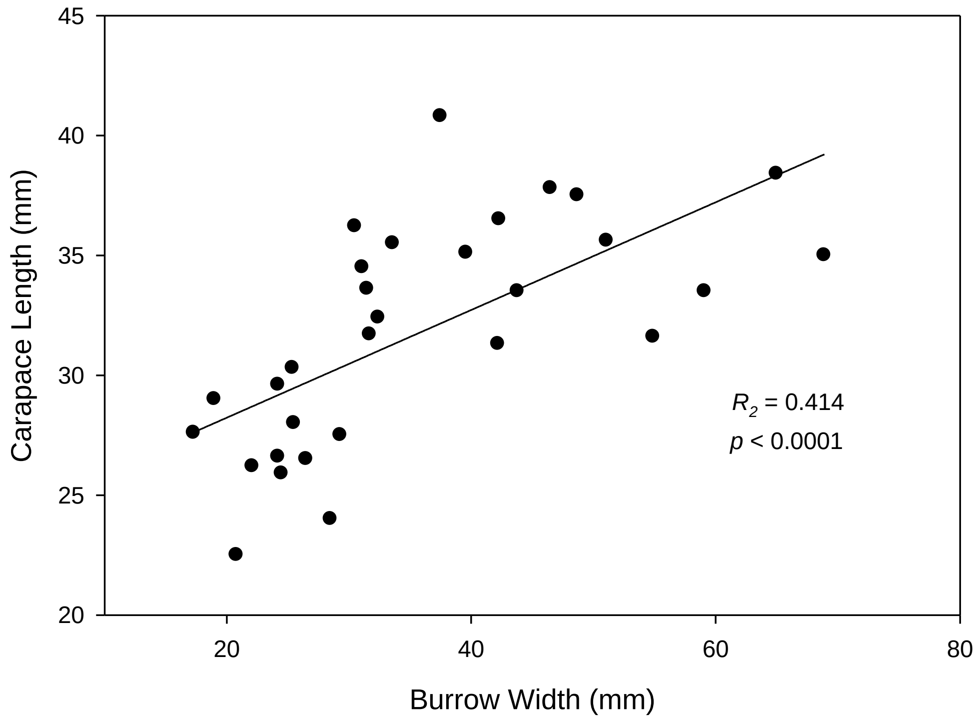

Figure 4. Regression between burrow width (mm) and crayfish carapace length (mm).

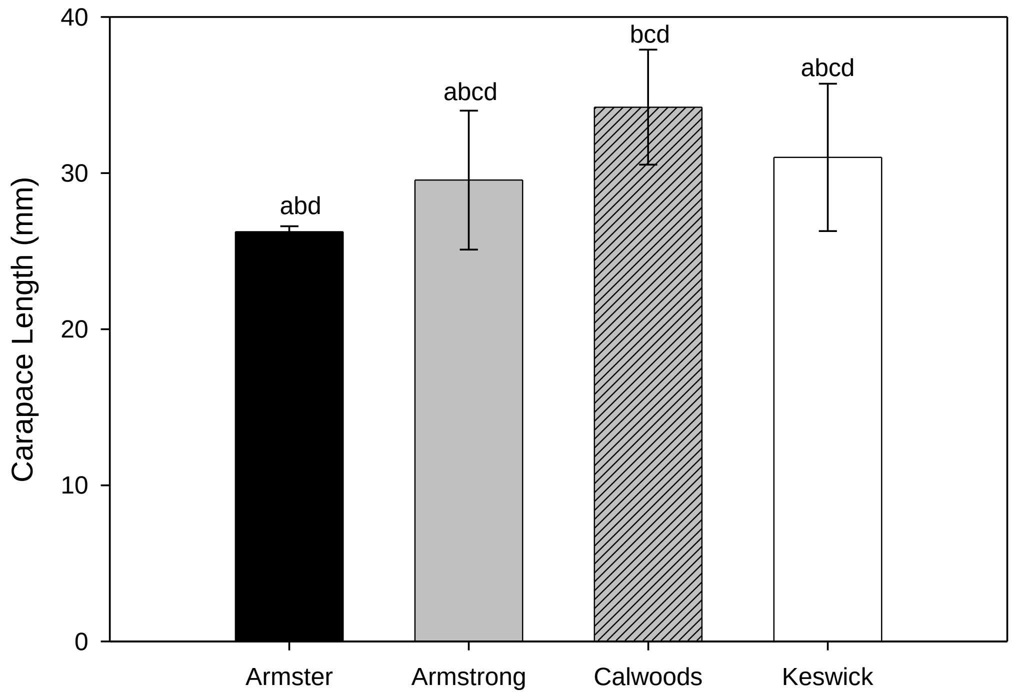

Figure 5. Carapace lengths (mm) of captured crayfish by soil type. Letters denote significant differences.

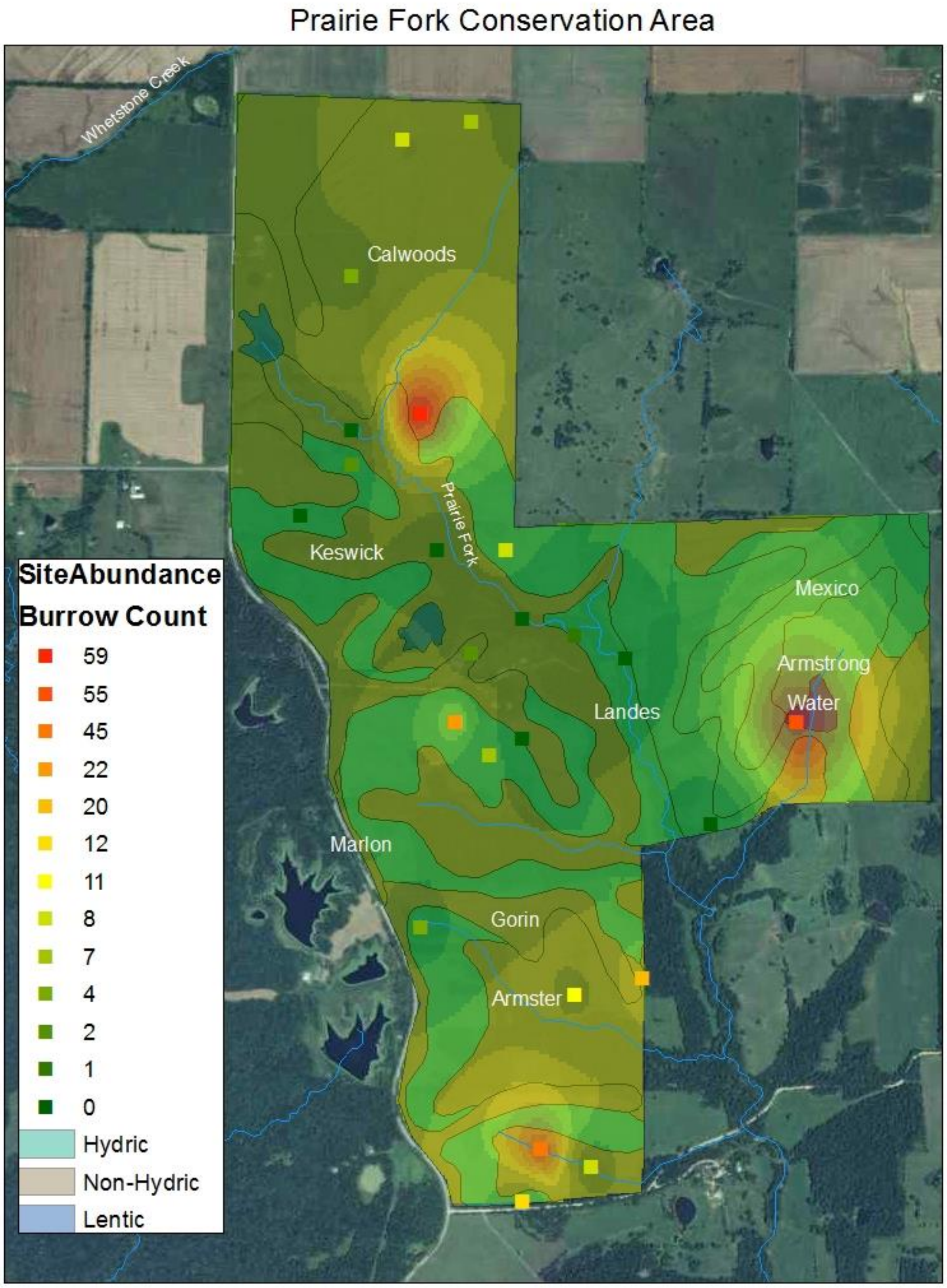

Figure 6. Map of Prairie Fork Conservation Area showing the sampled transects along with a colored gradient of burrow abundance. Transect types are delineated by line and color. Soil types are delineated by line and white labels. Inverse distance weighting contours for burrow abundance are displayed by color changes in contours.

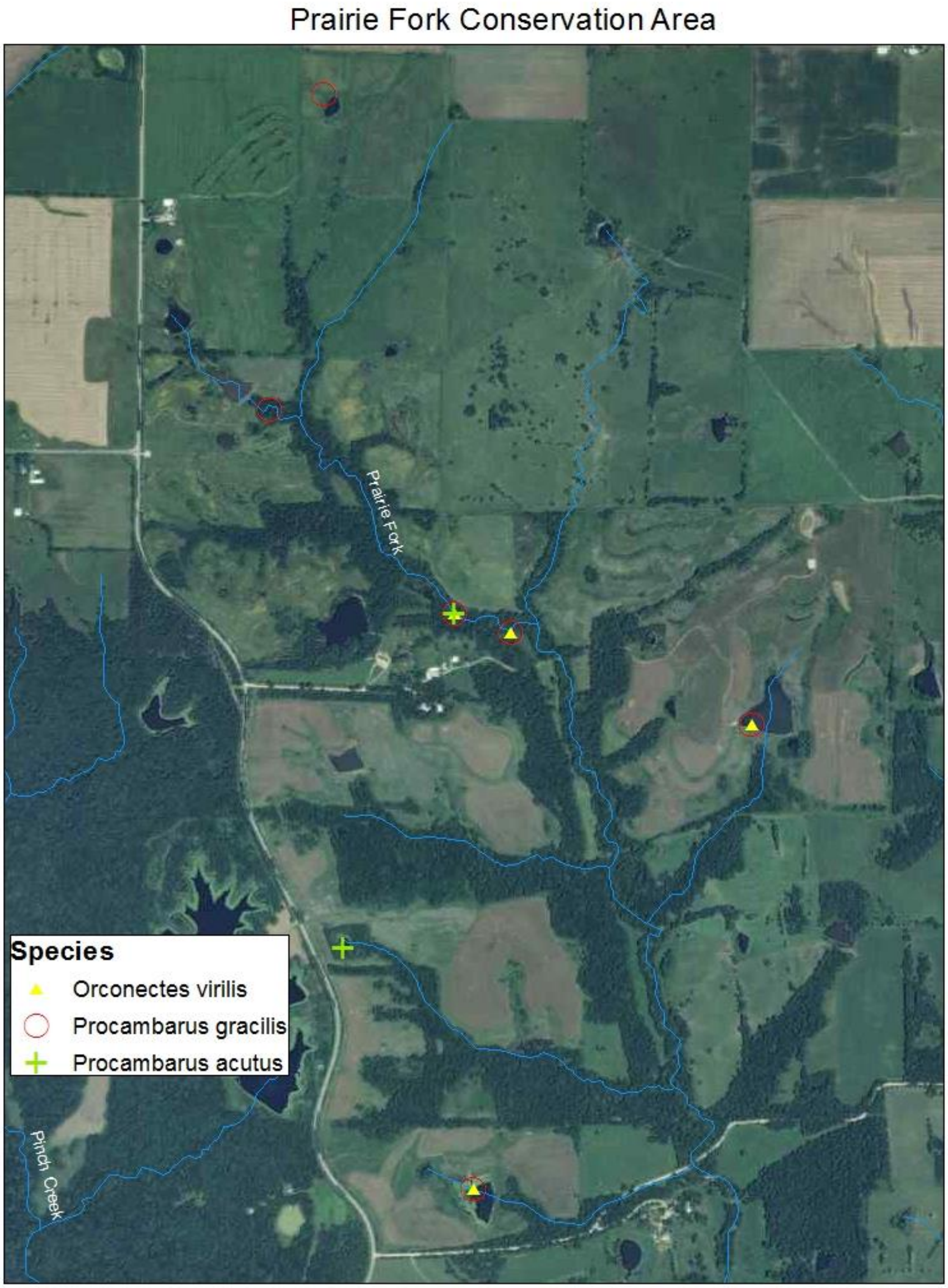

Figure 7. Map of Prairie Fork Conservation Area displaying species accounts and locations. *Orconectes virilis* (yellow triangle), *Procambarus gracilis* (red circle), and *P. acutus* (green cross).