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COMPARISON OF THREATENED NEOSHO MADTOM DENSITIES BETWEEN RIVERSCAPES DIFFERING IN ANTHROPOGENIC STRESSORS, WITH A PARTICULAR FOCUS ON RECOVERY FROM MINING-DERIVED METAL POLLUTION

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An Abstract of the Thesis by
Kali Lynn Boroughs

Water pollution imperils the Neosho Madtom (*Noturus placidus*), which is threatened in Kansas and federally. Within Kansas madtom densities were historically lower in the Spring River compared to the Cottonwood and Neosho Rivers, especially within the Spring River below tributary inputs that delivered cadmium, lead, and zinc pollution from the Tri-State Mining District. Studies suggest that madtoms are less numerous in waters containing elevated metal concentrations because of direct toxicity and lower benthic macroinvertebrate availability, which is also depressed by elevated metal concentrations. However, long-term reductions in metal concentrations in the Spring River have occurred, but to date no study has examined whether madtom and macroinvertebrate densities have responded to this improving water quality. We addressed this question by comparing madtom densities and macroinvertebrate biomass between the Neosho-Cottonwood and Spring Rivers, and within the Spring River above and below metal pollution inputs. However, madtoms are imperiled by environmental factors and anthropogenic stressors beyond mining-derived metal pollution, so we also examined if food availability (i.e., macroinvertebrate biomass), watershed characteristics (i.e., the upstream proximity of small and large dams, upstream watershed area, and percent open water in the upstream watershed), and local habitat variables (i.e., turbidity, depth, velocity, and percent gravel/pebble substrate) were related to madtom densities.

We found that madtom and macroinvertebrate population densities in the Spring River were similar to those of the Neosho-Cottonwood River system, and densities in the Spring River downstream of mining-impacted tributaries were similar to those upstream of pollution. Furthermore, macroinvertebrate availability and watershed characteristics were not associated with madtom abundance. However, turbidity and depth were associated with madtom densities, such that an increase in turbidity or decrease in depth resulted in higher madtom densities. Our results highlight the benefits that water quality improvements can have on stream organisms, especially those that are imperiled.

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CHAPTER I

INTRODUCTION

Heavy metals are a threat to fish and macroinvertebrate communities in lotic ecosystems globally (Wildhaber et al. 2000a; Courtney and Clements 2002; Iwasaki et al. 2009). Heavy metals enter flowing waterbodies via mining and industrial activities, municipal sewage effluent, and urban runoff (Malmqvist and Rundle 2002; Iwasaki et al. 2009). Once in the water, heavy metals can enter a fish directly through the gills and integument (Dallinger et al. 1987; Vinodhini and Narayanan 2008; Afshan et al. 2014), or indirectly via biotransference from contaminated diet items (e.g., macroinvertebrates, smaller fish, aquatic vegetation; Dallinger et al. 1987; Maret et al. 2003; Afshan et al. 2014). Toxic metals, such as lead (Pb), zinc (Zn), and cadmium (Cd) can reduce fish diversity and density (Wildhaber et al. 2000a; Maret et al. 2003; Afshan et al. 2014; Authman et al. 2015) by having deleterious effects on physiology, interfering with reproduction, and altering behavior (Wildhaber et al. 2000a; Authman et al. 2015). Finally, metal pollution can also harm fish indirectly via reduced food availability (Dallinger et al. 1987; Wildhaber et al. 2000a; Campbell et al. 2003; Kövecses et al. 2005; Landers 2016), as streams with elevated metal concentrations have lower macroinvertebrate abundance and diversity via the loss of sensitive species (Courtney and

Clements 2002; Maret et al. 2003; Qu et al. 2010). Macroinvertebrates are an important food source for fish, thus, a decrease in benthic macroinvertebrate density and biomass could have significant implications in food availability for benthic fish assemblages (Wildhaber et al. 2000a; Campbell et al. 2003; Kövecses et al. 2005; Mebane et al. 2015; Landers 2016).

The Neosho Madtom (*Noturus placidus*, Taylor 1969), is a small (< 75 mm total length) North American freshwater catfish that was listed as threatened under the Endangered Species Act (1973) in 1990, and has been a threatened species in Kansas since 1987 (Wilkinson and Fuselier 1997; Wildhaber et al. 2000a; Kansas Fishes Committee 2014). This species is native to the Illinois River in Oklahoma, the Neosho River (Kansas & Oklahoma), the Cottonwood River (Kansas), and the Spring River (Kansas, Oklahoma, and Missouri), where it inhabits riffles and bar habitats with loose pebble and gravel substrate, moderate to high water velocities, and relatively shallow depths (Ernsting et al. 1989; Wilkinson et al. 1996; Wilkinson and Fuselier 1997; Wildhaber et al. 2000a; Allen et al. 2001; Kansas Fishes Committee 2014). Neosho Madtoms are nocturnal predators that feed on immature benthic macroinvertebrates, such as caddisflies, mayflies, and midges (Allen et al. 2001; Kansas Fishes Committee 2014).

Historically, within Kansas the density of Neosho Madtoms was lower in the Spring River compared to the Cottonwood and Neosho Rivers (Wilkinson et al. 1996; Wildhaber et al. 1999a; 2000a). This pattern was observed in surveys conducted in the early 1990's that examined natural and anthropogenic factors limiting Neosho Madtom and other riffle-dwelling benthic fishes in the Spring River (Wildhaber et al 2000a). One proposed reason for the lower Neosho Madtom density was elevated concentrations of toxic metals in the Spring River compared to the Neosho-Cottonwood system (Wildhaber et al. 1999a; 2000a). Metal pollution in the Spring River was the consequence of metal inputs from the Tri-State Mining District, where extensive mining for Pb and Zn occurred during the mid-1800s through the 1950s (Barks 1986; Wildhaber et al. 1999b; 2000a; Brumbaugh et al. 2005). These metal inputs were derived from several of the Spring River's major tributaries, including Center Creek, Turkey Creek, and Shoal Creek (Figure 1). Furthermore, within the Spring River, Neosho Madtom densities below the first major input of metal pollution (i.e., Center Creek) were much lower than in the Spring River above this tributary (Wildhaber et al. 2000a). Previous studies suggested that Neosho Madtoms are less numerous in waters containing elevated Pb, Zn, and Cd concentrations because of direct toxicity and lower benthic macroinvertebrate availability (Wildhaber et al. 2000a; USFWS 2013).

A long-term reduction in Cd, Pb, and Zn concentrations in the Spring River and its tributaries has occurred since the 1990s which was revealed through the water quality monitoring program conducted by the Kansas Department of Health and Environment (KDHE) (Figure 2). However, even though metal concentrations have decreased, certain segments of the Spring River in Kansas are still listed under section 303(d) of the Clean Water Act (1972) (e.g., below Center Creek) (KDHE 2020). Improvements to water quality may not yet be substantial enough to elicit a response from Neosho Madtom and benthic macroinvertebrates, but the response of these stream organisms to improving water quality is presently unknown.

Abiotic habitat factors and anthropogenic stressors beyond mining-impaired watersheds and their resultant degradation of water quality may influence Neosho

Madtom densities. For instance, dams exert control over Neosho Madtom distribution and abundance by altering processes from regional to local scales. Large dams (i.e., >15 m in height; Poff and Hart 2002) negatively affect Neosho Madtom populations by fragmenting populations, creating lentic conditions upstream, and by altering habitat and natural flow regimes downstream from dams (Wildhaber et al. 2000b). For instance, extended bankfull flows released from large dams during the spawning season decreases the reproductive success of Neosho Madtoms (Kansas Fishes Committee 2014). These regional-scale modifications occurring from dams can alter local physicochemical conditions, as river segments downstream from dams have coarser substrate with greater embeddedness, greater water clarity, and altered depth and velocity (Wildhaber et al. 2000b; Tiemann et al. 2004). Madtoms are also negatively affected by small (i.e., <15m height), low-head dams that have similar negative effects as large dams, although the extent and magnitude of their impacts is lesser, and they do not alter the natural flow regime (Tiemann et al. 2004).

It is currently unknown whether Neosho Madtom and macroinvertebrate abundances in the Spring River have responded to decreasing metal concentrations, thus our first objectives were to compare contemporary 1) Neosho Madtom and 2) macroinvertebrate abundances among the Spring River above metal pollution, the Spring River below metal pollution, and the Neosho-Cottonwood River system. We predicted that Neosho Madtom and macroinvertebrate abundances would be similar among our three stream systems because of rebounding abundances in the lower Spring River that were the consequence of long-term water quality improvements. We emphasize that our investigation concerning responses to long-term water quality improvements examines whether or not a pattern that was present in the 1990s (i.e., depressed Neosho Madtom and macroinvertebrate abundance in the lower Spring River) still existed in 2019-2020 rather than looking directly at temporal trends in abundances, as long-term abundance data is not available. Furthermore, we do not directly compare our abundance estimates to those from the 1990s, as differences in sampling efficiency and methodology between time periods made direct abundance comparisons difficult. Regardless, we reasoned that if Neosho Madtom and macroinvertebrate abundances in the lower Spring River were now similar to the other two systems, that result would provide evidence for a positive biotic response to the long-term reduction of metal concentrations. Our final objective was to determine what environmental variables could best explain contemporary spatial variation in Neosho Madtom densities, which we assessed by comparing models that included invertebrate food availability, local physicochemical conditions, and upstream watershed characteristics as explanatory variables. We predicted that Neosho Madtom abundances would be positively associated with food availability but would be negatively affected by factors related to large and small dams. Our research will provide further information on the environmental factors influencing the prevalence of a federally-threatened fish species, while also yielding more general insights concerning the response of stream organisms to long-term reductions in water pollution.

METHODS

Study Area

Our study streams included the Cottonwood, Neosho, and Spring Rivers, as well as Lightning Creek, a major tributary of the Neosho River (Figure 1; Figure 3). All study streams were located within the Neosho River basin, which corresponds to a U.S.

Geological Survey level-6 hydrologic unit code (HUC-6 = 110702). We selected 10 sites each in the Spring and Neosho-Cottonwood River systems, for a total of 20 sites. Within the Spring River, five sites were located above and five below tributary inputs with elevated Cd, Pb, and Zn concentrations (Figures 1 and 2; Table 1). Site selection was based primarily on the presence of preferred Neosho Madtom habitat (i.e., riffles and gravel bars), followed by accessibility and our ability to obtain sampling permission. We used Google Earth™ to identify locations with preferred habitat. All sampling was conducted during June 2019 – August 2020, with 2019 and 2020 treated as separate sample years.

Fish Sampling

Fish sampling was conducted at each site once each year between late summer and early fall (i.e., two total samples per site). However, in 2019 severe flooding and extended high flows prevented us from conducting fish surveys at our two lower Spring River sites. Fish sampling using kick-seining (4.6 m x 1.8 m seine with 3.2 mm mesh) took place in riffle and moderate to low-velocity gravel bar habitats. Kick-seining was conducted by one or two individuals thoroughly disturbing the substrate beginning four meters upstream from a stationary seine and then kicking in a downstream direction to the seine's leadline. As such, each kick-seining effort yielded an 18.4 m^2 sample area (i.e., 4 m kicking length X 4.6 m seine width = 18.4 m^2). Kick-seining started at the downstream end of a habitat and proceeded laterally and then upstream with multiple kick-seine efforts until all habitat less than one meter deep at a site had been sampled. All fishes captured were identified to species, measured for total length (TL) to the nearest millimeter, counted, and then returned to the stream.

Overall Neosho Madtom densities at each site were calculated by dividing the total number of Neosho Madtoms captured by the total area sampled via kick-seining. We also calculated adult Neosho Madtom densities separately to address if young of year (YOY) individuals were influencing our results, as variation in sample timing between years impacted the number of YOY individuals captured at some sites. Using length-frequency histograms we determined adults were individuals >35 mm TL in July, >40 mm TL in August, >45 mm TL in September, and >50mm TL in October and November.

Benthic Macroinvertebrate Sampling

Benthic macroinvertebrate samples were collected at sites twice between late spring through fall during each sample year (i.e., four total sampling occasions per site). We sampled macroinvertebrates twice each year to help account for the high degree of intra-annual variability that is typical of aquatic macroinvertebrate communities (O'Connor 2010; Nava et al. 2015). A modified-Hess sampler (0.086 m^2 sample area, 363 μm mesh collection bag) was used to collect six replicate benthic invertebrate samples at most sample sites during each sampling occasion, although only three replicates were collected at two of our smaller sites. In the Neosho-Cottonwood River system we collected 108 replicate macroinvertebrate samples in 2019 and 120 samples in 2020, while in the Spring River we collected 99 and 117 replicates in 2019 and 2020, respectively. Fewer replicates were collected in 2019 compared to 2020 in both systems because high flows prevented us from collecting some samples. When benthic macroinvertebrate and fish sampling coincided at a sample site, benthic macroinvertebrate samples were collected prior to fish sampling so that substrates were not disturbed prior to macroinvertebrate collections. Within the Hess sampler, substrate down to a depth of five centimeters was disturbed for approximately two minutes to dislodge any macroinvertebrates into the collection net. Benthic invertebrate samples were preserved in 10% formalin and taken back to the lab.

Individuals were identified to family for insects (Merritt et al. 2008) and class or order for non-insect taxa (Huggins et al. 1985), measured for total length to the nearest mm using one mm square grid paper, and counted. Published length-mass relationships (Benke et al. 1999) were used to estimate benthic macroinvertebrate biomass, which was expressed as grams of dry mass (DM) per m^2. However, prior to density and biomass calculations we removed any non-bivalve invertebrate that had a length greater than ten millimeters and any bivalve with a length or height greater than five millimeters. We excluded large invertebrates from density and biomass calculations because we wanted to focus on macroinvertebrates that were available for consumption, thus providing a more accurate estimate of food availability.

Environmental Factors

We collected data describing local physicochemical conditions and upstream watershed characteristics at each sampling location. Prior to any macroinvertebrate or fish sampling, we used an empty 125 ml plastic bottle to collect a water sample, which was taken back to the lab and analyzed using a 2100P turbidimeter to calculate turbidity in nephelometric turbidity units (NTUs). To quantify physical habitat, three to thirteen transects were positioned per site, depending on site length. Along each transect, we quantified depth (m), water velocity (m/s), and substrate at five equally spaced points, for a total of 10-65 measurements per sample habitat. We used a Hach top-setting wading rod to measure depth and a Hach FH950 portable flow meter to measure water velocity.

Substrate classifications were based on a modified-Wentworth scale, including (from smallest to largest) clay, silt, sand, gravel, pebble, cobble, boulder, and bedrock (Cummins 1962). Upstream watershed characteristics included percent open water, distance to the nearest large dam (km), distance to nearest small dam (km), and watershed area (km^2). All open waterbodies in our study area are artificially-created by human activity (mostly impoundment; some excavation), thus percent open water helped us further quantify the upstream influence of dams on study sites (Stene 1946; Arruda and Fromm 1989). We calculated upstream watershed area to estimate stream size, which is an important predictor of stream fish distributions for most species (Horwitz 1978; Jackson et al. 2001; Zorn et al. 2002; Troia and Gido 2014). Percent open water and upstream watershed area were estimated using the StreamStats program for Kansas (USGS 2016), while upstream distances to large and small dams were measured using Google Earth. The Neosho-Cottonwood River system has three large dams (i.e., Marion, Council Grove, and John Redmond Reservoirs) while the Spring River system has none (Figures 1 and 3). Both systems have numerous small dams, although small dams are more prevalent in the Neosho-Cottonwood compared to the Spring River system (Tiemann et al. 2004; Kansas Fishes Committee 2014).

Data Analyses

We used analysis of variance (ANOVA) to compare total and adult Neosho Madtom densities as well as macroinvertebrate density and biomass among the Neosho-Cottonwood River, Spring River upstream of pollution, and Spring River downstream of pollution. Densities (i.e., madtom and macroinvertebrate) and macroinvertebrate biomass were square root transformed to help data conform to the normality and homoscedasticity assumptions. To perform our analyses, we began by constructing linear models (LMs) using the lm function from the R base package (R Core Team 2020), followed by conducting an ANOVA on the LM using the Anova function from the car package (Fox and Weisberg 2019). If a significant effect (i.e., $\alpha = 0.05$) was found, we used Tukey's honest significant difference (HSD) to determine which categories significantly differed from one another. We predicted that we would fail to reject the null hypothesis of no difference in abundances among our three stream categories.

We had three competing models concerning the factors that could explain spatial variation in either total or adult contemporary Neosho Madtom density, including 1) food availability, 2) local physicochemical habitat conditions, and 3) upstream watershed characteristics. We used macroinvertebrate biomass as the lone predictor variable for the food availability model, while turbidity, mean depth, mean water velocity, and percent gravel-pebble substrate served as predictor variables for the local habitat model. Finally, percent open water in the upstream watershed, upstream watershed area, and the inverse of the distances to nearest large and small dams multiplied by 1,000 were used as predictor variables for the upstream watershed characteristics model. We took the inverse of distances to nearest dams such that 0 = no upstream dams and larger numbers equated to greater dam proximity and presumably greater effects of a dam on a sample site. We multiplied inverse distance to dams by 1,000 so that numbers were not small decimals. We reasoned that both of these changes to dam distance would facilitate model interpretation. Prior to modeling we scaled variables between 0-1 for models that included multiple predictors. Furthermore, since models 2 and 3 had multiple predictor variables, we used multiple linear regression coupled with stepwise variable selection to determine if we could reduce the number of predictor variables within a model prior to comparison among the three models. We compared our three competing models as well as an intercept-only null model using adjusted R^2 and Akaike's Information Criterion corrected for small sample size (AIC_c).

RESULTS

Madtom and Macroinvertebrate Abundance Comparisons

In total we found 607 Neosho Madtoms across the Neosho-Cottonwood and Spring River systems during 2019-2020. During 2019 we captured 129 Neosho Madtoms, with 103 (80%) of those coming from 8/10 sample sites in the Cottonwood and Neosho Rivers (Figures 3 and 4). In the Spring River during 2019, we only found 26 Neosho Madtoms at 4/8 sample sites, which were all upstream of metal pollution (Figures 1 and 4). In 2020, we captured 478 Neosho Madtoms, with 359 (75%) of those coming from 7/10 sample sites in the Cottonwood and Neosho Rivers (Figures 3 and 4) and 119 individuals from 8/10 Spring River sites (Figures 1 and 4). In the Spring River in 2020, 86/119 (72%) madtoms occurred downstream of the first tributary input of metal pollution (i.e., Center Creek), although the two sites where we did not detect Neosho Madtom were downstream of metal inputs as well (Figure 1 and 4). Furthermore, 73/86 madtoms that were found downstream of metal pollution occurred at one site located just above the Willow Creek confluence, which we were unable to sample in 2019 because of high flows (Figure 1).

In accordance with our predictions, there were no differences in Neosho Madtom and macroinvertebrate abundances among our three stream categories. Specifically, total ($F_{2,35}$ = 1.32; P = 0.28) and adult madtom densities ($F_{2,35}$ = 1.05; P = 0.36) did not differ among the Neosho-Cottonwood, Spring River above pollution inputs, and Spring River below pollution (Figure 4), and macroinvertebrate biomass ($F_{2,37} = 3.25$; $P = 0.05$) and density ($F_{2,37} = 2.29$; $P = 0.12$) did not differ among systems either (Figure 4).

Explanatory Models of Neosho Madtom Density

The local habitat model was the top model that could best explain spatial variation in total ($R^2 = 0.25$; $F_{2,35} = 7.07$; $P = 0.003$) and adult ($R^2 = 0.30$; $F_{2,35} = 8.83$; $P = <0.001$) Neosho Madtom densities, although it only included turbidity and depth as predictor variables following variable selection (Table 2). An increase in turbidity (total madtom $\beta = 0.54$; adult madtom $\beta = 0.39$) or decrease in depth (total madtom $\beta = -0.33$; adult madtom $\beta = -0.22$) resulted in increasing madtom densities. In contrast, there was no relationship between benthic macroinvertebrate biomass and total ($r^2 = -0.03$; $F_{1,36} = 0.08$; $P = 0.78$) or adult Neosho Madtom densities ($r^2 = -0.01$; $F_{1,36} = 0.47$; $P = 0.50$; Table 2), and no watershed-scale variables were associated with total (adjusted $R^2 = 0.01$; $F_{4,33} = 1.11$; $P = 0.37$) or adult madtom densities (adjusted $R^2 = 0.01$; $F_{4,33} = 1.08$; $P = 0.38$). Both results defied our initial predictions concerning the influence of food availability and dams on Neosho Madtom density.

DISCUSSION

Historically, Neosho Madtom densities were lower in the Spring River compared to the Neosho-Cottonwood Rivers, especially in the Spring River below major tributary inputs of metal pollution (Wilkinson et al. 1996; Wildhaber et al. 1999a; 2000a). However, we found that Neosho Madtom densities throughout the Spring River were now comparable to the Neosho-Cottonwood system, even in the Spring River below tributaries with elevated metal concentrations. We reasoned that the disappearance of the historical pattern concerning depressed Neosho Madtom abundances in the Spring River was due to increasing abundances in the lower Spring River that resulted from long-term reductions in metal concentrations, rather than decreasing abundances in our other study systems. We support this reasoning with several lines of evidence. First, few madtoms were observed at a limited number of locations in the Spring River below metal pollution in the 1990s (Wilkinson et al. 1996; Wildhaber et al. 2000a), whereas we observed numerous Neosho Madtoms across multiple sites in this reach, which included the site with the greatest density within the Spring River (Figure 1). And although the two sites in the Spring River where we did not detect Neosho Madtom as part of this study were located below metal pollution, other studies have detected Neosho Madtoms at those sites during 2018-2020 (J. Whitney, personal communication). As such, Neosho Madtom now occur throughout the entirety of the Spring River in Kansas, and achieve some of their highest densities in the reach below metal pollution, contrasting strongly with patterns observed in the 1990s (Wilkinson et al. 1996; Wildhaber et al. 2000a). Furthermore, Neosho Madtom density remains relatively high in the Neosho-Cottonwood system (Figure 4), indicating that it is unlikely that reductions in density in this system over time would explain non-significant differences between the Neosho-Cottonwood and Spring Rivers. All of these lines of evidence support our assertion that Neosho Madtom distribution and abundance in the Spring River have increased, with long-term water quality improvements being the most plausible explanation for this result. As such, our results highlight the benefits that water quality improvements can have on stream organisms, especially those that are imperiled.

We found no differences in macroinvertebrate density or biomass between or within river systems, similar to our results for Neosho Madtom. These patterns are also likely a consequence of long-term decreases in metal concentrations throughout the Spring River subbasin. For instance, it is common for streams with elevated metal concentrations to exhibit reduced macroinvertebrate abundance compared to unpolluted streams (Clements et al. 2000; Courtney and Clements 2002; Maret et al. 2003; Qu et al. 2010). However, since we did not observe this pattern, this result suggested that metal pollution is no longer a major impairment to stream organisms in the lower Spring River. Furthermore, this result may explain why we found no evidence that macroinvertebrate availability was associated with madtom abundance. Wildhaber et al. (2000a) suggested that one of the primary reasons for lower madtom densities in the Spring River impacted by metal pollution was lower macroinvertebrate availability that resulted in diet limitations for madtoms. However, since at present there are no differences in food availability among systems, food availability may no longer be a limiting factor to contemporary Neosho Madtoms populations in Kansas.

Many studies have examined the effects of elevated metal concentrations on fish and macroinvertebrates populations in streams, but very few have examined how fish and macroinvertebrates respond to decreases in metal concentrations over time. However, the few studies that have been conducted have observed fish and macroinvertebrates responding positively to reductions in metal concentrations in streams, as would be expected. For instance, Mebane et al. (2015) observed Rainbow Trout (*Oncorhynchus mykiss*) and Chinook Salmon (*Oncorhynchus tshawytscha*) populations make a full recovery in four years after copper's chronic criteria had been met in Panther Creek and

Big Deer Creek in Idaho, USA. Furthermore, Panther Creek and Big Deer Creek also saw an increase in Shorthead Sculpin (*Cottus confusus*) distribution and density and benthic macroinvertebrate richness and biomass following decreases in copper concentration (Mebane et al. 2015). In another study in Idaho, the South Fork and mainstem of the Coeur d'Alene River experienced an increase in benthic macroinvertebrate richness and species diversity with a decrease in heavy metal concentrations (Hoiland et al. 1994). Lastly, benthic macroinvertebrate richness and biomass increased following a decline in zinc concentrations in the Ichi-kawa River of Japan (Watanabe et al. 2000). Our findings were similar to results from these previous studies, in that the Spring River had lower madtom and macroinvertebrate abundances when metal concentrations were elevated, but abundances rebounded when metal concentrations decreased.

Turbidity and depth were the only local habitat variables that were associated with madtom densities, such that increases in turbidity or decreases in depth resulted in higher madtom densities. Our results are similar to Wildhaber et al. (2000a) who found that Neosho Madtoms prefer habitats with higher turbidities. Wildhaber et al. (2000a) suggested that turbid habitats may provide Neosho Madtoms protection from predators and more hunting opportunities. Our results are also similar to Bulger and Edds (2001) in that Neosho Madtom densities increased as waters became shallower, although in Bulger and Edds (2001 this pattern occurred only for YOY and breeding adults, but not for non-breeding adults; we did not distinguish breeding from non-breeding adults. Regardless, Bulger and Edds (2001) suggested that YOY use these shallower depths for feeding, refuge from strong currents, and to avoid competitive interactions with other benthic fish species for limited resources (e.g., cavities; food), while it was posited that breeding adult Neosho Madtoms may use the shallower depths to decrease their predation risk while they care for their eggs and larvae.

Smaller, low-head dams can negatively affect Neosho Madtom populations immediately (i.e., ≤ 0.1 km) downstream of dams via increased water velocity and the subsequent coarsening and embedding substrates that results, ultimately lowering Neosho Madtom densities (Tiemann et al., 2004). However, we found no relationships between proximity to a lowhead dam and Neosho Madtom density, contrasting with Tiemann et al. (2004). A possible explanation for why we did not observe a negative impact of lowhead dams on Neosho Madtom density whereas Tiemann et al. (2004) did; may have occurred because of differences between studies in site proximity to lowhead dams. For instance, the mean distance from a lowhead dam for our study sites was 26.31 km (range 0.12 - 56.67 km), whereas in Tiemann et al. (2004) sites were located ≤ 0.1 km from a dam. If the effects of low-head dams on Neosho Madtom populations are immediate downstream from a dam but then rapidly dissipate, it would be more difficult for our study design to observe a negative effect of lowhead dams compared to Tiemann et al. (2004).

We found no evidence that large dams and reservoirs were associated with Neosho Madtom densities. This result was surprising, as previous studies have identified large dams as a major threat to Neosho Madtom populations (Wildhaber et al. 2000b; Tiemann et al. 2004). A possible explanation for why we did not see a significant effect of large dams on Neosho Madtoms densities was that our study included sites from both the Neosho-Cottonwood system and the Spring River, as there are three large dams in the

Neosho-Cottonwood system, but none in the Spring River. Had we only analyzed sites within the Neosho-Cottonwood system, we would have likely found a significant effect at least from John Redmond reservoir, as madtom abundance appeared to be lower downstream of this reservoir compared to upstream (Figure 3). Specifically, in the Neosho-Cottonwood system 87/103 (84%) of madtoms captured in 2019 and 342/359 (95%) individuals captured in 2020 were found upstream of John Redmond Reservoir, even though only 4/10 sample sites were upstream of this reservoir each year (Figure 1.3). However, it seems that Council Grove and Marion Reservoirs are not affecting madtom densities to the extent that John Redmond does, as madtom densities at sites below these reservoirs in our study were relatively high. This could be a consequence of these dams being positioned more in the headwaters of the basin, causing them to have less severe impacts on the natural flow regime and connectivity compared to John Redmond dam, which is located more intermediately within the basin. Although, our study sites may have been positioned too distant from Council Grove and Marion reservoirs to observe negative effects on Neosho Madtom densities.

Many imperiled fish species like the Neosho Madtom are simultaneously impacted by multiple stressors (e.g., habitat degradation, water pollution, large and small dams, etc.), and some of these stressors are easier to remove than others. Historically, major threats to the Neosho Madtom included large and small dams in the Neosho-Cottonwood system and toxic metal pollution in the Spring River (Wildhaber et al. 2000a). However, it appears at least one of these major threats has been ameliorated, as toxic metal concentrations in the Spring River have decreased, with concomitant increases occurring in Neosho Madtom distribution and abundance. However, the threat of dams will be much harder to remove compared to metal pollution, and as such Neosho Madtoms will likely continue to be imperiled by other stressors. This is especially true for John Redmond reservoir, as it is the water supply reservoir for Wolf Creek nuclear power plant. Regardless, we observed that reducing the intensity of even one stressor (e.g., metal pollution) can benefit imperiled stream organisms, enhancing their potential for continued persistence.

Table 1 Percentage decrease of metal concentrations across five stream segments within the Spring River subbasin from 1990 to 2016. We calculated percentage decrease from averages calculated for before and after 2000. Data are from the water quality monitoring program conducted by the Kansas Department of Health and Environment. Cadmium concentrations for Spring River upstream of pollution input and Shoal Creek were only measured during the 1990's.

	Stream Segments				
Metal Type	Spring River Upstream	Center Creek	Turkey Creek	Shoal Creek	Spring River Downstream
Cadmium	NA	47.40	23.17	NA	36.95
Lead	58.41	64.67	59.50	73.30	65.43
Zinc	81.70	55.40	31.75	59.80	40.77

Table 2 Results from competing models that sought to explain variation in total and adult Neosho Madtom densities in the Neosho, Cottonwood, and Spring Rivers of Kansas during 2019 and 2020. Models are compared and arranged according to Akaike's information criterion corrected for small sample size (AIC_c) and adjusted R^2. The local physicochemical habitat conditions model (top) included turbidity and depth as predictor variables, while the local physicochemical habitat conditions (global) model included turbidity, depth, velocity, and percent gravel/pebble substrate, and the upstream watershed characteristics (global) model included distance to large dam, distance to small dam, percent open water in the upstream watershed, and watershed area.

	Total Density				Adult Density			
Model	AIC_c	ΔAIC_c	Adjusted R^2	P-value	AIC_c	ΔAIC_c	Adjusted R^2	P-value
Local Physicochemical Habitat Conditions (Top)	116.70	0.00	0.25	< 0.01	82.22	0.00	0.30	< 0.01
Local Physicochemical Habitat Conditions (Global)	121.49	4.79	0.21	0.02	85.25	3.03	0.29	< 0.01
Null (Intercept Only)	125.33	8.63	——	0.28	93.49	11.27	——	0.36
Macroinvertebrate Biomass	127.25	10.55	- 0.03	0.78	96.94	14.72	- 0.01	0.50
Upstream Watershed Characteristics (Global)	129.84	13.14	0.01	0.37	98.14	15.92	0.01	0.38

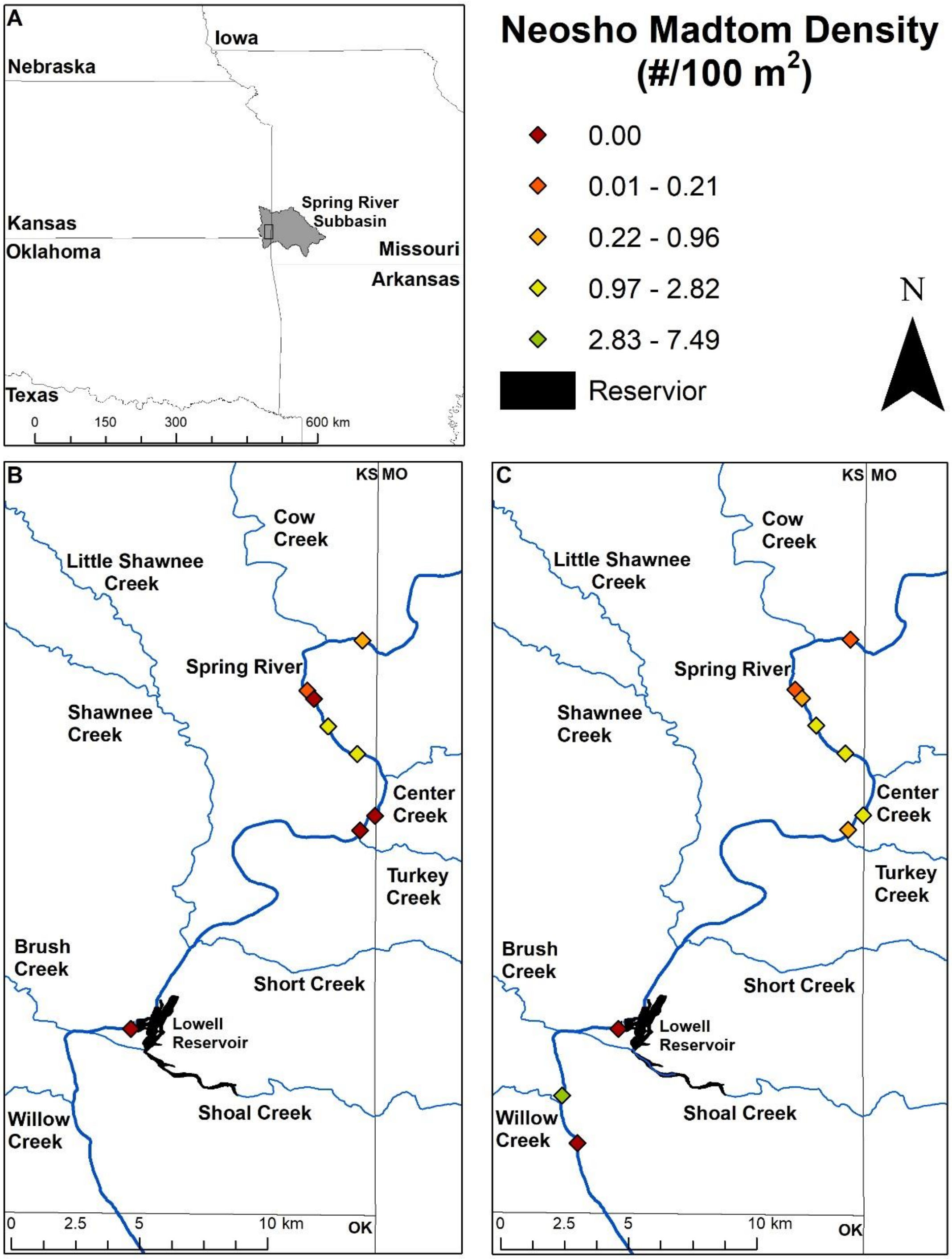

Figure 1 (A) Study area in the Spring River subbasin, with (B) Neosho Madtom densities across all age classes at sample sites in 2019 and (C) 2020. Sites were split with 5 above and 5 below the first metal-contaminated tributary input (i.e., Center Creek).

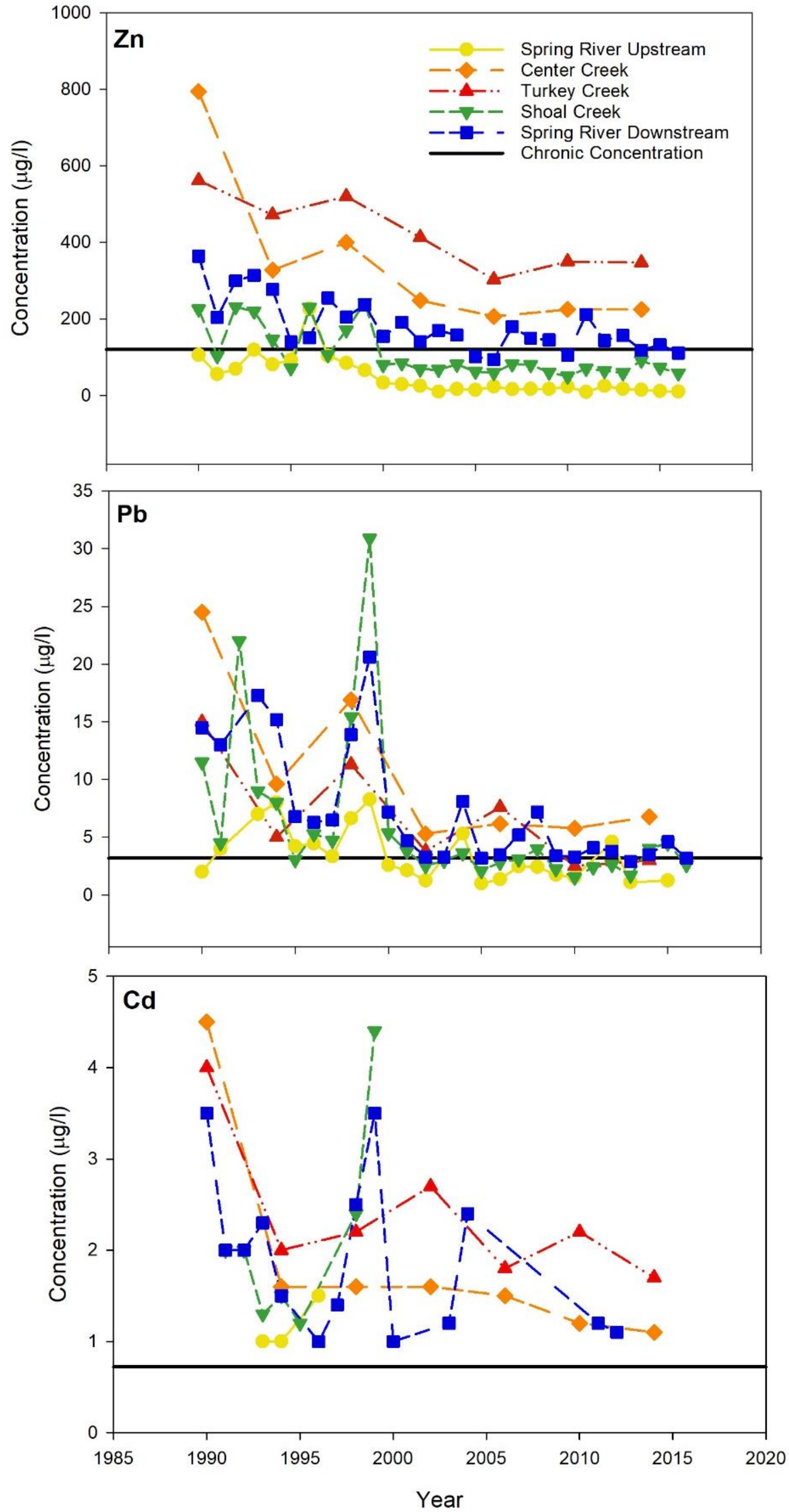

Figure 2 Trends in zinc (Zn), lead (Pb), and cadmium (Cd) concentrations during 1990-2016 for the Spring River and several of its tributaries. Data are from the water quality monitoring program conducted by the Kansas Department of Health and Environment. The horiztonal black line represents the chronic concentration for each metal as designated by the Environmental Protection Agency.

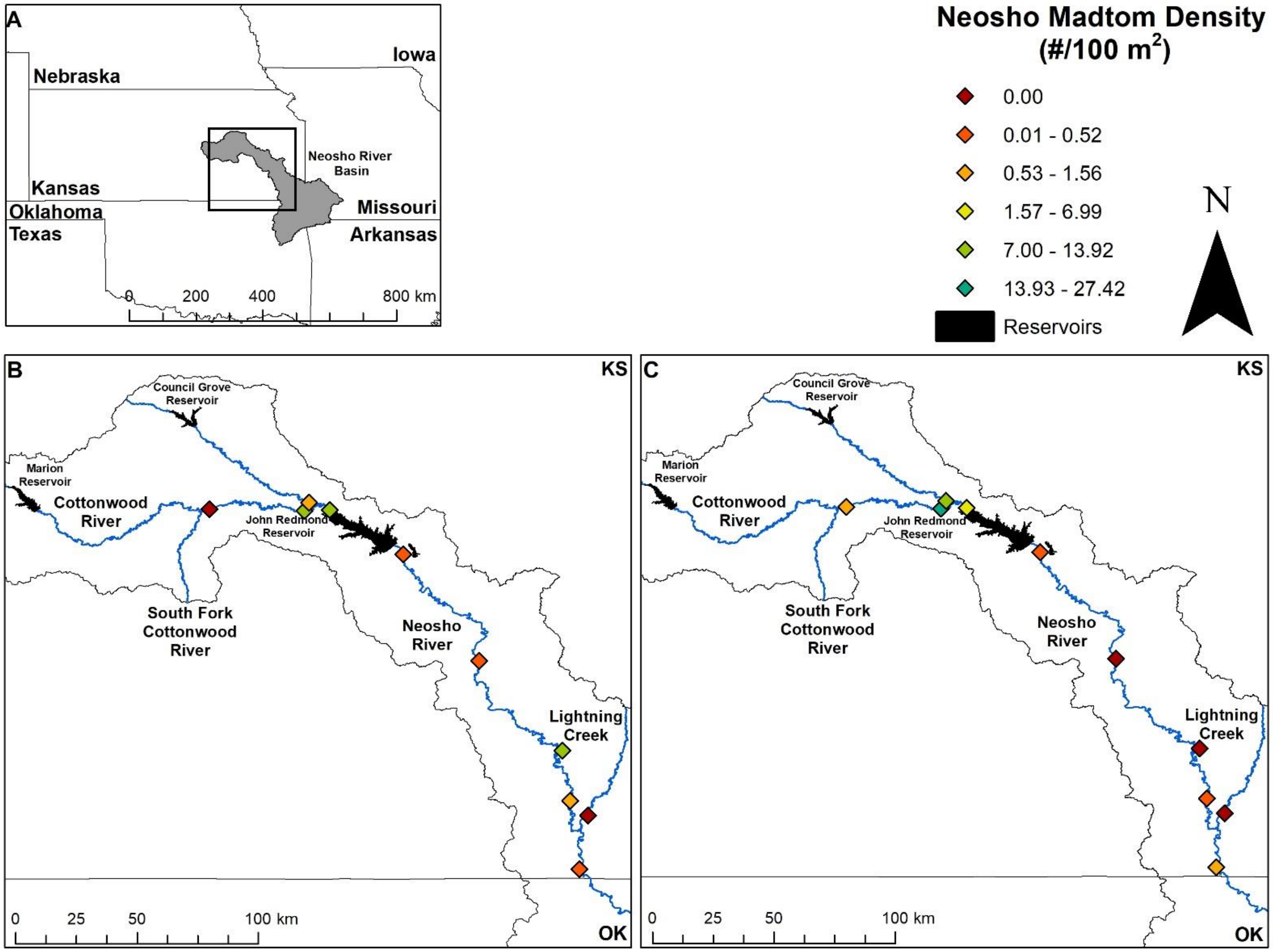

Figure 3 (A) Study area in the Neosho River basin, with (B) Neosho Madtom densities across all age classes in 2019 and (C) 2020.

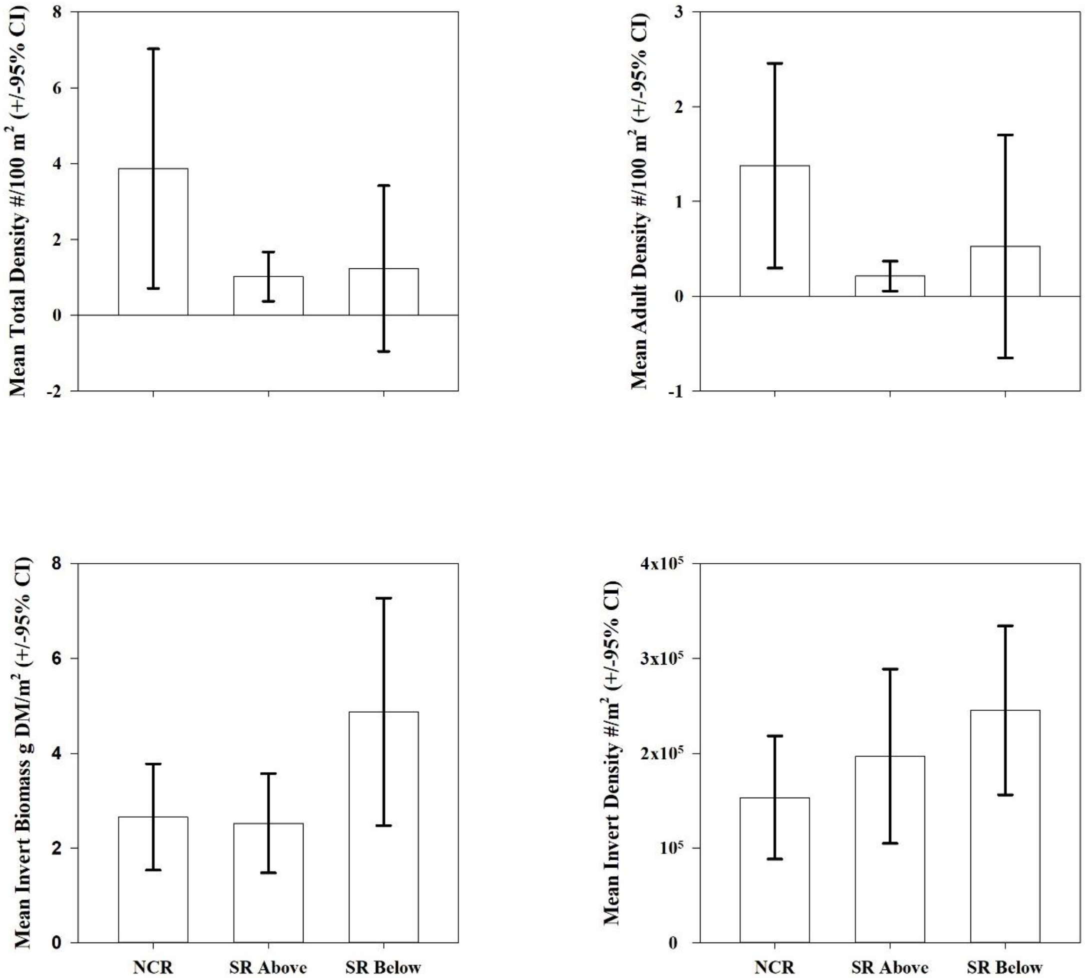

Figure 4 Bar chart with 95% confidence interval (CI) error bars comparing mean Neosho Madtom densities (i.e., total and adult) and macroinvertebrate biomass and densities among the Neosho-Cottonwood River system (NCR), the Spring River above metal pollution (SR Above), and the Spring River below metal pollution (SR Below). DM = dry mass.

REFERENCES

Afshan, S., S. Ali, U.S. Ameen, M. Farid, S. A. Bharwana, F. Hannan, and R. Ahmad. (2014). Effect of different heavy metal pollution on fish. *Research Journal of Chemical and Environmental Sciences*, 2(1), 74-79.

Allen, G.T., S. H. Blackford, V. M. Tabor, and M. S. Cringan. (2001). Metals, boron, and selenium in Neosho Madtom habitats in the Neosho river in Kansas, U.S.A. *Environmental Monitoring and Assessment*, 66(1), 1-21.

Arruda, J. A., and C. H. Fromm (1989). The relationship between taste and odor problems and lake enrichment from Kansas lakes in agricultural watersheds. *Lake and Reservoir Management*, 5(1), 45-52.

Authman, M.M., M. S. Zaki, E. A. Khallaf, and H. H. Abbas. (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquaculture Research & Development*, 6(4), 1-13.

Barks, J. H. (1986). Effects of abandoned lead and zinc mines and tailing piles on water quality in the Joplin area, Missouri. Guide to the geology and environmental concerns in the tri-state lead-zinc district, Missouri, Kansas, Oklahoma associations of Missouri geologists, 60-65.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. (1999). Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society*, 18(3), 308-343.

Bulger, A. G., and D. R. Edds. (2001). Population structure and habitat use in Neosho madtom (*Noturus placidus*). *The Southwestern Naturalist*, 46, 8-15.

Brumbaugh, W. G., C. J. Schmitt, and T. W. May. (2005). Concentrations of cadmium, lead, and zinc in fish from mining-influence waters of northeastern Oklahoma: Sampling of blood, carcass, and liver for aquatic biomonitoring. *Archives of Environmental Contamination and Toxicology*, 49(1), 76-88.

Campbell, P. G., A. Hontela, J. B. Rasmussen, A. Giguère, A. Gravel, L. Kraemer, J. Kovesces, A. Lacroix, H. Levesque, and G. Sherwood. (2003). Differentiating between direct (physiological) and food-chain mediated (bioenergetic) effects on fish in metal-impacted lakes. *Human and Ecological Risk Assessment: An International Journal*, 9(4), 847–866.

Courtney, L. A., and W. H. Clements. (2002). Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach. *Freshwater Biology*, 47(9), 1766–1778.

Cummins, K.W. (1962). An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. *The American Midland Naturalist*, 67(2), 477-504.

Dallinger, R., F. Prosi, H. Segner, and H. Back. (1987). Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia*, 73(1), 91-98.

Davis, N., and C. Paukert. (2008). Impacts of gravel bar scalping on Neosho Madtom (*Noturus placidus*) populations from the Lower Neosho River, Kansas. *Journal of Freshwater Ecology*, 23(4), 501-511.

EPA (Environmental Protection Agency). *National Recommended Water Quality Criteria Tables.* (2020). https://www.epa.gov/wqc/national-recommended-water-quality-criteria-tables accessed on 3 March 2020.

Ernsting, G. W., M. E. Eberle, and T. L. Wenke. (1989). Range extensions for three species of madtoms (*Noturus*: Ictaluridae) in Kansas. *Transactions of the Kansas Academy of Science*, 92, 206-207.

Fox, J., and S. Weisberg. (2019). *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks, California. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Hoiland, W. K., Rabe, F. W., and Biggam, R. C. (1994). Recovery of macroinvertebrate communities from metal pollution in the South Fork and mainstem of the Coeur d'Alene River, Idaho. *Water Environment Research*, 66(1), 84-88.

Horwitz, R. J. (1978). Temporal variability patterns and distributional patterns of stream fishes. *Ecological Monographs*. 48(3), 307-321.

Huggins, D. G, P. M. Liechti, and L. C. Ferrington Jr. (1985). *Guide to the freshwater invertebrates of the Midwest*, second edition. Technical Publication 11 of the Kansas Biological Survey, University of Kansas.

Iwasaki, Y., T. Kagaya, K.-I. Miyamoto, and H. Matsuda. (2009). Effects of heavy metals on riverine benthic macroinvertebrate assemblages with reference to potential food availability for drift-feeding fishes. *Environmental Toxicology and Chemistry*, 28(2), 354-363.

Jackson, D. A., P. R. Peres-Neto, and J. D. Olden. (2001). What controls who is where in freshwater fish communities: the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences*, 58(1), 157-170.

Kansas Fishes Committee. (2014). *Kansas fishes*. University Press of Kansas, Lawrence.

KDHE (Kansas Department of Health and Environment). (2020). *2020 303 (d) List of all impaired & potentially impaired waters.* https://www.kdheks.gov/tmdl/2020/2020_303_d_List_Approved.pdf. Accessed on 30 August 2020.

Kövecses, J., G. D. Sherwood, and J. B. Rasmussen. (2005). Impacts of altered benthic invertebrate communities on the feeding ecology of yellow perch (*Perca flavescens*) in metal-contaminated lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 62(1), 153-162.

Landers, J. E. (2016). *Aquatic food webs and heavy metal contamination in the upper Blackfoot River, Montana*. Thesis. Master of Science in Environmental Studies. University of Montana. Missoula, MT. USA https://scholarworks.umt.edu/etd/10719 accessed 2 November 2019.

Malmqvist, B., and S. Rundle. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation*, 29(2), 134-153.

Maret, T. R., D. J. Cain, D. E. Maccoy, and T. M. Short. (2003). Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. *Journal of the North American Benthological Society*, 22(4), 598-620.

Mebane, C. A., Eakins, R. J., Fraser, B. G. and Adams, W. J. (2015). Recovery of a mining-damaged stream ecosystem. *Elem Sci Anth*, 3.

Merritt, R. W., K. W. Cummins, and M. B. Berg (editors). (2008). *An introduction to the aquatic insects of North America*. Kendall Hunt, Dubuque, Iowa.

Nava, D., Restello, R. M., and Hepp, L. U. (2015). Intra- and inter-annual variations in Chironomidae (Insecta: Diptera) communities in subtropical streams. *Zoologia (Curitiba)*, 32(3), 207-214.

O'Connor, E. (2010). *Long-term Spatial and Temporal Variability of Benthic Macroinvertebrate Communities: Implications for Bioassessment of Lotic Systems.* Thesis. Master of Science. Virginia Commonwealth University. Richmond, VA. USA. http://scholarcompass.vcu.edu/edt/2064 accessed on 17 October 2020.

Poff, N. L., and D. D. Hart. (2002). How dams vary and why it matters for the emerging science of dam removal: An ecological classification of dams is needed to characterize how the tremendous variation in the size, operational mode, age, and number of dams in a river basin influences the potential for restoring regulated rivers via dam removal. *BioScience*, 52(8), 659-668.

Qu, X., N. Wu, T. Tang, Q. Cai, and Y.-S. Park. (2010). Effects of heavy metals on benthic macroinvertebrate communities in high mountain streams. *Annales de Limnologie - International Journal of Limnology*, 46(4), 291-302.

R Core Team. 2020. A language and environment for statistical computing and graphics. R Foundation for Statistical Computing, Vienna, Austria. URL:https//www.R-project.org/.

Stene, E. O. (1946). How lakes came to Kansas. *Transactions of the Kansas Academy of Science, 49*(2), 117-137.

Tiemann, J. S., Gillette, D. P., Wildhaber, M. L., and Edds, D. R. (2004). Effects of lowhead dams on riffle-dwelling fishes and macroinvertebrates in a midwestern river. *Transactions of the American Fisheries Society*, 133(3), 705-717.

Trioa, M. J., and K. B. Gido (2014). Towards a mechanistic understanding of fish species niche divergence along a river continuum. *Ecosphere*, 5(4), 1-18.

USFWS (US Fish and Wildlife Service). (2013). Neosho Madtom (*Noturus placidus*) 5-Year Review. US Fish and Wildlife Service, Denver, Colorado.

US Geological Survey (USGS) (2016) The StreamStats program for Kansas. http://water.usgs.gov/osw/streamstats/kansas.html. Accessed 20 August 2019.

Vinodhini, R. and M. Narayanan. (2008). Bioaccumulation of heavy metals in organs of freshwater fish *Cyprinus carpio* (Common carp). *International Journal of Environmental Science & Technology*, 5(2), 179-182.

Watanabe, N. C., Harada, S., and Komai, Y. (2000). Long-term recovery from mine drainage disturbance of a macroinvertebrate community in the Ichi-kawa River, Japan. *Hydrobiologia*, 429(1-3), 171-180.

Wildhaber, M. L., A. L. Allert, and C. J. Schmitt. (1999a). Potential effects of interspecific competition on Neosho Madtom (*Noturus placidus*) populations. *Journal of Freshwater Ecology*, 14(1), 19-30.

Wildhaber, M. L., C. J. Schmitt, and A. L. Allert. (1999b). Factors explaining the distribution and site densities of the Neosho Madtom (*Noturus placidus*) in the

Spring River, Missouri. US. Geology Survey Toxic Substances Hydrology Program. *Proceedings of the Technical Meeting*, Charleston, SC.

Wildhaber, M. L., A. L. Allert, C. J. Schmitt, V. M. Tabor, D. Mulhern, K. L. Powell, and S. P. Sowa. (2000a). Natural and anthropogenic influences on the distribution of the threatened Neosho Madtom in a Midwestern warmwater stream. *Transactions of the American Fisheries Society*, 129(1), 243-261.

Wildhaber, M. L., V. M. Taber, J. E. Whitaker, A. L. Allert, D. Mulhern, P. J. Lamberson, and K. L. Powell. (2000b). Icatalurid populations in relation to the presences of a main-stem reservoir in a midwestern warmwater stream with emphasis on the threatened Neosho madtom. *Transactions of the American Fisheries Society*, 128, 1264-1280.

Wilkinson, C., D. Edds, J. Dorlac, M. L. Wildhaber, C. J. Schmitt, and A. Allert. (1996). Neosho madtom distribution and abundance in the Spring River. *Southwestern Naturalist*, 41, 78-81.

Wilkinson, C., and L. Fuselier. (1997). Neosho Madtom in the South Fork of the Cottonwood River: implications for management of the species. *Transactions of the Kansas Academy of Science*, 100, 162-165.

Zorn, T. G., Seelbach, P. W., and Wiley, M. J. (2002). Distributions of stream fishes and their relationship to stream size and hydrology in Michigan's Lower Peninsula. Transactions of American Fisheries Society, 131(1), 70-85.