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The Impact of Road Crossings on Karst Headwater Streams in Northwest Arkansas

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Abstract

The karst region of NW Arkansas is home to many headwater endemic Species of Greatest Conservation Need (SGCN). This includes many species of darters, such as *Etheostoma cragini*, *E. microperca*, and *E. mihileze*, as well as crayfish such as *Faxonious meeki brevis* and *F. nana*. NW Arkansas is rapidly urbanizing, increasing the need to construct structures like culverts, bridges, and fords. These man-made road crossings can cause stream habitat degradation and fragmentation, as well as impair overall stream connectivity. To evaluate the impact that road crossings have on aquatic SGCN species and their habitat, 30 headwater streams were sampled throughout Benton and Washington counties. A series of Wilcoxon signed-rank tests were used to examine relationships between road crossing presence and stream habitat. Stream sites with road crossings had significantly higher water temperatures and conductivity, as well as more embedded substrates and increased levels of bank incision. Partial least squares regression was used to examine how road crossing presence and stream habitat variables influence SGCN abundance, density, condition, diversity indices, and community metrics. The composition of fine sediment and aquatic vegetation, which is preferred habitat for *E. cragini* and *E. microperca,* was significantly lower at sites with road crossings. SGCN darters tended to occur less frequently at sites with these structures, and at smaller abundances when they did occur. However, SGCN crayfish occurred at higher abundances at sites with road crossings, which typically had larger substrates. Additionally, the condition of F. nana, along with two other non-SGCN species, was significantly higher when these structures were present. These data suggest the need for

multispecies conservation approaches, as road crossings may affect SGCNs uniquely, especially across taxonomic groups.

Table of Contents

Chapter I

Introduction

Headwaters are integral and diverse aquatic ecosystems, encompassing springs, ephemeral, intermittent, and perennial flowing channels, as well as wetlands outside of floodplains (Colvin et al. 2019). They are defined as areas where water originates within a network that contributes to the development and maintenance of downstream navigable waters such as rivers, lakes, and oceans (Gomi et al. 2002; Colvin et al. 2019). Collectively, these systems comprise the majority of river networks globally (Datry et al. 2014) and are 79% of U.S. river length (Colvin et al. 2019). Headwaters help sustain aquifers, maintain natural flow regimes, regulate sediment transport, influence nutrient cycling, and provide habitat for a wide array of species (Gomi et al. 2002; Lowe and Likens 2005; Wohl 2017; Colvin et al. 2019; Tsuboi et al. 2022). They are also major contributors to the hydrologic and riverine connectivity of a water body, as well as the surrounding terrestrial landscape (Freeman et al. 2007). As a result, headwaters make substantial contributions to the water quality, biodiversity, and ecological integrity of their respective watersheds (Lowe and Likens 2005).

Karst headwaters are produced by the dissolution of carbonate rocks such as limestone and dolomite (White et al. 1995). This process results in the development of internal drainage and conduit flow systems, which can produce sinking streams, springs, and caves (White et al. 1995) Karst ecosystems have distinct physical, chemical, and biological characteristics. Typically they are isolated, small in size, and have stable temperatures and clear water (Seilheimer and Fisher 2010; Cantonati et al. 2012; Wagner et al. 2012). Karst systems also have relatively constant flow regimes, part of which may occur underground, creating stretches of dry streambed that only flow during storm events (Homan et al. 2005; Wohl 2017). The volume of surface flow in these systems can be reduced by half, or completely dry up in groundwater recharge zones (Hubbs 1995).

Springs are found wherever groundwater from aquifers permeates the surface, forming a three-way ecotone among the hyporheic zone, streams, and terrestrial ecosystems (Barquin and Scarsbrook 2008; Cantonati et al. 2012). As a result, they act as important drivers of the physiochemical characteristics of karst headwaters and influence many structural and functional attributes of freshwater ecosystems (Barquin and Scarsbrook 2008). The size of the springs and the volume of their flow influences downstream thermal stability (Hubbs 2001). For example, large springs can dampen the effects of storm events or the ambient air temperature downstream of their origin (Hubbs 1995). Springs also contribute downstream base flow, whether they run over a short distance with little residency underground, or span thousands of miles and have residency periods that last hundreds of thousands of years (Cantonati et al. 2021; Stevens et al. 2021). Due to their geologic origin, thermal stability, consistent flows, and microhabitat heterogeneity, springs support highly diverse communities, as well as highly endemic organisms (Hubbs 2001; Gomi et al. 2002; Barquin and Scarsbrook 2008; Cantonati et al. 2012).

The Arkansas Darter *Etheostoma cragini* and Least Darter *E. microperca* are Species of Greatest Conservation Need (SGCN) that inhabit the karst region of northwest Arkansas. The Arkansas Darter is found in the Arkansas River drainage which stretches across five states, from Colorado to Arkansas (Taber et al. 1986; Wagner and Kottmyer 2006; Wagner et al. 2011; CPW profile). The Least Darter is primarily distributed throughout the Mississippi Basin, with the Arkansas population at the southern extent of its range (Becker 1983; Wagner et al. 2012). The populations of both species found in the karst region of northwest Arkansas are genetically distinct from other populations in their ranges (Wagner et al. 2020). Arkansas and Least Darters can be found in small spring-fed streams with an open canopy, dense aquatic vegetation, and fine substrates (Wagner and Kottmyer 2006; Wagner et al. 2011; Wagner et al. 2012; Baker et al. 2018; Wagner et al. 2020; CPW Profile). In these habitats, they typically feed on small macro-invertebrates, and spawn between February and July (Burr and Page 1979; Taber et al. 1986).

Another SGCN darter species that occurs in northwest Arkansas is the Sunburst Darter *E. mihileze*. Endemic to the Arkansas River drainage in the Ozark Plateau, they are typically found in springs or spring-fed creeks dominated by coarse substrates and watercress *Nasturtium* spp. (Robison and Buchanan 2019). Though they can be locally abundant in this area, their habitat is declining rapidly due to extensive human population growth, warranting their conservation status in the state of Arkansas (Robison and Buchanan 2019).

The Midget Crayfish *Faxonius nana* and Meeks Shortpointed Crayfish *F. meeki brevis* are also SGCNs that inhabit the karst region of northwest Arkansas. Both species inhabit headwaters and spring runs of the Illinois River drainage in northeast Oklahoma and northwest Arkansas (Williams 1952). Additionally, both the Midget and Meeks Shortpointed Crayfish occur less frequently in highly disturbed streams (Mouser et al. 2018). Two other crayfish species that constitute the remainder of the assemblage in northwest Arkansas are the Northern *Faxonius virilis* and Ringed Crayfish *F. negelectus*. Although both are native to the state and throughout northern Mississippi drainages, they have successfully invaded adjacent watersheds and may hold a competitive advantage over other endemic crayfish due to their generalist nature and potential to attain larger body sizes (Simon and Stewart 2014; Rodger and Starks 2020).

Crayfish often act as keystone species, serving as important omivorous nutrient cyclers who process organic matter, and as prey items for a wide variety of organisms (Magoulick et al. 2017; Mouser et al. 2018; Taylor et al. 2019). They also serve as ecosystem engineers, influencing algal cover on substrate, bioturbation of sediments, and presence of interstitial spaces in the substrate (Creed and Reed 2004; Taylor et al. 2019). Crayfish as a taxonomic group are understudied. More than 50% of U.S. states identify the need for basic information such as distributions, life history, and ecology to carry out effective conservation (Taylor et al. 2019). Crayfish are also among the most at risk to be negatively impacted by anthropogenic change in lotic systems (Nolen et al. 2014).

One of the most persistent threats to SGCNs is the reduction and alteration of habitat. Spring-fed headwaters are highly susceptible to localized anthropogenic disturbances due to the interaction between surface and groundwater in these systems, their lack of adequate downstream buffering mechanisms, and linkage to the terrestrial environment (Lowe and Likens 2005; Wohl 2017). Northwest Arkansas is rapidly urbanizing, and populations of Benton and Washington counties have grown by over 100,000 since 2010, a population increase of over 20% for both counties (Nelson 2021). Land use practices can alter the hydrological, physiochemical, and biological conditions of an organism's habitat, and as a result, can change or restrict species distributions (Mouser et al. 2018).

Culverts, bridges, fords, and other road crossings can negatively impact aquatic ecosystems, potentially altering hydrologic and geomorphologic characteristics of the lotic environment as well as inhibiting sediment and nutrient transport downstream (Norman et al 2009; Birnie-Gauvin et al. 2017; van Puijenbroek et al. 2021). These structures also contribute to increased sedimentation due to the erosion of nearby sloped banks (Shields et al. 1994; Gal et al. 2019). This can occur even during low-magnitude precipitation events, and the common removal of riparian vegetation around the crossings further amplifies the potential for sedimentation (Shields et al. 1994; Gal et al. 2019). Along with the addition of sediment, road crossings can also introduce pollutants such as heavy metals, salts, fertilizers, and pesticides into the stream depending on land use practices (Gal et al. 2019). The temperature of these systems may also be impacted, which can affect the distribution, abundance, and persistence of ectotherms (Farless and Brewer 2017). Additionally, natural flow regimes can be impacted, further altering habitat. For example, culverts can degrade the stream benthos downstream of the road crossing due to increased water velocity, creating scour pools (Pluym et al. 2008). Similarly, if insufficient water can pass through the culvert, the upstream area may become ponded, also impacting habitat (Birnie-Gauvin et al. 2017).

Urbanization creates a higher prevalence of impermeable surfaces, which increases the input of surface flow into streams, leading to increased flooding and channelization (Poff et al. 1997). Riffle-run-pool ratios of the stream are also impacted, simplifying overall structural complexity (Pluym et al. 2008). This complexity is critical to the survival of aquatic communities, as it creates suitable habitat and influences interactions with their biotic and abiotic environment (Pluym et al. 2008). These concerns especially apply to spring-adapted species, which are specialized to these environments and are unable to compete with more tolerant generalists (Hubbs 2001).

Along with altering habitat, road crossings also limit or prevent the passage and dispersal of fish, crayfish, aquatic insects, and freshwater mussels within and between habitats (Norman et al. 2009). Without the ability to move through all of their habitat, foraging, avoiding predators, and finding spawning ground becomes more difficult, or even impossible (Warren and Pardew 1998; van Puijenbroek et al. 2021). Roadside crossings can also delay or prevent fish recovery or recolonization following other disturbances such as droughts, floods, or introductions of invasive species (Warren and Pardew 1998). Additionally, these barriers can cause habitat fragmentation. This process occurs when a large continuous stretch of habitat is converted into several smaller, isolated patches (Keyghobadi 2007). Habitat fragmentation can also take place when the total area of habitat decreases, when the area of non-habitat increases, or if the quality of habitat decreases (Keyghobadi 2007). Survival rates and the ability to move within and between these fragmented patches of habitat will vary, meaning some patches will become crucial for the persistence of species that use them (Tsuboi et al. 2022).

Habitat fragmentation can also impact entire populations. When existing populations become smaller and more isolated from each other, increased genetic drift with decreased gene flow occurs (Keyghobadi 2007). This results in an erosion of genetic diversity, which over time reduces fitness and hampers the population's ability to adapt to changes in their environment (Keyhobadi 2007; Fullerton et al. 2010; Baker et al. 2018). Fragmented, small populations have higher rates of local extinction, which in turn alters local community structure, ultimately resulting in the replacement of unique, rare species with more tolerant generalists (Benton et al. 2008, Briggs and Galarowicz 2013, Baker et al. 2018).

Culverts, bridges, and fords can also influence species distributions. Higher occurrence probabilities for Midget and Meeks Short Pointed Crayfish have been reported when stream disturbance levels are lower (Mouser et al. 2018). Gagen and Landrum 2000 as well as Nislow et al. 2011 found that stream reaches downstream of road crossings had almost twice the fish abundance and species richness of reaches upstream of the roadside crossing. Additionally, road crossings frequently do not meet established conservation requirements (if known) or are designed without taking fish passage and habitat into consideration (Gibson et al. 2005).

Roadside crossings also pose a threat to springs at the local level, along with agricultural practices and recreation (Cantonati et al. 2020). At the regional level, urbanization, excessive groundwater extraction, and pollution from mining, agriculture, and sewage contribute to potential problems (Cantonati et al. 2020). These disturbances and foreign inputs can alter the flow, thermal stability, and geochemistry of springs, all of which can lead to the loss of fish species and other highly adapted endemic fauna (Hubbs 2001). At the current rate groundwater is being harvested, 40-80% of the world's catchments will be below the minimum flow required to maintain ecological function by 2050 (Cantonati et al. 2020). Streamflow quantity and timing influence water quality and the ecological integrity of river systems, as it is correlated with water temperature, channel geomorphology, and habitat diversity (Poff et al. 1997). Furthermore, groundwater-dependent ecosystems require the surface expression of groundwater to maintain species composition and habitat quality (Seilheimer and Fisher 2010). Even minor declines in flow, whether that be from overdraft or flow alteration from road crossings, can be detrimental to fish populations (Hubbs 2001). In addition to supporting diverse life, springs also provide ecosystem services and hold important economic and cultural value. Considering that springs have reportedly been degrading and collapsing in the lower latitudes of North America as human influence has increased, it is crucial that we gain a better understanding of these systems (Stevens et al. 2021).

The Ozark Highlands has complex geology with karst topography and is vulnerable to anthropogenic impacts as a result of increased urbanization (Baker et al. 2018). The physiochemical and biotic integrity of these streams is at risk due to land use practices and increasing human populations. Stream banks are eroding and water quality is deteriorating due to infrastructure development (Robison and Buchanan 2019). Livestock production is also popular in this region (over 180,000 cattle in Benton and Washington counties as of 2022 according to USAD), further increasing the risk of contaminating waters with nitrates and phosphates (Robison and Buchanan 2019). Unfortunately, the karst topography lacks a developed soil profile, providing little means of filtering out inputs of sediment and pollutants, further amplifying this issue (Robison and Buchanan 2019). This study investigates (1) how road-crossing structures impact stream habitat (2) how road crossings and stream habitat impact SGCN darter abundance and density (3) how road crossings and stream habitat impact crayfish abundance, density, and condition.

Objective 1 Hypotheses:

H_O- Streams with road crossings and those without them have the same stream habitat

H_A- Streams with road crossings will be more incised, have higher water temperatures and conductivities, have less aquatic vegetation, and lower amounts of dissolved oxygen than streams without these structures.

Objective 2 Hypotheses:

H_O- Road crossings and stream habitat do not influence darter abundance and density

H_A- Streams with road crossings and that have less aquatic vegetation and lower levels of dissolved oxygen with higher water temperatures will have lower abundances and densities of SGCN darters

Objective 3 Hypotheses:

H_O- Road crossings and stream habitat do not influence crayfish abundance, density, diversity, and condition

H_A- Streams with road crossings and that have less coarse substrates and lower levels of dissolved oxygen with higher water temperatures will negatively influence SGCN crayfish abundance, density and condition while positively influence generalist crayfish parameters

Chapter II

Methodology

Site Information

Sampling was conducted from late May through early August of 2022 in Benton and Washington counties in the Northwest Karst Region of Arkansas. Sites were selected at or near locations where Arkansas and Least Darter have been documented (Wagner et al. 2020) (Figure 1), as well as in lesser surveyed sub-basins with similar habitat. The 30 sites occurred in the Interior Highlands of Arkansas and in the Ozark Plateau Ecoregion, specifically within the Springfield Plateau (Robison and Buchanan 2019). This area is mostly comprised of limestone, dolomite, sandstone, and shale, with its streams being spring-fed and clear (Robison and Buchanan 2019).

Fish and Crayfish Sampling

Sites consisted of stream reaches of roughly 30x average wetted width and no less than 150m, with road crossings serving as the middle point of each site if present. Fish and crayfish were sampled with dipnets of 2-mm mesh size. Multiple dip netters sampled for 20 minutes both upstream and downstream of barriers (40 minutes for the entire ~150m reach if no barrier was present). Dipnetters focused their effort on microhabitats known to be preferred by Arkansas and Least Darters, including near or within aquatic vegetation, submerged terrestrial vegetation, undercut banks, and backwaters with fine substrates (Wagner and Kottmyer 2006; Wagner et al. 2011; Wagner et al. 2012; Wagner et al. 2020; CPW Profile). In addition to dipnets, a minimum of five seine hauls were used to cover other areas of the streams and capture more of the overall fish assemblage.

Seine dimensions consisted of ~2.5m in length and ~1.25m in height, with a mesh size of 0.3175cm. Both techniques have been proven to be effective at sampling darters (Labbe and Fausch 2000; Wagner et al. 2020), and crayfish (Price and Welch 2009; Engelbert et al. 2016). Captured individuals were identified, weighed (grams), and measured (standard and total length in millimeters), with crayfish also being sexed before release. Bubblers were used to provide oxygen while individuals were processed.

Road Crossing Assessment

The Southeastern Aquatic Resource Partnership's Stream Crossing Survey protocol (2020) was used to evaluate road crossings. Various qualitative and quantitative data were collected on the road crossing structures, including crossing type, flow, crossing condition, structure alignment, and scour pool presence, as well as structure shape, grade, and dimensions (Table 1). Reference reach data, including measurements of bankfull width, water depth, and velocity were also collected to determine the extent of alteration in reaches influenced by road crossing presence. The location of each reference reach was dependent on the wetted width of the stream near the road crossing (15 times that measurement upstream of the structure).

Habitat Assessment

Stream habitat surveys were conducted across the length of each stream reach using the EPA's Environmental Monitoring and Assessment Program (Kaufman and Robinson 1998). This was done at eleven equidistant transects set a minimum of 15 meters apart, depending on the wetted width of the stream. Various physiochemical, hydrologic, and habitat data were collected, such as cross-sectional depths, substrate cover and embeddedness, bank dimensions, and densiometer readings, as well as stream discharge and sinuosity (Table 2). Stream incision was determined by measuring the height up from the water surface to the elevation of the first terrace of the floodplain (Kaufman and Robinson 1998). Stream thalweg was obtained by identifying the flow path of the deepest water in the channel (Kaufman and Robinson 1998). Riparian vegetation was estimated at three levels; canopy (>5m high), understory (0.5-5m high), and ground cover (<0.5m high). Averages of these measures were then calculated to be representative of each stream reach. The amount of coarse woody debris (CWD) > 2.5cm was also measured within half a meter upstream and downstream of each transect. Planar area was calculated by multiplying the diameter of each piece by its length, then dividing by the total area sampled within each transect (m^2 of CWD per m^2 of stream). This was then extrapolated to the entire stream reach of each site (Maloney et al. 2005). The use of an Onsite YSI Professional Plus™ unit was used to collect dissolved oxygen (mg/L), water temperature (°C), and conductivity (µs/cm) readings.

Parameter Calculations

Scaled mass index of crayfish was calculated using the formula developed by Peig and Green 2009 $\dot{M}_i = M_i[\frac{L_0}{L_i}]^{b_{SMA}}$, where $M_i$is the mass of an individual, L_0 is the average carapace length of the species, L_i is the carapace length of an individual, and b_{sma} is the slope of the relationship between M_i and L_i. SGCN densities were calculated by dividing the abundance of each species by the surface area of the stream reach (reach length x average wetted width). Crayfish abundances were also used to calculate diversity indices using the Past4 software (Hammer et al. 2001). These included richness estimators such as Shannon's, Brillouin's, and Margalef's Indices, as well as Fisher's alpha, and Chao1 estimator (Table 3). Evenness estimators such as Dominance, Simpson's Index, and Equitability were also incorporated (Table 3). These indices were chosen because they cover an array of distributional assumptions, and are affected differently by sample size or effort.

Species richness of crayfish at each site is typically small, so additional community metrics were incorporated in the analyses to help understand the effect of environmental predictors. These included the geographic range of each species (narrow, regional, widespread), as well as their habitat (lotic, lentic, generalist) and substrate (fine, coarse, generalist) preferences. Sources used to justify classifications in each category include Pflieger and Dryden 1996, Larson and Olden 2010, McAllister et al. 2015, and NatureServe (Table 4).

Data Analysis

Wilcoxon signed-rank tests were used to examine relationships between road-crossing presence (predictor) and stream habitat (response), while Kruskal Wallis tests were used to examine relationships between road-crossing type and severity (according to SARP protocols) impact on stream habitat. These tests were chosen because the data did not meet the assumption of normality for t-tests. Partial least squares regressions (PLS, R package pls) were used to examine how road crossing presence and stream habitat variables influence darter and crayfish abundance, and densities, as well as the condition, diversity indices, and community metrics of crayfish. This allowed for a holistic approach when analyzing the data, and also accounted for collinearity among predictors. All analyses were conducted in R version 3.4.3

Chapter III

Results

Impact of Road Crossings on Stream Habitat

Seventeen road crossings were assessed using SARP's Stream Crossing Protocol. Each crossing received a score (ranging from 0-1, with lower scores indicating more severe barriers) and classification determining how likely they were to impact aquatic organisms (Table 5 and Table 6). Stream habitat assessments were conducted at 12 of these structures, which included 3 bridges, 2 single-celled culverts, 5 multiple-celled culverts, and 2 fords. Thirty-three percent of these 12 structures were classified as having severe impacts on aquatic organisms. These included two multiple-celled culverts and both fords. One single-celled culvert was classified as having minor impacts, and the remaining structures were classified as having insignificant or no impact on aquatic organisms (Figure 2).

Many aspects of stream habitat were significantly different between sites with and without road crossings (Table 7). Wilcoxon sign-ranked tests found that the presence of road crossings was associated with higher water conductivity ($p = 0.02$), substrate embeddedness ($p = 0.07$), stream incision ($p = 0.04$), and water temperatures ($p = 0.02$). Fine and organic substrates ($p < 0.01$), aquatic vegetation ($p < 0.01$) canopy cover ($p < 0.01$), and tree roots ($p = 0.07$) were all found less frequently at sites that had these structures. No significant differences in these variables were found between the upstream and downstream portions of sites with road crossings. There were also no significant differences in the total number of riffles, runs, and pools located above and below these structures.

Impact of Road Crossing Type on Stream Habitat

A Kruskal Wallis test, followed by a post hoc Dunn test indicated that sites with multiple-celled culverts had significantly higher stream temperatures compared to other crossing types and sites that lacked structures, with the median being 22.9°C. A Wilcoxon sign-ranked test determined that differences in temperature upstream and downstream of these structures, as well as the single-celled culvert, were minimal (downstream reaches were < 0.25° warmer than upstream reaches). However, stream reaches downstream of fords ($p = 0.05$) and bridges ($p = 0.07$) were ~0.75 C warmer than reaches upstream of the structures (Figure 3). Dissolved oxygen was also significantly lower at sites with multiple-celled culverts than those with other structures, at a median of ~5 mg/L. Downstream portions of fords had significantly lower dissolved oxygen readings than upstream, with dissolved oxygen being more than 1mg/L lower ($p = 0.02$, Figure 4). Conductivity was highest at sites with fords, with median conductivity readings at 335.55 uS/cm (Figure 5). No differences in conductivity between upstream and downstream reaches were detected at any structure type.

Streams with bridges were deeper than all other sites, with a median thalweg of 0.56m and median cross-sectional depths of 0.34m. Cross-sectional depths downstream of these structures were double that of upstream portions ($p = 0.02$). Wetted and bankfull widths at sites with bridges were not significantly different from measures at streams with other road crossing types or sites that lacked structures, with the exception of those with single-celled culverts. The presence of aquatic vegetation was not different across road crossing types, as all had median percentages of 0. Streams with single-celled culverts had more aquatic vegetation upstream than downstream ($p = 0.08$). Sites with bridges and multiple-celled culverts did have 5% higher median amounts of fine and organic substrates, like silt, sand, and clay, than sites with single-celled culverts and fords, which was significant ($p < 0.01$).

Stream reaches with single-celled culverts had more fine substrates downstream ($p = 0.04$). Differences in fine and organic substrate prevalence upstream and downstream of road crossings were not detected across other structure types. Stream reaches with fords had the highest percentage of cobble with a median of 27.5%, followed by single-celled culverts at 20%. All other site types had medians of 5% or lower. Gravel substrate was most abundant at stream reaches with bridges, at a median of 62.5%. Differences between upstream and downstream reaches were seen at single-celled culverts, with a higher percentage of gravel found downstream ($p = 0.03$). Sites with multiple culverts had the most embedded substrates, at a median of 80%. The highest densiometer readings were found at stream reaches with bridges, with median readings of 14.75 out of 17. This was followed by sites with no road crossings at 10.16, and all other site types had medians of 3 or lower. Stream reaches with single-celled culverts exhibited the largest extent of incision, with median incised heights of the banks being 1.25 m. Banks downstream of these crossings had median incised heights ~0.6 m greater than upstream, which was close to but not significant ($p = 0.12$). All other site types had median incised heights of 0.52 m or less.

Impact of Road Crossing Severity on Stream Habitat

In stream reaches with road crossings, water temperatures were significantly lower at sites with structures classified as non-barriers than those with higher severity classifications (insignificant, minor, and severe barriers), being about 4°C cooler on average ($p < 0.01$). The same pattern was seen with conductivity, as median conductivity readings of 230.2 uS/cm were about 85 units lower than stream reaches with more severe road crossings ($p < 0.01$). Levels of dissolved oxygen did not vary across severity classifications. However, readings in downstream portions of severe crossings were ~1 mg/L lower than upstream ($p = 0.03$, Figure 6).

Stream reaches with road crossings classified as non-barriers were deeper than all sites, including those without any structures, with a median thalweg of 0.48m and median cross-sectional depths of 0.28m. Wetted and bankfull width at these sites was not significantly different among severity classifications or sites without road crossings. Percentages of fine and organic substrates did not vary across severity classifications. Sites with severe barriers had significantly less aquatic vegetation and embedded substrates than all other severity classifications. Stream reaches with severe barriers had the highest percentage of cobble with a median of 20%. All other site types had medians of 5% or lower. Gravel was found most abundantly at stream reaches with road crossings classified as non-barriers, at a median of 70%. The highest densiometer readings were found at stream reaches with road crossings classified as non-barriers, with median readings of 14.16. This was followed by sites with no road crossings at 10.16, and all other site types had medians of 3 or lower. Stream reaches with severe and minor barriers were significantly more incised than those that were classified as insignificant or non-barriers. No differences in any habitat variables between upstream and downstream portions were seen among severity classifications.

Impact of Road Crossings and Stream Habitat on SGCN Darters

Fish Assemblage Data

Using the combination of dipnets and seine, 6966 individuals across 27 species were captured. Cyprinidae (66%) and Percidae (19%) made up most of the individuals that were encountered. SGCN darters, which included *E. cragini*, *E. mihileze*, and *E. microperca*, accounted for 6.3% of the total catch and were found at 19 of the 30 field sites. Fish abundance across families was not different between sites with road crossings and those without them.

Arkansas Darter

Forty-eight *E. cragini* were captured at 8 of the 30 total field sites. A Wilcoxon sign-ranked test determined that their abundance at sites with road crossings was not significantly different from sites without those structures ($W = 120.5$, $p = 0.51$). PLS indicated that dissolved oxygen levels ($\beta = -0.17$, SE = 0.07, $p = 0.02$), flow ($\beta = -0.06$, SE = 0.03, $p = 0.06$), gravel cover ($\beta = -0.15$, SE = 0.04, $p < 0.01$), and stream wetted width ($\beta = -0.10$, SE = 0.03, $p < 0.01$) were all negatively correlated with *E. cragini* abundance, while fine and organic substrates ($\beta = 0.11$, SE = 0.05, $p = 0.03$) and overhanging vegetation ($\beta = 0.11$, SE = 0.04, $p = 0.02$) were positively correlated (Table 8). The same variables also influenced *E. cragini* densities (Table 9), with the exception of flow and the addition of a negative correlation with stream bankfull height ($\beta = -0.08$, SE = 0.04, $p = 0.04$, Table 9).

Sunburst Darter

Like the Arkansas Darter, *E. mihileze* was not locally abundant when captured, but was found slightly more often. Twenty-six individuals were captured across 11 of the 30 sites. Of these, only 2 were found across 2 stream reaches influenced by road crossings, while the remaining 24 individuals were found across 10 sites without structures. A Wilcoxon sign-ranked test found this to be significantly different (W = 147, p = 0.05, see Figure 7). PLS indicated that barrier presence, cobble cover, conductivity, dissolved oxygen, gravel cover, stream incision, and stream temperature all significantly impacted *E. mihileze* abundance (Table 10) and density (Table 11).

Least Darter

Though rare, *E. microperca* were locally abundant when captured. Three-hundred and sixty-two individuals were captured at 6 of the 30 sites. Of these fish, only 7 were found across 2 stream reaches influenced by road crossings, while the remaining 355 were found across 4 reaches without structures. PLS indicated that undercut banks, barrier presence, cobble cover, dissolved oxygen, substrate embeddedness, gravel cover, ground cover, and stream incision, all significantly influenced *E. microperca* abundance and densities (Table 12 & Table 13).

Impact of Road Crossings and Stream Habitat on Crayfish

Crayfish Assemblage and Sampling

One thousand nine hundred and three individuals were captured using both sampling methods. These included *F. virilis*, *F. neglectus, F. meeki brevis, F. nana*, and one White River Crayfish *Procambarus acutus*. Wilcoxon sign-ranked tests indicated that there were no significant differences in the amount of crayfish caught between dipnets and kick seines and that neither sampling method was biased towards capturing individuals of certain sizes. A chi-squared test also indicated that male and female crayfish were not caught disproportionately by either gear type. SGCN crayfish (*F. neglectus*, *F. meeki brevis*) made up 53% of the total catch, while the remaining individuals consisted mostly of Ringed crayfish (41%).

Northern Crayfish

A total of 97 *F. virilis* were captured at 8 of the 30 field sites. Of these crayfish, only 10 were found across 4 stream reaches influenced by road crossings, which was not significantly different from the 87 found across 4 without those structures. PLS indicated that cobble substrate ($\beta = -0.08$, SE = 0.02, $p < 0.01$), dissolved oxygen ($\beta = -0.22$, SE = 0.09, $p = 0.03$), and stream incision ($\beta = -0.08$, SE = 0.03, $p < 0.01$) were negatively correlated with *F. virilis* abundance, while fine and organic substrates ($\beta = 0.14$, SE = 0.04, $p < 0.02$) and stream temperature ($\beta = 0.08$, SE = 0.05, $p = 0.08$) were positively correlated (Table 14). Northern Crayfish density was negatively correlated with road crossing presence ($\beta = -0.06$, SE = 0.03, $p = 0.06$) and gravel substrate ($\beta = -0.13$, SE = 0.02, $p < 0.01$), and positively correlated with ground cover ($\beta = 0.10$, SE = 0.04, $p = 0.03$), overhanging vegetation ($\beta = 0.14$, SE = 0.05, $p = 0.01$), and wood density ($\beta = 0.24$, SE = 0.08, $p < 0.01$, Table 15). PLS did not find any correlations between *F. virilis* condition and aspects of stream habitat or road crossing presence.

Ringed Crayfish

F. neglectus was found throughout most of the study area, with a total of 788 individuals captured at 24 of the 30 field sites. Two-hundred and thirteen of these were found across 9 stream reaches impacted by road crossings, which was not significantly different from the 575 found at those without them. PLS indicated that *F. neglectus* abundance (Table 16) had significant negative relationships with cobble substrate ($\beta = -0.09$, SE = 0.03, $p < 0.01$), substrate embeddedness ($\beta = -0.15$, SE = 0.05, $p < 0.01$), and thalweg ($\beta = -0.08$, SE = 0.03, $p = 0.02$), and positive correlations with fine and organic substrates ($\beta = 0.16$, SE = 0.03, $p < 0.01$) and ground cover ($\beta = 0.13$, SE = 0.04, $p < 0.01$). Ringed Crayfish density was negatively correlated with aquatic vegetation ($\beta = -0.10$, SE = 0.04, $p < 0.04$), bankfull width ($\beta = -0.02$, SE = 0.04, $p < 0.01$), cobble cover ($\beta = -0.07$, SE = 0.04, $p = 0.06$), gravel cover ($\beta = -0.10$, SE = 0.03, $p < 0.01$), thalweg ($\beta = -0.10$, SE = 0.04, $p = 0.03$), tree roots ($\beta = -0.07$, SE = 0.02, $p < 0.01$) and wetted width ($\beta = -0.10$, SE = 0.02, $p < 0.01$), while fine and organic substrates ($\beta = 0.16$, SE = 0.03, $p < 0.01$), ground cover ($\beta = 0.11$, SE = 0.06, $p = 0.06$) and overhanging vegetation ($\beta = 0.16$, SE = 0.06, $p = 0.02$) had positive relationships (Table 17).

PLS on *F. neglectus* condition indicated that aquatic vegetation ($\beta = 0.03$, SE = 0.01, $p < 0.01$), bankfull height ($\beta = 0.03$, SE = 0.01, $p < 0.01$), bankfull width ($\beta = 0.03$, SE = 0.01, $p < 0.01$), road crossing presence ($\beta = 0.04$, SE = 0.01, $p < 0.01$), dissolved oxygen (coeff = 0.06, SE = 0.01, $p < 0.01$), flow ($\beta = 0.02$, SE = 0.01, $p < 0.01$), thalweg ($\beta = 0.02$, SE = 0.01, $p = 0.05$), and wetted width ($\beta = 0.02$, SE = 0.01, $p < 0.01$) were all positively correlated. Aspects of stream habitat such as boulder substrate ($\beta = -0.03$, SE = 0.02, $p = 0.06$), canopy cover ($\beta = -0.02$, SE = 0.01, $p = 0.01$), conductivity ($\beta = -0.02$, SE = 0.01, $p < 0.01$), fine and organic substrates ($\beta = -0.07$, SE = 0.01, $p < 0.01$), overhanging vegetation ($\beta = -0.05$, SE = 0.01, $p < 0.01$), stream temperature ($\beta = -0.02$, SE = 0.01, $p = 0.08$), understory ($\beta = -0.02$, SE = 0.01, $p = 0.09$), and wood density ($\beta = -0.06$, SE = 0.01, $p < 0.01$) were correlated with smaller values of scaled mass index (Table 18).

Meeks Short-pointed Crayfish

A total of 185 individuals were captured at 12 of the 30 field sites. Of these crayfish, 80 were caught across 3 stream reaches with road crossings, which was not significantly different from the 105 caught at 9 sites without them. PLS found that substrate embeddedness (Figure 10), fine and organic substrates, and wood density were negatively correlated with *F. meeki brevis* abundance (Table 19), and density (Table 20), while cobble cover, conductivity, and dissolved oxygen were positively correlated.

PLS on *F. meeki brevis* condition (Table 21) was negatively associated with aquatic vegetation ($\beta = -0.03$, SE = 0.01, $p < 0.01$), canopy cover ($\beta = -0.04$, SE = 0.01, $p = 0.01$), gravel substrate ($\beta = -0.02$, SE = 0.01, $p < 0.01$), overhanging vegetation ($\beta = -0.03$, SE = 0.01, $p < 0.01$), tree roots ($\beta = -0.04$, SE = 0.01, $p < 0.01$), and understory ($\beta = -0.03$, SE = 0.01, $p < 0.01$). Scaled mass index values were positively correlated with bankfull height ($\beta = 0.02$, SE = 0.01, $p < 0.01$), cobble cover ($\beta = 0.04$, SE = 0.01, $p < 0.01$), conductivity ($\beta = 0.02$, SE = 0.01, $p = 0.05$), stream depth ($\beta = 0.03$, SE = 0.01, $p < 0.01$), incision (coeff = 0.02, SE = 0.01, $p = 0.04$), sand ($\beta = 0.03$, SE = 0.01, $p = 0.01$), temperature ($\beta = 0.02$, SE = 0.01, $p < 0.01$), and thalweg ($\beta = 0.02$, SE = 0.01, $p < 0.01$). Males also had smaller SMI values than females ($\beta = -0.02$, SE = 0.01, $p = 0.05$).

Midget Crayfish

A total of 835 individuals were captured at 19 of the 30 field sites. Of these crayfish, 525 were caught across 8 stream reaches with road crossings, which was not significantly different from the 307 caught at 11 streams without them. PLS found that aquatic vegetation ($\beta = -0.08$, SE = 0.03, $p = 0.01$), substrate embeddedness ($\beta = -0.14$, SE = 0.04, $p < 0.01$), fine and organic substrates ($\beta = -0.11$, SE = 0.03, $p < 0.01$), and overhanging vegetation ($\beta = -0.07$, SE = 0.04, $p = 0.05$) were negatively correlated with *F. nana* abundance, while % gravel substrate ($\beta = 0.13$, SE = 0.04, $p < 0.01$) and stream incision ($\beta = 0.09$, SE = 0.03, $p = 0.01$) were positively correlated (Table 22). Midget Crayfish density (Table 23) displayed similar correlations, with the exception of overhanging vegetation and the addition of positive relationships with dissolved oxygen ($\beta = 0.07$, SE = 0.03, $p = 0.02$) and wood density ($\beta = 0.07$, SE = 0.04, $p = 0.08$).

F. nana condition (Table 24) was negatively associated with canopy cover ($\beta = -0.02$, SE = 0.01, $p = 0.04$), overhanging vegetation ($\beta = -0.02$, SE = 0.01, $p < 0.01$), silt coverage ($\beta = -0.02$, SE = 0.01, $p = 0.01$), stream temperature ($\beta = -0.02$, SE = 0.01, $p = 0.01$), tree roots ($\beta = -0.02$, SE = 0.01, $p < 0.01$), and understory cover ($\beta = -0.02$, SE = 0.01, $p = 0.03$). Scaled mass index values were positively correlated with road crossing presence ($\beta = 0.05$, SE = 0.01, $p = 0.01$), bank angle ($\beta = 0.01$, SE = 0.01, $p = 0.02$), dissolved oxygen ($\beta = 0.02$, SE = 0.01, $p = 0.02$), flow ($\beta = 0.03$, SE = 0.01, $p < 0.01$), gravel cover ($\beta = 0.02$, SE = 0.01, $p = 0.02$), ground cover ($\beta = 0.02$, SE = 0.01 $p = 0.05$), and sand ($\beta = 0.01$, SE = 0.01 $p = 0.05$).

Crayfish Community Metrics

PLS (Table 25) found that the proportion of substrate generalists (Northern and Ringed Crayfish) compared to the entire crayfish assemblage was negatively correlated with canopy cover ($\beta = -0.07$, SE = 0.04, $p = 0.09$), cobble cover ($\beta = -0.14$, SE = 0.04, $p < 0.01$), dissolved oxygen ($\beta = -0.18$, SE = 0.05, $p < 0.01$), gravel cover ($\beta = -0.15$, SE = 0.05, $p < 0.01$), and stream incision ($\beta = -0.10$, SE = 0.04, $p = 0.04$). The proportion of substrate generalists was found to be positively associated with substrate embeddedness ($\beta = 0.13$, SE = 0.05, $p = 0.02$) and fine and organic substrates ($\beta = 0.13$, SE = 0.04, $p < 0.01$).

PLS found that the proportion of crayfish affiliated with lotic habitat (all excluding the Northern Crayfish, Table 26) was positively correlated with cobble cover ($\beta = 0.10$, SE = 0.04, $p = 0.01$), dissolved oxygen ($\beta = 0.22$, SE = 0.05, $p < 0.01$), gravel cover ($\beta = 0.18$, SE = 0.04, $p < 0.01$), flow ($\beta = 0.04$, SE = 0.02, $p = 0.06$), stream incision ($\beta = 0.09$, SE = 0.03, $p < 0.01$) and wetted width ($\beta = 0.09$, SE = 0.03, $p < 0.01$). Significant negative relationships were present with substrate embeddedness ($\beta = -0.14$, SE = 0.04, $p < 0.01$) and fine and organic substrates ($\beta = -0.12$, SE = 0.04, $p < 0.01$). Analyses evaluating the relationship between stream habitat and the proportion of species whose range is widespread (Northern Crayfish) yielded inverse results to those including of proportion of lotic species.

PLS found that the proportion of species whose range is considered regional (Ringed Crayfish, Table 27) had a negative correlation with aquatic vegetation ($\beta = -0.11$, SE = 0.06, $p = 0.08$), and positive relationships with dissolved oxygen ($\beta = 0.16$, SE =

0.04, $p < 0.01$), gravel cover ($\beta = 0.13$, SE = 0.05, $p = 0.01$) and stream wetted width ($\beta = 0.10$, SE = 0.05, $p = 0.03$). PLS found that the proportion of species whose range is considered narrow (Meeks Short-pointed and Midget Crayfish, Table 28) yielded significant positive relationships with canopy cover ($\beta = 0.07$, SE = 0.04, $p = 0.08$), cobble cover ($\beta = 0.04$, SE = 0.04, $p < 0.01$), dissolved oxygen ($\beta = 0.19$, SE = 0.05, $p < 0.01$) and gravel ($\beta = 0.16$, SE = 0.05, $p < 0.01$). The proportion of narrow-range crayfish species was negatively correlated with embedded substrates ($\beta = -0.14$, SE = 0.06, $p = 0.02$) and those of fine particle size ($\beta = -0.14$, SE = 0.05, $p < 0.01$).

Crayfish Diversity Indices

PLS on Simpson's Index, Shannon's Diversity, and Brullion's Index showed positive correlations with dissolved oxygen and gravel cover while being negatively correlated with substrate embeddedness. Margalef's index displayed the same relationships, with the addition of a negative correlation to fine and organic substrates ($\beta = -0.10$, $p = 0.02$). Dominance, as well as Shannon's Equitability, displayed inverse relationships compared to the aforementioned indices. Chao's richness estimator was negatively correlated with bankfull width ($\beta = -0.13$, SE = 0.06, $p = 0.04$), embedded substrates ($\beta = -0.19$, SE = 0.07, $p < 0.01$), and fine and organic substrates ($\beta = -0.09$, SE = 0.04, $p = 0.04$). It was positively correlated with dissolved oxygen ($\beta = 0.15$, SE = 0.05, $p = 0.01$) and gravel cover ($\beta = 0.14$, SE = 0.05, $p < 0.01$ (Table 29). Fisher's alpha was positively correlated with thalweg ($\beta = 0.15$, SE = 0.07, $p = 0.04$).

V. Discussion

Impact of Road Crossings on Stream Habitat

Results support the alternative hypothesis for objective one. Stream reaches with road crossings have higher conductivity and temperatures, with more embedded substrate and stream banks with greater incision. These sites also have less fine and organic substrates, aquatic vegetation, canopy cover, and tree roots. Stream habitat and riffle-run-pool ratios were not different between upstream and downstream locations. However, different structure types had different impacts on stream habitat, which were also seen between the stream reaches found above and below these structures.

When evaluating road crossing presence on stream habitat, our results align with previous work. Neal et al. 2007 found no differences in substrate size and embeddedness, undercut banks, overhanging vegetation, pool habitat, aquatic macrophytes, and woody debris between upstream and downstream portions of road crossings. In contrast, when evaluating impacts on stream habitat among crossing types, some of our results are contradictory to what has been found in similar systems. Bouska et al. 2010 found that riffle spacing, particle size, and bankfull depth did not vary among prairie streams with box culverts and low water crossings in eastern Kansas. Subsequently, these measures were not different between upstream and downstream reaches (Bouska et al. 2010). However, the majority of their road crossing sites were more incised than those that lacked these structures, which was evident in our data with the exception of bridges. In our study area, sites with single-celled culverts were the most incised, and were so at a greater extent downstream, which is consistent with what previous research has determined (see Wargo and Weisman 2006; Neal et al. 2007). Streams with higher incision tend to have less canopy cover, woody debris, organic material, pool habitat, and stable substrates, all of which degrade habitat for aquatic organisms (Shields et al. 1994). Additionally, these systems tend to have flashier hydrology, and increased sediment loads (Shields et al. 1994). Similarly, our sites with road crossings were negatively correlated with fine and organic substrates, aquatic vegetation, canopy cover, and tree roots, while being positively correlated with embedded substrates. The construction of road crossings tends to constrict stream flow and remove riparian vegetation, leading to higher water velocities and increased bank scouring. This can increase the rate of sediment transport downstream. Faster flow coupled with banks that lack stability can lead to increased sediment loads. More sedimentation can result in altered substrate and channel characteristics downstream, which may alter the spawning success of fishes and density of macroinvertebrates (Jackson 2003, Bouska et al. 2010). Furthermore, increased sediment loads can lead to higher turbidity levels (Jackson 2003). This can reduce the ability of sunlight to reach macrophytes, as well as the foraging success of filter feeders and visual predators (Jackson 2003).

Although our data suggested that sites with bridges were just as incised as those without road crossings, these streams had deeper thalwegs and cross-sectional depths than those impacted by other structure types. Although these bridges are often constructed across larger streams, our results seem to be independent of stream order, as the wetted and bankfull widths of sites with bridges were not significantly different from that of multi-celled culverts and fords. Scouring was also present to a greater extent downstream of bridges. Wellman et al. 2011 reported similar results and postulated that the pillars or piers that support some of these structures could lead to more local scouring around them (also see Southard 1993). Additionally, Douglas 1985 noted that the constriction of the stream under the bridge also contributes to scouring seen downstream of the structure. Both of these characteristics of bridges can alter flow around and downstream of the structure, potentially leading to more scouring and transport of unstable sediments downstream (Douglas 1985; Southard 1993; Wellman et al. 2011; Roy and Sahu 2018). These deeper habitats then have a greater potential to house predatory fish, which may lead to a decrease in the abundance of lower tropic fish (Wellman et al. 2011). Though they may not physically restrict fish passage, bridges do alter stream habitat, and when evaluating a road crossing's impact on aquatic organisms, the influence of scour pools should be considered.

Impact of Road Crossings and Stream Habitat on SGCN Darters

The alternative hypothesis for objective two was supported for *E. microperca* and *E. mihileze*. Scarcity of Arkansas Darters may have restricted our ability to detect significant impacts of road crossings on their abundance and density. Considering the habitat data we collected and the results for other SGCN darters, we would predict road crossings to have negative effects on this species. Arkansas Darters prefer small spring-fed streams with fine and organic substrates, which held true in our models as they were positively correlated with their abundance and density. Overhanging vegetation also had this effect, which may be analogous to an affiliation with riparian prairie habitat that used to be prevalent in the watershed. However, fine and organic substrates, as well as aquatic vegetation, were less abundant at sites with road crossings, and stream temperatures were higher. The flashiness of these systems may be washing out their preferred habitat, and a reduced riparian zone around the structure may be leading to increases in water temperature. Dissolved oxygen was also found to be negatively associated with the abundance of Arkansas Darter. Many of the streams with these fish were adjacent to cattle pastures that see high inputs of organic matter, which would result in reduced levels of dissolved oxygen compared to other spring runs without that type of land use (Thomas et al. 2004).

Sunburst Darters seemed to prefer more pristine spring-fed headwaters. Their abundance and density were negatively correlated with the presence of road crossings, which may explain the relationships of these parameters with stream habitat. Dissolved oxygen and aquatic vegetation were positively correlated with their abundance and density, while conductivity, stream incision, and water temperature were all negatively correlated. These aspects of stream habitat had inverse relationships with the presence of road crossings, relative to their influence on the two Sunburst Darter parameters. Similar patterns were seen with the Least Darter, which displayed the same relationships with road crossing presence, dissolved oxygen, and stream incision.

Habitat selection can influence the movement of fish (Benton et al. 2008). Arkansas and Least Darters have been found to have low historic and contemporary migration rates (Labbe and Fausch 2000; Baker et al. 2018). Darters as a whole are territorial and as a result, have smaller probabilities of large-scale movement (Nislow et al. 2011). This would imply that immigration is less important relative to local survival and recruitment for the persistence of these fish (Nislow et al. 2011). If road crossings are damaging the preferred habitat of these fish, and fragmenting habitat patches by restricting any movement of darters that may exist (Labbe and Fausch 2000; Baker et al.

2018), populations may be subject to further declines if conservation efforts are not taken.

Impact of Road Crossings and Stream Habitat on Crayfish Assemblages and Condition

Results somewhat supported the alternative hypothesis for objective three. The proportion of narrow-ranged crayfish, which includes the SGCN Midget and Meeks Short-pointed Crayfish, was positively correlated with the presence of coarser substrates and higher levels of dissolved oxygen. PLS also indicated positive associations with gravel for *F. nana* and cobble for *F. meeki brevis* respectively. *F. nana* is smaller bodied in comparison to *F. meeki brevis,* which may explain this difference in substrate association. Both species, as well as the Ringed Crayfish, are also *Faxonius* taxa, or stream-dwelling crayfish, providing support as to why they were classified as lotic species. This potentially explains the correlation of narrow-ranged species with dissolved oxygen exists, along with the positive relationship between lotic crayfish and flow. *F. nana* and *F. meeki brevis* were also negatively associated with embedded substrates and those of fine particle size. This benthic habitat may limit the use of interstitial spaces, and provide less cover for them to hide from predators.

Road crossing presence was positively correlated with *F. nana* scaled mass index. Individuals at these sites may need higher energy reserves to pass through these structures or to avoid being swept downstream during high-flow events. This could be attributed to the flashier hydrology of streams with road crossings, and the fact that Midget Crayfish are smaller bodied compared to the rest of those found in the assemblage. Alternatively, habitat conditions may be contributing to higher food availability at these sites. Differences in scaled mass index between sexes did exist for *F. meeki brevis*, as males had smaller values than females. Sexual dimorphism typically exists in crayfish, as males display increased growth of chelae (Wang et al. 2011). However, females have been seen to exhibit larger abdomens than equal sized males, hypothesized to aid in carrying eggs (Wang et al. 2011). Although no crayfish in berry was found, eggs are more costly to produce than sperm, which may explain why female *F. meeki brevis* had higher relative energy reserves.

The proportion of substrate generalists, which includes the Ringed and Northern Crayfish, displayed inverse relationships to those seen with the narrow range endemics. Lynch et al. 2018 found that the percentage of generalist crayfish was positively associated with erosion and negatively correlated with measures of substrate size. Although we did find that *F. neglectus* and *F. virilis* were positively related to fine and organic substrates, they were negatively related to stream incision. *F. virilis* abundance was also found to be negatively correlated with dissolved oxygen, while their density displayed a negative relationship with road crossing presence. These crayfish were often found at sites similar to *E. cragini*, which saw high inputs of organic matter from cattle pastures, which may explain reduced levels of dissolved oxygen. *F. virilis* are also secondary burrowers, compared to the rest of the assemblage being tertiary, as they frequently could be found along the soft banks in dug holes or on the banks in burrows. The construction of the crossing itself, as well as any hydrologic change that may accompany it, could reduce the amount of suitable habitat to burrow, translating to lower densities of this species in stream reaches within close proximity to the structure.

The presence of road crossings was also positively correlated with the condition of *F. neglectus*. No differences in scaled mass index existed between sexes in both species. Additionally, aspects of stream habitat that displayed negative relationships with *F. neglectus* density, such as coarser substrates, aquatic vegetation, and stream size, displayed inverse relationships with their condition, potentially illustrating density-dependent interactions in this species.

A majority of the diversity indices we evaluated had significant positive correlations with dissolved oxygen and gravel while displaying negative relationships with substrate embeddedness. This may be explained by the fact that our models looking at SGCN crayfish abundance and density displayed the same relationship with these aspects of stream habitat. Indices such as Dominance, and Shannon's equitability had inverse relationships, which is typical, as communities dominated by one or two taxa are usually less diverse.

A species' tolerance to environmental conditions can influence its distribution (Mouser et al. 2018). Previous research has shown that local habitat variables may be more informative when predicting crayfish distributions and abundance than landscape level variables (Nolen et al. 2014, Magoulick et al. 2017). The impoundment of streams can impact crayfish by altering their habitat, as well as increasing the density of their predators (Taylor et al. 2007). Changes to crayfish assemblages can be followed by shifts in algal, macrophyte, macroinvertebrate, or fish populations, significantly altering the aquatic ecosystem (Mouser et al. 2018). This is because crayfish can have direct and indirect top-down or bottom-up effects on food webs (Adams 2013). As of 2019, conservation concerns exist for half of North American crayfish (Taylor et al. 2019).

Meanwhile, major knowledge gaps exist in areas such as life history and impacts of altered flow regimes for the taxa group as a whole (Taylor et al. 2019). Therefore, it is imperative that more research is done on these taxa.

Conclusions and Recommendations

Results indicate that road crossings alter stream habitat. Stream sites with these structures contained less aquatic vegetation, an aspect of preferred habitat for the three SGCN darters discussed in this paper, as well as less fine and organic substrates, which both *E. microperca* and *E. cragini* prefer. These streams also had less canopy cover and submerged tree roots which can provide thermal refugia via shade and act as cover that aquatic organisms can use respectively. Additionally, stream sites with road crossings had higher temperatures and conductivities, which can increase metabolic stress on aquatic organisms and inhibit their ability to persist (Barquin and Scarsbrook 2007; Farless and Brewer 2017). The Southeastern Aquatic Resource Partnership's database alone currently has over 100,000 road-related barriers inventoried in the state of Arkansas, and over four million across most of the country. Given that human population growth and urbanization are on the rise, these numbers will likely continue to climb. With it being a logistically impossible feat of assessing all of these structures for detrimental impacts, managers should aim to prioritize work that they can do and keep these considerations in mind:

- The area (location and scale) you wish to work in and the species of concern there
- The number and condition of road crossings in that area
- The miles of stream or floodplain being impacted and that may be restored

- The support (opinion, funds…) or opposition will you receive, and from whom

The impact that road crossings have on stream habitat also may influence the distribution, abundance, density, and condition of aquatic species. Impacts are dependent upon the species being examined, as well as the taxa group of interest. For example, *F. nana* and *F. neglectus* scale mass index values, or their relative size and energy reserves, were positively correlated with the presence of road crossings, while no such relationship existed for *F. meeki brevis* and *F. virilis*. Additionally, road crossing presence did not have a significant effect on crayfish abundances or densities, while *E. microperca* and *E. mihileze* were negatively affected. Managers should seek to employ multi-species management, as targeted conservation efforts may impact species differently.

When drawing conclusions about the influence that road crossings and stream habitat had on darter and crayfish parameters, some considerations need to be taken. It is oftentimes difficult to attain a statistically appropriate sample size when working with rare and imperiled species. This can be further exacerbated when working on the edge of a species' geographic range, which was the case for *E. microperca* and *E. cragini*. Obtaining data on these taxa and streams in adjacent states such as Oklahoma and Missouri could strengthen the claims made in this paper, as well as better describe how road crossings are impacting aquatic organisms and their habitat.

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Appendix A. Tables

TABLE 1. List of data collected using the Southeast Aquatic Resource Partnership Stream Crossing Survey protocol with descriptions.

Data Type	**Description**
Crossing Type	Bridge, culvert, ford…
Bridge/Culvert Cells	# of Bridge/Culvert Cells
Flow Condition	None, typical low, moderate, high
Crossing Condition	Ok, poor, new…
Alignment	Is the barrier aligned with the flow direction of the stream or is it skewed
Bankfull width	Description
Constriction	Ratio of wetted width inside of structure to the structure's width…severe, moderate…
Tailwater Scour Pool	Ratio of dimensions of the pool directly below the barrier to the ration of dimensions of reference reach pool upstream of the structure… none, small, large
Tailwater Riparian Vegetation	Composition of the overstory, understory, and ground level vegetation in the riparian area directly below the structure… low, high
Inlet Scour Pool	Ratio of dimensions of the tailwater pool directly above the barrier to the ration of dimensions of reference reach pool upstream of the structure… none, small, large
Inlet Riparian Vegetation	Composition of the overstory, understory, and ground level vegetation in the riparian area directly above the structure… low, high
Structure Material	Metal, concrete, plastic…
Outlet Shape	Round, box…
Outlet Grade	At stream grade, freefall, cascade…
Outlet Dimensions	Width, height, substrate/water width, water depth (ft)

Outlet Undermining	Scouring underneath this end of the structure… Y/N
Outlet Armoring	Presence of material placed downstream of the outlet to diffuse flow and minimize scouring… None, not extensive, extensive
Inlet Shape	Round, box…
Inlet Grade	At stream grade, freefall, cascade…
Inlet Type	Projecting, headwalls, wingwalls…
Inlet Dimensions	Width, height, substrate/water width, water depth (ft)
Inlet Undermining	Scouring underneath this end of the structure… Y/N
Inlet Armoring	Presence of material placed upstream of the structure to diffuse flow and minimize scouring… None, not extensive, extensive
Structure Length	At both the top and bottom of the structure (ft)
Internal Structures	None, baffles/weirs…
Dry Passage Through Structure	If an animal can walk through the structure without getting its feet wet…Y/N
Structure Substrate (Inside) Matches Stream's	Comparable, contrasting
Structure Substrate Type	None, silt, sand…
Structure Substrate Coverage (%)	0,25,50,75,100
Water Depth (Inside Structure) Matches Stream's	Yes, no (shallower, deeper, dry)
Water Velocity (Inside Structure) Matches Stream's	Yes, no (faster, slower, dry)
Physical Barriers	None, debris, dry streambed… inside or structure or restricting access through inlets/outlets
Physical Barrier Severity	None, minor, moderate, severe

TABLE 2. List of data collected using the EPA’s Environmental Monitoring and Assessment Protocol with descriptions.

Data Type	**Measurement**
Cross-sectional Depths	5 across channel, left bank, 25% ww, 50%ww, 75% ww, right bank (cm)
Thalweg	Flow path of the deepest water in channel (cm)
Substrate	Sand, silt, organic, cobble, gravel… (%)
Embeddedness	%
Densiometer Readings	6 across channel, lb,cu,cd,cl,cr,rb
Bank Angle	°
Bank Undercut	Horizontal distance of undercutting (cm)
Bankfull Height and Width	Height of bankfull flow above the present waterline (cm)
Incised Height	Height up from the water surface to the elevation of the first terrace of the floodplain (cm)
Fish Cover	Algae, tree roots, macrophytes… (%)
Stream Discharge	15-20 across the wetted width (m/s)
Sinuosity	Bearings recorded while backsighting downstream between transects
Riparian Vegetation	% canopy (>5m high), understory (0.5-5m high) and ground cover (<0.5m high)

TABLE 3. List of diversity indices calculated in Past4, with formulas and descriptions.

Biotic Index	**Description**
Dominance	0 = all taxa are equally present 1 = one taxon dominates the community
Simpson's Index	Takes into account the # of species, as well as their relative abundances Measures evenness in the community 0 = no diversity 1 = infinite diversity Sample size independent (Somerfield, P. J., K. R. Clarke, and R. M. Warwick. 2008. Simpson index. Elsevier The weighted mean of proportional abundances More weight given to dominant species (rare species have little effect)
Shannon's Index	Diversity index taking into account the # of taxa and the # of individuals of each taxon Does not reflect sample size

	Higher when richness and evenness is higher Main source of error is failure to include all taxa in its random sample
Brillouin's Index	Does not assume random sampling in an infinite population, used in non-random sampling
Margalef's Index	(# of taxa – 1)/ln(# of individuals) Attempt to correct for sample size
Equitability	Shannon's Index/log(# of taxa), measures evenness with which individuals are divided among the taxa present How similar are abundances of different species in the community The evenness with which individuals are divided among taxa present
Fisher's alpha	Most applicable in situations where one or few factors dominate the ecology of a community Assumes the abundance of species follows the log series distribution

	Independent of sample sie when its over 1000
Chao1 estimator	Bias corrected richness estimator Assumes a poisson distribution and corrects for variance Useful for datasets skewed toward low abundance calls Nonparametric Assumes rare species are informative of unobserved species

TABLE 4. Crayfish community metric classifications by species encountered. Sources used to justify the classifications in each category include Larson and Olden 2010, McAllister et al. 2015, Crayfishes of Missouri, and Natureserve

Category	*F. meeki brevis*	*F. nana*	*F. neglectus*	*F. virilis*
Range	narrow	narrow	regional	widespread
Habitat	lotic	lotic	lotic	generalist
Substrate	coarse	coarse	generalist	generalist

TABLE 5. Component scores for each variable used in calculating the overall crossing score in the Southeastern Aquatic Resources Partnership's Stream Crossing Assessment Protocol. Scores closer to 0 represent worse conditions while scores closer to 1 represent more ideal conditions.

Parameter	Level	Score
Constriction	severe	0
Constriction	moderate	0.5
Constriction	spans only bankfull/active channel	0.9
Constriction	spans full channel and banks	1
Inlet grade	at stream grade	1
Inlet grade	inlet drop	0
Inlet grade	perched	0
Inlet grade	clogged/collapsed/submerged	1
Inlet grade	unknown	1
Internal structures	none	1
Internal structures	baffles/weirs	0
Internal structures	supports	0.8
Internal structures	other	1
Outlet armoring	extensive	0
Outlet armoring	not extensive	0.5
Outlet armoring	none	1
Physical barriers	none	1
Physical barriers	minor	0.8
Physical barriers	moderate	0.5
Physical barriers	severe	0
Scour pool	large	0
Scour pool	small	0.8
Scour pool	none	1
Substrate coverage	none	0
Substrate coverage	25%	0.3
Substrate coverage	50%	0.5
Substrate coverage	75%	0.7
Substrate coverage	100%	1
Substrate matches stream	none	0
Substrate matches stream	not appropriate	0.25
Substrate matches stream	contrasting	0.75
Substrate matches stream	comparable	1
Water depth	no (significantly deeper)	0.5
Water depth	no (significantly shallower)	0
Water depth	yes (comparable)	1
Water depth	dry (stream also dry)	1
Water velocity	no (significantly faster)	0
Water velocity	no (significantly slower)	0.5
Water velocity	yes (comparable)	1
Water velocity	dry (stream also dry)	1

TABLE 6. Weights associated with each variable in the Southeastern Aquatic Resources Partnership's Stream Crossing scoring algorithm.

Parameter	Weight
Outlet drop	0.161
Physical barriers	0.135
Constriction	0.090
Inlet grade	0.088
Water depth	0.082
Water velocity	0.080
Scour pool	0.071
Substrate matches stream	0.070
Substrate coverage	0.057
Openness	0.052
Height	0.045
Outlet armoring	0.037
Internal structures	0.032

TABLE 7. Results of Wilcoxon Sign-Ranked tests of road crossing presence on aspects of stream habitat. Response column dictates whether road crossing presence is positively or negatively correlated with each aspect of stream habitat.

Variable	W	Response	p-value
Aquatic Vegetation	14573	-	<0.01
Canopy Cover	15100	-	<0.01
Cobble	10716	…	0.17
Conductivity	5710.5	+	0.02
Depth	11998	…	0.65
Dissolved Oxygen	10498	…	0.29
Fine and Organic Substrates	14208	-	<0.01
Gravel	11534	…	0.87
Stream Incision	10829	+	0.04
Substrate Embeddedness	10060	+	0.07
Temperature	10227	+	0.02
Tree Roots	13080	-	0.07
Wetted Width	14346	-	<0.01

TABLE 8. Results of the Partial Least-Squares Regression of road crossing presence and stream habitat on *E. cragini* abundance.

Variable	Coeff	p-value
Aquatic Vegetation	0.041	0.383
Bank Angle	0.024	0.613
Bank Undercut	-0.011	0.816
Bankfull Height	-0.029	0.643
Bankfull Width	0.016	0.899
Barrier Presence	-0.004	0.946
Canopy cover	-0.017	0.821
Cobble	-0.057	0.185
Conductivity	0.021	0.598
Depth	-0.013	0.847
Dissolved Oxygen	-0.173	0.016
Embeddedness	0.070	0.159
Fine and Organic Substrate	0.113	0.033
Flow	-0.057	0.057
Gravel	-0.146	0.001
Ground Cover	-0.013	0.819
Incision	-0.051	0.188
Overhanging Vegetation	0.105	0.021
Temperature	0.106	0.124
Thalweg	-0.034	0.551
Tree Roots	0.111	0.130
Understory	-0.004	0.958
Wetted Width	-0.099	0.002
Wood Density	0.030	0.637

TABLE 9. Results of the Partial Least-Squares Regression of road crossing presence and stream habitat on *E. cragini* density.

Variable	Coeff	p-value
Aquatic Vegetation	-0.010	0.807
Bank Angle	0.077	0.220
Bank Undercut	-0.019	0.683
Bankfull Height	-0.077	0.043
Bankfull Width	0.024	0.872
Barrier Presence	0.020	0.762
Canopy cover	-0.042	0.550
Cobble	-0.067	0.116
Conductivity	0.004	0.901
Depth	-0.065	0.195
Dissolved Oxygen	-0.165	0.002
Embeddedness	0.099	0.042
Fine and Organic Substrate	0.126	0.054
Flow	-0.028	0.329
Gravel	-0.170	<0.001
Ground Cover	0.024	0.685
Incision	-0.040	0.332
Overhanging Vegetation	0.109	0.017
Temperature	0.082	0.258
Thalweg	-0.079	0.106
Tree Roots	0.098	0.352
Understory	0.036	0.701
Wetted Width	-0.111	0.002
Wood Density	0.068	0.264

TABLE 10. Results of the Partial Least-Squares Regression of road crossing presence and stream habitat on *E. mihileze* abundance.

Variable	Coeff	p-value
Aquatic Vegetation	0.136	0.134
Bank Angle	-0.099	0.311
Bank Undercut	0.017	0.892
Bankfull Height	-0.001	0.990
Bankfull Width	0.024	0.748
Barrier Presence	-0.129	<0.001
Canopy cover	0.073	0.158
Cobble	-0.090	0.068
Conductivity	-0.124	0.018
Depth	0.047	0.416
Dissolved Oxygen	0.096	0.051
Embeddedness	-0.004	0.958
Fine and Organic Substrate	0.038	0.293
Flow	-0.052	0.100
Gravel	0.105	0.042
Ground Cover	0.011	0.924
Incision	-0.097	0.026
Overhanging Vegetation	-0.052	0.495
Temperature	-0.117	0.051
Thalweg	0.038	0.483
Tree Roots	0.063	0.528
Understory	0.010	0.946
Wetted Width	0.113	0.343
Wood Density	-0.013	0.878

TABLE 11. Results of the Partial Least-Squares Regression of road crossing presence and stream habitat on *E. mihileze* density.

Variable	Coeff	p-value
Aquatic Vegetation	0.151	0.099
Bank Angle	-0.102	0.290
Bank Undercut	-0.008	0.961
Bankfull Height	0.008	0.915
Bankfull Width	0.028	0.720
Barrier Presence	-0.121	<0.001
Canopy cover	0.069	0.181
Cobble	-0.091	0.044
Conductivity	-0.121	0.034
Depth	0.041	0.524
Dissolved Oxygen	0.099	0.033
Embeddedness	0.002	0.987
Fine and Organic Substrate	0.029	0.423
Flow	-0.048	0.120
Gravel	0.112	0.022
Ground Cover	0.004	0.973
Incision	-0.099	0.025
Overhanging Vegetation	-0.053	0.566
Temperature	-0.113	0.050
Thalweg	0.036	0.560
Tree Roots	0.062	0.499
Understory	-0.002	0.994
Wetted Width	0.114	0.351
Wood Density	-0.016	0.842

TABLE 12. Results of the Partial Least-Squares Regression of road crossing presence and stream habitat on *E. microperca* abundance.

Variable	Coeff	p-value
Aquatic Vegetation	0.062	0.179
Bank Angle	-0.028	0.733
Bank Undercut	0.068	0.059
Bankfull Height	-0.030	0.249
Bankfull Width	-0.007	0.847
Barrier Presence	-0.088	0.003
Canopy cover	-0.009	0.951
Cobble	-0.115	0.001
Conductivity	-0.011	0.782
Depth	0.031	0.680
Dissolved Oxygen	0.114	0.001
Embeddedness	-0.086	0.011
Fine and Organic Substrate	0.016	0.663
Flow	-0.029	0.408
Gravel	0.109	0.010
Ground Cover	0.149	0.034
Incision	-0.076	0.010
Overhanging Vegetation	-0.034	0.262
Temperature	-0.064	0.407
Thalweg	0.004	0.919
Tree Roots	-0.031	0.866
Understory	-0.022	0.913
Wetted Width	0.054	0.431
Wood Density	-0.025	0.780

TABLE 13. Results of the Partial Least-Squares Regression of road crossing presence and stream habitat on *E. microperca* density.

Variable	Coeff	p-value
Aquatic Vegetation	0.060	0.222
Bank Angle	-0.026	0.736
Bank Undercut	0.071	0.058
Bankfull Height	-0.035	0.230
Bankfull Width	-0.008	0.819
Barrier Presence	-0.087	0.016
Canopy cover	-0.004	0.980
Cobble	-0.116	0.003
Conductivity	-0.011	0.763
Depth	0.023	0.779
Dissolved Oxygen	0.118	0.001
Embeddedness	-0.093	0.014
Fine and Organic Substrate	0.013	0.743
Flow	-0.028	0.420
Gravel	0.115	0.013
Ground Cover	0.147	0.050
Incision	-0.079	0.008
Overhanging Vegetation	-0.031	0.367
Temperature	-0.070	0.334
Thalweg	0.002	0.970
Tree Roots	-0.023	0.896
Understory	-0.010	0.969
Wetted Width	0.055	0.407
Wood Density	-0.022	0.795

TABLE 14. Results of the partial least squares regression of road crossing presence and stream habitat on *F. virilis* abundance.

Variable	Coeff	p-value
Aquatic Vegetation	0.040	0.631
Bank Angle	-0.021	0.703
Bank Undercut	0.059	0.633
Bankfull Height	0.074	0.655
Bankfull Width	-0.047	0.116
Barrier Presence	-0.077	0.114
Canopy cover	0.099	0.404
Cobble	-0.101	<0.001
Conductivity	0.068	0.098
Depth	0.087	0.540
Dissolved Oxygen	-0.216	0.032
Embeddedness	-0.061	0.378
Fine and Organic Substrate	0.136	0.002
Flow	-0.065	0.167
Gravel	-0.065	0.377
Ground Cover	0.045	0.575
Incision	-0.083	0.009
Overhanging Vegetation	0.083	0.271
Temperature	0.082	0.082
Thalweg	0.043	0.731
Tree Roots	0.073	0.422
Understory	-0.073	0.419
Wetted Width	-0.046	0.403
Wood Density	0.136	0.474

TABLE 15. Results of the partial least squares regression of road crossing presence and stream habitat on *F. virilis* densities.

Variable	Coeff	p-value
Aquatic Vegetation	-0.047	0.293
Bank Angle	0.049	0.744
Bank Undercut	0.021	0.809
Bankfull Height	-0.046	0.358
Bankfull Width	-0.077	0.117
Barrier Presence	-0.059	0.062
Canopy cover	0.043	0.29
Cobble	-0.107	<0.001
Conductivity	0.030	0.219
Depth	-0.054	0.430
Dissolved Oxygen	-0.166	0.004
Embeddedness	-0.002	0.987
Fine and Organic Substrate	0.177	<0.001
Flow	-0.018	0.708
Gravel	-0.128	<0.001
Ground Cover	0.097	0.033
Incision	-0.049	0.453
Overhanging Vegetation	0.138	0.015
Temperature	0.026	0.746
Thalweg	-0.085	0.136
Tree Roots	0.010	0.824
Understory	-0.034	0.487
Wetted Width	-0.097	0.004
Wood Density	0.238	0.005

TABLE 16. Results of the partial least squares regression of road crossing presence and stream habitat on *F. neglectus* abundance.

Variable	Coeff	p-value
Aquatic Vegetation	-0.089	0.173
Bank Angle	-0.093	0.407
Bank Undercut	-0.046	0.785
Bankfull Height	-0.046	0.772
Bankfull Width	-0.117	0.228
Barrier Presence	-0.082	0.095
Canopy cover	-0.048	0.713
Cobble	-0.089	0.005
Conductivity	0.065	0.494
Depth	-0.032	0.411
Dissolved Oxygen	0.009	0.942
Embeddedness	-0.152	0.005
Fine and Organic Substrate	0.160	<0.001
Flow	-0.060	0.235
Gravel	-0.065	0.603
Ground Cover	0.134	0.002
Incision	-0.055	0.748
Overhanging Vegetation	0.155	0.122
Temperature	0.053	0.541
Thalweg	-0.077	0.018
Tree Roots	-0.108	0.365
Understory	-0.045	0.783
Wetted Width	-0.063	0.447
Wood Density	0.344	0.264

TABLE 17. Results of the partial least squares regression of road crossing presence and stream habitat on *F. neglectus* densities.

Variable	Coeff	p-value
Aquatic Vegetation	-0.098	0.036
Bank Angle	-0.021	0.927
Bank Undercut	-0.023	0.877
Bankfull Height	-0.065	0.256
Bankfull Width	-0.113	0.009
Barrier Presence	-0.056	0.172
Canopy cover	-0.010	0.781
Cobble	-0.074	0.062
Conductivity	0.046	0.112
Depth	-0.070	0.238
Dissolved Oxygen	-0.060	0.307
Embeddedness	-0.080	0.613
Fine and Organic Substrate	0.161	<0.001
Flow	-0.031	0.400
Gravel	-0.098	0.006
Ground Cover	0.113	0.059
Incision	-0.046	0.691
Overhanging Vegetation	0.156	0.022
Temperature	0.032	0.741
Thalweg	-0.099	0.027
Tree Roots	-0.072	0.006
Understory	-0.033	0.670
Wetted Width	-0.095	0.001
Wood Density	0.337	0.003

TABLE 18. Results of the partial least squares regression of road crossing presence and stream habitat on *F. neglectus* scaled mass index.

Variable	Coeff	p-value
Aquatic Vegetation	0.027	0.009
Bank Angle	-0.021	0.185
Bank Undercut	0.009	0.359
Bankful Height	0.029	<0.001
Bankful Width	0.026	0.002
Barrier Presence	0.037	<0.001
Boulder	-0.034	0.057
Canopy Cover	-0.018	0.014
Cobble	0.004	0.726
Conductivity	-0.022	0.007
Depth	0.016	0.127
Dissolved Oxygen	0.062	<0.001
Embeddedness	0.007	0.604
Fine and Organic Substrates	-0.072	<0.001
Flow	0.023	0.004
Gravel	0.069	<0.001
Ground Cover	-0.011	0.232
Incision	0.004	0.689
Overhanging Vegetation	-0.047	<0.001
Sex	0.006	0.565
Temperature	-0.018	0.083
Thalweg	0.020	0.047
Tree Roots	0.011	0.169
Understory	-0.020	0.087
Wetted Width	0.023	0.008
Wood Density	-0.060	<0.001

TABLE 19. Results of the partial least squares regression of road crossing presence and stream habitat on *F. meeki brevis* abundance.

Variable	Coeff	p-value
Aquatic Vegetation	0.073	0.711
Bank Angle	-0.053	0.262
Bank Undercut	-0.080	0.268
Bankfull Height	-0.041	0.451
Bankfull Width	-0.069	0.178
Barrier Presence	0.029	0.748
Canopy cover	0.032	0.609
Cobble	0.172	0.044
Conductivity	0.082	0.061
Depth	-0.105	0.177
Dissolved Oxygen	0.109	0.008
Embeddedness	-0.167	0.045
Fine and Organic Substrate	-0.177	0.005
Flow	0.005	0.948
Gravel	0.163	0.233
Ground Cover	-0.036	0.665
Incision	0.024	0.686
Overhanging Vegetation	0.133	0.548
Temperature	-0.119	0.196
Thalweg	-0.068	0.423
Tree Roots	-0.056	0.225
Understory	0.091	0.459
Wetted Width	-0.022	0.711
Wood Density	-0.072	0.025

TABLE 20. Results of the partial least squares regression of road crossing presence and stream habitat on *F. meeki brevis* density.

Variable	Coeff	p-value
Aquatic Vegetation	0.043	0.803
Bank Angle	-0.030	0.588
Bank Undercut	0.005	0.971
Bankfull Height	-0.045	0.369
Bankfull Width	-0.070	0.172
Barrier Presence	0.068	0.504
Canopy cover	0.011	0.841
Cobble	0.199	0.050
Conductivity	0.094	0.045
Depth	-0.079	0.312
Dissolved Oxygen	0.101	0.018
Embeddedness	-0.127	0.166
Fine and Organic Substrate	-0.191	0.007
Flow	-0.040	0.419
Gravel	0.168	0.145
Ground Cover	-0.087	0.453
Incision	-0.005	0.92
Overhanging Vegetation	0.141	0.412
Temperature	-0.093	0.266
Thalweg	-0.030	0.683
Tree Roots	-0.024	0.619
Understory	0.087	0.351
Wetted Width	-0.009	0.875
Wood Density	-0.063	0.056

TABLE 21. Results of the partial least squares regression of road crossing presence and stream habitat on *F. meeki brevis* scaled mass index.

Variable	Coeff	p-value
Aquatic Vegetation	-0.034	<0.001
Bank Angle	0.010	0.306
Bank Undercut	0.010	0.228
Bankfull Height	0.022	0.002
Bankfull Width	0.013	0.172
Barrier Presence	0.000	0.997
Boulder	0.006	0.476
Canopy Cover	-0.037	0.010
Cobble	0.042	<0.001
Conductivity	0.017	0.054
Depth	0.025	<0.001
Dissolved Oxygen	0.013	0.163
Embeddedness	-0.011	0.459
Flow	0.004	0.693
Gravel	-0.023	0.005
Ground Cover	-0.011	0.296
Incision	0.020	0.040
Overhanging Vegetation	-0.031	<0.001
Sand	0.028	0.011
Sex	-0.019	0.048
Silt	-0.009	0.447
Temperature	0.022	0.001
Thalweg	0.024	0.001
Tree Roots	-0.036	0.003
Understory	-0.032	0.002
Wetted Width	0.003	0.785
Wood Density	-0.007	0.541

TABLE 22. Results of the partial least squares regression of road crossing presence and stream habitat on *F. nana* abundance.

Variable	Coeff	p-value
Aquatic Vegetation	-0.082	0.010
Bank Angle	0.020	0.774
Bank Undercut	0.016	0.838
Bankfull Height	0.072	0.123
Bankfull Width	-0.039	0.357
Barrier Presence	0.084	0.172
Canopy cover	0.087	0.065
Cobble	0.066	0.392
Conductivity	0.000	0.993
Depth	0.015	0.793
Dissolved Oxygen	0.059	0.118
Embeddedness	-0.135	0.004
Fine and Organic Substrate	-0.107	0.001
Flow	0.150	0.125
Gravel	0.125	0.009
Ground Cover	0.009	0.870
Incision	0.091	0.012
Overhanging Vegetation	-0.074	0.052
Temperature	0.012	0.843
Thalweg	0.027	0.624
Tree Roots	-0.024	0.577
Understory	-0.040	0.411
Wetted Width	-0.032	0.458
Wood Density	0.006	0.864

TABLE 23. Results of the partial least squares regression of road crossing presence and stream habitat on *F. nana* density.

Variable	Coeff	p-value
Aquatic Vegetation	-0.102	0.001
Bank Angle	0.043	0.715
Bank Undercut	0.036	0.782
Bankfull Height	0.049	0.388
Bankfull Width	-0.053	0.218
Barrier Presence	0.083	0.170
Canopy cover	0.098	0.097
Cobble	0.031	0.735
Conductivity	-0.026	0.724
Depth	0.021	0.801
Dissolved Oxygen	0.070	0.024
Embeddedness	-0.178	<0.001
Fine and Organic Substrate	-0.092	0.003
Flow	0.217	0.207
Gravel	0.129	0.013
Ground Cover	0.051	0.447
Incision	0.088	0.020
Overhanging Vegetation	-0.060	0.294
Temperature	0.026	0.683
Thalweg	0.029	0.664
Tree Roots	-0.042	0.247
Understory	-0.034	0.446
Wetted Width	-0.047	0.249
Wood Density	0.071	0.077

TABLE 24. Results of the partial least squares regression of road crossing presence and stream habitat on *F. nana* scaled mass index.

Variable	Coeff	p-value
Aquatic Vegetation	-0.012	0.162
Bank Angle	0.013	0.022
Bank Undercut	0.007	0.402
Bankfull Height	-0.007	0.423
Bankfull Width	-0.008	0.409
Barrier Presence	0.046	0.001
Canopy Cover	-0.018	0.037
Cobble	0.001	0.894
Conductivity	0.009	0.401
Depth	0.001	0.889
Dissolved Oxygen	0.020	0.024
Embeddedness	-0.005	0.583
Flow	0.027	<0.001
Gravel	0.019	0.024
Ground Cover	0.018	0.046
Incision	0.012	0.268
Overhanging Vegetation	-0.018	0.008
Sand	0.012	0.045
Sex	-0.015	0.101
Silt	-0.022	0.011
Temperature	-0.023	0.013
Thalweg	-0.001	0.913
Tree Roots	-0.019	0.004
Understory	-0.022	0.029
Wetted Width	-0.011	0.159
Wood Density	-0.015	0.142

TABLE 25. Results of the partial least squares regression of road crossing presence and stream habitat on the proportion of substrate generalists (*Faxonius negelectus & virilis*).

Variable	Coeff	p-value
Aquatic Vegetation	0.022	0.724
Bank Angle	0.031	0.685
Bank Undercut	-0.009	0.874
Bankfull Height	-0.047	0.472
Bankfull Width	0.060	0.374
Barrier Presence	0.046	0.378
Canopy cover	-0.065	0.085
Cobble	-0.141	0.003
Conductivity	-0.055	0.491
Depth	-0.016	0.799
Dissolved Oxygen	-0.181	0.001
Embeddedness	0.129	0.015
Fine and Organic Substrate	0.131	0.003
Flow	-0.026	0.371
Gravel	-0.152	0.003
Ground Cover	0.044	0.276
Incision	-0.096	0.035
Overhanging Vegetation	0.035	0.452
Temperature	0.001	0.989
Thalweg	-0.046	0.455
Tree Roots	0.045	0.563
Understory	0.045	0.414
Wetted Width	-0.052	0.133
Wood Density	0.027	0.455

TABLE 26. Results of the partial least squares regression of road crossing presence and stream habitat on the proportion of crayfish affiliated with lotic environments (all excluding the *F. virilis*).

Variable	Coeff	p-value
Aquatic Vegetation	-0.068	0.262
Bank Angle	-0.080	0.348
Bank Undercut	0.055	0.096
Bankfull Height	0.014	0.878
Bankfull Width	-0.086	0.320
Barrier Presence	-0.072	0.240
Canopy cover	0.073	0.141
Cobble	0.107	0.010
Conductivity	-0.030	0.623
Depth	-0.022	0.779
Dissolved Oxygen	0.218	<0.001
Embeddedness	-0.135	0.002
Fine and Organic Substrate	-0.119	0.006
Flow	0.043	0.056
Gravel	0.180	<0.001
Ground Cover	-0.081	0.153
Incision	0.092	0.006
Overhanging Vegetation	-0.038	0.490
Temperature	-0.084	0.174
Thalweg	-0.014	0.863
Tree Roots	-0.110	0.109
Understory	-0.073	0.222
Wetted Width	0.088	0.007
Wood Density	-0.024	0.513

TABLE 27. Results of the partial least squares regression of road crossing presence and stream habitat on the proportion of species whose range is considered regional (Ringed Crayfish).

Variable	Coeff	p-value
Aquatic Vegetation	-0.114	0.082
Bank Angle	-0.126	0.328
Bank Undercut	0.107	0.189
Bankfull Height	-0.054	0.670
Bankfull Width	-0.086	0.373
Barrier Presence	-0.080	0.307
Canopy cover	0.047	0.493
Cobble	-0.019	0.764
Conductivity	-0.170	0.278
Depth	-0.079	0.367
Dissolved Oxygen	0.159	<0.001
Embeddedness	-0.069	0.278
Fine and Organic Substrate	-0.026	0.744
Flow	0.048	0.272
Gravel	0.126	0.011
Ground Cover	-0.099	0.123
Incision	0.032	0.692
Overhanging Vegetation	-0.021	0.711
Temperature	-0.187	0.044
Thalweg	-0.116	0.222
Tree Roots	-0.166	0.024
Understory	-0.084	0.282
Wetted Width	0.103	0.030
Wood Density	-0.003	0.944

TABLE 28. Results of the partial least squares regression of road crossing presence and stream habitat on the proportion of crayfish with narrow ranges (Meeks Short-pointed and Midget Crayfish).

Variable	Coeff	p-value
Aquatic Vegetation	0.000	0.995
Bank Angle	-0.047	0.558
Bank Undercut	-0.008	0.874
Bankfull Height	0.047	0.515
Bankfull Width	-0.071	0.311
Barrier Presence	-0.047	0.419
Canopy Cover	0.071	0.076
Cobble	0.136	0.004
Conductivity	0.066	0.449
Depth	-0.005	0.936
Dissolved Oxygen	0.189	0.001
Embeddedness	-0.139	0.019
Fine and Organic Substrates	-0.135	0.006
Flow	0.022	0.402
Gravel	0.169	0.003
Ground Cover	-0.041	0.362
Incision	0.072	0.130
Overhanging Vegetation	-0.024	0.656
Temperature	-0.047	0.436
Thalweg	0.032	0.621
Tree Roots	-0.047	0.586
Understory	-0.046	0.462
Wetted Width	0.061	0.104
Wood Density	-0.036	0.343

TABLE 29. Results of the partial least squares regression of road crossing presence and stream habitat on Chao's richness estimator on crayfish assemblages.

Variable	Coeff	p-value
Aquatic Vegetation	-0.042	0.564
Bank Angle	-0.046	0.567
Bank Undercut	-0.050	0.491
Bankfull Height	0.081	0.373
Bankfull Width	-0.127	0.042
Barrier Presence	-0.015	0.834
Canopy cover	0.064	0.294
Cobble	0.077	0.173
Conductivity	0.090	0.341
Depth	-0.053	0.530
Dissolved Oxygen	0.147	0.012
Embeddedness	-0.194	0.007
Fine and Organic Substrate	-0.094	0.038
Flow	0.005	0.902
Gravel	0.142	0.008
Ground Cover	0.025	0.690
Incision	0.098	0.064
Overhanging Vegetation	0.075	0.356
Temperature	-0.041	0.600
Thalweg	-0.026	0.743
Tree Roots	-0.083	0.296
Understory	-0.048	0.491
Wetted Width	0.002	0.960
Wood Density	0.056	0.502

Appendix C: Figures

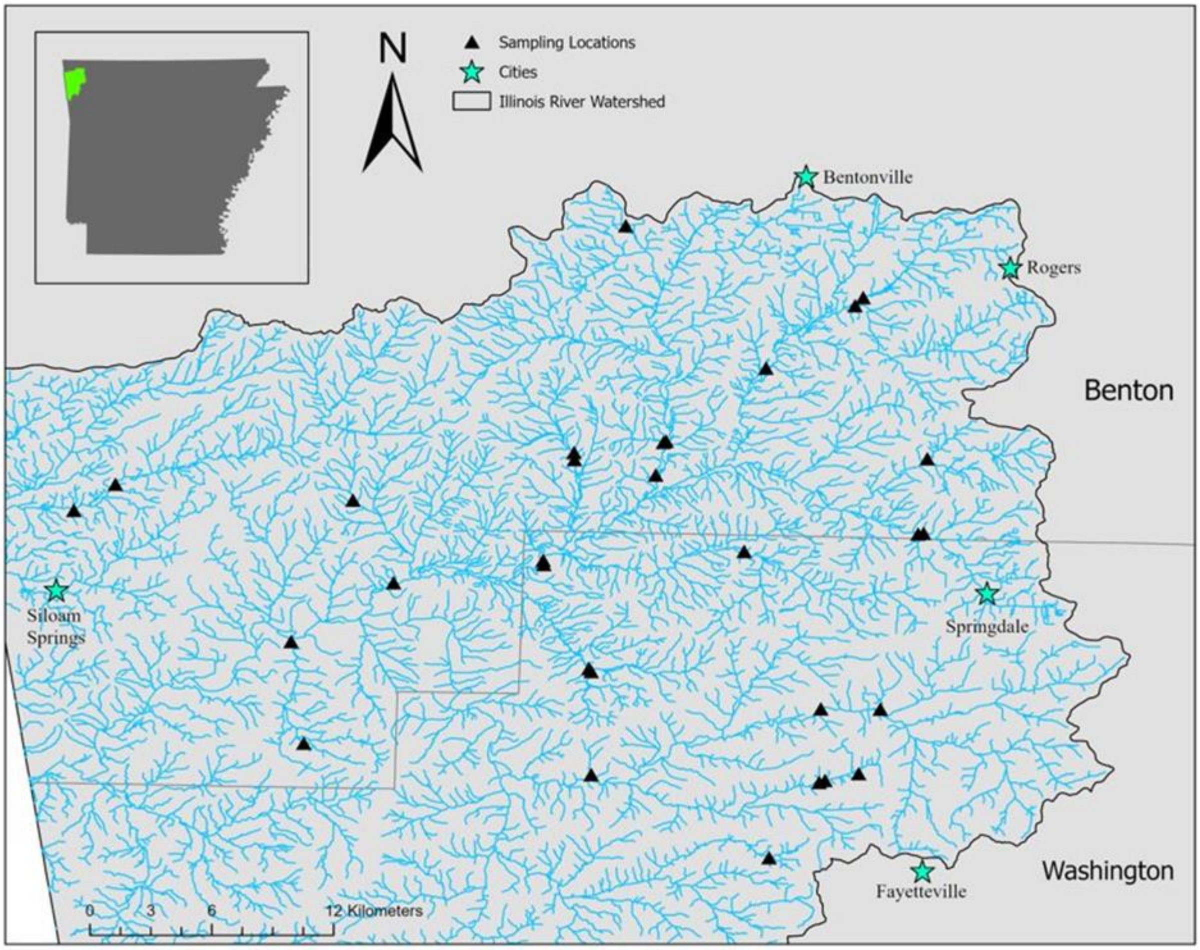

FIGURE 1. Map of study sites within the Illinois River watershed, Northwest Arkansas.

FIGURE 2. Examples of severe (top right), minor (top left), and insignificant (bottom) barriers according to the Southeastern Aquatic Resource Partnership's Stream Crossing Assessment Protocol.

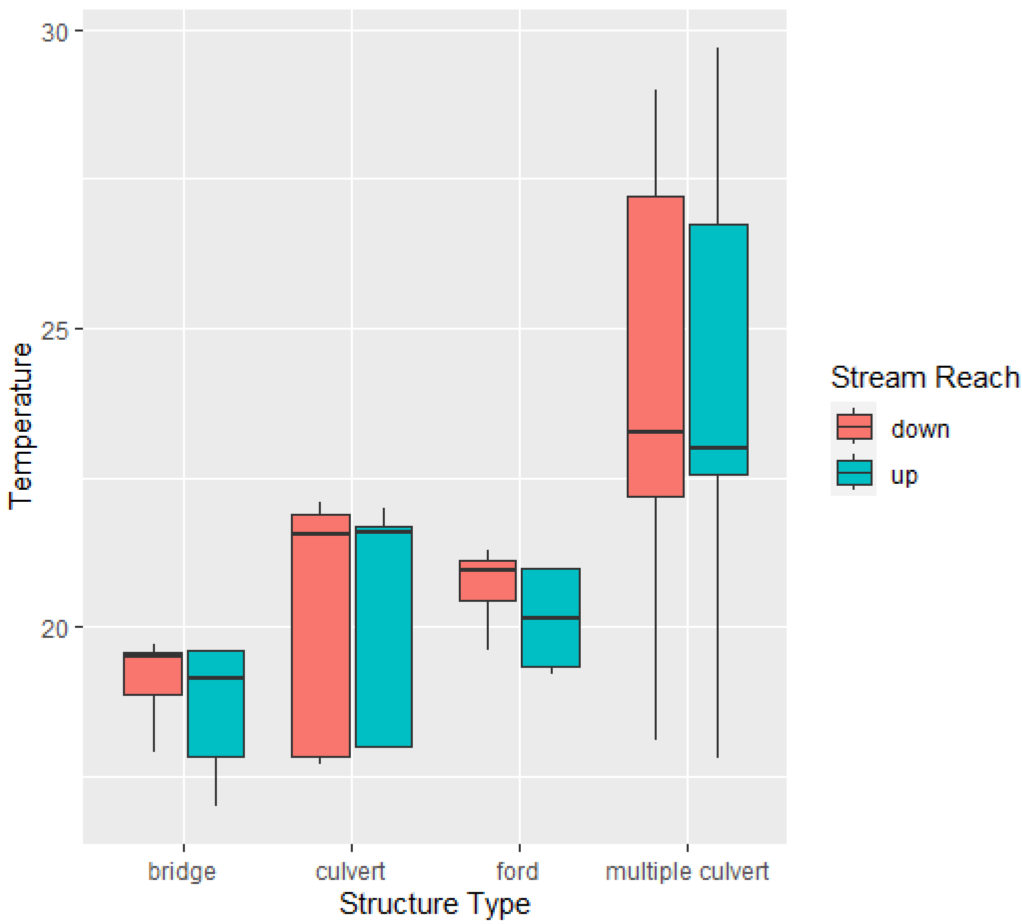

FIGURE 3. Average water temperatures at each road crossing type, filtered by stream reaches upstream and downstream of the structures.

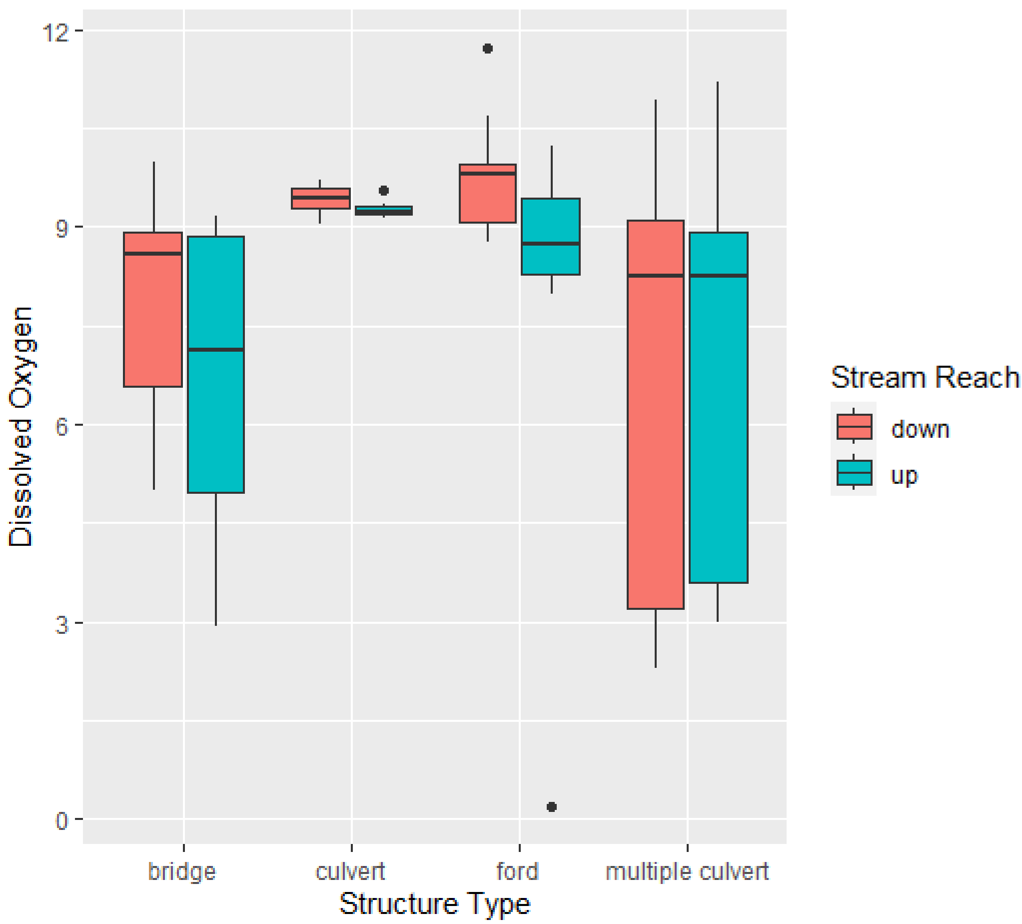

FIGURE 4. Average dissolved oxygen at each road crossing type, filtered by stream reaches upstream and downstream of the structures.

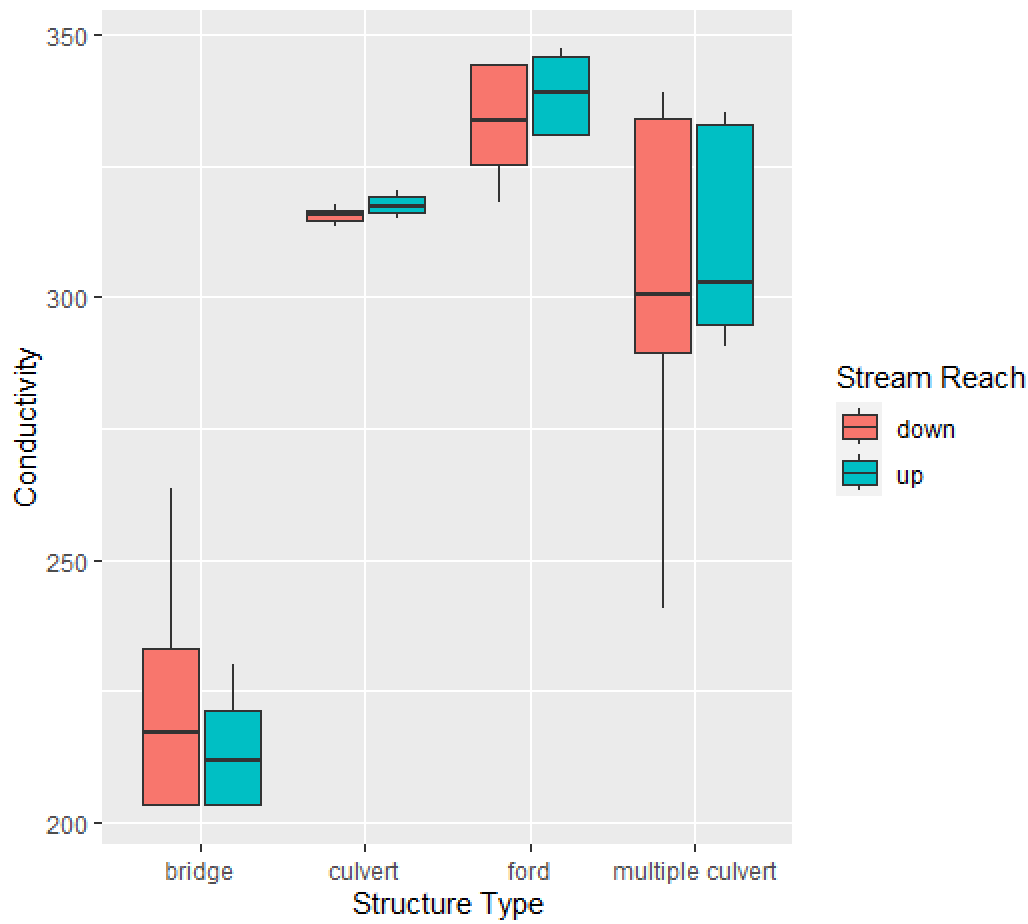

FIGURE 5. Average conductivity at each road crossing type, filtered by stream reaches upstream and downstream of the structures.

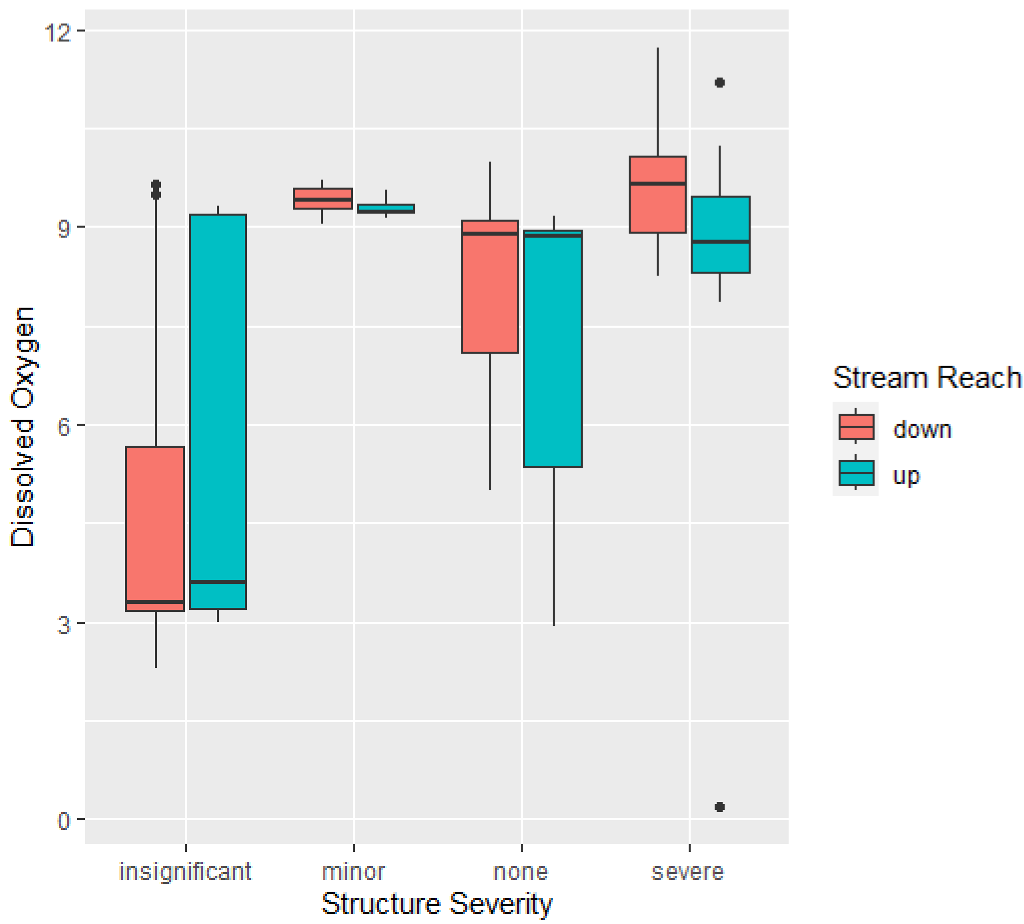

FIGURE 6. Average dissolved oxygen at each road crossing severity, filtered by stream reaches upstream and downstream of the structures.

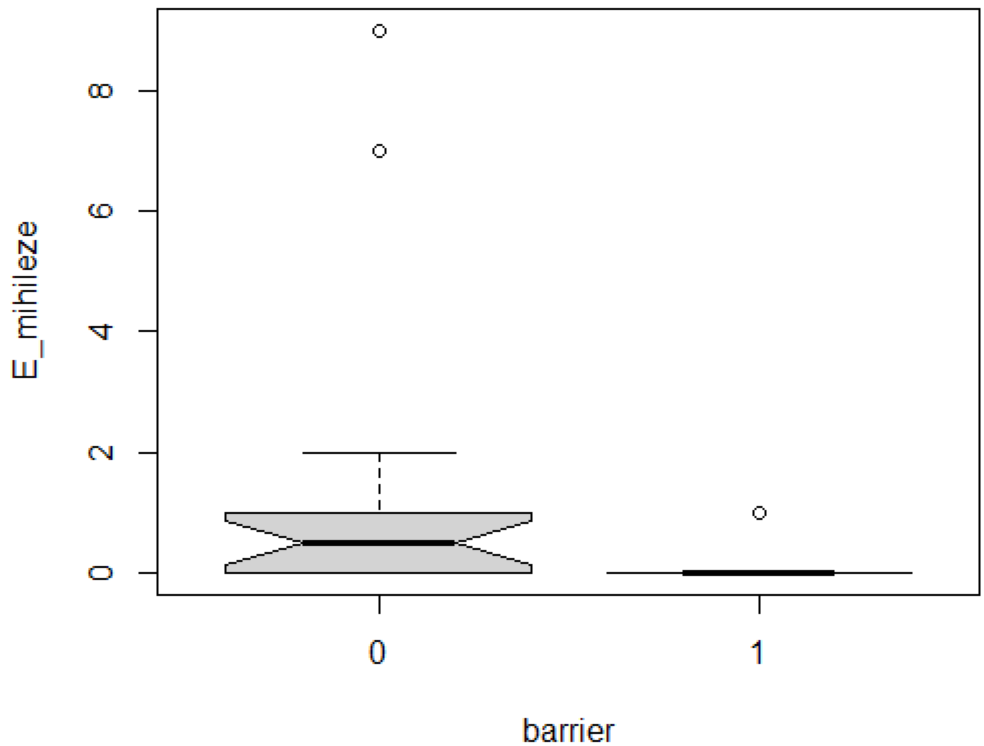

FIGURE 7. Abundance of Sunburst Darter at sites with (1) and without (0) road crossings. Wilcoxon signed-rank test determined this difference to be significant ($W = 147$, $p = 0.05$).