Title: Utilizing native isopods to assess the connectivity and quality of Oklahoma groundwater

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Post Doc	0	
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Hess, A.J. and R. M. Bonett. 2018. Utilizing native isopods to assess the connectivity and quality of Oklahoma groundwater. NSF EPSCoR Annual State Conference. Poster presentation.

Hess, A.J., and R.M. Bonett. 18 July 2018. Predicting groundwater hydrology and water chemistry using native isopods. Water Research Advisory Board Summer Conference. Oral Presentation.

Hess, A.J., and R.M. Bonett. 2018. Biogeography and ecology of North American aquatic isopods. Society of Systematic Biologists Conference. Poster Presentation.

Hess, A.J. 2017. River ramblings and interesting isopods. Spring Creek Coalition. Oral Presentation. (Outreach: included specimen displays and stream excursion).

Problem and Research Objectives:

This study aims to assess native groundwater isopod distributions as a method to delineate watershed boundaries, as a tool to identify surface-groundwater interactions, and as a possible indicator of water quality. Understanding the distribution and connectivity of groundwater and its relationship to surface flow is critical for the management and conservation of this invaluable resource. The Ozark aquifer system occurs in parts of four states and is subject to range-wide extraction. Therefore aquifer and watershed border delimitation is essential to accurately measure rates of recharge and sustainable withdrawal limits. Aquifer borders typically follow the extent of drainage basins, but subterranean karstic boundaries like those of the Ozark Plateau are not necessarily correlated with surface relief and can change with fluctuating water tables. Regional declines in Ozark groundwater have been noted repeatedly since the turn of the century and local depressions have been observed around major pumping centers (reviewed Pope et al. 2009).

Receding well levels increase concerns about the sustainability of the water supply and the risk of declining water quality. With the reduction in regional water levels the risk of saline water migration from western regions of the aquifer or upwelling from lower geologic strata increases (Pope et al. 2009). Furthermore, as surface disturbance grows concurrent with population, agricultural contaminants present a growing risk. Runoff can rapidly enter groundwater due to the thin soil and extensive network of near surface karstic faults and fractures (Imes and Emmett 1994). Degrading regional water quality presents risks to human health and ecosystem integrity.

Due to their abundance and the ease of distinction between surface and subterranean species, isopods present a potentially powerful tool for assessing watershed connectivity and quality. The geographic genetic distribution of isopod diversity will likely mirror the hydrologic connectivity and discontinuity within the region. By developing distributional maps of both surface and subterranean species throughout the Oklahoma Ozarks, the limits of surface and sub-surface drainage systems can be delineated. In conjunction with water quality data, identification of species-level environmental limits can be identified to evaluate their use as a tool for understanding water quality. Continued monitoring of isopod species composition and density could serve as an indicator of changing groundwater quality.

Methodology:

To examine isopod abundance in relation to various water chemistry features we surveyed 38 Ozark streams for isopods and quantified local water chemistry. The Oklahoma Ozarks were divided into Hydrologic Unit Codes for Watersheds (HUC10) watersheds using Quantum Geographical Information Systems (QGIS). To maintain consistency of habitat type, low order streams were chosen in each watershed for sampling. At each sampling locality, bottle traps outfitted with iButton dataloggers were deployed to measure isopod abundance and temperature respectively. All sites were checked for abundance at 4 time points between October 2017 and June 2018. Concurrent with isopod collection, a water quality meter (YSI proDSS) was used to measure water chemistry parameters. These included pH, conductivity, pressure, nitrates, dissolved oxygen, and total dissolved solids. These measurements were collected during each isopod sampling period. We analyzed the data using a repeated measures linear mixed effects model in the program R (R Core Team 2018) using the package *lme4* (Bates et al. 2015) to determine which environmental features were significant predictors of isopod abundance. Site and season were treated as random effects. AIC values were used to identify the best fitting model for each genus. Genera were analyzed separately to reflect distinct surface and subsurface ecologies. Concurrent with measuring abundance, all isopods found at each sampling locality were collected and stored for genetic analyses, providing sampling resolution across both aquifers and all watersheds within the Oklahoma Ozarks.

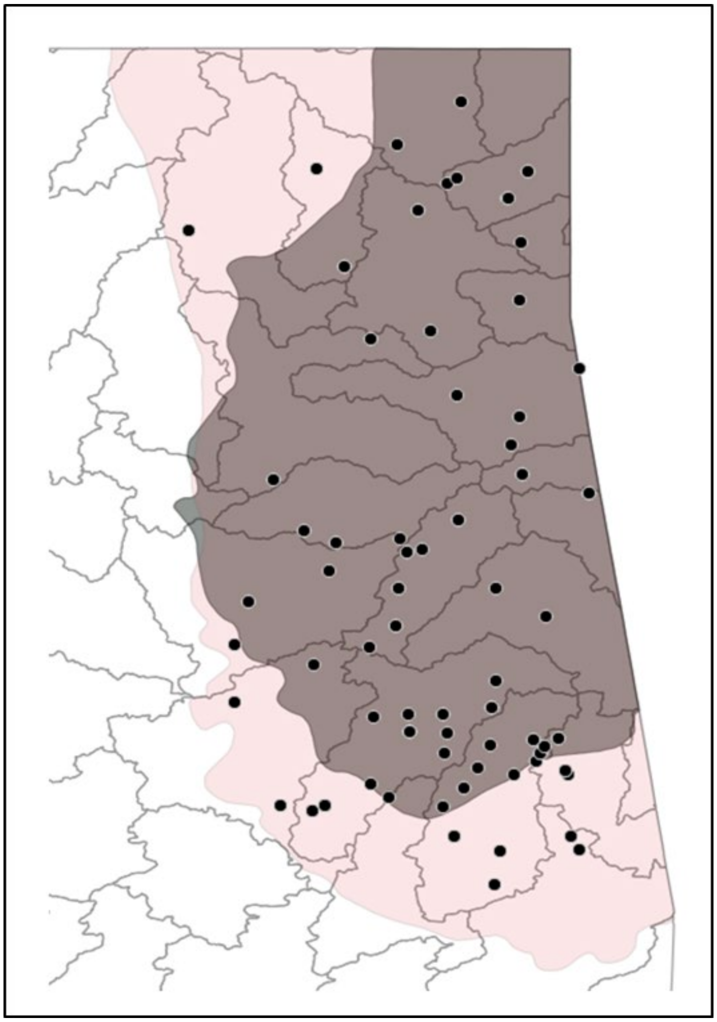


Figure 1: Sampling localities used for biogeographic analyses. Boone (black) and Roubidoux (pink) aquifers are shown, along with HUC10 watersheds.

To assess species and population level relationships across the Ozark Plateau, we derived a dataset of Asellidae specific myBaits primers for the amplification of ~500 nuclear genes. These baits were then tested on 61 *Lirceus* and 38 *Caecidotea* collected from 67 sites in the Oklahoma Ozarks (Figure 1), as well as a European family member, *Asellus aquaticus*, which was used as an out-group for genetic analyses. DNA was extracted with Qiagen DNeasy kits and ultrasonicated with a Covaris M220. Sequence adaptors were added using the Illumina DNA Truseq protocol. myBaits were used to isolate 100s of genes per sample, which were sequenced on the Illumina MiSeq at the University of Tulsa. Captured sequences were imported into CLC Genomics Workbench and assembled into contigs, which were then used to derive consensus sequences. Orthologous sequences were aligned using Sequencher.

A subset of 49 alignments of nuclear genomic regions (exons and introns) were imported into Sequence Matrix and used to construct a time calibrated Bayesian phylogeny in BEAST (Figure 2). Subphylogenies corresponding to individual genera were analyzed using the Dispersal-Extinction-Cladogenesis (DEC) model (Ree et al. 2008) in RASP (Yu et al. 2015) to reconstruct watershed colonization history. Ancestral aquifer inhabitance was reconstructed using the Mk1 model (Pagel 1999) in Mesquite (Maddison and Maddison 2018). This allows us to examine movement over time between major aquatic boundaries. Divergence time estimation was performed using a calibration point from Morvan et al. (2013).

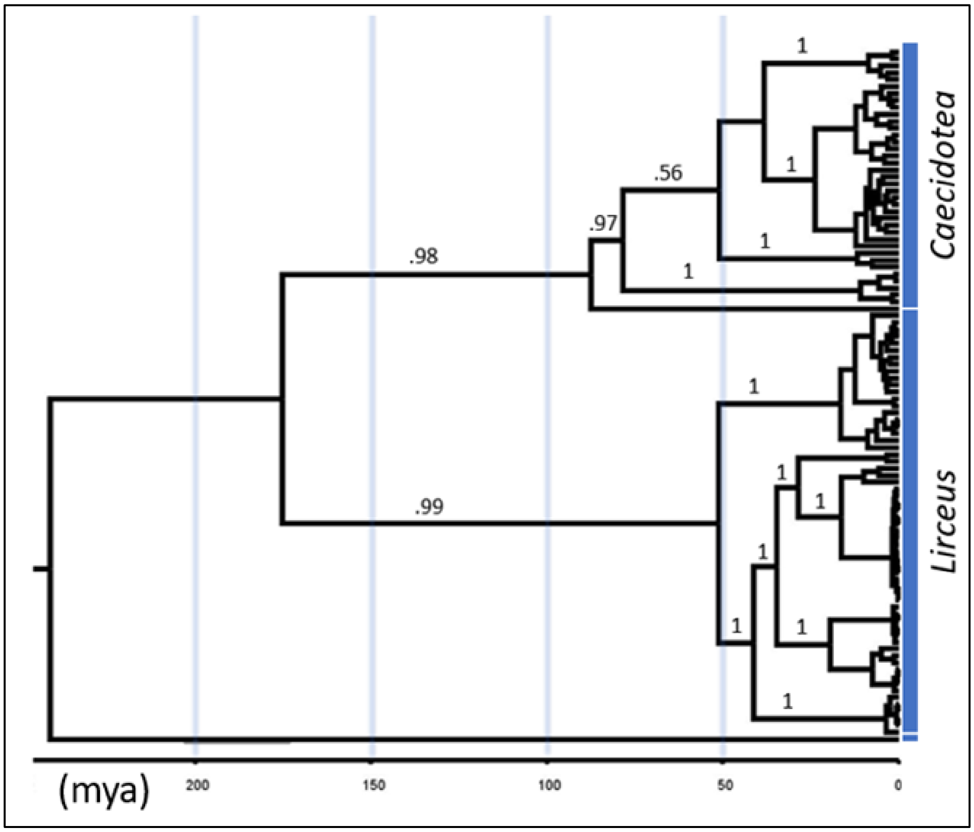


Figure 2: Time calibrated phylogeny produced in BEAST of interior highland *Caecidotea* and *Lirceus*. *Asellus aquaticus* was used as an out-group, calibration point provided by Morvan et al. 2013. Posterior probability values are provided for major clades, which indicate statistical support for relationships (>0.95 is significant).

Principle Findings and Significance:

The Oklahoma Ozarks consist of a deep aquifer of Roubidoux sediment comprised of sandstone and dolomite. This is overlain by the chert limestone of the Boone aquifer. The region is further bisected by the Neosho (northern) and Illinois (southern) Rivers, both of which flow into the Arkansas River, forming a coarse border for the Oklahoma portion of the Ozarks and further bisecting the Interior Highlands. This region can be subdivided into 6 primary (HUC8) watersheds: “Lake o’ the Cherokees”, “Elk”, “Lower Neosho”, “Dirty-Greenleaf”, “Illinois”, and the “Robert S. Kerr Reservoir” (Figure 3).

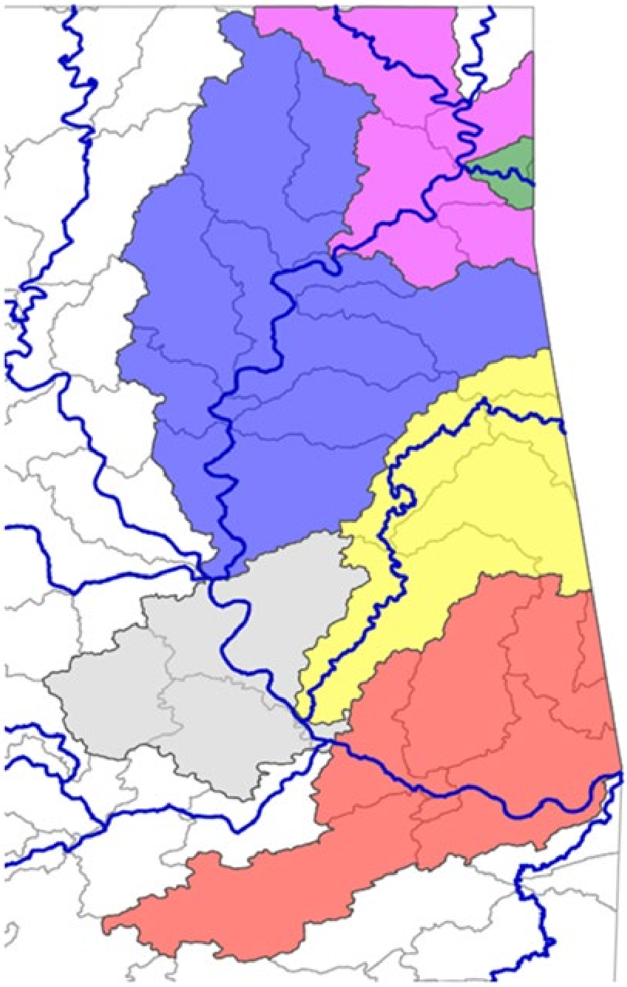


Figure 3: Surface Rivers and HUC10 watersheds overlying the Boone and Roubidoux aquifers of the Oklahoma Ozarks. Color corresponds to aquifer. Magenta: Lake o’ the Cherokees, Pale Green: Elk, Blue: Lower Neosho, Grey: Dirty-Greenleaf, Yellow: Illinois, and Red: Robert S. Kerr Reservoir.

Due to their abundance, high frequency of occurrence, and obligately aquatic life history, isopods have potential utility for measuring water quality and hydrological connectivity. Within Oklahoma there are 16 named species (Graening et al. 2007) distributed across two genera, *Lirceus* (4 species) and *Caecidotea* (12 species). North American isopods have benefited from extensive taxonomic work based on morphology (e.g. Lewis et al. 2006; Hubricht and Mackin 1949), but these hypotheses have not been tested with molecular data. This is in stark contrast to European taxa (Morvan et al. 2013; Verovnik et al. 2004), where native species have been shown to have utility in examining historic watershed and aquifer connectivity (Verovnik et al. 2005).

In this study we reconstructed a molecular phylogeny of 99 Oklahoma Ozark isopods using 49 genomic regions (Figure 2). The isopods *Lirceus* (surface morphology) and *Caecidotea* (subterranean morphology) form two well-resolved clades that are highly divergent (Figure 2). *Caecidotea* displays deeper divergences among lineages, indicating both high species level diversity in the region as well as that an older history in the Oklahoma Ozarks. The increased genetic structure within *Caecidotea* suggests that groundwater connectivity has been reduced relative to surface watersheds.

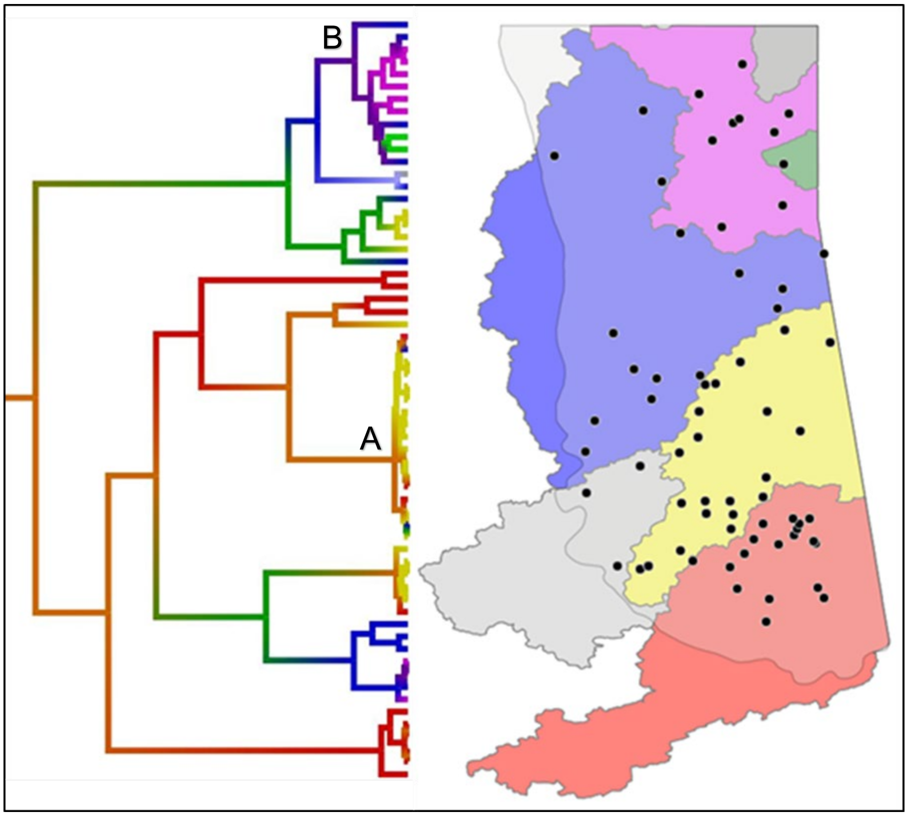


Figure 4: Ancestral area reconstruction of *Lirceus* based on a DEC model. Color corresponds to HUC8 watershed. Magenta: Lake o' the Cherokees, Pale Green: Elk, Blue: Lower Neosho, Grey: Dirty-Greenleaf, Yellow: Illinois, and Red: Robert S. Kerr Reservoir. Blended colors on branches correspond to lineages historically occupying multiple drainages

Ancestral area reconstruction was performed for both *Lirceus* (Figure 4) and *Caecidotea* (Figure 5) to assess patterns of water connectivity. *Lirceus* displays a pattern consistent with a recent, rapid expansion of two lineages across the Illinois River watershed (Figure 4; Clade "A"). Rapid lineage expansion, as observed in the *Lirceus* of the Illinois River watershed, suggests a recent or ongoing period of high connectivity within the drainage. In contrast, a primarily northern clade in the Lower Neosho, Lake o' the Cherokees, and Elk watersheds shows more structure (Figure 4; Clade "B"), which suggests a period of limited connectivity and reduced dispersal to allow for lineage differentiation.

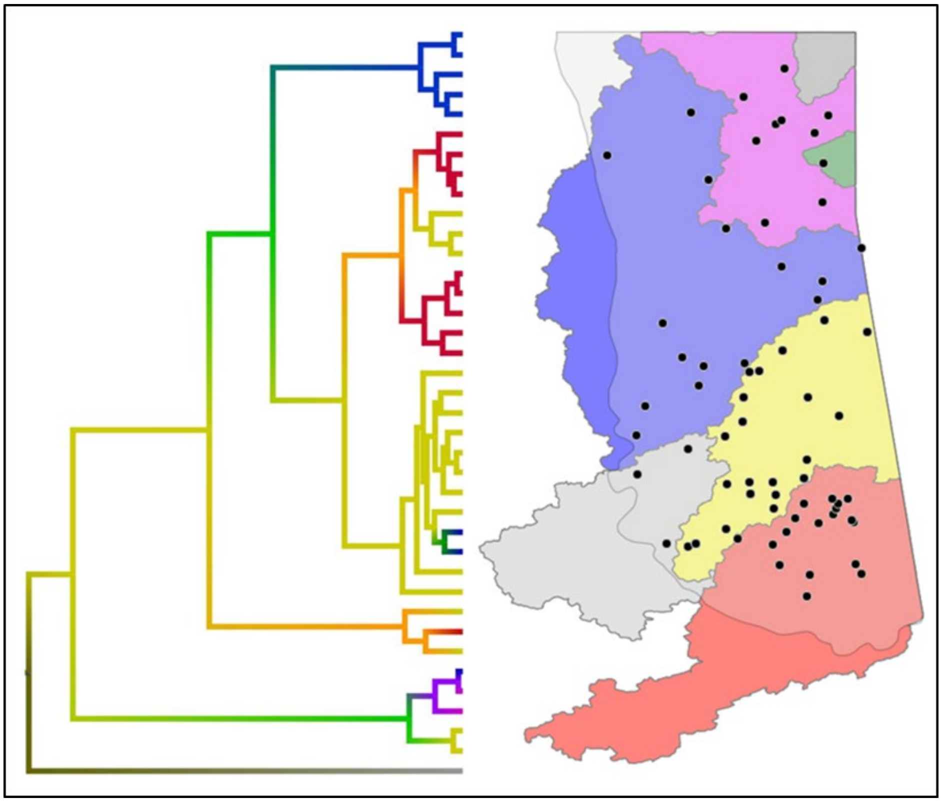


Figure 5: Biogeographic reconstruction of *Caecidotea* based on a DEC model. Color corresponds to HUC8 watershed. Magenta: Lake o' the Cherokees, Pale Green: Elk, Blue: Lower Neosho, Grey: Dirty-Greenleaf, Yellow: Illinois, and Red: Robert S. Kerr Reservoir. Blended colors on branches correspond to lineages historically occupying multiple drainages

A similar pattern can be observed in *Caecidotea*, with the oldest lineage associated with the Dirty-Greenleaf watershed followed by a transition to the Illinois River drainage. Subsequent colonization events expanded from the Illinois River to the Robert S. Kerr and Lower Neosho watersheds. A recent stream capture from the Illinois to the Lower Neosho Watershed reinforces a need for better resolution in the Illinois and Lower Neosho border region.

Reconstructions of aquifer colonization for both *Caecidotea* (Figure 6A) and *Lirceus* (Figure 6B) reflect a pattern of colonization consisting of repeated invasions of the Roubidoux Aquifer from the limestone chert of the Boone. Interestingly, the most deeply divergent lineage within *Caecidotea* is associated with the Roubidoux, suggesting this aquifer may have been colonized earlier. This also might reflect biased sampling effort, as cave associated isopods were frequently found in the hyporheic zone around streams and may not be representative of the biogeographic history of obligate deep aquifer inhabitants.

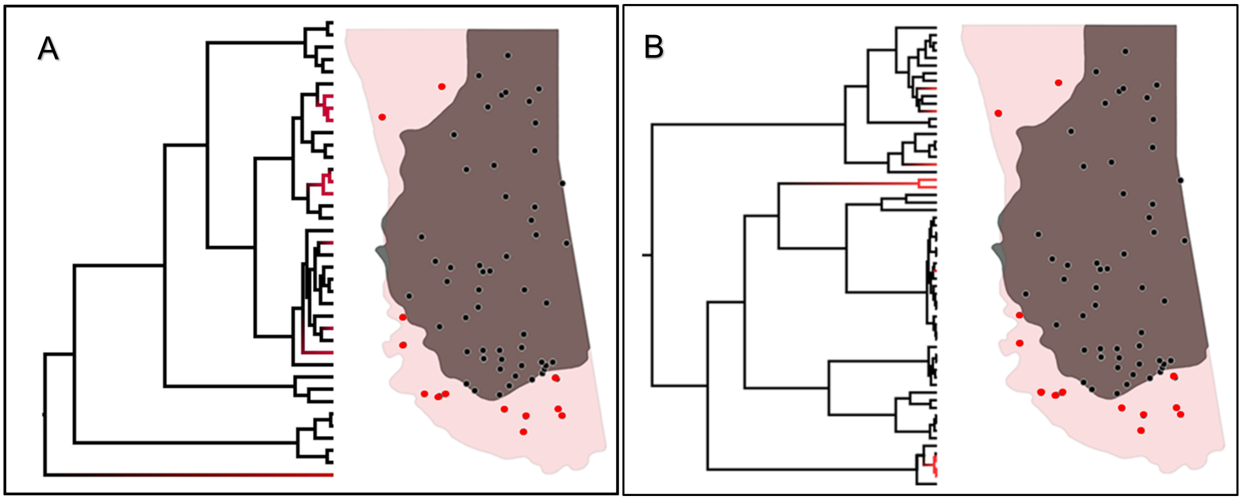
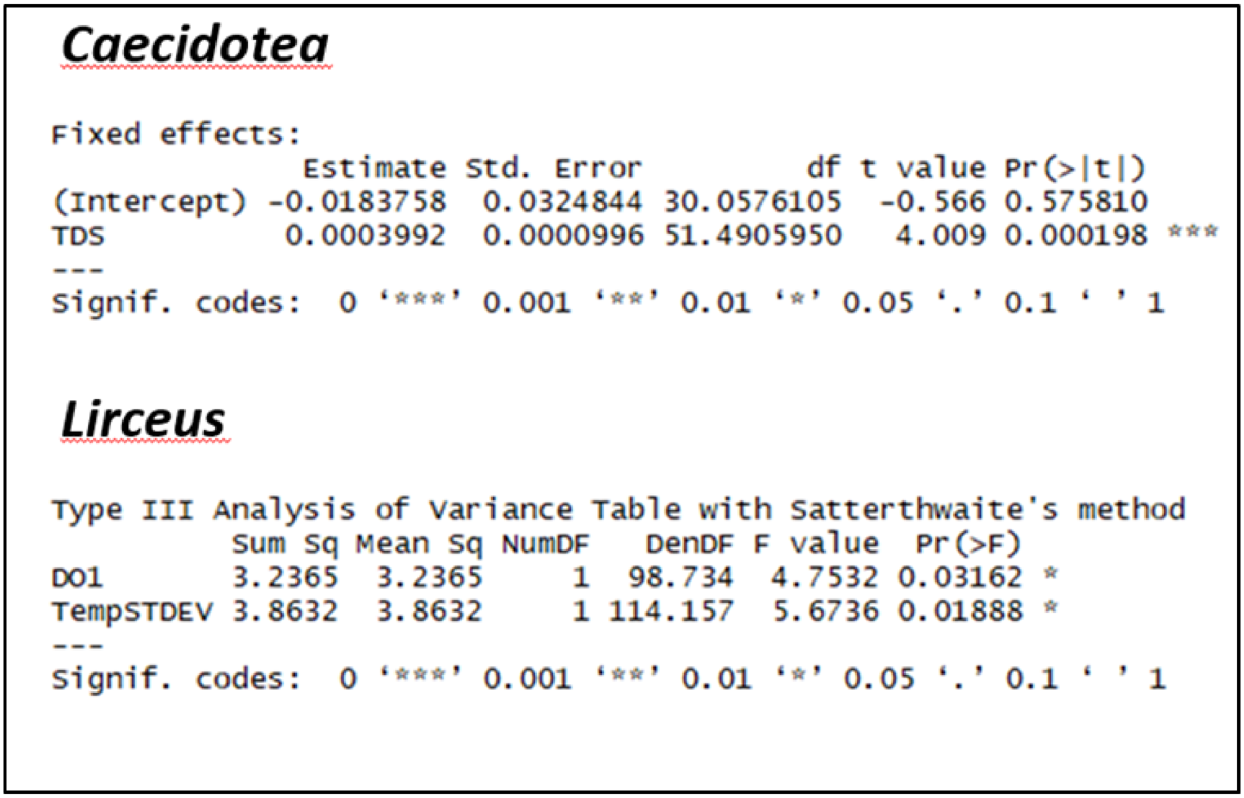


Figure 6: Reconstructions of aquifer colonization history in *Caecidotea* (A) and *Lirceus* (B) using the Mk1 model implemented in Mesquite. Black lineages correspond to the Boone aquifer, red to the Roubidoux.

Both *Lirceus* and *Caecidotea* show some recent transitions across the border of the Illinois and Lower Neosho Watersheds. This suggests either higher permeability between these watersheds than expected, the isopods can to some degree disperse across watershed borders, or they may be the signatures of recent stream captures.

Models of isopod stream abundance in relation to water chemistry (Figure 7) supported total dissolved solids (TDS) as the best-fit model for predicting abundance of *Caecidotea*. As a measure of total dissolved content of all inorganic and organic substances, TDS is likely representative of higher mineral content in the groundwater outputs associated with *Caecidotea*. However, sample size was low, as *Caecidotea* infrequently colonized our bottle traps. In contrast, *Lirceus* abundance declined with increasing thermal instability (TempSTDEV) and declining dissolved oxygen (DO1). *Lirceus* abundance appears to be indicative of high stream quality and well-developed riparian buffers.



Caecidotea

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Fixed effects:
              Estimate Std. Error        df t value Pr(>|t|)
(Intercept) -0.0183758  0.0324844 30.0576105  -0.566 0.575810
TDS          0.0003992  0.0000996 51.4905950   4.009 0.000198 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Lirceus

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Type III Analysis of Variance Table with Satterthwaite's method
          Sum Sq Mean Sq NumDF   DenDF F value  Pr(>F)
DO1       3.2365  3.2365     1  98.734  4.7532 0.03162 *
TempSTDEV 3.8632  3.8632     1 114.157  5.6736 0.01888 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 7: Significance products from a repeated measures linear mixed effects model run in R using package lme4 for both *Caecidotea* and *Lirceus*. Best fitting models determined by AIC values are displayed here. Total dissolved solids (TDS), thermal instability (TempSTDEV) and dissolved oxygen (DO1).

Closing Thoughts:

This project demonstrated that genetic structure of aquatic isopods follows broad patterns of regional hydrological history in the Oklahoma Ozarks. Therefore, these organisms are promising for examining patterns of hydrologic connectivity and discontinuity. As our broad regional analysis encompassed many species, more in depth local genetic analyses of a single species are needed to test hydrologic patterns at a finer scale.

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