Assessment of Potential Neosho Madtom (*Noturus placidus*) Habitat in Tributaries of the Spring River of Kansas and Missouri, USA

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Executive Summary

The Neosho madtom (*Noturus placidus*: Pisces, Ictaluridae; henceforth Np) is a federally listed (threatened) fish endemic to the Neosho (Grand) River system of Missouri, Kansas, and Oklahoma. The species is known to be present in the main stem of the Spring River in Kansas and Missouri upstream from the reach contaminated by metals from historical lead-zinc mining in the Tri-State Mining District and at several locations further downstream, but there is only one report of Np being present in Spring River tributaries flowing westward from Missouri (Center, Turkey, and Shoal Creeks). Mining-related contamination of area streams, which began in the 1870s, might have extirpated some populations before biological surveys were undertaken and before Np was recognized as a species in 1969 (Taylor, 1969).

The initial objective of this assessment was to conduct a statistical analysis of previously reported data to determine whether Shoal, Turkey, and Center Creeks in Missouri and Kansas (contaminated tributaries of the Spring River) would be physically suitable as Np habitat absent mining-related contamination. Favorable habitat comparisons could strengthen aquatic injury claims in Missouri. The approach initially proposed was to analyze fish, physical habitat, and water-quality data obtained in 1995 and 2009 from the Spring River system with a regression model developed from 1991 data for the Neosho and Cottonwood Rivers in east-central Kansas (not shown) by Wildhaber et al. (2000). The initial modeling approach had to be modified because one variable in the model (dissolved chloride) was not measured in 2009. The Wildhaber et al. (2000) model was used to evaluate 1995 habitat data, and new regression models were developed from variables measured in all sampling years (1991, 1994, 1995, and 2009) to evaluate the 1995 and 2009 data. Sites also were evaluated based on present and historical habitat conditions known to be inhabited by Np and by principal components modeling.

All the methods indicated that the lower reaches of the westward-flowing Spring River tributaries could support Np; that is, the habitat conditions represented by the data analyzed should not preclude the presence of Np. Although the regression models differed with respect to the variables they contained and the sites where Np was predicted to be present, they all predicted that Np would be present at some tributary sites. Many of the habitat variables were highly intercorrelated. Consequently, all of the regression models contained one or more terms related to substrate texture and dissolved constituents. The regression models (including the original model published by Wildhaber et al., 2000) also were somewhat counterintuitive because they included positive terms for variables that can be affected by mining and other sources of pollution, which probably reflects the fact that the models contained no metals data. In addition, the range represented by the modeled variables probably was not wide enough to represent a gradient on which fish density would respond. These variables were probably included because they are correlated with other variables that were not measured.

Most of the sites investigated fell within the range of conditions (occurrence envelope) developed from sites where Np was previously collected. The occurrence envelope analyses indicated that Np presently (2010) inhabits a wide range of habitat conditions that was wider still when it was present in the lower Illinois River before the completion of Tenkiller Ferry Dam in 1953. Only a few of the sites sampled in 1991−95 and 2009 were outside the occurrence envelope, adding support to the hyposthesis that contaminants from mining limit Np distribution in the Spring River system.

Extant information on the present (2010) and historical distribution of Np indicates that its geographic range can rapidly expand and contract in response to habitat changes, and that it can tolerate a wide range of physical and chemical conditions. It is therefore reasonable to consider Np a vagrant species that can invade the lower parts of the westward-flowing tributaries of the Spring River when conditions are favorable, with the term "favorable" implying the absence of mining-related contaminants at harmful concentrations. Collectively, the results indicate that Np probably inhabited the lower reaches of the larger tributaries (Shoal Creek and Center Creek) at least occasionally before the advent of mining in the Tri-States Mining District, and that re-establishment of populations in these streams is feasible.

1. Introduction

1.1 Neosho Madtom Distribution and Habitat

The Neosho madtom (*Noturus placidus*: Pisces, Ictaluridae; Np) is endemic to the Neosho (Grand) River system of Missouri, Kansas, and Oklahoma. It was recognized as unique among madtoms in the early 1950s (Cross, 1954), but Np was not recognized as a distinct species until a 1969 morphological review of the genus *Noturus* (Taylor, 1969). Subsequent reviews based on biochemical methods and chromosome morphology (LeGrande, 1981; Grady, 1987; Grady and LeGrande, 1992; Hardman, 2004) have confirmed the status of Np as a distinct species.

The U.S. Fish and Wildlife Service (USFWS) listed Np as a threatened species in 1990, primarily because of hydrologic alteration and habitat loss in the Neosho River system (USFWS, 1990; Wenke and Eberle,1991). At that time, the geographic range of Np was identified as the main stem of the Neosho River from near Miami, Oklahoma, upstream to the confluence of the Cottonwood River in Lyon County, Kansas; the Cottonwood River upstream to the confluence of Middle Creek in Chase County, Kansas; and one reach of the Spring River in Jasper County, Missouri and Cherokee County, Kansas (Cross, 1967; Moss, 1981; USFWS, 1990; Pflieger, 1970). A subsequent investigation also found Np in the Oklahoma waters of the Neosho River upstream from Grand Lake, north and west of Miami in Ottawa County (Luttrell et al., 1992). A population previously known from the lower Illinois River in Sequoyah County, Oklahoma (Moore and Paden, 1950; Taylor, 1969) was extirpated by cold water releases from Tenkiller Ferry Dam, which was completed in 1953 (Moss, 1981; USFWS, 1990). Studies conducted since Np was federally listed as threatened have expanded the known geographic range of Np further upstream and downstream in the Spring River in Missouri and Kansas (Wilkinson et al., 1996, 2000; USFWS, 2007) and upstream in the Neosho River in Kansas (Ernsting et al., 1989). The presence of Np in tributaries of the Neosho and Cottonwood Rivers also has been documented (Branson et al., 1969; Ernsting et al., 1989; Wilkinson and Fuselier, 1997), and two specimens were reportedly collected from the lowermost reaches of Shoal Creek near Galena, Kansas, in 1963 (Branson et al., 1969; Fig. 1).

The Neosho madtom is a “riffle fish” (Taylor, 1969) that inhabits flowing-water riffles composed of unconsolidated sand and pebbles with moderate flows and depths (Moss, 1981). The fish are nocturnal (Bryan et al., 2006; Bulger and Edds, 2001); during the day, they commonly inhabit interstitial spaces within the streambed (Powell and Tabor, 1992). Population density at 11 sites in the Neosho-Cottonwood River system was positively correlated with the percentage of fine sediments, turbidity, hardness, specific conductance, and other inter-correlated water-quality properties (e.g., nutrients, dissolved chloride, dissolved sulfate; Powell and Tabor, 1992; Wildhaber et al., 2000). A statistical model that estimated Np density as a function of habitat variables was developed from 1991 USFWS monitoring data by Wildhaber et al. (2000). Because of the high degree of inter-correlation among habitat variables, only two were statistically significant when the data were analyzed by stepwise multiple linear regression: the weight-proportion of substrate >38 mm diameter ($p_{>38}$) and dissolved chloride concentration (henceforth chloride). The model was statistically significant (P <0.05) and explained 72% of the total variation in Np density at the Neosho-Cottonwood sites. Application of this model to 1994 data accurately predicted Np density at Neosho-Cottonwood sites and sites on the Spring River upstream from the confluence of Center Creek. Measured densities at sites downstream from Center Creek were lower than predicted from habitat measurements, the implication being that differences between observed and expected densities were because of contaminants from mining. Sites on the Spring River and its westward-flowing tributaries also were sampled for habitat and fish in 1995 (Allert et al., 1997), but these data were not evaluated relative to the habitat model developed by Wildhaber et al. (2000). During the 1994−95 studies, Np was collected only in the main stems of the Neosho, Cottonwood, and Spring Rivers; it was not found in any tributaries, in the North Fork of the Spring River, or in the main stem upstream from its confluence with the North Fork (Fig. 1).

1.2 Objectives

The historical (prior to 1953) occurrence of Np in the lower Illinois River, together with more recent collections in Neosho and Cottonwood River tributaries and the reported capture of Np in lower Shoal Creek, indicate that Np may have inhabited the lower reaches of the larger Spring River tributaries in Kansas and Missouri (Shoal Creek and Center Creek; Fig. 1) before the advent of lead-zinc mining. Accordingly, the initial objective of this investigation was to apply the statistical model developed for the Np polulation inhabiting the Neosho-Cottonwood system (Wildhaber et al., 2000) to 1995 and 2009 habitat data (Allert et al., 1997, 2011) for the purpose of evaluating the lower reaches of Shoal, Turkey, and Center Creeks as potential Np habitat, exclusive of contaminants. Favorable habitat comparisons could strengthen the aquatic injury claim in Missouri. Not all the variables incorporated into the model developed by Wildhaber et al. (2000) were measured in 2009, however. A secondary objective, therefore, was to develop and evaluate additional models based only on variables measured in all studies (1991, 1994, 1995, and 2009) and apply them to the habitat data for the tributaries.

2. Methods

2.1 Study Sites

The Np model (Wildhaber et al., 2000) was developed from 1991 USFWS data for the Neosho and Cottonwood Rivers (Powell and Tabor, 1992). Note: As indicated in Table 1, some that were sampled in more than one year were numbered differently each time they were sampled. Throughout the remainder of this report, sites are identified by the year they were sampled (i.e., 91, 94, 95, and 09) followed by the site number assigned the year they were sampled. Twelve sites were sampled in 1991, but water-quality parameters were not measured at one; only the 11 sites with complete data were analyzed by Wildhaber et al. (2000). Of the 28 sites sampled in 1994, six (sites 94-1–6) were on the Neosho and Cottonwood Rivers; of these, three (sites 94-4–6) had been sampled in 1991, and three were previously un-sampled (Schmitt et al., 1997; Fig. 1, Table 1). The remaining 22 sites sampled in 1994 were located on the main stem of the Spring River, from just below Interstate 44 in Ottawa County, Oklahoma, upstream to the confluence of the North Fork in Jasper County, Missouri (18 sites); on Turkey Creek (site 94-28) and Center Creek (site 94-17) near their confluences with the Spring River; and on Shoal Creek (sites 94-15 and 94-20) in Galena, Kansas (Fig. 1, Table 1). Twelve sites on the Spring River and its westward-flowing tributaries were sampled in 1995 (Allert et al., 1997). Of these, six sites sampled in 1995, including those near the mouth of Center Creek (site 95-12) and on lower Shoal Creek (site 95-2), had been sampled in 1994 (sites 94-17 and 94-20, respectively; Table 1). The other six were located further upstream on the main stem and in the lower reaches of tributaries, including the North Fork (Fig. 1, Table 1). In 2009, 16 sites on Shoal, Turkey, and Center Creeks in Kansas and Missouri were sampled. Of these, the downstream-most sites on each stream (sites 09-16, 09-5, and 09-6) had been sampled in 1994 (sites 94-20, 94-28, and 94-7, resepctively). The lower sites on Shoal Creek (site 09-16) and Center Creek (site 09-6) also were sampled in 1995 (sites 95-2 and 95-12, respectively), as was the Shoal Creek site upstream from the Joplin wastewater treatment plant (WWTP) in Newton County, Missouri (sites 09-10 and 95-3). None of the other 2009 sites had been previously sampled (Fig. 1, Table 1).

2.2 Field and Laboratory Methods

All field and laboratory methods used in this investigation are fully described elsewhere (Powell and Tabor, 1992; Allert et al., 1997, 2011; Schmitt et al., 1997; Wildhaber et al., 2000). The fish and physical habitat methods were developed from those employed by USFWS in 1991 (Powell and Tabor, 1992) and formed the basis of the analyses conducted by Wildhaber et al. (2000). In September 1994 and 1995, gravel bars at each site were sampled for fish, water quality, and substrate composition. At each site, 3 to 5 transects, depending on the length of the gravel bar and collection year, were established perpendicular to the thalweg. Three to five sampling stations, depending on gravel bar width and water depth, were spaced at roughly equal distances along each transect. At each station, a substrate sample for particle size analysis was obtained with a 1.1-L, 100-cm (dia.) cylindrical grab sampler. Substrate samples were sieved (38 mm, 19 mm, 9.5

mm, and 2 mm) and weighed. All material passing the finest sieve (2 mm) were returned to the laboratory, dried, and analyzed for fines (sand-silt-clay) by the hydrometer method; however, because the 1991 samples were not analyzed for fines, these data were not included in the statistical analyses. Fish were collected by kick-seining a 4.5-m^2 area of the stream bottom and identified on-site. Water depth and velocity at 60% depth were measured at each station with a current meter and wading staff. After all stations were sampled, temperature, dissolved oxygen, pH, turbidity, and specific conductance were determined at the upstream end of the site with portable instruments, and a grab-sample of stream water was obtained for laboratory analysis of alkalinity, hardness, chloride, dissolved sulfate (henceforth sulfate), nutrients [various forms of dissolved nitrogen (N) and phosphorous (P)], and metals (not evaluated in the habitat models). Concentrations of all dissolved constituents were reported as milligrams per liter (mg/L) in filtered (0.45 µm) samples. Pore-water samples also were collected at each station and analyzed in 1994 and 1995, and the 1994 pore-water data were included in the analyses reported by Wildhaber et al. (2000); however, different collection methods were employed in 1995 than in 1994, and pore-water samples were not collected in 2009. Consequently, only surface-water data were used in most of the analyses presented in this report.

A slightly modified field protocol was developed for the 2009 study, which was focused on crayfish. Three riffles were sampled at each of the 16 sites during July. At each riffle, crayfish were kick-seined at eight randomly selected stations. Water depth and velocity at 60% depth were measured at each station, after which water-quality sampling was conducted and additional depth and velocity measurements were made at multiple transects. A single substrate sample was collected from near the center of each riffle for sediment texture analysis. The substrate samples were collected and processed in the same manner as those collected in 1991−95. The depth and velocity measurements associated with the crayfish sampling were judged to more closely approximate the 1991−95 protocol than the post-sampling measurements and were used to evaluate the sites as potential Np habitat.

2.3 Statistical Analyses

Substrate size category means at each site were computed by dividing the total weight of a size category by total weight of all size categories. The geometric mean, 25th percentile, and 75th percentile particle size and fredle index (geometric mean adjusted for distribution of particle sizes) also were computed for evaluation by regression analysis (Wildhaber et al., 2000). The fredle index relates potential permeability of sediment to water, and hence is an indirect index of dissolved oxygen transport within sediment; it has been correlated with the emergence success of salmonid alevins (Platts et al., 1983, citing other sources; McMahon et al., 1996). Nevertheless, and as also was true in the original analyses (Wildhaber et al., 2000), regression models incorporating the fredle index and other computed variables provided no greater precision or accuracy than models based on the original weight proportions, and the computed variables were eliminated from further consideration. Preliminary inspection of the data also indicated that field measurements of water temperature and dissolved oxygen concentrations, which vary seasonally and respond rapidly to changing local weather conditions, differed substantially among years because of differing sampling times (July vs. September) and antecedent conditions. These variables also were eliminated from consideration.

Pore water was collected at each station in 1991−95, but variables associated with surface water, which were included in the statistical models, were measured only once at each site. In addition, only arithmetic site means were available for the 1991 USFWS data. Consequently, all regression analyses conducted by Wildhaber et al. (2000) and for pre-2009 data in this study were based on arithemetic site means rather than station- or transect-level measurements. In these analyses, Np counts (numbers per seine haul) were summed for each site, converted to densities by dividing the counts by the total area seined at the site, then multiplied by 100 and expressed as number per 100 m^2. In 2009, fish were not sampled and three riffles were sampled at each site. The 2009 data could, therefore, have been analyzed at the site and riffle levels; however, only one substrate sample was collected at each riffle. Therefore, the 2009 data were analyzed as the means of the three riffles sampled at each site.

All variables were transformed as described by Wildhaber et al. (2000) to meet normality and other assumptions inherent in the statistical analyses employed ($\log_{10} + 1$ for Np density, $\log_{10}$ weight proportion for sediment texture). All analyses were performed with the Statistical Analysis System (Version 9.1; SAS Institute, Cary, North Carolina). Stepwise multiple linear regression of $\log_{10}$ (Np density + 1.0) against physical and chemical habitat variables were performed with SAS PROC REG. Models containing the largest numbers of independent variables that significantly ($P < 0.10$) reduced the unexplained sum-of-squares after all other variables had been fit were retained, which is essentially the approach used by Wildhaber et al. (2000). Additional analyses were performed using PROC REG with variable selection based on Akaike's Information Criterion (AIC; Burnham and Anderson, 2002). In these analyses, models were evaluated relative to each other based on corrected AIC values (AICc). The AICc values were adjusted upward from the AIC values based on sample size relative to the number of independent variables, which protects against over-fitting (Burnham and Anderson, 2002). Models with the smallest (most negative) AICc were judged "most parsimonious" (i.e., most efficient), and those with AICc values that differed by <2.0 were considered equivalent (Burnham and Anderson, 2002). In most instances the models identified by stepwise and AIC regression were identical, so the stepwise results are reported unless otherwise indicated. Models were developed from the 1991 and 1994 datasets and were evaluated relative to predictions from the model reported by Wildhaber et al. (2000) before application to the 1995 and 2009 data. In the application of regression models, predicted densities >1 fish/100 m^2 were considered indicative of potential Np habitat without regard to the precision (i.e., confidence limits) of the predicted values.

Additional analyses based on occurrence envelope approaches were also employed. In this report, occurrence envelope is defined as the range of conditions at sites where Np was either collected during 1991−95 or, for the Illinois River, was known to have occurred historically. Habitat measurements for sites where Np was either absent during 1991−95 or not collected (2009) were compared to the occurrence envelope. Historical (1947) and contemporary (1994−2009) water-quality data for the Illinois River were retrieved from the USGS National Water Information System (NWIS;

http://nwis.waterdata.usgs.gov/nwis) for these analyses. Two of the specimens examined by Taylor (1969) were obtained from the Illinois River: one from the reach immediately downstream from the present site of Tenkiller Ferry Dam, and one from near the confluence with the Neosho River. Water quality data from the summer of 1947, when construction of Tenkiller Ferry Dam began, were available for USGS gage site 07196000 (Gore, Oklahoma), which is near the downstream Np collection site. Contemporary data (1991−2009) were available for USGS site 07196500 (Tahlequah, Oklahoma), which is located upstream of Lake Tenkiller (not shown). Summer data (June-September) were retrieved for these locations to maintain consistency with the Spring-Neosho-Cottonwood data.

In addition to regression modeling, Wildhaber et al. (2000) performed several multivariate statistical analyses of the 1994 data that successfully separated sites with and without Np. However, many of the variables incorporated into these analyses (e.g., concentrations of metals in a variety of media, benthic invertebrate species richness) were not available for 1995 or 2009, and the intent of the analyses presented here was to evaluate habitat without regard to mining-related contaminants. Accordingly, a principal component (PC) analysis was conducted that was restricted to the habitat variables available for all sites and years. In contrast to multiple regression analysis, which can be problematic in situations where the independent variables are highly correlated, PCs are orthogonal to each other and uncorrelated (Cooley and Lohnes, 1971). Habitat variables for sites where Np was collected (1991−95) were characterized with SAS PROC FACTOR. PCs (eigenvalues) greater than one were considered statistically significant (Cooley and Lohnes, 1971) and were used to generate component scores with SAS PROC SCORES. Sites where Np was either not present (1991−95) or where fish were not collected (2009) were then scored in the same manner and compared to the range of scores (occurrence envelope) for sites where the species was present.

3. Results

3.1 Regression Model Development and Evaluation

The 1991−94 data indicated that streams in the Neosho-Cottonwood and Spring River systems differ in their physical habitat, water chemistry, and nutrient concentrations (Wildhaber et al., 2000). Although there were exceptions, Spring River substrates were typically coarser than those in streams of the Neosho-Cottonwood system (Fig. 2). Pore- and surface-waters in the Neosho-Cottonwood system were generally warmer, harder, had higher ammonia-N and sulfate concentrations, and were more conductive, alkaline, and turbid than those in the Spring River system (Wildhaber et al., 2000; Fig. 3). Concentrations of dissolved constituents were especially high in the Cottonwood River (Fig. 3). Among sites supporting Np, densities were typically higher in the Neosho-Cottonwood system than in the Spring River system (Fig. 4), and were highest at sites where the substrate texture was relatively fine and not dominated by coarse material (Figs. 2, 4). In the 1991 USFWS data set used to derive the Wildhaber et al. (2000) model, chloride was correlated with sulfate ($r = 0.89$), specific conductance ($r = 0.83$), and hardness ($r = 0.82$; all $n = 11$, $P < 0.01$), and chloride was therefore the only water-quality variable that was statistically significant in the regression model. This model (original 1991 chloride model), which was based on site means from the 11 USFWS sites with complete data sampled in 1991, included only an intercept, a negative coefficient for $\log_{10}$-transformed $p_{>38}$, and a positive coefficient for chloride,

$$\log_{10} (\text{density} + 1) = -1.447 - 0.892 \log_{10} (p_{>38}) + 0.0897 \text{ chloride}$$

(Note: the sign of the chloride coefficient was incorrectly shown as negative and the constant added to density was not shown in the journal article equation.) The model was statistically significant ($P < 0.01$) and explained 72% of the variation in Np density, but inspection of the 1991 and 1994 datasets revealed some minor discrepancies in both. Re-analysis of corrected data yielded a nearly identical model,

$$\log_{10}(\text{density} + 1) = -1.3772 - 0.7429 \log_{10}(p_{>38}) + 0.0327 \text{ chloride.}$$

This model (revised 1991 chloride model) also was statistically significant ($F_{2,8} = 17.14$, $P < 0.01$) and explained 81% of the variation in Np density (Fig. 5); $p_{>38}$ explained 42% of the variation, chloride explained 39%, and both were statistically significant ($P < 0.01$). As expected, the results of the original and revised chloride models were closely correlated when applied to the corrected 1994 data (Fig. 6), and the plot of measured vs. predicted 1994 Np densities from the revised 1991 chloride model (Fig. 7) resembles Fig. 3 of Wildhaber et al. (2000). The model accurately predicted Np density at two of the previously sampled Neosho-Cottonwood sites and one of the previously un-sampled sites, but it underestimated the density at the other three Neosho-Cottonwood sites (Fig. 7). Nevertheless, the model indicated the presence of potential Np habitat at Spring River main-stem sites upstream from Center Creek, and that some of these sites could support higher than measured densities. It also indicated that potential habitat was present at several sites downstream from Center Creek where Np was not collected in 1994 (Fig. 7), as did the original 1991 chloride model (Wildhaber et al., 2000). In addition, the model indicated that greater than measured Np densities could be supported in the Spring River at Willow Creek (site 94-29); and that potential habitat was present in the lower reaches of Turkey Creek (site 94-28) and Center Creek (site 94-17), but not at either site on Shoal Creek (sites 94-15 and 94-20; Fig. 7). [Note: The tributary sites were not shown in the Wildhaber et al. (2000) illustration]. The revised 1991 chloride model was retained for evaluation of the 1995 data.

As previously noted, chloride was not measured in 2009, which necessitated development of alternative models. Stepwise multiple regression analysis of the 1991 data with chloride excluded indicated that the model

$$\log_{10}(\text{density} + 1) = -0.7828 - 0.8113 \log_{10}(p_{>38}) + 0.0129 \text{ sulfate}$$

(1991 sulfate model) was statistically significant ($F_{2,8} = 8.14$, $P < 0.02$) and explained 67% of the total variation in Np density (Fig. 8). Application of this model to the 1994 data also overestimated Np density at three of the six sites in the Neosho-Cottonwood system, but underestimated Np density at most Spring River sites; the Spring River below Willow Creek (site 94-29) was the only site downstream from Center Creek at which potential NP habitat was indicated (Fig. 9). Nevertheless, and similar to the revised 1991 chloride model, the 1991 sulfate model indicted the presence of potential Np habitat at sites on lower Center Creek (site 94-17) and Turkey Creek (site 94-28), but not at either site on Shoal Creek (sites 94-15 and 94-20; Fig. 9). The predicted densities from the revised 1991 chloride model and the 1991 sulfate models were generally in agreement except for the Spring River at Willow Creek (site 94-29), where the 1991 sulfate model predicted lower density (Figs. 7, 9).

Concentrations of most dissolved constituents (e.g., sulfate, chloride) and specific conductance were inter-correlated at sites in the Neosho-Cottonwood system sampled in 1994, as they were in 1991 (Wildhaber et al., 2000). However, these variables were not as correlated when examined for the complete 1994 data set (Fig. 10). Although concentrations of most dissolved constituents (and hence specific conductance) were typically greatest in the Cottonwood River in both years, the relative contributions of some constituents (especially chloride and sulfate) differed substantially among sites (Figs. 10, 11). Particularly noteworthy were comparatively high chloride concentrations in surface water and pore-water in the Spring River at Willow Creek (site 94-29), high sulfate in lower Turkey Creek (site 94-28), and low chloride and sulfate concentrations in Shoal Creek (sites 94-15 and 94-20) and in the Spring River at all sites upstream from Center Creek and downstream from Shoal Creek (Figs. 3, 10, 11). The high chloride concentration in the Spring River at Willow Creek (site 94-29), which exceeded Cottonwood River concentrations, was responsible for the previously noted difference between the NP densities predicted by the revised 1991 chloride and 1991 sulfate models. In addition, chloride concentrations just upstream from Willow Creek in the Spring River at Riverton (site 94-11) were not elevated (Figs. 10, 11). The presence of potential Np habitat in lower Turkey Creek (site 94-28) indicated by the models also was partly related to high sulfate and chloride concentrations. These anomalies and the lack of overall correlation between dissolved constituents indicate that chloride might be better represented by more than one variable across the Neosho-Cottonwood-Spring River system, and additional regression models were sought.

Further stepwise regression analysis of the 1991 data with chloride excluded indicated that the model

$$\log_{10}(\text{density} + 1) = 3.9223 - 0.0500 \text{ alkalinity} + 0.0112 \text{ hardness}$$
$$- 1.3970 \log_{10}(p_{>38}) - 1.1978 \log_{10}(p_{19\text{-}38}),$$

where $p_{19\text{-}38}$ = the weight-proportion of 19–38 mm (diameter) substrate, was statistically significant ($F_{4,6}$ = 44.54, P <0.01) and explained 97% of the variation in Np density (Fig. 12). Although this model (1991 alkalinity model) contained parameters representing 5 variables extracted from only 11 observations, all were statistically significant; $p_{19\text{-}38}$ explained 37% of the variation (P = 0.06), alkalinity 21% (P <0.01), $p_{>38}$ 17% (P <0.01), and hardness 2% (P <0.01). Lower-order models (including the 1991 sulfate model) that explained 67–88% of the variation in Np density contained sulfate and $p_{>38}$, but sulfate was replaced by alkalinity and hardness in the stepwise regression. The 1991 alkalinity model was also the most parsimonious based on its AICc value, which was the smallest among all possible models fit to the 1991 data. Predicted Np densities from the 1991 alkalinity model were correlated with those from the revised 1991 chloride model when applied to the 1994 data (r = 0.62, n = 11, P <0.01) and indicated the presence of potential Np habitat in lower Center Creek (site 94-17) but not in lower Turkey Creek (site 94-28) or Shoal Creek (sites 94-15 and 94-20; Fig. 13). However, the 1991 alkalinity model did not accurately predict Np density at four of the six Neosho-Cottonwood sites sampled in 1994, including the sites that had been sampled in 1991, or at many of the Spring River sites upstream from Center Creek where Np was collected (Fig. 13). This model did accurately predict the density in the Spring River at Willow Creek (site 94-29), however, and both the 1991 sulfate and 1991 alkalinity models were retained for evaluation of the 1995 and 2009 habitat data.

Although three of the six Neosho-Cottonwood sites sampled in 1994 (sites 94-4, 94-5, and 94-6) were sampled in 1991, the others (sites 94-1, 94-2, and 94-3) had not been previously sampled. The 1994 data from these six sites were combined with the 1991 data and used to develop additional models based on 17 total observations. Stepwise regression indicated that the model

$$\log_{10}(\text{density}+1) = 0.1612 + 1.7174\ \text{vel} + 2.790\ \text{cond} + 1.9107 \log_{10}(p_{9.5\text{-}19}),$$

where vel = velocity (m/sec) at 60% water depth, cond = specific conductance, and $p_{9.5\text{-}19}$ = weight-proportion of 9.5–19 mm dia. substrate, was statistically significant ($F_{3,\,13}$ = 6.96, P <0.01) and explained 62% of the variation in Np density. This model (1991-94 three-variable model) also had the lowest AICc value among all possible models, indicating that it was the most parsimonious. Application of this model to the 1994 data predicted the occurrence of Np at the six Neosho-Cottonwood sites reasonably well (Fig. 14). The model also indicated the presence of potential habitat in Center Creek (site 94-17), Turkey Creek (94-28), and lower Shoal Creek (94-20), but not at the site further upstream on Shoal Creek (site 94-15; Fig. 14). It also accurately predicted the occurrence of Np in the Spring River at Willow Creek (site 94-29) and several of the Spring River sites upstream from Center Creek, but both overestimated and underestimated density at other sites (Fig. 14). Stepwise regression analysis of this data set also indicated that the model

$$\log_{10}(\text{density}+1) = 4.8193 - 0.05298 \log_{10}(p_{>38}) - 2.2376 \log_{10}(p_{19\text{-}375})$$
$$+ 3.5594 \log_{10}(p_{9.5\text{-}19}) - 2.6261 \log_{10}(p_{2\text{-}9.5}) + 3.8488\ \text{vel} + 5.4364\ \text{cond},$$

where $p_{2\text{-}9.5}$ is the weight-proportion of 2–9.5 mm dia. substrate, also was statistically significant ($F_{6,\,10}$ = 8.84, P <0.01); it explained 84% of the variation in Np density. All six variables in this model (1991-94 six-variable model) were statistically significant (most P <0.01, one P = 0.06) and it had an AICc value only 0.09 greater than the 1991-94 3-variable model, indicating that it was equally parsimonious. Application of this model to the 1994 data slightly underestimated Np density at the six Neosho-Cottonwood sites, but substantially underestimated densities at most of the Spring River sites upstream from the confluence of Center Creek and at Willow Creek (site 94-29; Fig. 15). The model indicated the presence of potential Np habitat in Center Creek (site 94-17) and in lower Shoal Creek (site 94-20), but not in upper Shoal Creek (site 94-15) or Turkey Creek (site

94-28; Fig. 15). Both 1991-94 models were retained for evaluation of the 1995 and 2009 habitat data.

The high alkalinity, hardness, chloride, and sulfate concentrations of the Cottonwood River relative to the Spring River system derive primarily from natural sources (Wildhaber et al., 2000). Nevertheless, concentrations of these constituents also can be affected by anthropogenic sources such as sulfide-containing mine wastes, which oxidize and contribute sulfate, and WWTP effluents and urban runoff, which are sources of chloride. Acid mine drainage also affects hardness and alkalinity. Increases in these constituents are reflected as increased specific conductance. Due to the presence of mining and other pollution sources in the Spring River watershed, a model that included only physical habitat variables was sought. The data set available for this analysis was the combined 1991-94 data used in the preceding analyses with the addition of data from the 1991 site at which water quality was not measured (total n = 18). Stepwise regression analysis of this data indicated that the model

$$\log_{10} (\text{density} +1) = 2.3207 + 1.5390 \log_{10} (p_{19\text{-}375}) - 1.0429 \text{ depth},$$

where depth is water depth (m), was statistically significant ($F_{2,\,15} = 5.26$, $P < 0.02$), but it explained only 41% of the variation in density, substantially less than any of the models that included water quality variables. This model was not retained.

3.2 Regression Model Application

3.2.1. Application to the 1995 Data

Substrate composition spanned a wide range at the sites sampled in 1995. As expected, the substrate in the upper tributary reaches, as represented by sites in the North Fork (site 95-8), Center Creek (site 95-10), and Shoal Creek (site 95-11), was dominated by coarse material (>19 mm dia.) whereas the substrate at sites in the main stem of the Spring River and in downstream tributary reaches contained proportionally more finer material (Fig. 16). Water-quality differed less across the 1995 Spring River sites (Fig. 17) compared to the Neosho-Cottonwood-Spring River system as a whole (Fig. 2). Nevertheless, concentrations of dissolved constituents were lower in Shoal Creek than elsewhere in the Spring River system in 1995 (Fig. 17), as they were in 1994 (Fig. 3). The comparatively high chloride concentration in the Spring River at Willow Creek (site 94-29) did not recur in 1995 (site 95-1), but the sulfate concentration in the North Fork (site 95-8) was higher than all others sampled in 1995 (Figs. 17, 18). Turkey Creek, where sulfate concentrations also were comparatively high in 1994, was not sampled in 1995. Neosho madtoms were present at only 4 of the 12 sites sampled in 1995: in the Spring River at Willow Creek (site 95-1, same as 94-29) and at three main stem Spring River sites upstream from Center Creek (sites 95-4, 95-5, and 95-9; Fig. 1, Table 1). Np had been previously collected at or near all of these sites. Among the four, Np density was greatest at the Willow Creek site.

All five models successfully predicted the occurrence of Np in the Spring River at Willow Creek in 1995 (site 95-1; Figs. 19–23). All except the 1991 alkalinity model predicted that Np would be present at the four Spring River sites where they were found in 1995; the 1991 alkalinity model predicted only three (Fig. 20). In addition, none of the models indicated the presence of potential Np habitat in the North Fork (site 95-8), where Np was not found in 1995. Due to the lower chloride concentration in 1995, and in contrast to 1994, the revised 1991 chloride model successfully predicted the occurrence of Np in the Spring River at Willow Creek (site 95-1), but it underestimated Np density at the other three sites where the species was collected (Fig. 19). Nevertheless, the revised 1991 chloride model indicated the presence of potential Np habitat in lower Shoal Creek (site 95-2, same as 94-20), lower Center Creek (site 95-12, same as 94-17), and in the Spring River above the confluence of the North Fork (site 95-7; Fig. 19). Application of the 1991 alkalinity model to the 1995 data also indicated the presence of potential Np habitat in lower Center Creek (site 95-12), but not at any other tributary sites (Fig. 20). However, this model greatly overestimated the density at one Spring River site where Np was collected (Fig. 20). The 1991 sulfate model also indicated potential Np habitat at the four sites where it was found in 1995, and that potential habitat was present in lower Center Creek (site 95-12) and possibly in the Spring River above the North Fork (site 95-7), but not elsewhere (Fig. 21).

Both models based on combined 1991 and 1994 data successfully predicted the occurrence of Np at the four Spring River sites where it was found in 1995 (Figs. 22, 23), but the 1991–94 three-variable model overestimated the measured density at all four sites (Fig. 22). Nevertheless, this model indicated the presence of potential Np habitat at two of the Center Creek sites (sites 95-10 and 95-12); at all three Shoal Creek sites (sites 95-2, 95-3, and 95-12); and in the Spring River upstream from the confluence of the North Fork (site 95-7; Fig. 22). Of all the models evaluated, the 1991–94 six-variable model most accurately estimated Np density at all four Spring River sites where the species was found and indicated the presence of potential habitat in lower Shoal Creek (site 95-2) and lower Center Creek (site 95-7), but not in the Spring River above the North Fork (site 95-7; Fig. 23). The 1991–94 six-variable model also indicated the presence of potential Np habitat at one site located further upstream on Shoal Creek (site 95-11) and at all three Center Creek sites (Fig. 23).

3.2.2. Application to the 2009 Data

With one exception, substrate texture at the 2009 sites was similar to that at the sites sampled in 1994 and 1995 (Fig. 24). The exception was in Shoal Creek above the WWTP (site 09-10, same as site 95-3), where the substrate at all three riffles comprised mostly coarse (>19 mm dia.) material (Fig. 24). The substrate was finer when measured at this site in 1995 (Fig. 16). Conversely, the substrate at the lowermost sites on all three tributaries (sites 09-5, 09-6, and 09-16) comprised mostly material <19 mm dia. (Fig. 24). Substrates in Sites 09-5 (lower Turkey Creek (site 09-5, same as 94-28) and lower Center Creek (09-6, same as 94-17 and 95-12) also were comparatively fine in 1994 (Fig. 2); however, substrate at the Shoal Creek WWTP site (09-10) was coarser in 2009 than in 1994 (Fig. 2). Substrate texture in lower Center Creek and Shoal Creek (sites 09-05 and 09-16, respectively) was also finer when these sites were sampled in 1995 (sites 95-12, 95-2; Fig. 24) than in 2009 (Fig. 24).

Concentrations of dissolved constituents in 2009 (Fig. 25) were also similar to those in 1994 (Fig. 3) and 1995 (Fig. 17). Concentrations of the constituents included in the regression models (i.e., sulfate, alkalinity, and hardness) were generally lower in Shoal Creek (all sites) and in the upper reaches of Turkey Creek and Center Creek (including Jenkins Creek; Fig. 25). Conversely, concentrations were higher in the lower reaches of Center Creek and Turkey Creek. Chloride concentrations were not measured in 2009.

Densities of Np also were not measured in 2009, so densities predicted by the four models that excluded chloride (1991 alkalinity, 1991 sulfate, 1991−94 three-variable, 1991−94 six-variable) were evaluated relative to each other and to previous data. In addition, many of the upstream tributary sites were outside the known range of Np, and the high proportion of coarse substrate at the Shoal Creek WWTP site (site 09-10) was well beyond the range of the data from which the models were developed. Nevertheless, and consistent with the 1994 and 1995 results, several of the models indicated the presence of potential Np habitat in the lower reaches of all three tributaries (Fig. 26). The models based on 1991 data were the most consistent. In addition, and in contrast to results predicted from application of these models to the 1994 and 1995 data, the 1991 models indicated only marginal habitat in the tributaries, even at the downstream-most sites (Fig. 26). However, these models also contain terms that can be affected by the high concentrations of mining-related dissolved constituents in the lower reaches of the tributaries (Fig. 25). Both models based on the combined 1991−94 data sets indicated potential Np habitat at the downstream-most sites on Center Creek (site 09-6) and Shoal Creek (site 09-16), but only the 1991−94 three-variable model indicated the presence of Np habitat at the downstream Turkey Creek site (site 09-5; Fig. 26). The 1991−94 three-variable model also indicated progressively lower-quality habitat with distance upstream from the mouth of all three tributaries, which is consistent with the distribution and habitat preference of Np. However, because this model includes a positive coefficient for specific conductance, the declining predicted density also parallels the ionic strength gradient of the tributaries. The 1991−94 six-variable model, which contains four coefficients associated with sediment texture, produced the most diverse estimates, especially for upstream sites (Fig. 26). This model also indicated the presence of potential Np habitat in Center Creek below Hwy. JJ (site 09-10), which is an artifact resulting from application of the model to substrate composition data outside the range from which it was developed.

3.3 Occurrence Envelopes

The mean values of most physical habitat variables were within the occurrence envelopes at most sites in the Neosho-Cottonwood-Spring River system in all years (Table 2). The only obvious exception was for Shoal Creek at the WWTP (site 09-10), where $p_{>38}$ was substantially greater than at all other sites (Fig. 24), which resulted in this site being outside the occurrence envelope for all substrate categories (Table 2). Center Creek below Hwy. JJ (site 95-10) was also outside the occurrence envelope for most substrate categories, and several sites were slightly below the envelope for substrate fines (i.e., <2 mm, $p_{<2}$; Table 2). Mean depth was above the envelope in the Spring River above the North Fork (site 95-7) whereas all the Turkey Creek sites were at or near the lower limit of the depth envelope, as were some Center Creek sites (Table 2). Velocity exceeded the occurrence envelope at most Shoal Creek sites and at several sites on Center Creek, but two Neosho River sites (sites 91-HB and 94-2) were below (Table 2). Nevertheless, it is important to note that these are means; i.e., deeper, shallower, faster, and slower-moving water was present at all sites, and substrate texture was variable. It is therefore likely that some habitat within the occurrence envelopes of all the variables was present at all or most of the sites.

Most sites in the Neosho-Cottonwood-Spring River system also were within or near the Np occurrence envelopes for water-quality (Table 2). No sites were outside the pH envelope, but several tributaries were below the Neosho-Cottonwood-Spring turbidity envelope; one Cottonwood River site was above (Table 2). Two sites on Shoal Creek sampled in 1995 (sites 95-2 and 95-3) were below the specific conductance envelope, as was the Spring River above the North Fork (site 95-7), but all Shoal Creek sites and all other 1995 sites were within the occurrence envelope when they were sampled in 1994 and 2009 (Table 2). These differences no doubt reflect antecedent weather conditions in the Spring River basin. Sites on the Spring River in Oklahoma sampled in 1994 were below the occurrence envelope for alkalinity, as was one Spring River site upstream from

Center Creek (site 94-6), and site 09-1 (Jenkins Creek) was at the lower limit (Table 2). However, only one 1995 site on Center Creek (site 95-6) was below the hardness envelope (Table 2). Many tributary sites were below the Neosho-Cottonwood-Spring river occurrence envelope for sulfate, but not for chloride (Table 2).

The Illinois River originates in northwestern Arkansas and flows westward to its confluence with the Arkansas River near Gore, Oklahoma. It is a clear, gravel-bottomed stream with water quality that more closely resembles similar streams in the Missouri Ozarks than the Neosho or Cottonwood Rivers. Water quality concerns in the Illinois River watershed are primarily focused on increasing nutrient concentrations and turbidity associated with poultry farms and urban growth. Ionic strength and suspended solids, as indicated by specific conductance and turbidity at Tahlequah, Oklahoma are typically higher than they were historically, but nevertheless lower than most streams in the Neosho-Cottonwood-Spring River system except for turbidity in upper Shoal Creek. Consequently, inclusion of data for the Illinois River had the net effect of further broadening the Np occurrence envelope. Historical (1947) data from NWIS indicate that ionic strength was even lower in the reach formerly inhabited by Np before the construction of Tenkiller Ferry Dam (data not shown). Consequently, only three sites on upper Turkey Creek (sites 09-5, 09-11, and 09-12) were below the turbidity occurrence envelope relative to the Illinois River near Tahlequah. The other dissolved constituents for which contemporary or historical data were available (hardness, alkalinity, chloride, and sulfate) also reflect the generally lower ionic strength in the Illinois River than most streams in the Spring-Neosho-Cottonwood River system, but the pH was similar. All the 1991−2009 sites were therefore within or near the broader occurrence envelopes when the Illinois River was included in the comparisons.

3.4 Principal Components Analysis

Data representing the 26 site-years in which Np was collected during 1991, 1994, and 1995 were available for PC analysis; data were incomplete for one 1991 and one 1994 site. Five PCs, which together explained >84% of the variation in the 17 habitat variables measured in all years, met the eigenvalue >1 criterion for retention (Table 3).

Communality values for the variables ranged from 0.6717 for ammonia N (NH_3) to 0.9673 for specific conductance, indicating that all 17 variables contributed substantially to the PCs. PC 1, which explained about 37% of the total variation, loaded negatively for coarse substrate, depth, velocity, and nitrate + nitrite N ($NO_{2\&3}$), with all others loading positively (Table 3). The largest positive loadings were for turbidity, fine substrate ($p_{<2}$), specific conductance, and sulfate. The absolute values of the loading factors for PC 1 spanned a relatively narrow range (from 0.3389 for alkalinity to 0.7606 for turbidity), indicating substantial contributions by all variables. PC 2, which explained about 21% of the total variation in the habitat data, loaded most negatively for fine substrate and total P and most positively for coarse substrate ($p_{>38}$), specific conductance, hardness, alkalinity, and sulfate (Table 3). In contrast to PC 1, some variables loaded weakly on PC 2 (absolute value <0.10). PC 3 loaded negatively on coarse substrate and total P and positively on medium-sized substrate ($p_{19\text{-}38}$ and $p_{9.5\text{-}19}$), depth, and $NO_{2\&3}$ (Table 3). None of the loadings on PC 3, which explained about 10% of the total variation in the habitat data, were particularly strong (absolute value <0.6) and many were weak (<0.1). Together, PC 1, PC 2, and PC 3 accounted for almost 68% of the total variation (81% of the explained variation) in the habitat data. PC 4, which accounted for an additional 9%, loaded strongly on pH and moderately on NH_3 (both positive), but all other variables loaded less strongly (absolute value <0.33). PC 5 accounted for only about 7% of the total variation; it loaded negatively for one of the coarse substrate variables, positively for depth, velocity, and alkalinity, and weakly for all others (Table 3).

Score plots on the first three principal components separated the 26 Np site-years into three groups, mostly according to river, and sites sampled in multiple years grouped together on all axes (Fig. 27). The Cottonwood River sites scored high on PC 1 and PC 2, the Neosho River sites were high on PC 1 and low on PC 2, and the Spring River sites were low on PC1 and intermediate on PC 2. The Neosho River near Burlington, Kansas (site 94-3) was an exception; it scored with the Spring River sites on PC 1 and PC 2, largely because the substrate comprised a greater proportion of coarse material than most Neosho-Cottonwood sites (Fig. 27). Scores plotted on PC 3, which was weighted negatively for $p_{>38}$ and positively for intermediate and fine substrate, added little further separation for most sites. The exceptions were the Spring River below Hwy. 96 (site 94-

18), which scored lower on PC 3 than all other sites due to a preponderance (87%) of >38 mm substrate ($p_{>38}$); and the Neosho River at Neosho Wildlife Area (site 94-1), which scored high on PC 3 due to a complete absence of substrate >38 mm (Figs. 2, 27).

Scores developed from PC 1−PC 5 for the 1991−95 sites where Np was not present and the 2009 sites (at which fish were not collected) were examined relative to the range of scores (i.e., occurrence envelope) for each PC (Table 4). Of the 40 site-years evaluated, 18 were within the occurrence envelopes on all five axes. These included two of the three 1991 USFWS sites on the Neosho River and all of the 1994 and 1995 sites on the main stem of the Spring River (including site 95-7, upstream from the North Fork confluence) except the one located southeast of Lawton, Kansas (site 94-26), which was slightly outside the PC 1 occurrence envelope (Table 4). Among tributaries, both Shoal Creek sites sampled in 1994 (sites 94-16 and 94-25) were inside the occurrence envelope on all PCs, as were lower Turkey Creek when sampled in 1994 (site 94-28) and both Center Creek sites sampled in 1995 (sites 95-6 and 95-12). Also insided the envelope were two tributary sites sampled in 2009: Center Creek E. of Dogwood Rd. (site 09-9), and Turkey Creek at Schifferdecker Rd. (site 09-11; Table 4). Five other tributary sites sampled in 2009 were within the occurrence envelope defined by the first three PCs, which explained most of the variation in the habitat data. These were Center Creek at Oronogo (site 09-3, below CR 230), lower Turkey Creek (site 09-5, same as site 94-28), and three sites on Shoal Creek (sites 09-14, 09-15, and 09-16; Table 4). Among the Shoal Creek sites, three (sites 09-14 and 09-16, same as 94-20 and 95-2, respectively) were on lower Shoal Creek, but one (site 09-15) was the farthest upstream (Fig. 1, Table 1).

In contrast to the previously noted sites, and as expected, many of the tributary sites sampled in 2009 were outside the occurrence envelopes defined by multiple PCs (Table 4). In 1994, lower Center Creek (site 94-17) was above the occurrence envelope on PC 3, which weights positively for $NO_{2\&3}$ (Table 3); nitrate concentrations have been historically elevated in Center Creek (Schmitt et al., 1997) and were the highest measured in 1994 (Fig. 11). However, this site was within the occurrence envelope on all five PCs in 1995 (Site 95-12), when $NO_{2\&3}$ concentrations were lower, but in 2009 it was outside the envelope on four of five axes (site 09-6; Table 4). Center Creek below Hwy JJ (site

95-10) was below the occurrence envelope on PC 1 and PC 3 due to the preponderance of coarse substrate, as noted earlier (Fig. 16), and Center Creek at Carl Junction (site 09-2) was outside the envelope defined by all five PCs (Table 4). In addition, and although not within the occurrence envelope defined by PC 1−PC 3, several of the 1994 and 1995 sites on the lower reaches of the tributaries were not far outside (Table 4).

4. Discussion

Linear regression assumes quantifies linear or at least monotonically increasing or decreasing relations between dependent and independent variables and quantifies the rate at which variables change relative to each other; however, it does not infer cause-effect. Organisms generally tolerate a range of conditions within which there are optima. Therefore, the existence (or not) of a relation and its direction (positive or negative) depend on the range sampled relative to the total range for the species on each variable and the shape of the relationship. The ranges of many of the water quality variables included in this study were narrow relative to their total possible ranges. The plausibility of monotonically increasing or decreasing Np densities due to water quality differences within the ranges spanned by the Neosho-Cottonwood-Spring Rivers is therefore suspect. In addition, the positive associations between Np density and dissolved sulfate and chloride concentrations, which tend to increase as a result of mining, seems counter-intuitive. These positive associations reflect the high mineral content of the prairie streams of the Neosho-Cottonwood Basin and the fact that no metals data were included in the models. It is therefore likely that the water quality variables are surrogates for something not measured, such as temperature, dissolved oxygen or discharge during some key time of the year, or some other physical habitat attribute not characterized. It should also be noted that all the data analyzed here represented the means of point measurements made in mid- to late summer or early fall, from which conditions throughout the year cannot be ascertained.

Only limited inferences can be drawn from correlational analyses, including regression and PC analysis; studies that span broad geographic areas are exclusively exploratory, not explanatory. Although the PCs are orthogonal to each other and uncorrelated, they are nevertheless extracted from the correlation matrix. The empirical relations that result often generate more questions than answers, but they may also suggest testable hypotheses that can be evaluated through subsequent laboratory research and focused field studies. To date, controlled studies of physical Np habitat have been conducted (Moss, 1981; Bulger and Edds, 2001; Bryan et al., 2006), but not of water quality (including temperature). Worthwhile topics for further research would therefore include documenting the seasonal ranges of water quality conditions in streams that support Np populations relative to those that do not, more thorough spatial and temporal characterization of physical Np habitat, and the tolerance of Np to a range of water quality conditions.

The fact that nearly all the Spring River sites were within the occurrence envelopes regardless of whether or not Np had been collected at them supports previous contentions that Np absence from some sites downstream from Center Creek is related to metals rather than other habitat conditions or the presence of other species (Wildhaber et al., 2000). However, Np also was not present at some sites upstream from Center Creek that were within the occurrence envelopes. This illustrates that the occurrence envelope approaches define the minimum and maximum values at points in time and space where Np has been collected, but does not preclude its occurrence elsewhere. As previously noted, the analyses were based on site means that do not reflect the full range of conditions or the variability at the sites.

Moss (1981) reported that Neosho madtoms were only abundant on riffles containing abundant 8−16 mm dia. gravel that is "loose" (i.e., not compacted). As illustrated by the 2−9.5 mm and 9.5−19 mm substrate categories in Figs. 2, 16, and 24, many sites on the lower reaches of Spring River tributaries contained substantial proportions of such gravel. Moss (1981, p. 10) also noted that

> "Neosho R. riffles are typical of most streams in that there is great variety in bottom material and water velocity. The Neosho is atypical in that it downcuts across geological substrata forming many riffles over bedrock. The >258 mm substrate (bedrock) is more common than in many medium-sized rivers".

This description applies equally to much of the Spring River and its tributaries in Missouri, especially Shoal Creek. Cross and Collins (1995) also described Np as occurring primarily in riffles and along sloping gravel bars in moderate to strong currents and preferring deep deposits of loose, rounded chert gravel. This description indicates that the depth and shape of the gravel is also important (i.e., smooth and round vs. sharp and angular). However, Fuselier and Edds (1995) noted that artificial gravel bars constructed of quarried limestone supported densities of Np and other riffle fishes equivalent to those of natural riffles, indicating that gravel shape may be less important than substrate depth and texture. These results also demonstrated the feasibility of restoring Np habitat.

Differences among years also are not surprising. Some of the variables incorporated into both the regression and PC models, such as depth and velocity, can vary from year-to-year depending on antecedent rainfall. In addition to varying hydrologic and meteorological conditions that are reflected in the habitat variables (depth, velocity, water-quality), Np is short-lived (1−2 y; Moss 1981; Bulger and Edds, 2001). In the Neosho River, Np expanded into some reaches during periods of high flow, then disappeared during droughts (Cross 1967; Cross and Brasch, 1968). Such a scenario is equally plausible for the lower reaches of the westward-flowing tributaries of the Spring River, which may all contain at least some potential Np habitat. Recent upstream population expansion by Np into the South Fork of the Cottonwood River and downstream in the Spring River may reflect both improving waterpquality and higher flows. Wilkinson and Fuselier (1997) noted that in Kansas, Np collections typically occurred in the lower 5 km of tributaries. However, the upstream-most site on the Illinois River at which Np was found was 12 km from the mouth (Taylor, 1969), indicating that the species can populate reaches farther upstream. Collectively, these results indicate that Np can inhabit the lower reaches of tributaries during periods of favorable hydrologic conditions.

5. Summary and Conclusions

Based on the variables included in the analyses, all the methods evaluated indicated that the lower reaches of the westward-flowing Spring River tributaries could support Np. Although the regression models differed with respect to the variables they contained and the sites at which Np was predicted to occur, they all indicated that Np could inhabit some tributary sites. The models accurately predicted the occurrence of Np at sites where it had been found. The models also indicated that Np could inhabit some sites where it was either not found (in 1994-95) or where fish were not collected (in 2009). In addition, many of the sites investigated (including those on the lower reaches of tributaries) were within the occurrence envelopes developed for sites where Np had been found. These results agree with previous studies in the Spring River indicating that absent contaminants from mining, the physical and chemical conditions represented by the data analyzed should not preclude the presence of Np, and that Np could inhabit a wider geographic range than it presently (2010) does. Many of the habitat variables were highly inter-correlated, however. Consequently, and although the variables included in the regression models differed, they all contained one or more terms related to substrate texture and total ionic strength (as indicated by specific conductance and concentrations of dissolved constituents), which generally reflect differences between the Ozark streams of the Spring River system and the prairie streams farther west. The regression-based models (including the original 1991 chloride model) also were counter-intuitive in that they included positive terms for variables that tend to increase as a result of mining and other sources of pollution, which is related to the fact that metals data were not incorporated into the models evaluated in this study. In addition, the range of conditions represented by the measured variables does not seem wide enough to represent a gradient on which fish density should respond. It is more probable that the water quality variables were included because they are correlated with other variables that were not measured. Potential candidates include water temperature and flow during certain times of the year, which were not evaluated; only point measurements during late summer-early fall were included in the analyses reported here. Another possibility is the depth and shape of the unconsolidated gravel in riffles, including the extent of substrate in size categories larger than 38 mm, the maximum quantified by the procedures used in 1991−2009. This would include bedrock and cobble, which would probably be avoided by Np.

Extant information on the present and historical distribution of Np indicates that its geographic range can expand and contract rapidly in response to habitat changes, and that it can tolerate a wide range of habitat conditions. It is therefore resonable to that Np is capable of inhabiting the lower parts of westward-flowing Spring River tributaries when conditions are favorable, with "favorable" defined as absent harmful concentrations of contaminants associated with lead-zinc mining. Collectively, the results of this study indicate that Np may have inhabited the lower reaches of the larger Spring River tributaries (Shoal Creek and Center Creek) at least occasionally before the advent of the Tri-States Mining District, it apparently did in Shoal Creek in 1963; and that re-establishment of populations in streams from which Np may have been extirpated is feasible.

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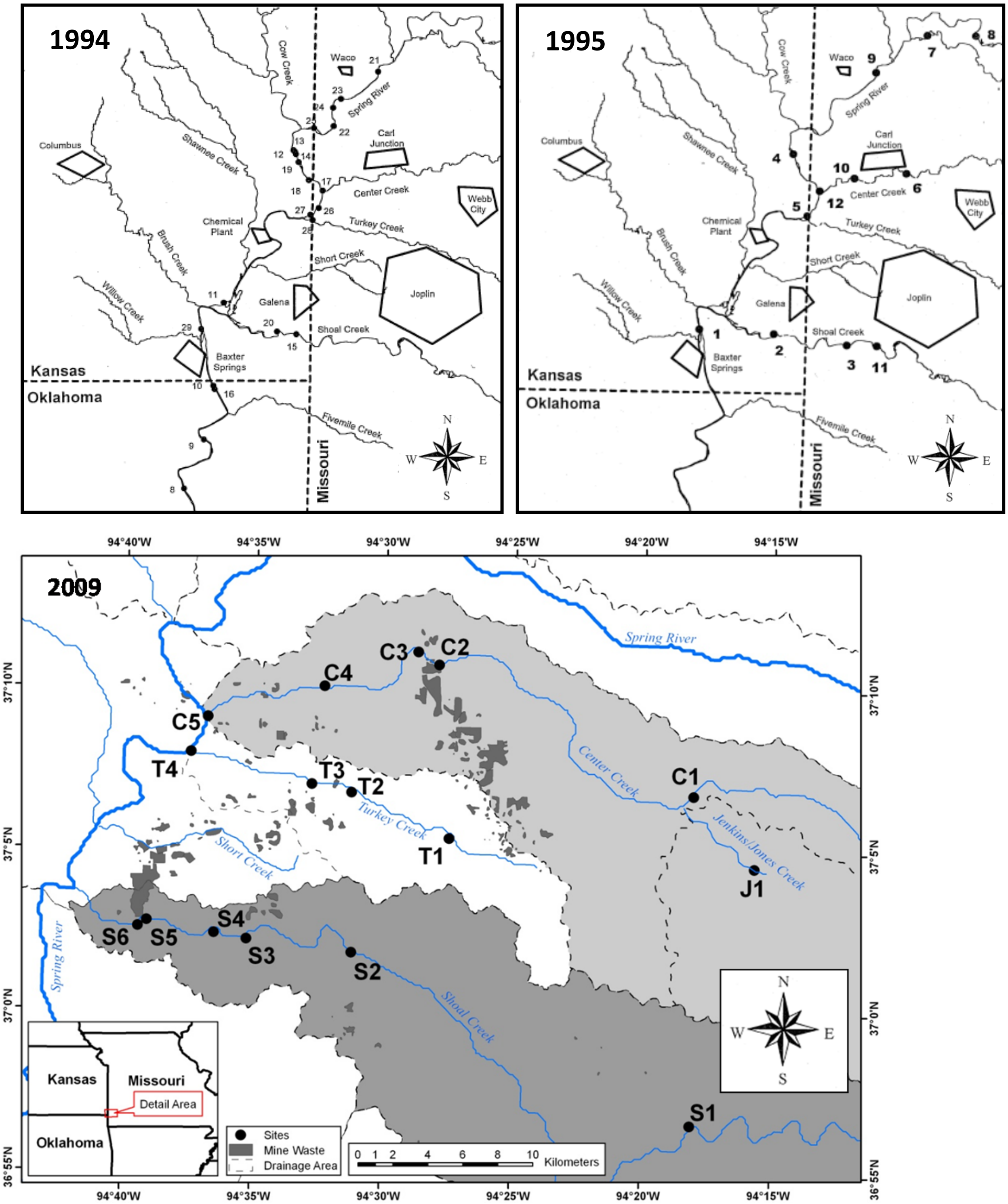

Figure 1. Sites in the Spring River system sampled in 1994 (Schmitt et and others, 1995), 1997 (Allert and others, 1997), and 2009 (Allert and others, 2011). Sites in the Neosho River basin sampled in 1991 and 1994 (Wildhaber and others, 2000) are not shown. In addition to mine waste sites (dark gray polygons), the 2009 map also shows the Center Creek (light gray) and Shoal Creek (medium gray) watersheds. See Table 1 for additional information.

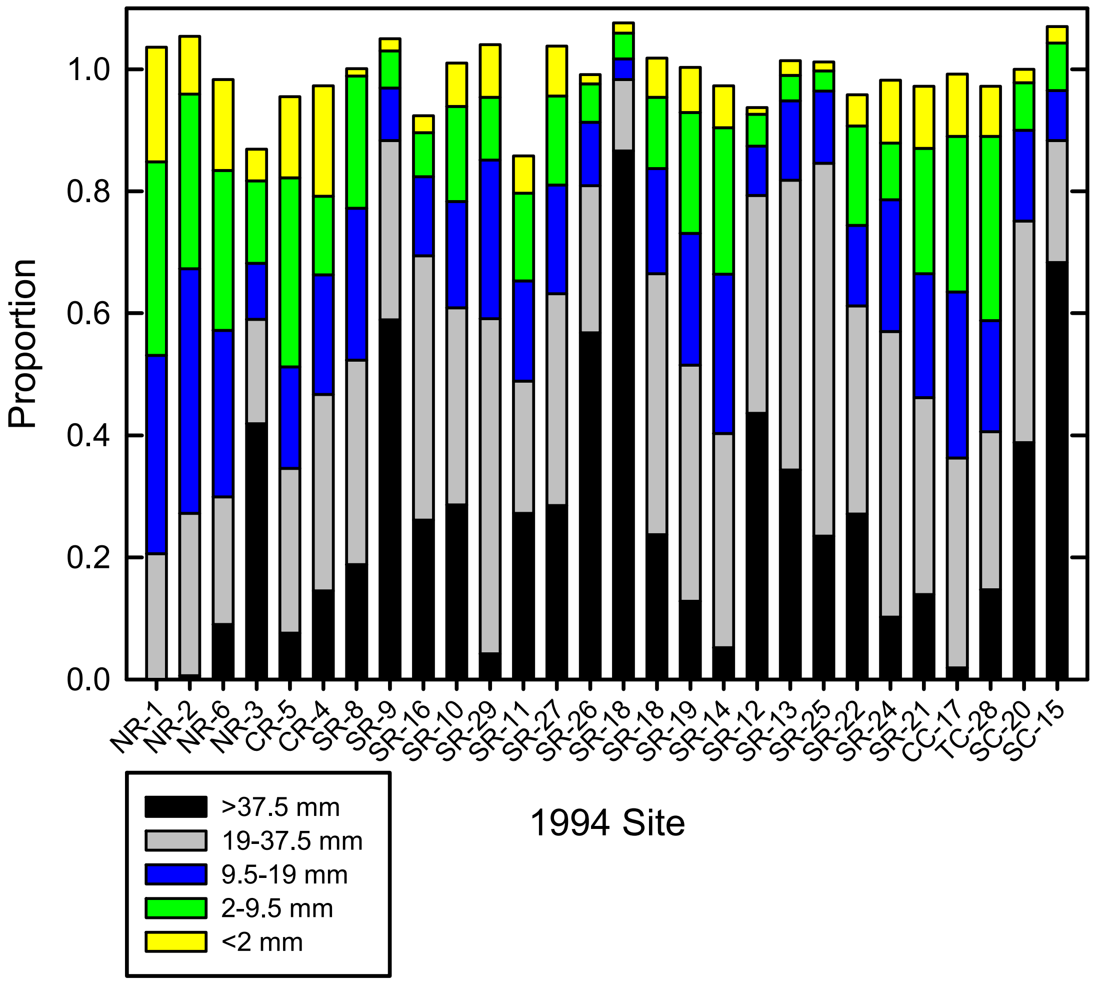

Figure 2. Mean weight-proportional substrate composition at sites on the Neosho River (NR), Cottonwood River (CR), Spring River (SR), Center Creek (CC), Turkey Creek (TC), and Shoal Creek (SC) sampled in 1994. Sites are ordered from downstream to upstream within each stream; numbers correspond to 94-x notation in the text and tables. (Note: Means were computed from multiple samples after angular transformation; means back-transformed to the linear scale may not sum to 1.0). See Fig. 1 and Table 1 for additional site information.

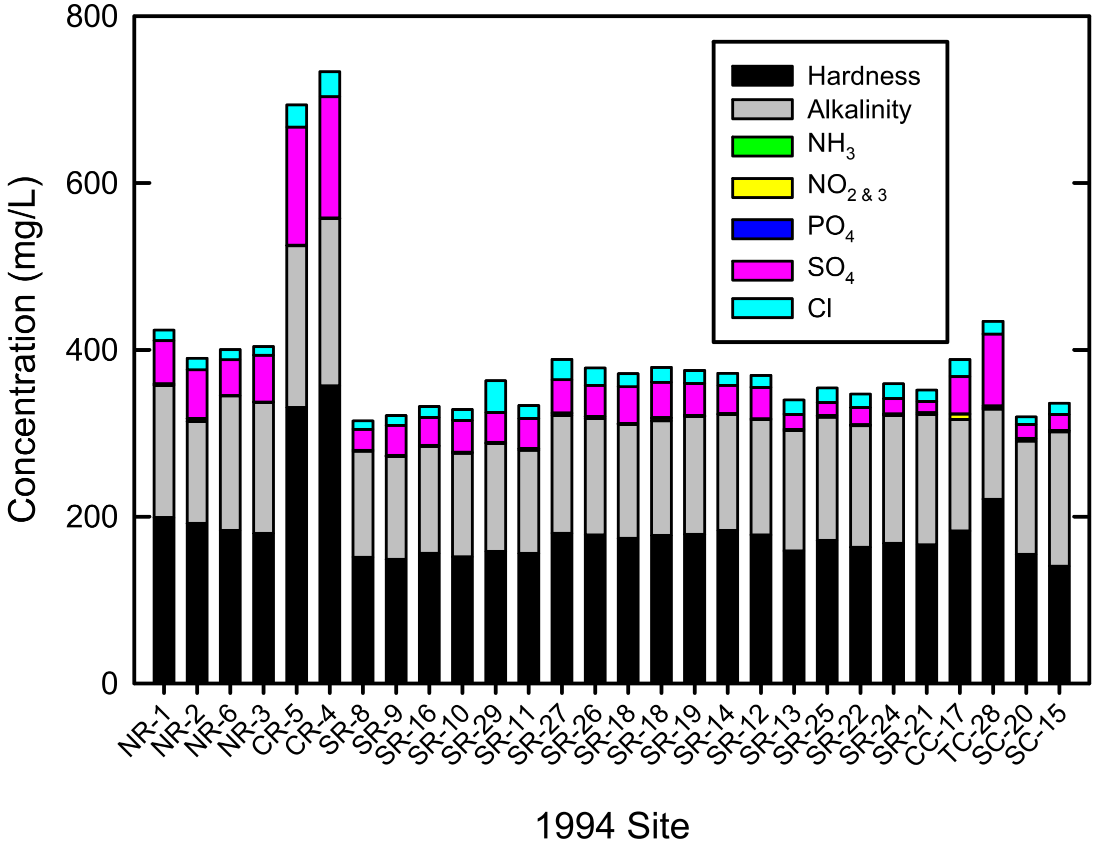

Figure 3. Mean hardness, alkalinity, ammonia-nitrogen (NH_3), nitrate + nitrite nitrogen ($NO_{2\ \&\ 3}$), phosphate (PO_4), sulfate (SO_4), and chloride (Cl) concentrations (all mg/L) in filtered surface water at sites on the Neosho River (NR), Cottonwood River (CR), Spring River (SR), Center Creek (CC), Turkey Creek (TC), and Shoal Creek (SC) sampled in 1994. Sites are ordered from downstream to upstream within each stream; numbers correspond to 94-x notation in the text and tables. See Fig. 1 and Table 1 for additional site information.

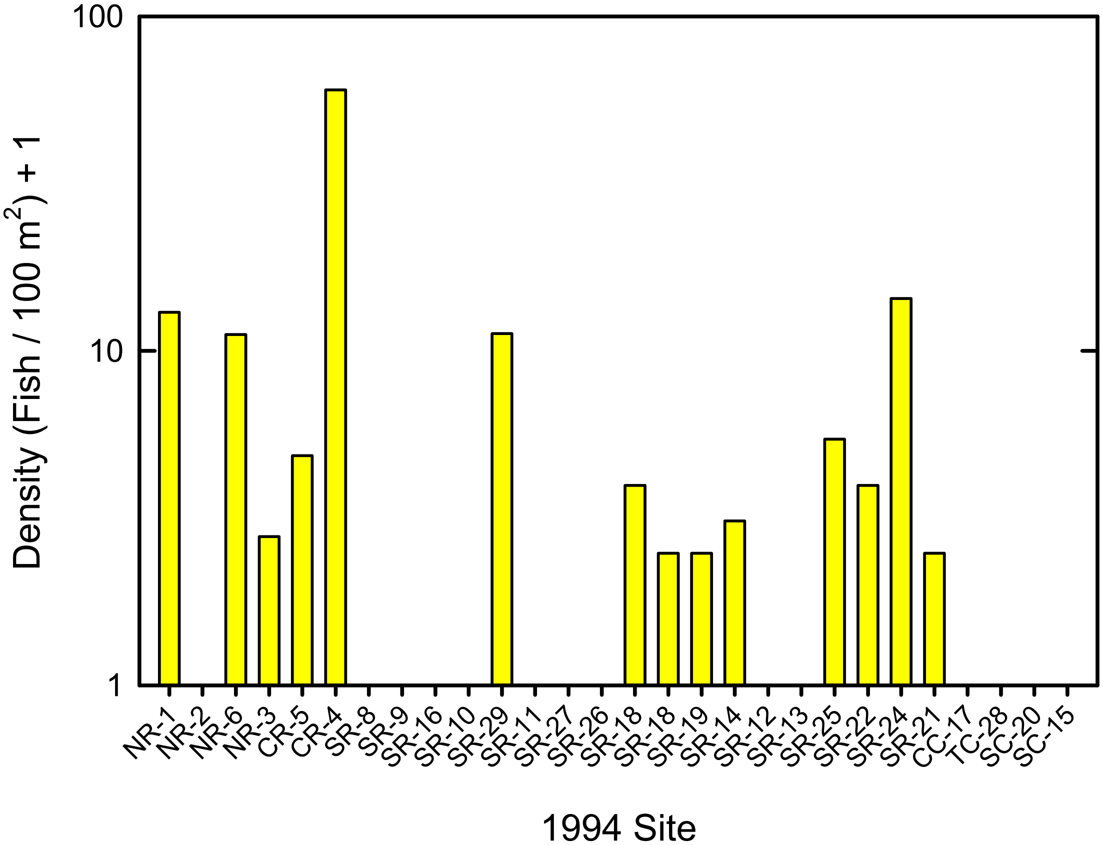

Figure 4. Mean Neosho madtom density at sites on the Neosho River (NR), Cottonwood River (CR), Spring River (SR), Center Creek (CC), Turkey Creek (TC), and Shoal Creek (SC) sampled in 1994. Sites are ordered from downstream to upstream within each stream. Sites are ordered from downstream to upstream within each stream; numbers correspond to 94-x notation in the text and tables. See Fig. 1 and Table 1 for additional site information.

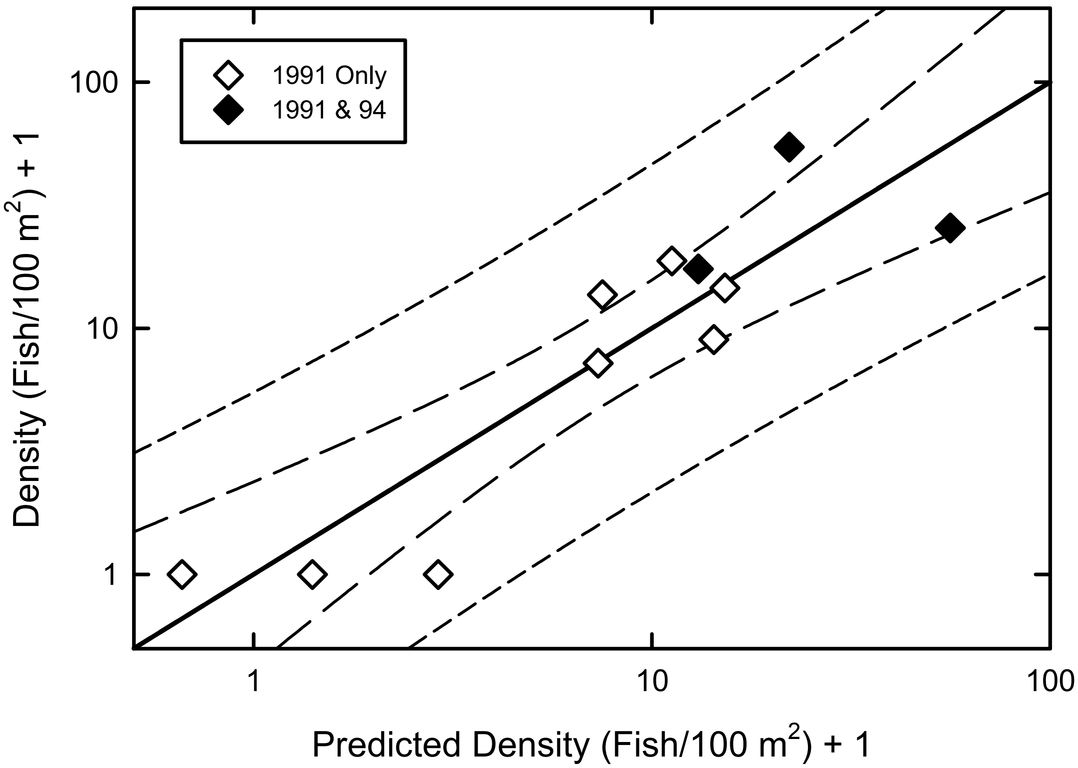

Figure 5. Measured Neosho madtom density (*Y* axis) vs. density predicted by the revised 1991 chloride model (*X* axis) at sites in the Neosho-Cottonwood system sampled in 1991. Solid line, $Y = <0.001 + 1.000\ X$, $n = 11$, $P < 0.01$, $r^2 = 0.81$; long-dashed lines, 95% confidence limit of the regression; short-dashed lines, 95% confidence limits of the prediction region. Sites shown with dark symbols were also sampled in 1994.

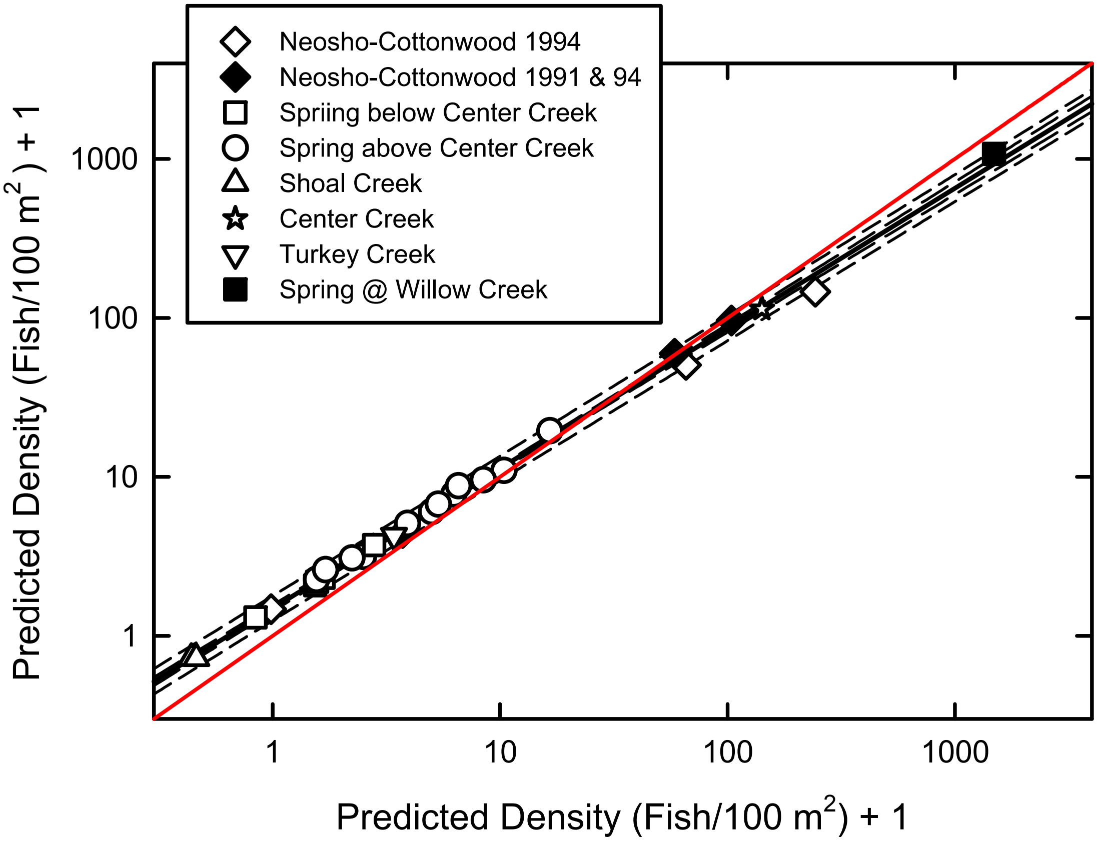

Figure 6. Neosho madtom density at sites in the Spring-Neosho-Cottonwood system sampled in 1994 predicted by the original 1991 chloride model (*X* axis, Wildhaber and others, 2000) and the revised 1991 chloride model (*Y* axis). Solid black line, $Y = 0.173 + 0.881\ X$, $n = 28$, $P < 0.01$, $r^2 > 0.99$; dashed lines, 95% confidence limits of the prediction region; solid red line, $Y = X$.

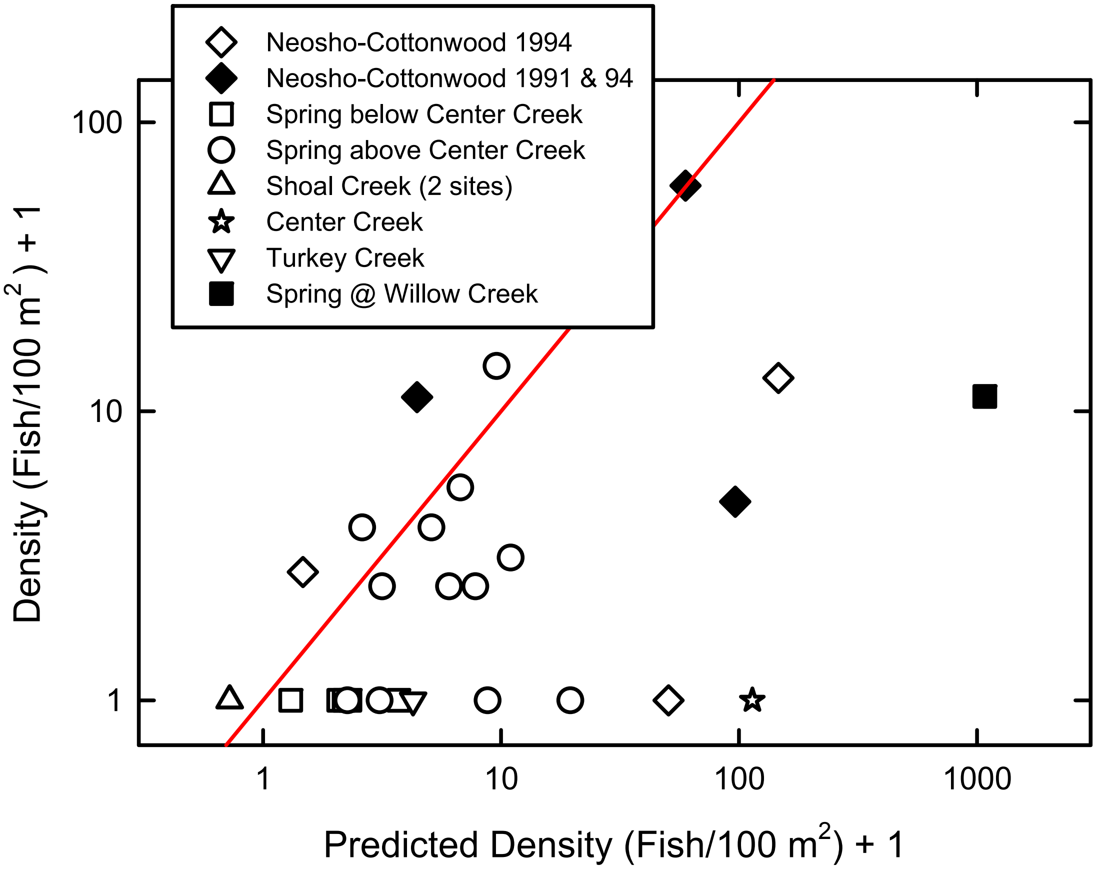

Figure 7. Measured Neosho madtom density (*Y* axis) vs. density predicted by the revised 1991 chloride model (*X* axis) at sites in the Spring-Neosho-Cottonwood River system sampled in 1994. Solid red line, *Y* = *X*.

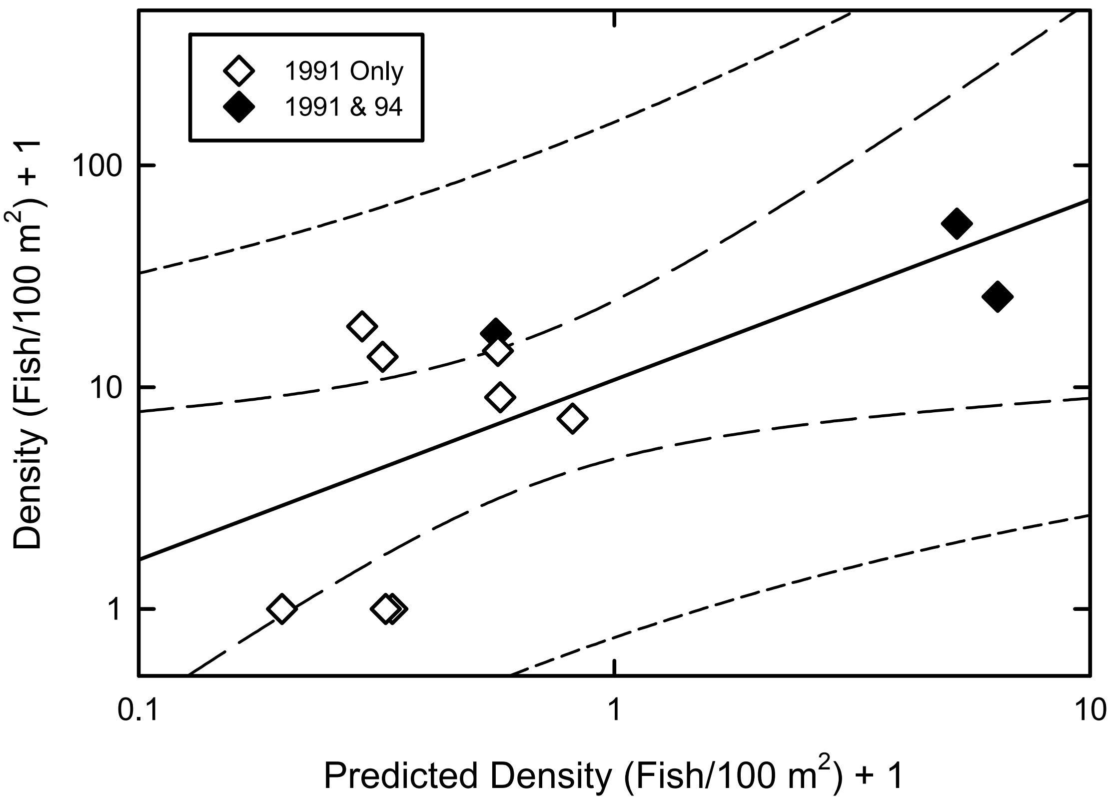

Figure 8. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991 sulfate model (*X* axis) at sites in the Neosho-Cottonwood system sampled in 1991. Solid line, $Y = <0.001 + 1.000\,X$, $n = 11$, $P < 0.01$, $r^2 = 0.67$; long-dashed lines, 95% confidence limit of the regression; short-dashed lines, 95% confidence limits of the prediction region. Sites shown with dark symbols were also sampled in 1994.

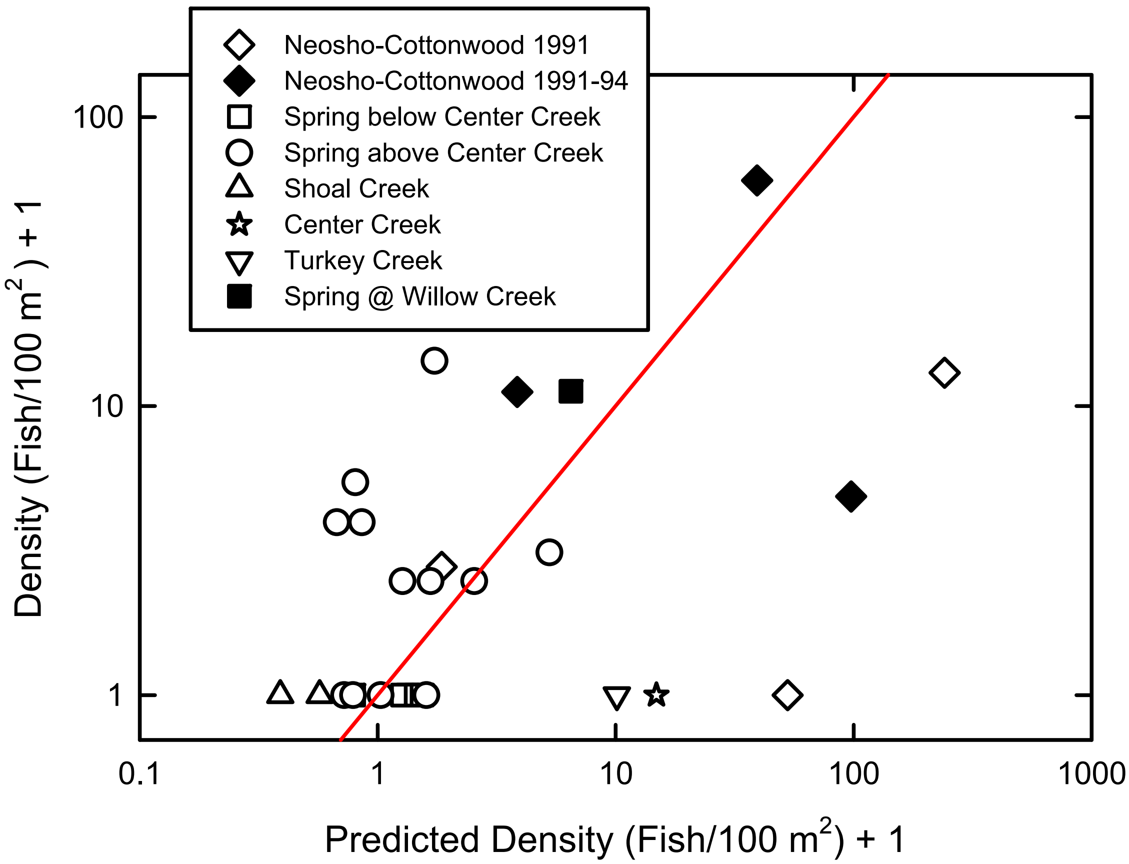

Figure 9. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991 sulfate model (*X* axis) at sites in the Spring-Neosho-Cottonwood River system sampled in 1994. Solid red line, $Y = X$.

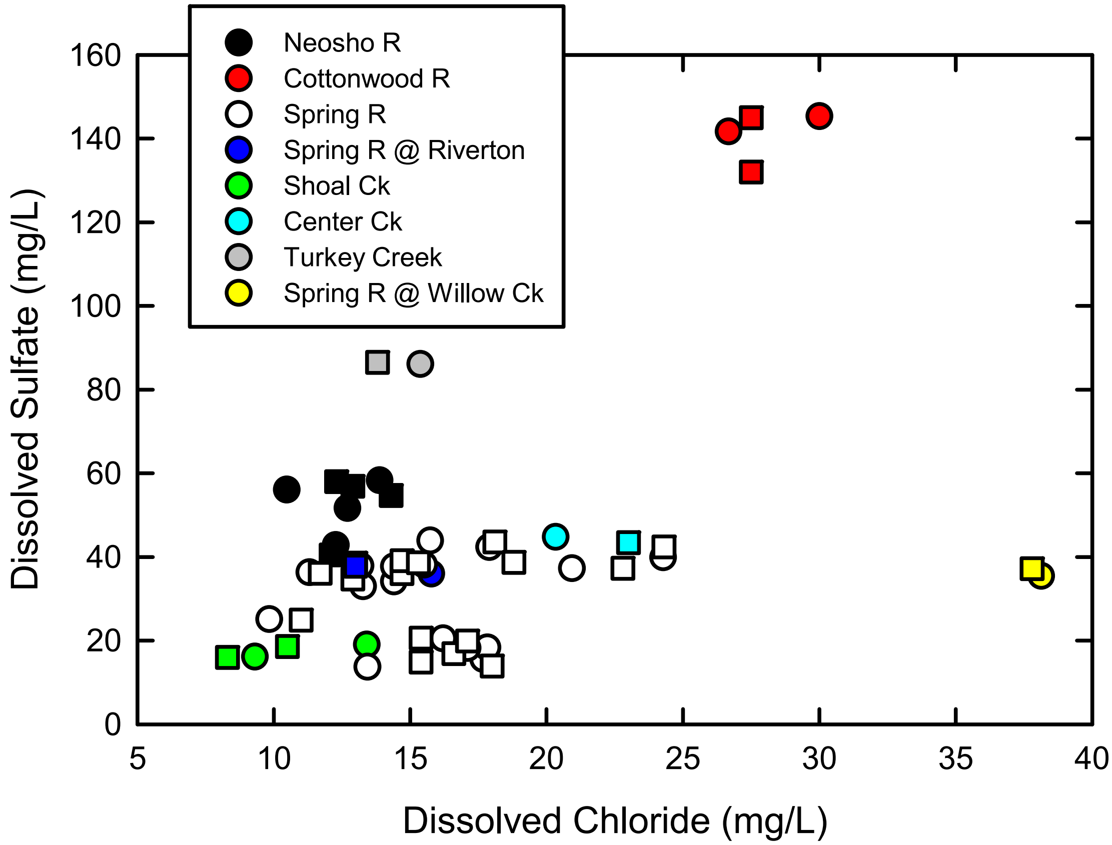

Figure 10. Concentrations of dissolved sulfate and chloride in surface water (circles) and pore-water (squares) at sites in the Neosho-Cottonwood-Spring River system sampled in 1994.

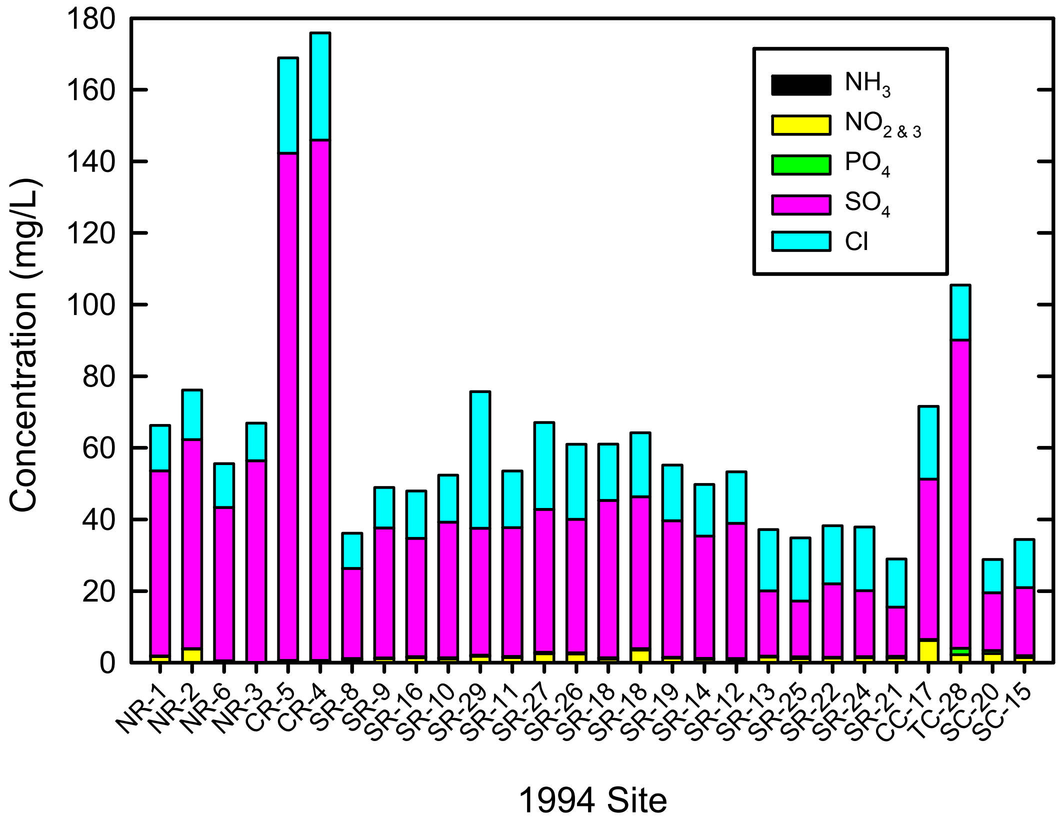

Figure 11. Mean ammonia-nitrogen (NH_3), nitrate + nitrite nitrogen ($NO_{2\&3}$), total phosphate (PO_4), sulfate (SO_4), and chloride (Cl) concentrations (all mg/L) in filtered surface water at sites on the Neosho River (NR), Cottonwood River (CR), Spring River (SR), Center Creek (CC), Turkey Creek (TC), and Shoal Creek (SC) sampled in 1994. Sites are ordered from downstream to upstream within each stream; numbers correspond to 94-x notation in the text and tables. See Fig. 1 and Table 1 for additional site information.

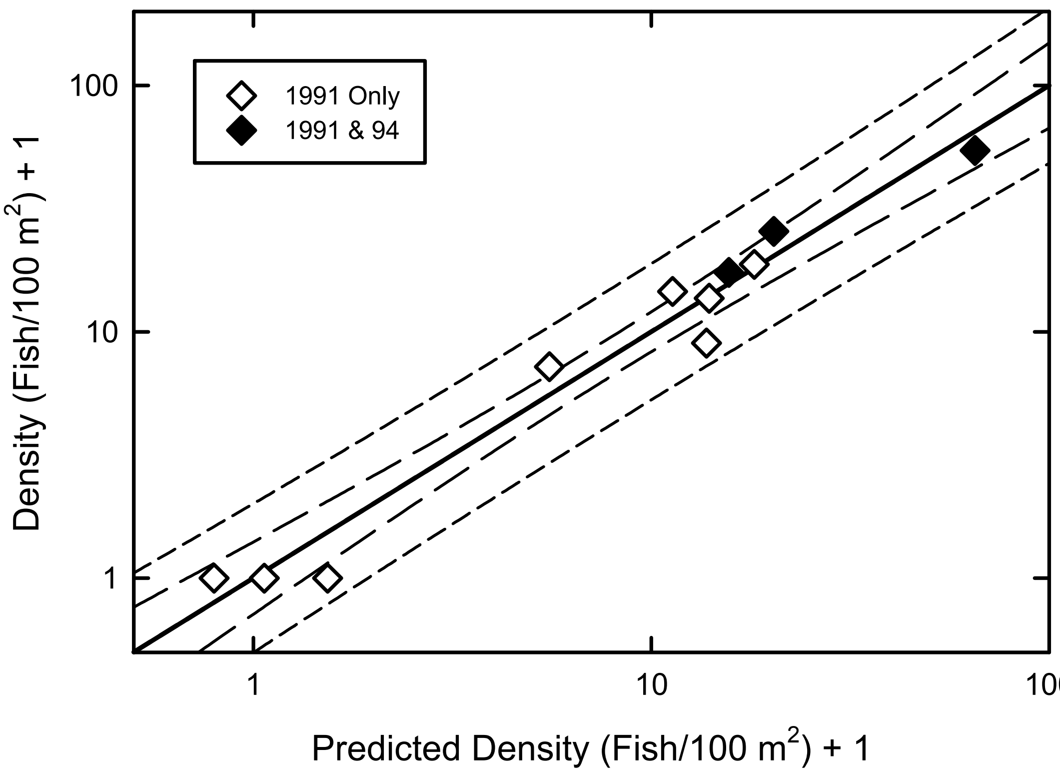

Figure 12. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991 alkalinity model (*X* axis) at sites in the Neosho-Cottonwood system sampled in 1991. Solid line, $Y = <0.001 + 1.000\ X$, $n = 11$, $P < 0.01$, $r^2 = 0.97$; long-dashed lines, 95% confidence limit of the regression; short-dashed lines, 95% confidence limits of the prediction region. Sites shown with dark symbols were also sampled in 1994.

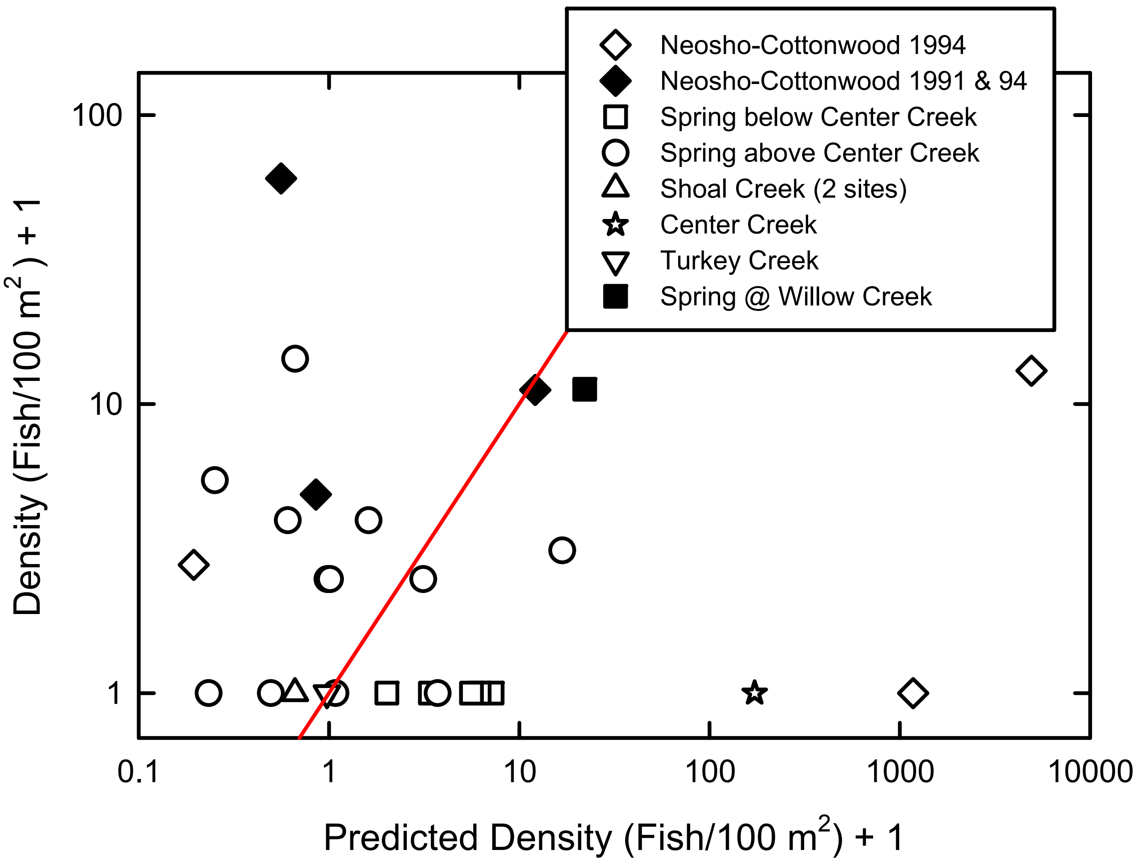

Figure 13. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991 alkalinity model (*X* axis) at sites in the Spring-Neosho-Cottonwood River system sampled in 1994. Solid red line, *Y* = *X*.

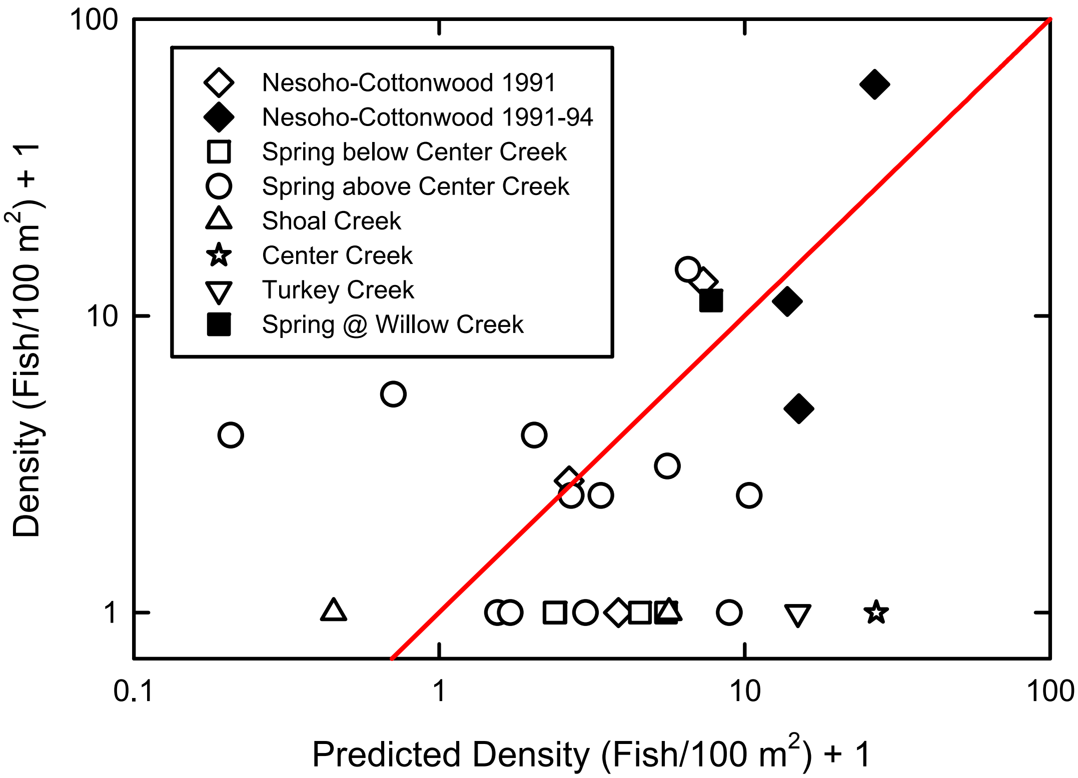

Figure 14. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991-94 three-variable model (*X* axis) at sites in the Spring-Neosho-Cottonwood River system sampled in 1994. Solid red line, *Y* = *X*.

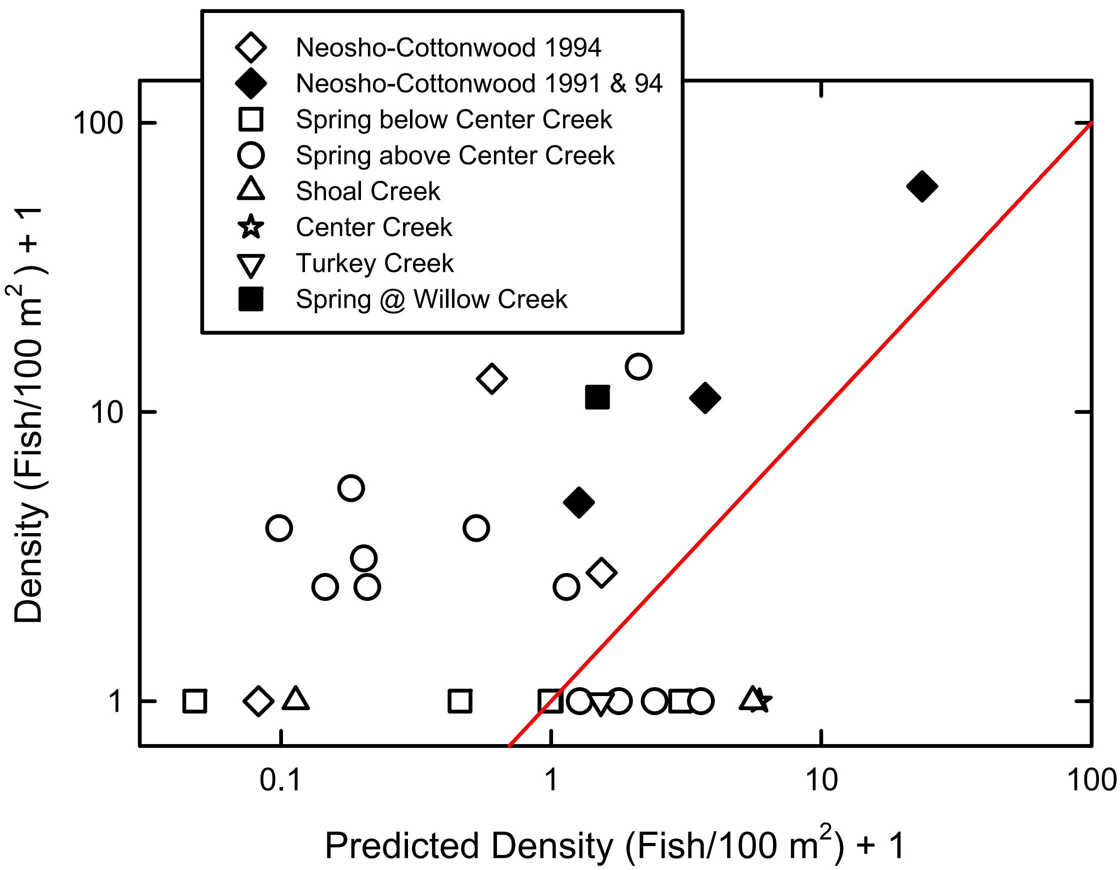

Figure 15. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991-94 6-variable model (*X* axis) at sites in the Spring-Neosho-Cottonwood system sampled in 1994. Solid red line, *Y* = *X*.

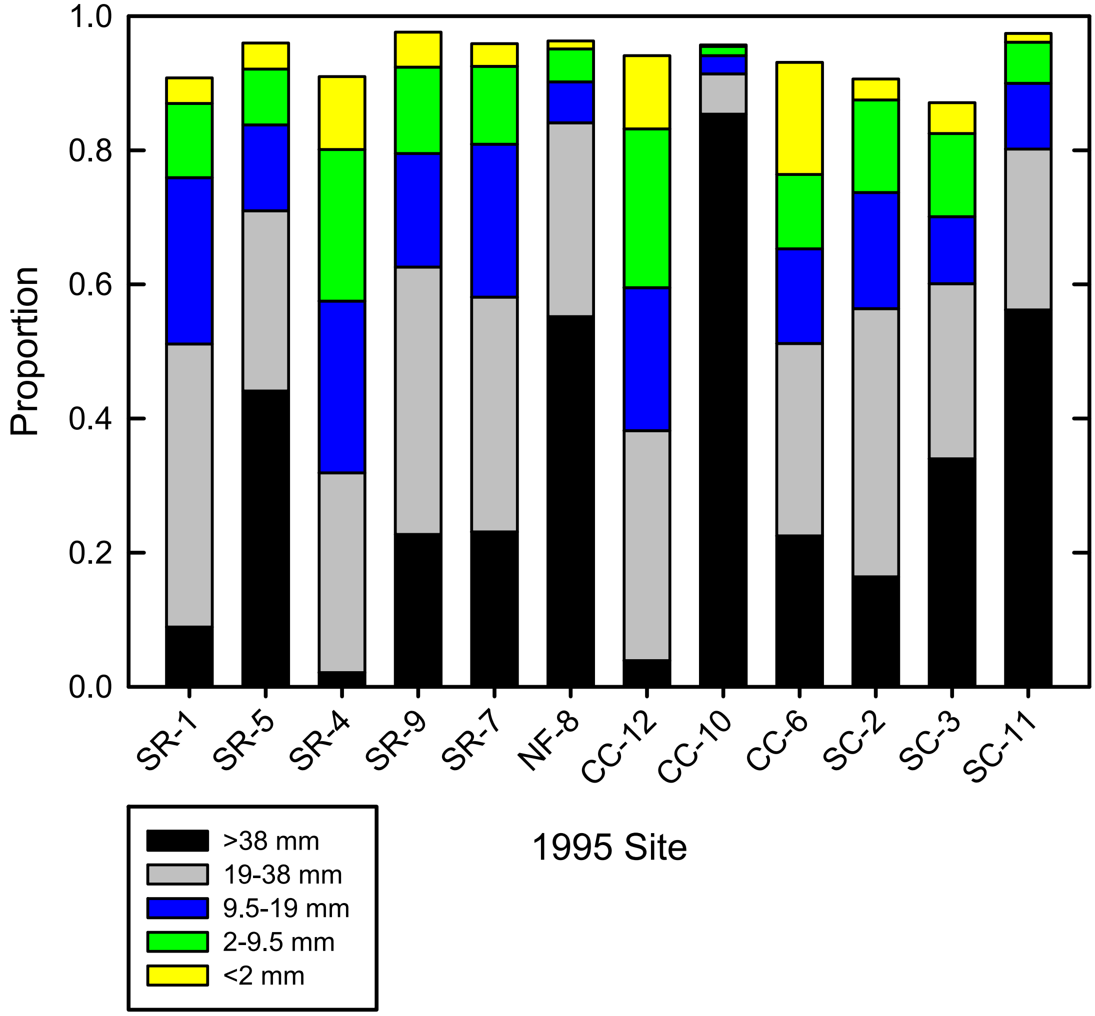

Figure 16. Mean weight-proportional substrate composition at sites on the Spring River (SR), North Fork Spring River (NF), Center Creek (CC), and Shoal Creek (SC) sampled in 1995. Within streams, sites ordered from downstream to upstream. Sites are ordered from downstream to upstream within each stream; numbers correspond to 95-x notation in the text and tables. (Note: Means were computed from multiple samples after angular transformations back-transformed to the linear scale and may not sum to 1.0). See Fig. 1 and Table 1 for additional site information.

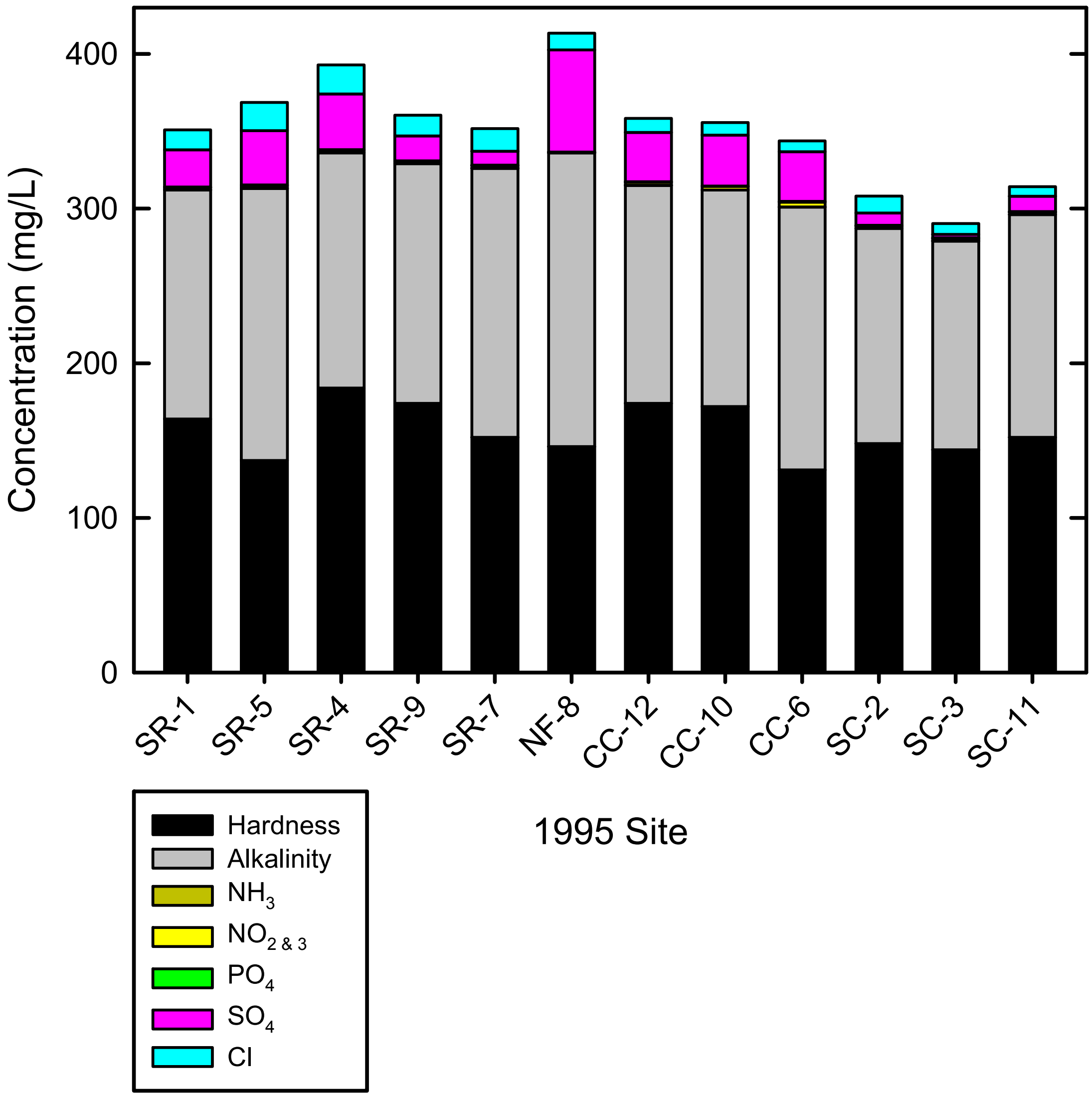

Figure 17. Mean hardness, alkalinity, ammonia-nitrogen (NH_3), nitrate + nitrite-nitrogen ($NO_{2\&3}$), phosphate (PO_4), sulfate (SO_4), and chloride (Cl) concentrations (all mg/L) in filtered surface water at sites on the Spring River (SR), North Fork Spring River (NF), Center Creek (CC), and Shoal Creek (SC) sampled in 1995. Sites are ordered from downstream to upstream within each stream; numbers correspond to 95-x notation in the text and tables. See Fig. 1 and Table 1 for additional site information.

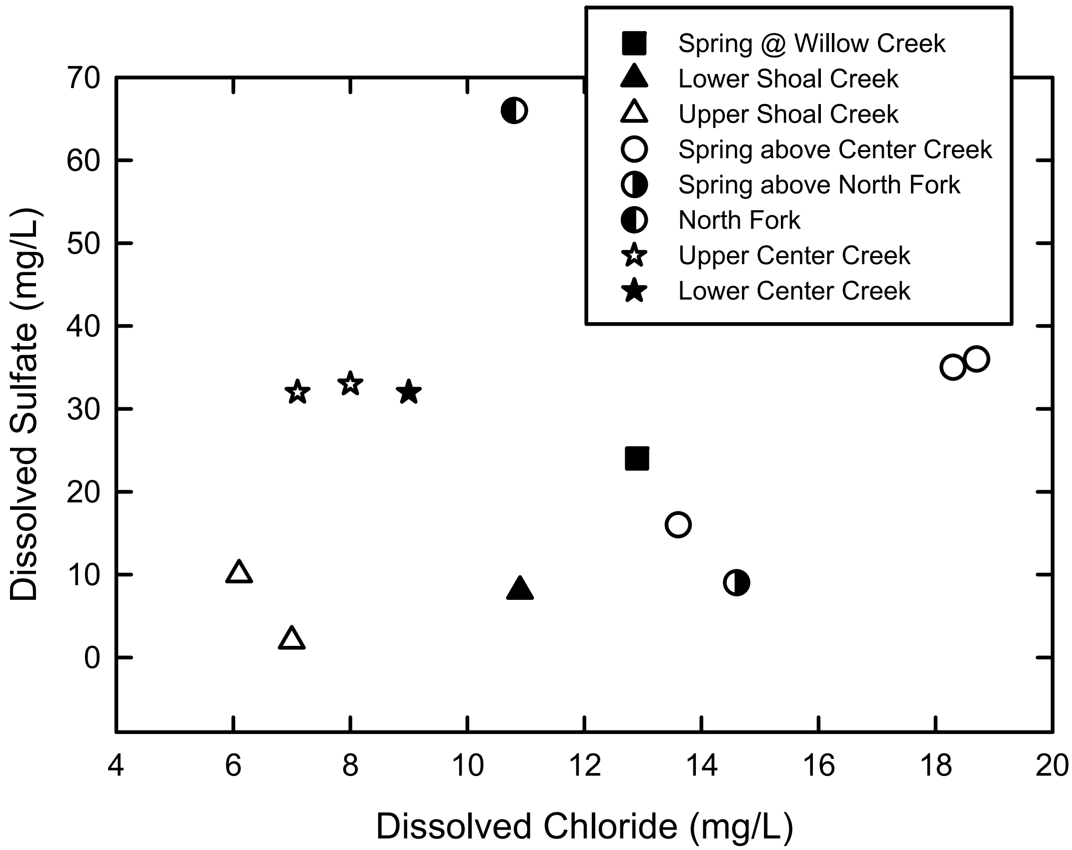

Figure 18. Concentrations of dissolved sulfate and chloride in surface water at sites in the Spring River system sampled in 1995.

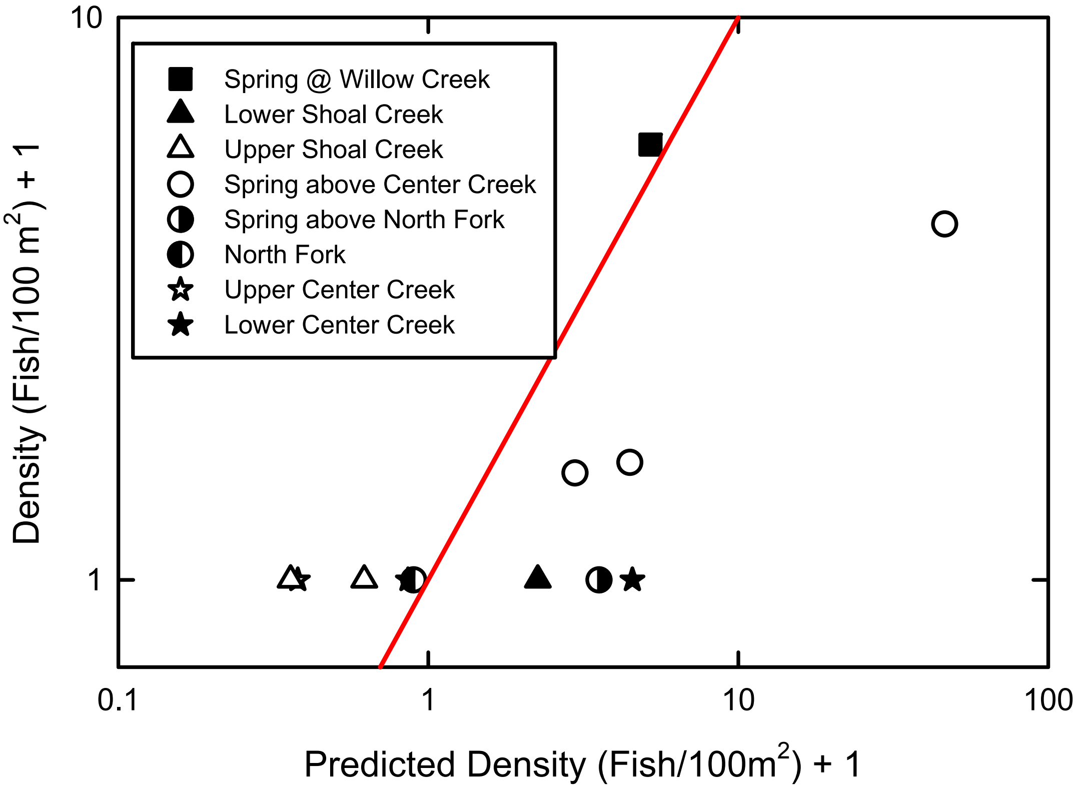

Figure 19. Measured Neosho madtom density (*Y* axis) vs. density predicted by the revised 1991 chloride model (*X* axis) at sites in the Spring River system sampled in 1995. Solid red line, $Y = X$.

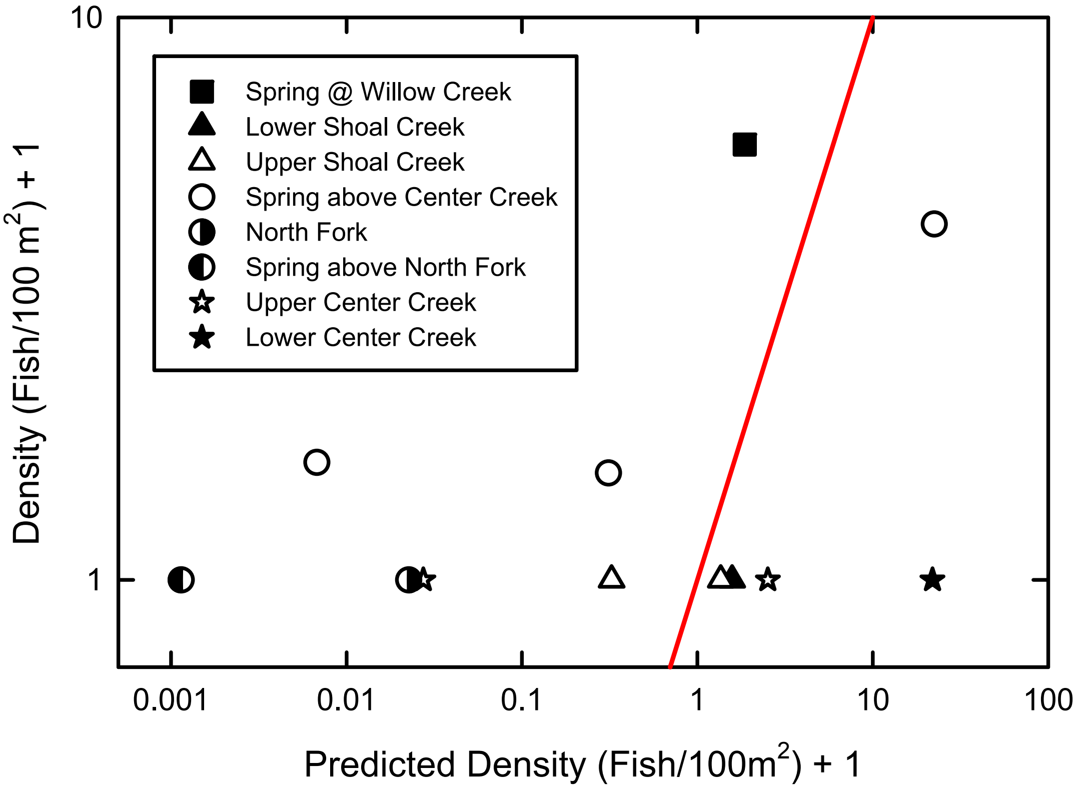

Figure 20. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991 alkalinity model (*X* axis) at sites in the Spring River system sampled in 1995. Solid red line, *Y* = *X*.

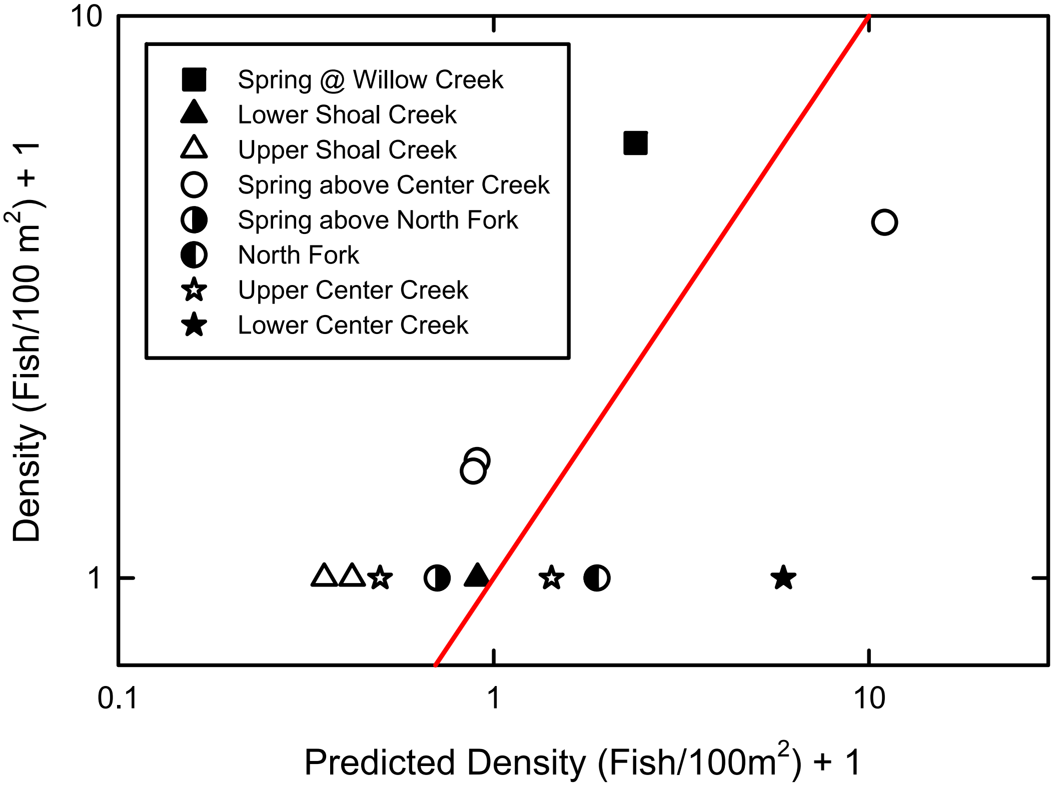

Figure 21. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 91 sulfate model (*X* axis) at sites in the Spring River system sampled in 1995. Solid red line, $Y = X$.

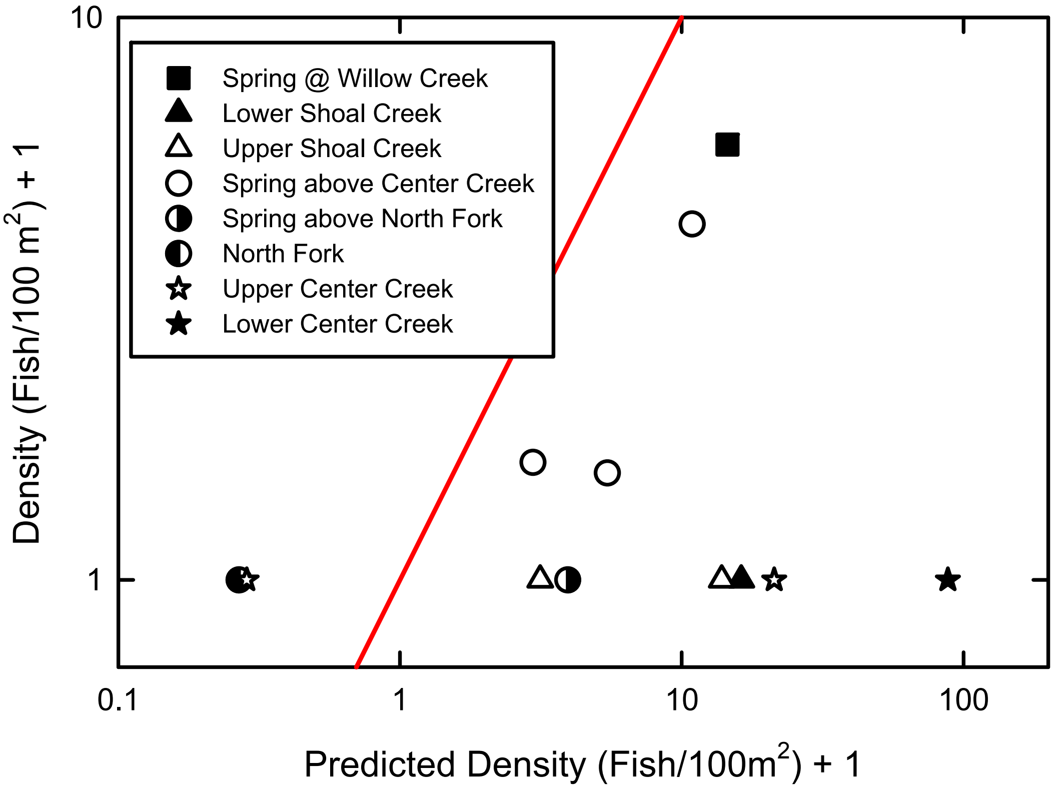

Figure 22. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991-94 three-variable model (*X* axis) at sites in the Spring River system sampled in 1995. Solid red line, *Y* = *X*.

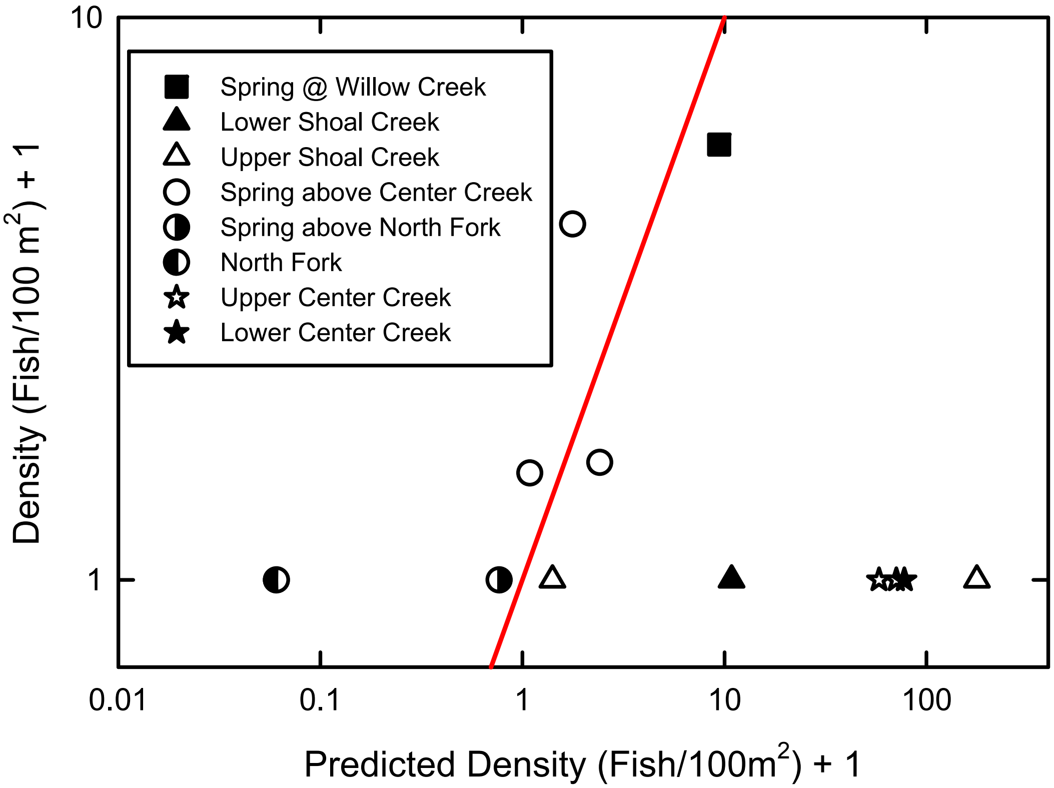

Figure 23. Measured Neosho madtom density (*Y* axis) vs. density predicted by the 1991-94 six-variable model (*X* axis) at sites in the Spring River system sampled in 1995. Solid red line, *Y* = *X*.

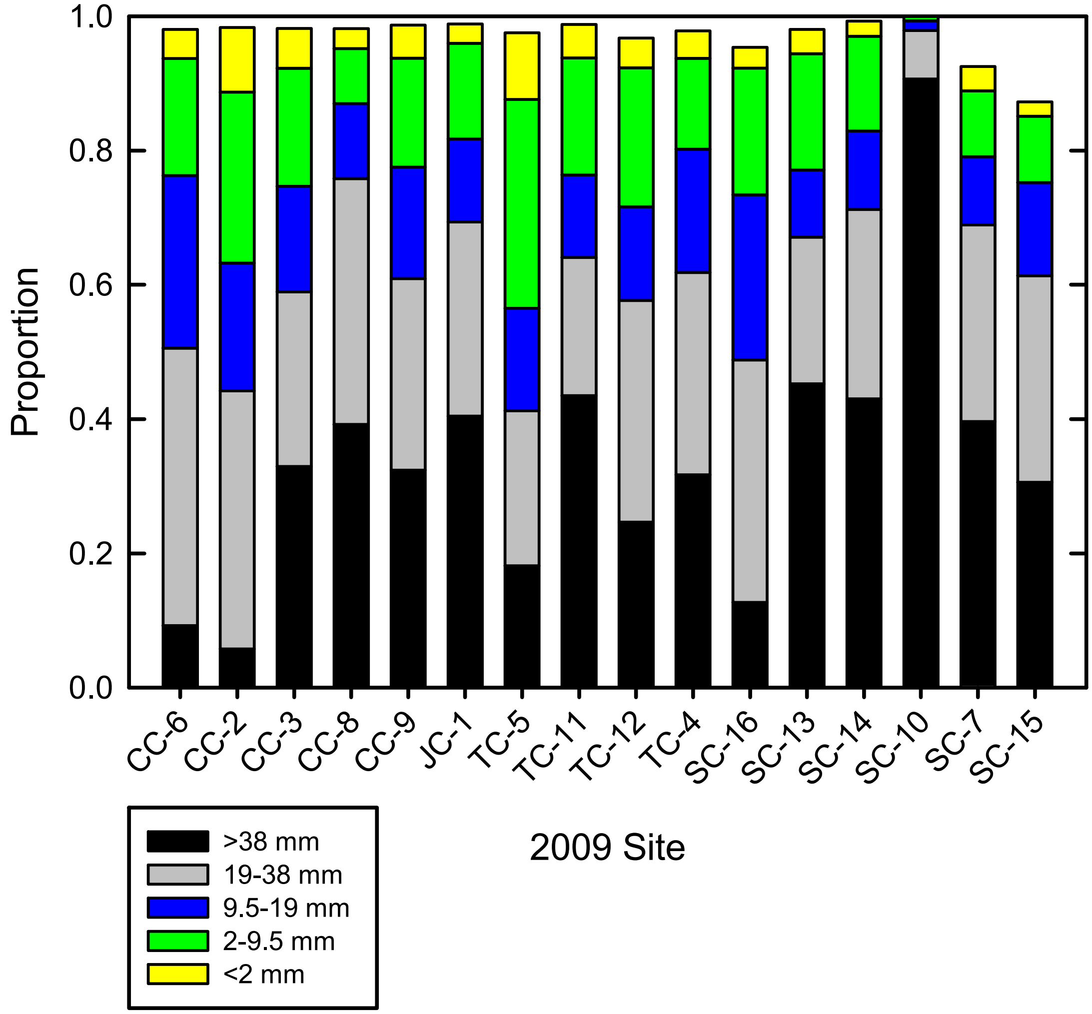

Figure 24. Mean weight-proportional substrate composition at sites on Center Creek (CC), Jenkins Creek (JC), Turkey Creek (TC), and Shoal Creek (SC) sampled in 2009. Sites are ordered from downstream to upstream within each stream; numbers correspond to 09-x notation in the text and tables. (Note: Means were computed from multiple samples after angular transformations back-transformed to the linear scale and may not sum to 1.0). See Fig. 1 and Table 1 for additional site information.

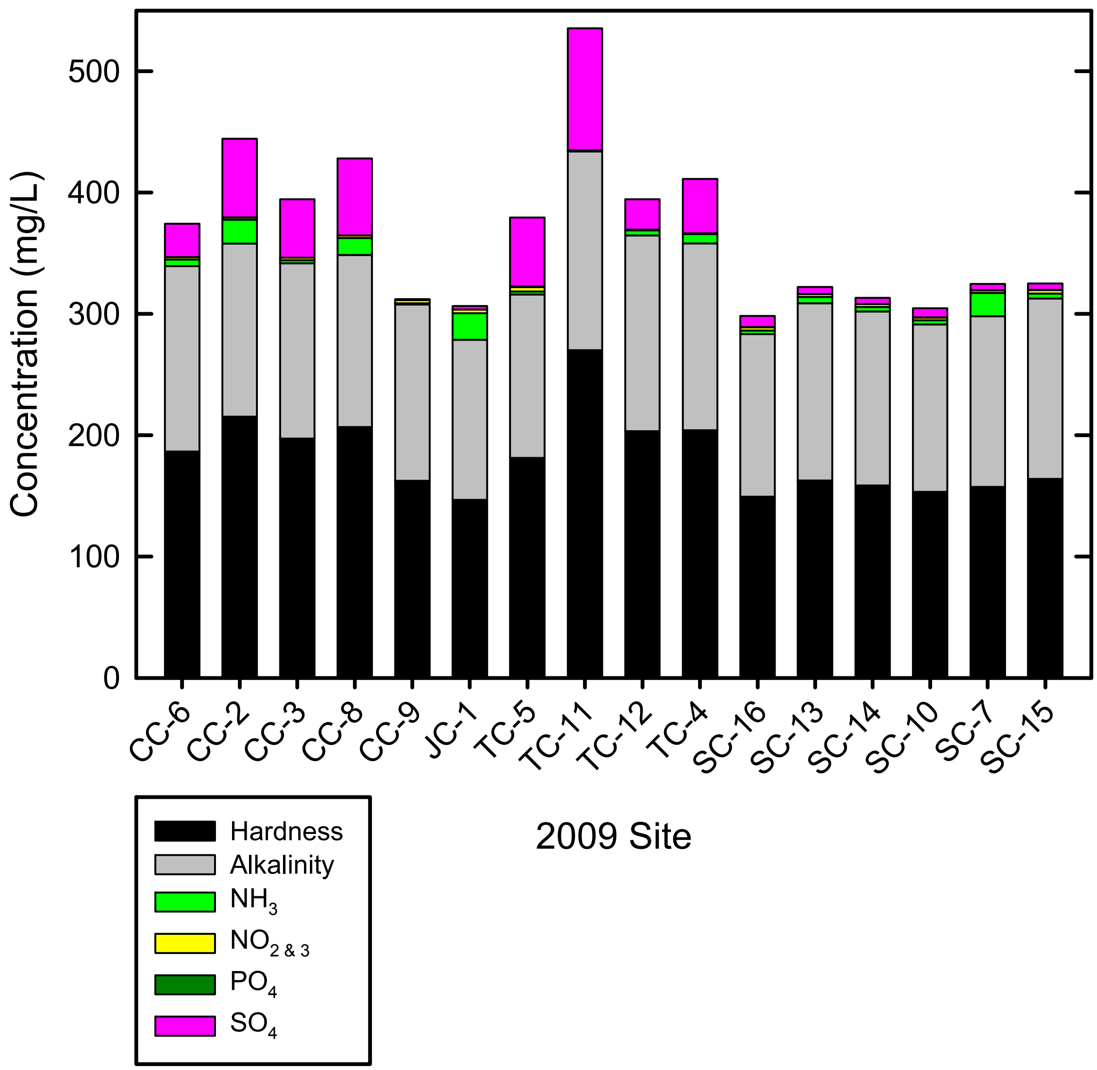

Figure 215. Mean hardness, alkalinity, ammonia-nitrogen (NH_3), nitrate + nitrite-nitrogen ($NO_{2\&3}$), phosphate (PO_4), and sulfate (SO_4) concentrations (all mg/L) in filtered surface water at sites on Center Creek (CC), Jenkins Creek (JC), Turkey Creek (TC), and Shoal Creek (SC) sampled in 2009. Sites are ordered from downstream to upstream within each stream; numbers correspond to 09-x notation in the text and tables. See Fig. 1 and Table 1 for additional site information.

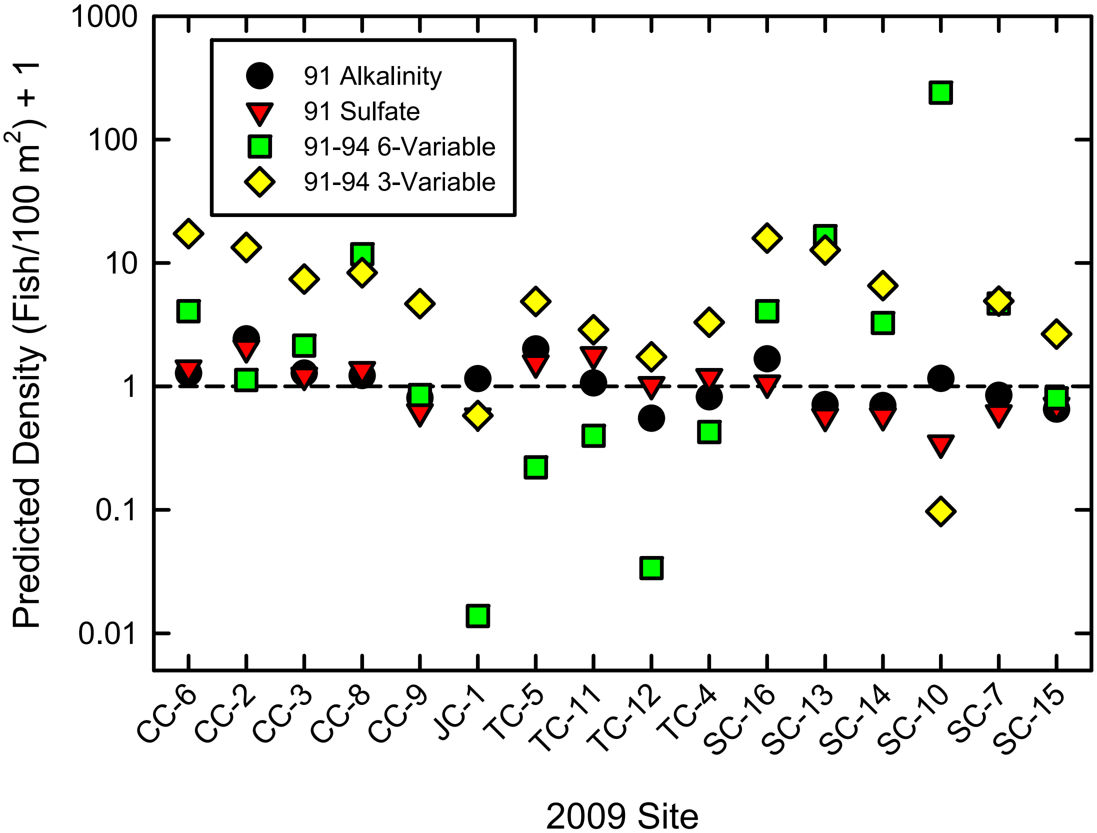

Figure 26. Neosho madtom density predicted by the 1991 alkalinity, 1991 sulfate, 1991-94 six-variable, and 1991-94 three variable models at sites on Center Creek (CC), Jenkins Creek (JC), Turkey Creek (TC), and Shoal Creek (SC) sampled in 2009. Sites are ordered from downstream to upstream within each stream; numbers correspond to 09-x notation in the text and Table 1. See Fig. 1 and Table 1 for additional site information.

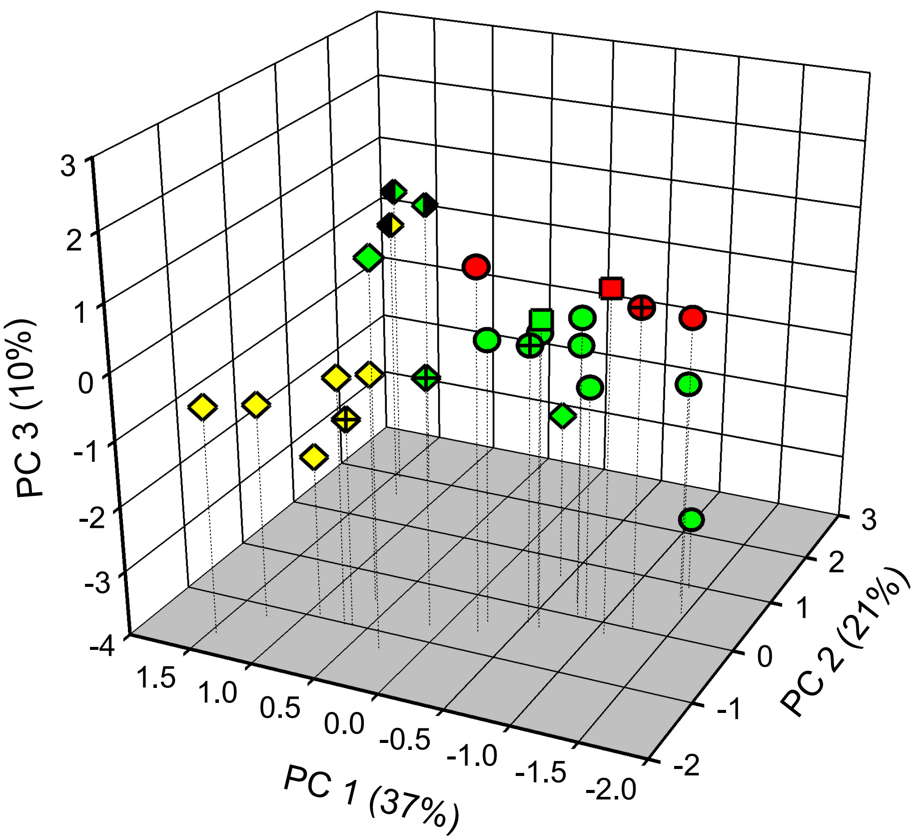

Figure 27. Scores on the first three principle components (PC 1, PC 2, PC 3) for the 26 site-years in which Neosho madtoms were present at sites in the Spring-Neosho-Cottonwood River system. Colors: Yellow, 1991; green, 1994; red, 1995. Shapes: Open diamonds, Neosho River; half-filled diamonds, Cottonwood River; squares, Spring River @ Willow Creek; circles, Spring River above Center Creek. Filled symbols (left shading, right shading, cross): Sites sampled in multiple years. Open symbols: Sites sampled only once.

Table 1. Sites sampled in 1994 (94-x sites) [a], 1995 (95-x sites) [b], and 2009 (09-x sites) [c].

Year and site	Alternate site number 1994	1995	2009	River or stream	Location	County, state	Legal	Latitude, longitude [d]
1994								
94-0A	0A	–	–	Neosho R.	E. of Oswego	Cherokee, KS	SW 1/4, Sec 13, T33S, R21E	37° 09' 56.3" N, 95° 03' 45.8" W
94-0B	0B	–	–	Neosho R.	N. of Oswego	Labette, KS	NW 1/4, Sec 15, T33S, R21E	37° 10' 34.1" N, 95° 06' 15.3" W
94-1	1	–	–	Neosho R.	NR NWR, lower	Neosho, KS	NW 1/4, Sec 32, T29S, R21E	37° 28' 33.1" N, 95° 08' 21.1" W
94-2	2	–	–	Neosho R.	NR NWR, upper	Neosho, KS	NE 1/4, Sec 31, T29S, R21E	37° 28' 50.6" N, 95° 08' 35.4" W
94-3	3	–	–	Neosho R.	NE of Burlington	Coffey, KS	SW 1/4, Sec 23, T21S, R15E	38° 12' 18.1" N, 95° 43' 47.2" W
94-4	4	–	–	Cottonwood R.	W of Emporia	Chase, KS	NW 1/4, Sec 26, T19S, R8E	38° 22' 27.3" N, 96° 29' 36.0" W
94-5	5	–	–	Cottonwood R.	W of Emporia	Chase, KS	SW 1/4, Sec 25, T19S, R8E	38° 21' 50.6" N, 96° 28' 41.2" W
94-6	6	–	–	Neosho R.	S of Humbolt	Allen, KS	SW 1/4, Sec 4, T26S, R18E	37° 48' 36.4" N, 95° 26' 50.1" W
94-6A	6A	–	–	Neosho R.	S of Humbolt	Allen, KS	NW 1/4, Sec 9, T26S, R18E	37° 47' 57.1" N, 95° 26' 48.5" W
94-7A	7A	–	–	Neosho R.	E of Emporia	Lyon, KS	NW 1/4, Sec 23, T19S, R12E	38° 23' 27.1" N, 96° 03' 26.0" W
94-7B	7B	–	–	Neosho R.	E of Emporia	Lyon, KS	NE 1/4, Sec 23, T19S, R12E	38° 23' 12.1" N, 96° 02' 57.6" W
94-8	8	–	–	Spring R.	Below I-44	Ottawa, OK	NE 1/4, Sec 8, T28N, R24E	36° 55' 27.5" N, 94° 44' 26.0" W
94-9	9	–	–	Spring R.	NE of Quapaw	Ottawa, OK	SW 1/4, Sec 28, T29N, R24E	36° 57' 40.3" N, 94° 43' 21.1" W
94-10	10	–	–	Spring R.	Above KS-OK line	Cherokee, KS	NE 1/4, Sec 18, T35S, R25E	37° 00' 07.4" N, 94° 42' 52.0" W
94-11	11	–	–	Spring R.	S of R.ton	Cherokee, KS	SE 1/4, Sec 19, T34S, R25E	37° 03' 54.2" N, 94° 42' 21.7" W
94-12	12	–	–	Spring R.	Above Hwy 96	Cherokee, KS	SW 1/4, Sec 11, T33S, R25E	37° 10' 52.2" N, 94° 38' 36.0" W
94-13	13	4	–	Spring R.	Above Hwy 96	Cherokee, KS	SW 1/4, Sec 11, T33S, R25E	37° 10' 56.7" N, 94° 38' 40.7" W
94-14	14	–	–	Spring R.	Above Hwy 96	Cherokee, KS	SW 1/4, Sec 11, T33S, R25E	37° 10' 46.1" N, 94° 38' 32.4" W
94-15	15	–	13	Shoal Creek	Schermerhorn Park	Cherokee, KS	NW 1/4, Sec 35, T34S, R25E	37° 02' 30.0" N, 94° 38' 22.0" W
94-16	16	–	–	Spring R.	Above KS-OK line	Cherokee, KS	NE 1/4, Sec 18, T35S, R25E	36° 59' 57.3" N, 94° 42' 47.2" W
94-17	17	12	6	Center Creek	Nr mouth	Jasper, MO	SW 1/4, Sec 14, T28N, R34W	37° 09' 05.1" N, 94° 36' 59.6" W
94-18	18	–	–	Spring R.	Blw Hwy 96	Cherokee, KS	NW 1/4, Sec 24, T33S, R25E	37° 09' 34.0" N, 94° 37' 47.3" W
94-19	19	–	–	Spring R.	Blw Hwy 96	Cherokee, KS	NE 1/4, Sec 14, T33S, R25E	37° 10' 23.7" N, 94° 38' 23.0" W
94-20	20	2	16	Shoal Creek	SW of Galena (Sprague)	Cherokee, KS	NW 1/4, Sec 34, T34S, R25E	37° 02' 36.8" N, 94° 39' 26.1" W
94-21	21	9	–	Spring R.	E of Waco	Jasper, MO	NE 1/4, Sec 18, T29N, R33W	37° 14' 35.4" N, 94° 34' 00.9" W
94-22	22	–	–	Spring R.	S of Waco	Jasper, MO	NE 1/4, Sec 35, T29N, R34W	37° 12' 03.8" N, 94° 36' 25.7" W
94-23	23	–	–	Spring R.	S of Waco	Jasper, MO	SE 1/4, Sec 23, T29N, R34W	37° 13' 19.9" N, 94° 36' 03.1" W
94-24	24	–	–	Spring R.	S of Waco	Jasper, MO	NW 1/4, Sec 26, T29N, R34W	37° 12' 55.2" N, 94° 36' 28.6" W
94-25	25	–	–	Spring R.	SE of Lawton	Cherokee, KS	NE 1/4, Sec 1, T33S, R25E	37° 11' 58.2" N, 94° 37' 32.7" W
94-26	26	–	–	Spring R.	SE of Lawton	Cherokee, KS	SE 1/4, Sec 25, T33S, R25E	37° 08' 17.6" N, 94° 37' 13.2" W
94-27	27	–	–	Spring R.	NW of Belleville	Cherokee, KS	NW 1/4, Sec 36, T33S, R25E	37° 07' 58.8" N, 94° 37' 40.1" W

94-28	28	–	5	Turkey Creek	Near mouth	Cherokee, KS	NW 1/4, Sec 36, T33S, R25E	37° 07' 44.9" N, 94° 37' 32.5" W
94-29	29	1	–	Spring R.	N of Baxter Springs	Cherokee, KS	NE 1/4, Sec 36, T34S, R24E	37° 02' 42.6" N, 94° 43' 35.7" W
1995	–	–	–					
95-1	29	1	–	Spring R.	N of Baxter Springs	Cherokee, KS	NE 1/4, Sec 36, T34S, R24E	37° 02' 42.0" N, 94° 43' 35.4" W
95-2	20	2	16	Shoal Creek	SW of Galena (Sprague)	Cherokee, KS	NW 1/4, Sec 34, T34S, R25E	37° 02' 33.2" N, 94° 39' 23.9" W
95-3	–	3	10	Shoal Creek	Above WWTP	Newton, MO	NE 1/4, Sec 25, T27N, R34W	37° 02' 07.7" N, 94° 35' 14.2" W
95-4	13	4	–	Spring R.	Above Hwy 96	Cherokee, KS	SW 1/4, Sec 11, T33S, R25E	37° 10' 45.9" N, 94° 38' 32.5" W
95-5	27	5	–	Spring R.	W of MO-KS line	Cherokee, KS	NW 1/4, Sec 36, T33S, R25E	37° 07' 57.3" N, 94° 37' 39.4" W
95-6	–	6	–	Center Creek	Blw. Hwy 171	Jasper, MO	NE 1/4, Sec 09, T28N, R33W	37° 10' 00.0" N, 94° 32' 10.1" W
95-7	–	7	–	Spring R.	NW of Galesburg	Jasper, MO	NW 1/4, Sec 10, T29N, R33W	37° 16' 18.2" N, 94° 31' 11.2" W
95-8	–	8	–	North Fork	E of Hwy 43	Jasper, MO	SE 1/4, Sec 01, T29N, R34W	37° 16' 22.4" N, 94° 28' 06.6" W
95-9	21	9	–	Spring R.	E of Waco	Jasper, MO	NE 1/4, Sec 18, T29N, R33W	37° 14' 33.2" N, 94° 34' 00.3" W
95-10	–	10	–	Center Creek	Blw. Hwy JJ	Jasper, MO	SE 1/4, Sec 12, T28S, R34W	37° 09' 43.3" N, 94° 35' 03.9" W
95-11	–	11	–	Shoal Creek	Blw. Hwy P	Newton, MO	NE 1/4, Sec 29, T27N, R34W	37° 02' 07.0" N, 94° 33' 34.3" W
95-12	17	12	6	Center Creek	Nr. mouth	Jasper, MO	SW 1/4, Sec 14, T28N, R34W	37° 09' 06.2" N, 94° 36' 58.5" W
2009								
09-1	–	–	1 (J1)	Jenkins Creek	Jenkins Creek	Jasper, MO	–	37° 04’ 34.9” N, 94° 15’ 37.8” W
09-2	–	–	2 (C4)	Center Creek	Carl Junction Park	Jasper, MO	–	37° 10’ 03.1” N, 94° 32’ 21.0” W
09-3	–	–	3 (C3)	Center Creek	Blw. CR230 (Oronogo)	Jasper, MO	–	37° 10’ 47.3” N, 94° 28’ 44.8” W
09-4	–	–	4 (T1)	Turkey Creek	Quail Drive	Jasper, MO	–	37° 05’ 25.6” N, 94° 27’ 25.1” W
09-5	28	–	5 (T4)	Turkey Creek	Nr. mouth	Cherokee, KS	–	37° 07’ 44.5” N, 94° 37’ 33.0” W
09-6	17	12	6 (C5)	Center Creek	Nr. mouth	Jasper, MO	–	37° 09’ 06.0” N, 94° 36’ 43.0” W
09-7	–	–	7 (S2)	Shoal Creek	Wildcat Glade	Newton, MO	–	37° 01’ 24.1” N, 94° 31’ 04.5” W
09-8	–	–	8 (C2)	Center Creek	Above CR230	Jasper, MO	–	37° 10’ 49.0” N, 94° 27’ 51.8” W
09-9	–	–	9 (C1)	Center Creek	Dogwood Rd.	Jasper, MO	–	37° 06’ 47.3” N, 94° 18' 01.5” W
09-10	–	3	10 (S3)	Shoal Creek	Above WWTP	Newton, MO	–	37° 02’ 07.6” N, 94° 35’ 14.3” W
09-11	–	–	11 (T3)	Turkey Creek	Schifferdecker Rd.	Jasper, MO	–	37° 06’ 50.9” N, 94° 32’ 43.6” W
09-12	–	–	12 (T2)	Turkey Creek	Soccer Field	Jasper, MO	–	37° 06’ 39.1” N, 94° 31’ 12.6” W
09-13	15	–	13 (S5)	Shoal Creek	Martin	Cherokee, KS	–	37° 02’ 28.1” N, 94° 39” 00.2” W
09-14	–	–	14 (S4)	Shoal Creek	SW of Galena (Scorse)	Cherokee, KS	–	37° 02’ 23.7” N, 94° 36’ 27.1” W
09-15	–	–	15 (S1)	Shoal Creek	E of Galena (Wright)	Newton, MO	–	36° 56’ 37.1” N, 94° 17’ 59.2” W
09-16	20	2	16 (S6)	Shoal Creek	SW of Galena (Sprague)	Cherokee, KS	–	37° 02’ 35.4” N, 94° 39’ 27.0” W

[a] From Schmitt and others (1997)
[b] From Allert and others. (1997)
[c] From Allert and others. (2011)
[d] World Geodetic System, 1984 (WGS84)

Table 2. Mean values of habitat variables for sites where Neosho madtoms were either not captured in 1991 (91-x sites), 1994 (94-x sites), or 1995 (95x- sites) or where fish were not sampled in 2009 (09-x sites) relative to the range for sites where Neosho madtoms were present (the occurrence envelope) during 1991−95. Values in **red** are equal to or below the minimum, those in **blue** equal to or above the maximum. Cond, specific conductance; Alk, alkalinity; $NO_{2\&3}$, nitrate + nitrate N; TP, total phosphorous; NH_3, ammonia N; nd, not determined. See Fig. 1 and Table 1 for additional site information.

Site	Weight proportion (mm)								Depth (m)	Velocity (m/sec)	pH	Turbidity (NTU)	Cond (mS/cm)	Alk (mg/L)	Hardness (mg/L)	$NO_{2\&3}$ (mg/L)	TP (mg/L)	NH_3 (mg/L)	Chloride (mg/L)	Sulfate (mg/L)
	$p_{>37.5}$	$p_{19–37.5}$	$p_{9.5–19}$	$p_{2–9.5}$	$p_{<2}$	$p_{<9.5}$	$p_{<19}$	$p_{<37.5}$												
91-0B	0.40	0.17	0.16	0.14	0.12	0.26	0.42	0.60	0.25	0.38	8.4	20.0	0.440	147	190	0.10	1.75	0.125	8.0	50.0
91-HB	0.69	**0.11**	0.11	0.05	0.04	0.09	0.19	0.31	1.09	**0.01**	**8.7**	25.0	0.460	150	188	0.00	3.50	0.063	14.0	50.0
91-HD	0.03	0.18	0.36	0.21	0.23	0.43	0.80	0.97	0.55	**0.07**	8.4	70.0	0.400	171	170	0.00	1.50	0.375	**5.0**	25.0
94-2	0.01	0.27	0.40	0.29	0.10	0.38	0.78	**1.00**	0.60	**0.02**	8.3	**75.0**	0.412	134	184	2.34	0.13	0.021	14.3	54.7
94-8	0.19	0.34	0.25	0.22	**0.01**	0.23	0.48	0.81	0.42	0.26	7.9	12.0	0.332	**126**	148	0.73	0.31	0.015	11.0	24.9
94-9	0.59	0.29	0.09	0.06	**0.02**	0.08	0.17	0.46	0.43	0.58	nd	**4.5**	nd	**120**	148	1.06	0.20	0.025	13.0	38.5
94-10	0.29	0.32	0.17	0.16	0.07	0.23	0.40	0.72	0.46	0.62	**8.6**	8.0	0.347	**124**	154	1.08	0.26	0.024	12.9	34.7
94-11	0.27	0.22	0.16	0.14	0.06	0.21	0.37	0.59	0.32	0.41	8.2	24.0	0.363	**130**	156	1.15	0.26	0.036	13.0	37.8
94-13	0.44	0.36	0.08	0.05	**0.01**	0.06	0.14	0.50	0.34	0.59	7.7	27.0	0.395	146	182	0.93	0.30	0.051	14.7	39.1
94-15	0.68	0.20	0.08	0.08	0.03	0.11	0.19	0.39	0.52	0.40	7.8	17.0	0.316	176	142	1.37	0.50	0.033	10.5	18.6
94-16	0.26	0.43	0.13	0.07	0.03	0.10	0.23	0.66	0.34	0.68	8.2	8.0	0.366	134	164	1.29	0.41	0.028	15.3	38.6
94-17	0.02	0.34	0.27	0.26	0.10	0.36	0.63	0.97	0.37	0.67	8.0	**3.5**	0.430	134	184	6.62	0.24	0.026	23.0	43.4
94-20	0.39	0.36	0.15	0.08	**0.02**	0.10	0.25	0.61	0.41	**0.72**	7.7	7.9	0.335	138	152	1.97	0.74	0.033	8.3	16.0
94-25	0.34	0.48	0.13	0.04	**0.02**	0.07	0.20	0.67	0.36	0.49	8.3	13.0	0.419	148	162	1.26	0.26	0.068	15.4	20.6
94-26	0.57	0.24	0.10	0.06	**0.02**	0.08	0.18	0.42	0.38	0.44	7.7	9.0	0.428	128	176	2.45	0.24	0.033	22.8	37.3
94-27	0.29	0.35	0.18	0.15	0.08	0.23	0.41	0.75	0.37	0.58	7.5	10.0	0.439	146	188	2.62	0.44	0.026	24.3	42.4

Site	Weight proportion (mm)								Depth (m)	Velocity (m/sec)	pH	Turbidity (NTU)	Cond (mS/cm)	Alk (mg/L)	Hardness (mg/L)	$NO_{2\&3}$ (mg/L)	TP (mg/L)	NH_3 (mg/L)	Chloride (mg/L)	Sulfate (mg/L)
	$p_{>37.5}$	$p_{19-37.5}$	$p_{9.5-19}$	$p_{2-9.5}$	$p_{<2}$	$p_{<9.5}$	$p_{<19}$	$p_{<37.5}$												
94-28	0.15	0.26	0.18	0.30	0.08	0.38	0.57	0.83	**0.16**	0.37	7.4	**5.0**	0.642	166	224	2.23	2.46	0.042	13.8	86.5
95-2	0.16	0.40	0.17	0.14	0.03	0.17	0.34	0.74	1.30	**0.98**	8.1	8.0	**0.295**	139	148	1.60	0.52	0.062	10.9	8.0
95-3	0.34	0.26	0.10	0.12	0.05	0.17	0.27	0.53	1.20	**0.87**	8.2	9.5	**0.270**	135	144	1.40	0.76	0.035	7.0	2.0
95-6	0.23	0.29	0.14	0.11	0.17	0.28	0.42	0.71	1.01	**0.83**	7.9	4.5	0.490	170	**131**	**3.10**	0.47	0.060	7.1	32.0
95-7	0.23	0.35	0.23	0.12	0.03	0.15	0.38	0.73	1.57	0.48	7.9	7.2	**0.300**	174	152	1.60	0.35	0.134	14.6	9.0
95-8	0.55	0.29	0.06	0.05	**0.01**	0.06	0.12	0.41	0.43	0.34	7.6	11.0	0.360	190	146	0.30	0.13	0.083	10.8	66.0
95-10	0.85	**0.06**	**0.03**	**0.01**	**<0.01**	**0.02**	0.04	0.10	0.96	0.36	8.0	**5.0**	0.600	140	172	**2.30**	0.22	0.030	8.0	33.0
95-11	0.56	0.24	0.10	0.06	**0.01**	0.07	0.17	0.41	1.28	0.80	8.3	7.0	0.550	144	152	1.50	0.45	0.034	6.1	10.0
95-12	0.04	0.34	0.21	0.24	0.11	0.35	0.56	0.90	0.97	0.73	7.6	**4.0**	0.650	141	174	**2.00**	0.25	0.021	9.0	32.0
09-1	0.40	0.29	0.12	0.14	0.03	0.17	0.30	0.58	**0.17**	0.27	7.7	**3.6**	0.316	**132**	147	**2.97**	**0.03**	0.022	nd	3.1
09-2	0.06	0.38	0.19	0.25	0.10	0.35	0.54	0.93	0.28	0.64	7.8	15.6	0.447	143	215	**1.90**	0.14	**0.020**	nd	64.7
09-3	0.33	0.26	0.16	0.18	0.06	0.23	0.39	0.65	0.22	0.64	7.8	15.3	0.411	144	197	**1.97**	**0.07**	**0.003**	nd	48.2
09-4	0.32	0.30	0.18	0.14	0.04	0.18	0.36	0.66	0.14	0.32	7.7	8.6	0.438	154	204	0.70	**0.03**	**0.008**	nd	44.7
09-5	0.18	0.23	0.15	0.31	0.10	0.41	0.56	0.79	0.19	0.41	7.8	0.4	0.496	135	181	3.61	0.57	**0.002**	nd	56.8
09-6	0.09	0.41	0.26	0.17	0.04	0.22	0.47	0.89	0.21	0.65	8.2	9.8	0.391	153	187	1.97	0.07	**0.006**	nd	27.4
09-7	0.40	0.29	0.10	0.10	0.04	0.13	0.24	0.53	0.43	**0.87**	8.0	19.8	0.338	141	157	2.13	0.16	**0.019**	nd	5.2
09-8	0.39	0.37	0.11	0.08	0.03	0.11	0.22	0.59	0.24	**0.80**	7.8	17.5	0.430	142	207	1.98	**0.06**	**0.014**	nd	63.5
09-9	0.32	0.29	0.17	0.16	0.05	0.21	0.38	0.66	0.22	0.63	8.1	11.8	0.331	145	162	2.66	**0.05**	**0.001**	nd	0.7
09-10	**0.91**	**0.07**	**0.01**	**0.00**	**0.00**	**0.00**	**0.02**	**0.09**	0.40	**0.82**	8.0	14.2	0.331	138	153	2.26	0.18	**0.003**	nd	7.4

Site	Weight proportion (mm)								Depth (m)	Velocity (m/sec)	pH	Turbidity (NTU)	Cond (mS/cm)	Alk (mg/L)	Hardness (mg/L)	$NO_{2\&3}$ (mg/L)	TP (mg/L)	NH_3 (mg/L)	Chloride (mg/L)	Sulfate (mg/L)
	$p_{>37.5}$	$p_{19–37.5}$	$p_{9.5–19}$	$p_{2–9.5}$	$p_{<2}$	$p_{<9.5}$	$p_{<19}$	$p_{<37.5}$												
09-11	0.43	0.21	0.12	0.17	0.05	0.22	0.35	0.55	**0.16**	0.28	7.9	**0.8**	0.558	164	270	0.30	**0.01**	**0.001**	nd	100.4
09-12	0.25	0.33	0.14	0.21	0.04	0.25	0.39	0.72	**0.11**	0.30	7.9	**1.0**	0.430	161	203	0.43	**0.03**	**0.004**	nd	25.2
09-13	0.45	0.22	0.10	0.17	0.04	0.21	0.31	0.53	0.34	**1.07**	7.8	11.1	0.363	146	163	**2.23**	0.22	**0.005**	nd	5.8
09-14	0.43	0.28	0.12	0.14	**0.02**	0.16	0.28	0.56	0.25	**0.83**	8.2	9.5	0.361	143	159	**2.31**	0.24	**0.004**	nd	5.0
09-15	0.31	0.31	0.14	0.10	**0.02**	0.12	0.26	0.57	0.24	0.52	7.9	13.2	0.365	149	164	**2.88**	0.17	**0.004**	nd	5.3
09-16	0.13	0.36	0.25	0.19	0.03	0.22	0.47	0.83	0.29	**0.72**	8.0	12.5	0.345	134	149	**2.67**	0.26	**0.003**	nd	9.1
Minimum	**<0.01**	**0.12**	**0.03**	**0.03**	**0.02**	**0.05**	**0.09**	**0.21**	**0.19**	**0.07**	**7.3**	**5.7**	**0.310**	**132**	**137**	**<0.01**	**0.08**	**0.020**	**7.0**	**13.9**
Maximum	**0.87**	**0.61**	**0.49**	**0.32**	**0.29**	**0.52**	**0.85**	**1.00**	**1.49**	**0.69**	**8.6**	**70.0**	**0.780**	**202**	**350**	**1.90**	**4.00**	**0.490**	**37.8**	**145.0**

Table 3. Results of principal components analysis of habitat variables for sites in the Spring-Neosho-Cottonwood basin (*n* = 26) where Neosho madtoms were collected during 1991, 1994, and 1995. Shown for each variable are the mean, standard deviation (SD), and communality. Also shown are the eigenvalues, relative loadings of each variable, and percentages (individual and cumulative) of the total variation explained by the first five principal components (PC1−PC5). Substrate particle size (mm) proportions (p) $\log_{10}$-transformed. $NO_{2\&3}$, nitrate + nitrate N; P, phosphorous; N, nitrogen; nd, not determined.

Variable	Mean	SD	Communality	PC1	PC2	PC3	PC4	PC5
$p_{>38}$	−1.081	0.599	0.8266	−0.4768	0.6009	−0.4521	0.1217	0.1376
$p_{19–38}$	−0.549	0.189	0.8526	−0.3625	0.3452	0.5539	0.3273	−0.4337
$p_{9.5–19}$	−0.674	0.238	0.9127	0.5676	−0.5496	0.4295	0.2563	−0.1954
$p_{2–9.5}$	−0.810	0.247	0.8112	0.6513	−0.4161	0.2965	−0.3297	0.1313
$p_{<2}$	−1.075	0.318	0.8305	0.7548	−0.3581	0.2556	−0.2532	0.0557
Depth (m)	0.49	0.37	0.8978	−0.5484	−0.0347	0.4339	0.2737	0.5768
Velocity (m/s)	0.40	0.17	0.7989	−0.6731	0.0055	0.1771	−0.2469	0.5035
pH	8.07	0.32	0.9037	0.5873	0.0693	−0.0564	0.7290	0.1391
Turbidity (NTU)	27.8	18.7	0.7707	0.7606	−0.2485	−0.0434	−0.3238	0.1538
Specific conductance (mS/cm)	0.446	0.138	0.9673	0.6928	0.6905	0.0603	−0.0336	−0.0754
Alkalinity (mg/L)	153.5	16.6	0.7852	0.3389	0.6822	0.2615	−0.0553	0.3655
Hardness (mg/L)	198.1	64.2	0.9583	0.5982	0.7540	0.1713	−0.0181	−0.0479
$NO_{2\&3}$ (mg/L)	0.802	0.683	0.7783	−0.7385	−0.1738	0.4308	0.0893	−0.0960
Total P (mg/L)	0.766	0.930	0.7698	0.5135	−0.4573	−0.4254	0.3271	0.0950
Ammonia N (NH_3, mg/L)	0.144	0.143	0.6717	0.5833	−0.1736	0.0227	0.4475	0.3172
Sulfate (mg/L)	49.8	38.6	0.9155	0.6462	0.6887	0.1070	−0.1102	0.0010
Eigenvalue	–	–	–	5.8717	3.4059	1.5534	1.4646	1.1552
Proportion	–	–	–	0.367	0.213	0.097	0.092	0.072
Cum. proportion	–	–	–	0.367	0.580	0.677	0.768	0.841

Table 4. Scores on the first five principal components (PC 1–PC 5) for sites where Neosho madtoms were either not captured (1991, 1994, 1995; 91-xx, 94-xx, and 95-xx sites) or where fish were not sampled (2009; 09-xx sites) relative to the range of scores for sites where Neosho madtoms were present during 1991−95. Values in **red** are equal to or below the minimum, those in **blue** equal to or above the maximum. See Fig. 1 and Table 1 for additional site information.

Site	PC 1	PC 2	PC 3	PC 4	PC 5
91-0B	0.269	-0.004	-1.712	0.352	0.349
91-HB	0.150	0.296	**-3.166**	2.256	1.041
91-HD	1.342	-0.978	-0.256	0.494	0.882
94-2	0.510	-1.148	1.367	-0.259	-1.469
94-8	-0.832	-0.329	-0.732	0.285	-1.535
94-10	-0.746	-0.252	-0.377	0.712	-0.176
94-11	-0.538	-0.271	-0.898	-0.110	-0.482
94-13	-1.339	0.876	-1.367	-0.257	-0.315
94-15	-1.159	0.563	-1.290	-0.304	0.634
94-16	-1.227	0.333	-0.566	0.572	-0.551
94-17	-1.433	-0.912	**3.214**	-0.094	-1.261
94-20	**-1.619**	0.029	-0.459	-0.012	-0.335
94-25	-1.143	0.666	-0.732	1.225	-0.790
94-26	**-1.543**	0.430	-1.082	-0.009	-1.015
94-27	-1.057	0.091	0.628	-0.899	-0.684
94-28	-0.003	0.420	0.152	-1.052	-0.727
95-2	**-1.652**	-0.326	0.830	0.501	1.707
95-3	**-1.554**	-0.340	-0.279	0.337	1.885
95-6	-1.284	0.134	1.412	-0.307	1.602
95-7	-1.247	0.056	1.062	0.975	1.530
95-8	-1.101	1.548	-1.580	-0.283	0.297
95-10	**-2.086**	1.564	**-3.488**	0.757	1.015
95-11	**-1.636**	0.685	-0.651	1.039	1.721
95-12	-0.818	-0.125	1.670	-0.960	0.256
09-1	**13.657**	**-8.020**	1.775	**46.350**	**40.016**

Site	PC 1	PC 2	PC 3	PC 4	PC 5
09-2	**13.137**	**-6.979**	**3.095**	**40.879**	**37.063**
09-3	0.919	-0.708	0.275	**4.510**	**4.626**
09-4	**4.792**	**-2.157**	0.190	**15.728**	**13.470**
09-5	0.938	-1.115	0.874	**4.463**	**3.578**
09-6	**3.004**	-1.944	1.392	**11.804**	**9.882**
09-7	**11.822**	**-6.846**	1.632	**40.733**	**37.581**
09-8	**8.365**	**-4.195**	1.258	**29.005**	**26.453**
2009-9	-0.565	-0.587	0.252	**1.855**	1.480
09-10	-0.830	0.439	**-4.339**	**7.835**	**7.698**
09-11	0.470	1.226	-0.807	0.562	0.629
09-12	**2.512**	-0.941	-0.129	**8.579**	**7.103**
09-13	**2.073**	**-1.906**	0.324	**10.119**	**11.512**
09-14	1.083	-1.238	0.005	**7.898**	**7.413**
09-15	1.363	-1.345	0.263	**8.690**	**7.002**
09-16	0.722	-1.593	0.936	**6.103**	**4.941**
Minimum	-1.467	-1.640	-2.890	-2.490	-1.646
Maximum	**1.456**	**2.076**	**1.782**	**1.857**	**2.139**