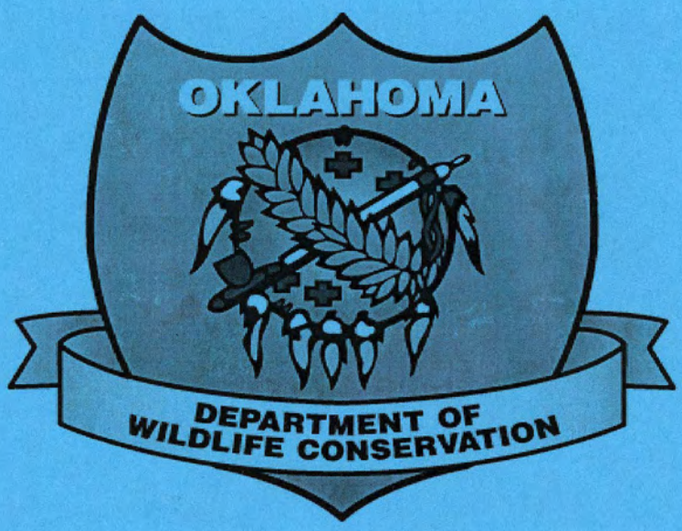
FINAL PERFORMANCE REPORT



FEDERAL AID GRANT NO. T-40-P-1

STATUS OF MACROINVERTEBRATE AND FISH ASSEMBLAGES IN THE SMALL RIVERS OF THE TALLGRASS PRAIRIE

OKLAHOMA DEPARTMENT OF WILDLIFE CONSERVATION

July 01, 2006 through December 30, 2009

FINAL ANNUAL REPORT

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GRANT PROGRAM: State Wildlife Grants

GRANT NAME: Status of Macroinvertebrate and Fish Assemblages in the Small Rivers of the Tallgrass Prairie

GRANT PERIOD: 1 July 2006 – 30 December 2009

PRINCIPLE INVESTIGATORS: Joseph R. Bidwell, Chad J. Boeckman, and William L. Fisher

NOTE: *This report contains information on the location and abundances of sensitive mussel fauna in the Verdigris, Caney, and Neosho Rivers of northeastern Oklahoma. Dissemination of this report should be handled with care.*

I. ABSTRACT

This project was largely conducted in the Oklahoma sections of the Verdigris, Caney and Neosho Rivers in northeastern Oklahoma. Its objectives were to: 1) update the native mussel community assemblage data for these rivers, 2) provide further assessments of general stream health using standard macroinvertebrate metrics, 3) determine fish species present in each river with emphasis on fishes that may serve as hosts for native mussel glochidia, 4) update zebra mussel distributions in these rivers, 5) use stable isotope techniques to characterize relative trophic positions of biota in these systems and determine zebra mussel position and potential competitors, and 6) construct a GIS database containing information about sampling sites to assist in conservation planning and highlight areas of special interest.

Mussel survey sites were selected to correspond with previous surveys conducted by Vaughn (1998). In the Verdigris River, a significant increase in mussel abundance and richness was noted over the previous survey. Two species of special interest were detected in the Verdigris River, the rabbitsfoot mussel (*Quadrula cylindrica*), a Tier I species in the Oklahoma Comprehensive Wildlife Conservation Strategy (OCWCS, (ODWC, 2005)) and a species that is currently being considered for listing as federally endangered, and the western fanshell (*Cyprogenia aberti,* OCWCS Tier I species), which was previously thought to have been extirpated from Oklahoma. The rabbitsfoot population may represent one of the largest concentrations in this region, although it is currently located below Oologah Lake in an

area also infested with zebra mussels. Abundances of the western fanshell were low (two individuals) and located near the OK-KS border. This species was one of 10 noted to be on the increase in Kansas (Miller and Lynott, 2006), therefore the improvement in Kansas may be driving the reintroduction into Oklahoma portions of the Verdigris River.

Mussel richness and abundance in the Caney and Neosho Rivers did not differ significantly from the previous survey by Vaughn (1998). Several sites on the Caney River located within the city of Bartlesville, OK were found to contain native mussels despite industrialized water use and public access to the river. On the Neosho River, one freshly dead Neosho mucket (*Lampsilis rafinesqueana,* OCWCS Tier I species) was discovered, although unfortunately no live specimens were detected.

The Verdigris River had the most abundant, diverse, and sensitive macroinvertebrate community of the three Rivers. Of the 67 invertebrate taxa identified in the study as a whole, 14 were unique to the Verdigris River, 10 were unique to the Neosho, and seven taxa were only found in the Caney River. The Verdigris and Caney Rivers had the most similar macroinvertebrate communities with a Jaccard similarity index of 0.51. (Values close to 1 indicate more similar communities, while values near 0 indicate dissimilarity) The Verdigris and Neosho communities were most dissimilar with a Jaccard value of 0.41.

Interestingly, macroinvertebrate abundance was significantly, positively correlated with mussel abundance and richness in the Verdigris River and macroinvertebrate taxa richness was correlated with mussel richness. This indicates sites with an abundant and diverse macroinvertebrate community, also supported more mussel species. These associations were more evident for the Caney River where macroinvertebrate taxa richness, % Ephemeroptera, Plecoptera, Trichoptera (%EPT), and Shannon diversity index were all significantly positively correlated with mussel richness. Furthermore, the macroinvertebrate biotic index that was calculated for this study was significantly negatively correlated with both mussel abundance and richness (the lower the biotic index score, the better the community). Similar significant correlations were not observed for the Neosho River, although this system was not sampled as extensively as the other rivers due to pervasive high water during the project period. The observed associations between macroinvertebrate and freshwater mussel community metrics suggest that

data generated from macroinvertebrate monitoring programs undertaken by state agencies and community groups could be used to help track the status of mussel beds near sampling sites and trigger focused mussel surveys if key metrics indicate a change in water quality.

Thirty-nine species of fish were identified from the three rivers, with the Caney River containing the most diverse assemblage at 26 total species, followed by the Neosho with 25 species, and the Verdigris with 23 species. Red shiners (*Notropis lutrensis*) and gizzard shad (*Dorosoma cepedium*) were among the most common taxa collected. Due to excessive numbers of shad in the Neosho River, our objectives in that system shifted from a quantitative to a qualitative sampling approach. As such, the abundance and catch per unit effort reported for the Neosho River are probably underestimates. The blue sucker (*Cycleptus elongates*), a species assigned Tier II status in the OCWCS, was collected from the Neosho River. Encouragingly, fish hosts for mussel glochidia were identified for every mussel species within each river.

While conducting mussel surveys, the presence or absence of zebra mussels was noted. In the Verdigris River, zebra mussels were not detected at any site above Oologah Lake, although they were present at every site below the lake. While densities of zebra mussels were low during the survey, evidence of previous attachment of zebra mussels to native Unionids (in the form of byssal threads on the native mussel shells) was noted. The low densities of zebra mussels is most likely due to a significant die-off within Oologah Lake just months before conducting mussel surveys in September 2006. Since that time, zebra mussels have slowly increased again in the lake.

One individual zebra mussel was collected in a macroinvertebrate sample taken from the Neosho River, although further sampling at that site revealed no additional zebra mussels. However, Marion Lake, KS, is infested with zebra mussels and is aquatically linked to the Neosho River. As this population increases, more zebra mussels may be transported into the Neosho River in Oklahoma. Overall, the most significant evidence for an interaction between zebra mussels and native mussels was observed in the Verdigris River downstream of Oologah Lake, although there was no clear indication of a negative effect on the native mussels. Sites in the Neosho River are currently not threatened by zebra mussels, however

the presence of zebra mussels in Marion Lake, KS represents a likely future threat. Lastly, the entire Caney River could be at risk if the zebra mussel were to be introduced into either Hulah or Copan Lakes.

Analysis of stable isotope concentrations of representative biota indicated three trophic levels in the Caney River and four in the Verdigris River. Based on ^{15}N concentrations, seston comprised the first level in both systems, with chironomids making up the second level in the Verdigris River. In the Caney River, riffle beetles, dragonflies and native mussels occupied the second trophic level while these organisms were positioned at the third tropic level in the Verdigris River. Various fish taxa occupied the 3rd and 4th trophic levels in the Caney and Verdigris River, respectively. Fish were also more enriched in ^{13}C than the other collected organisms in both rivers, indicating fish are relying more on autochthonous food resources than allochthonous sources.

Since zebra mussel numbers were low to non-existent in the rivers that were sampled, zebra mussels and other biota were collected for isotope analyses from Sooner Lake, OK, a site known to support a large zebra mussel population. Zebra mussels and the Asian clam, *Corbicula fluminea,* had very similar isotope compositions within Sooner Lake, which suggests that *Corbicula* may have use as a surrogate for determining approximate zebra mussel trophic position within a system. This may allow for identification of native species that may compete for food resources with zebra mussels and help prioritize biological assessments after the invasive establishes. Generally, filter feeding invertebrates and shad were positioned near zebra mussels or *Corbicula* in stable isotope investigations within these systems, indicating potential food resource competition between these taxa.

Geographic information system (GIS) software was used to map mussel sampling locations and to help identify potential threats to mussel beds. In the Verdigris River, three sites located below Oologah Lake that support the Tier I rabbitsfoot mussel were highlighted as at risk sites, due to zebra mussel infestation. In the Caney River, many mussel beds were located near public access areas. This accessibility could result in zebra mussel introduction in areas near boat ramps or may allow for human disturbance of mussel beds. Additionally, Hulah and Copan Lakes were identified as potential sources of zebra mussels for the entire Caney River, if they should become established in either lake. Finally, the

Stepps Ford Bridge was identified as an access area for the Neosho River. One site immediately downstream from the bridge supported the greatest abundance of mussels during the summer survey. A freshly dead Neosho mucket (*Lampsilis rafinesqueana*, OCWCS Tier I species) was also collected at this site.

II. OBJECTIVES

1. Determine spatial trends in the relative abundance of species present and overall community diversity along the Verdigris, Caney, and Neosho Rivers within the Tallgrass Prairie Region.
2. Determine spatial variation in the number of trophic levels (increasing N^{15} with increasing tophic level) alone each river.
3. Determine the range and densities of existing zebra mussel populations within the Verdigris (Oologah Lake and Verdigris River), Neosho (Grand Lake) and Arkansas (Kaw Lake, Keystone Lake) drainages.

III. NEED

The Oklahoma Comprehensive Wildlife Conservation Strategy (OCWCS) indicates that small rivers (Caney, Verdigris and Neosho) in the Tallgrass Prairie Region represent very high priority conservation landscapes. These habitats historically supported nine Tier I and II species of native unionid mussel and six Tier I and II fish species. However, a lack of basic data on the composition of fish and invertebrate assemblages in these rivers represent a significant impediment for conservation planning and implementation. In addition, invasive exotic species threaten to destabilize the resident aquatic communities.

The Verdigris, Caney, and Neosho rivers once supported diverse assemblages of native freshwater mussels (Isely, 1924). A number of mussel species that have been assigned a Tier I conservation rank in the OCWCS occurred in these rivers, including the Butterfly Mussel (*Ellipsaria lineolata*) , Neosho Mucket (*Lampsilis rafinesqueana*), Ouachita Kidneyshell (*Ptychobranchus occidentalis*), Rabbitsfoot (*Quadrula cylindrica*), and Western Fanshell (*Cyprogenia aberti*). Tier II species include the Elktoe (*Alasmidonta marginata*), Plain Pocketbook (*Lampsilis cardium*), Wartyback Mussel (*Quadrula nodulata*) and the Ohio River Pigtoe (*Pleurobema cordatum*). A survey conducted in the mid-1990's by Vaughn (1998) indicated that the majority of these species were either in a state of decline or had been extirpated from the rivers. Additional surveys of these systems will not only indicate the current status of the mussels, but evaluations for the entire macroinvertebrate assemblage provides insights about the current condition of streams in this area. Given the ecological importance of macroinvertebrates, community composition of these organisms is often used to determine the general health of aquatic systems. Sensitivity data are also available for many common invertebrate taxa, which allows for the determination of the relative proportions of sensitive versus resistant organisms in an assemblage. Data such as these are important to establish baseline conditions and track habitat quality over time.

The small rivers of the Tallgrass Prairie ecoregion also include fishes that have been assigned a Tier I rank such as the Kiamichi shiner (*Notropis ortenburgeri*), Neosho madtom (*Noturus placidus*) and shovelnose sturgeon (*Scaphirhynchus platorynchus*). As with the mussels, fish surveys can indicate the status of species of concern and the current data can be compared to historic collections to evaluate trends in abundance. Since freshwater mussels require a fish host to complete their lifecycle, fish surveys also indicate the status of those species known to serve as mussel hosts. This can provide a further indication of the potential for a mussel population to remain viable. If the fish hosts are absent, recruitment of juveniles into the mussel population will not be possible. This information is also important for any future programs to re-establish mussel taxa that have been extirpated from the rivers.

In addition to determining the type and abundance of fish and invertebrate taxa in the systems, comparison of the ratios of specific stable isotopes in biological samples can be used to indicate the number of trophic levels that occur in a system (Vander Zanden et al., 1999; Nichols and Garling, 2000; Vander Zanden and Rasmussen, 2001). The samples required for these analyses can often be obtained through non-terminal biopsy, which means individuals do not have to be sacrificed to generate the data. This information is useful when tracking changes in the status of aquatic communities and quality of habitat since it can indicate changes in the trophic complexity of a system.

The final issue to be addressed in this study is the status of the zebra mussel (*Dreissena polymorpha*), which is one of the most significant aquatic invasive species in North America due to the trophic effects and other impacts it can have on the communities it becomes a part of. Zebra mussels have been found in four of the major reservoirs in the Tallgrass Prairie ecoregion (Oologah, Kaw, Grand, and Keystone Lakes). Any management plans for rivers in the ecoregion should take into account the actual or potential presence of these invasive bivalves. Also, since one of the primary vectors for overland transport of zebra mussels is recreational boaters, knowledge of the location of boating access points in relation to native mussel beds will further support invasive species management plans by helping target signage and other efforts to increase public awareness of the problem.

IV. APPROACH

MUSSEL SURVEYS

Mussel surveys in the Verdigris, Caney, and Neosho Rivers largely followed sites previously sampled by Vaughn (1998). Previously surveyed site locations were marked on 7.5 minute topographical maps (USGS) and used as a reference while in the field. Each river was accessed at bridge crossings, private land with landowner consent, or public parks. A canoe was used to travel from access areas to sampling locations, as sections of each river were largely unnavigable with outboard engines during base flow. Upon reaching the sampling location, relic mussel shells were gathered for identification and global position system coordinates were recorded using a Garmin Geko 301 hand-held GPS unit (Garmin International Inc. Olathe, KS. USA). General habitat characteristics were recorded including riffle, run,

or gravel bar, bank height, riparian characteristics and land use, substrate composition as well as any outstanding or atypical characteristics such as excessive periphyton or evidence of human disturbance. Basic water chemistry data (dissolved oxygen, pH, temperature, and conductivity) were recorded using a Hydrolab Quanta multi-parameter probe (Hydrolab Corporation, Austin TX. USA). Additionally, 125-mL of water was collected in acid-washed polyethylene bottles and transported back to the laboratory on ice for determination of titratable alkalinity and hardness (APHA 1998). Sampling locations were numbered to correspond to the previous survey by Vaughn (1998).

Sample sites in the Verdigris and Caney Rivers largely consisted of riffle areas and runs immediately downstream from the riffle; however in the Neosho River, most sites were located on gravel bars on the inside curve of bends in the river. Mussel surveys were conducted through timed snorkel searches at least 15 minutes in duration (2 person x 15 minutes = 30 minutes total search time), with longer time intervals devoted to locations that contained greater area of stable gravel substrate with good flow and low sediment. In some areas of the Caney and Neosho Rivers, visibility was too poor to allow snorkel searches and 'grubbing' techniques were employed, where searchers combed the top few inches of substrate with their hands to find mussels. Once located, mussels were carefully removed from the substrate and placed in mesh bags that were kept in the water during the search. After each survey, mussels were sorted, measured to the nearest 0.01 mm maximum length using digital calipers (Fisher Scientific, Pittsburgh PA.), and identified to species using keys by Cummings and Mayer (1992), Oesch (1995), Couch (1997), and Mather (2005). Mussels were then carefully returned to the river throughout the search area by placing them anterior end down into the substrate. Live voucher specimens were not collected due to limited representatives of some species. Photo documentation and relic shell material were collected for rare species, and are currently held at the Ecotoxicology and Water Quality Research Laboratory (EWQRL) at Oklahoma State University.

Generally, each site was sampled on one occasion; however several sites in the Verdigris and most sites in the Neosho River were sampled twice. In the Verdigris River, initial mussel sampling occurred in the summer of 2006. In the summer of 2007, major flooding caused a spill of an estimated

42,000 gallons of crude oil from Coffeeville Resources Refinery in Kansas, which ultimately reached Oologah Lake. To assess the effects of this spill on the native mussel community above Oologah Lake, several sites were revisited in the fall of 2007 (sites 1-3 and 11-14). In the Neosho River, mussel surveys initially occurred in March 2009. As sampling conditions improved in August and September 2009, these sites were resurveyed to better assess the mussel community.

INVERTEBRATE SURVEYS

At each mussel sampling site, additional benthic macroinvertebrates were collected with a D-frame kicknet (400 μm mesh size). Three replicate kicknet samples were taken by holding the net downstream while the sampler disturbed approximately $1 m^2$ of the substrate with their feet. Each sample was placed in a clean 500-mL polyethylene container filled with river water and held on ice until they could be preserved. Preservation occurred within 15 h of taking the sample, and was completed by filtering the collected river water through a 64μm mesh to retain invertebrates, rinsing the collected material on the mesh back into the container with 70% ethyl alcohol, and subsequently filling the container with 70% ethyl alcohol. Once in the laboratory, kicknet samples were placed in a 400μm mesh sieve and washed with deionized water to remove fine sediment. Aliquots of sample material were then placed in cross-hatched plastic petri-dishes and observed under 1X magnification with a dissecting microscope. Invertebrates removed from the sample were placed in 8-mL glass vials filled with 70% ethyl alcohol. This process continued until all substrate material had been observed under magnification. Processed substrate material was placed back in the original 500mL polyethylene containers with alcohol and is currently held at the EWQRL at Oklahoma State University. Benthic invertebrates were subsequently enumerated and identified to genus (except chironomids, which were identified to family) using keys by Pennak (1953), Edmondson (1959), Voshell (2002) and Merritt et al. (2008).

FISH SURVEYS

In wadeable habitat, fish sampling was conducted using a Smith-Root Inc. 2.5 gas-powered pulsator (GPP, Smith-Root, Inc. Vancouver, WA USA.) attached to a generator. This system was placed in a 12ft. aluminum boat which acted as the cathode. Three hand-held anodes were connected to a

junction box which delivered 60 pulses per second of DC current to the water at 4-5 amps. One individual guided the boat, while three individuals directed the anodes with either 2 or 3 people netting fish immediately downstream from the anodes. Netted fish were placed in holding containers filled with river water on the boat until sampling was complete. Block nets were erected downstream of the sampling site using t-posts driven into the substrate at multiple intervals across the river. Sampling was initiated upstream and continued until the surveyors reached the block nets where time spent sampling was recorded. Sites consisted of wadeable stretches of river generally between 100-200 meters in length and surveyors weaved across the river focusing on multiple habitat types or woody debris where it existed. Fish collected in the block nets were included in the results.

As wadeable habitat in the Neosho River was limited, boat electrofishing was employed to further assess fish assemblage in this river. Boat access was obtained at Riverview Park in Miami, OK. Surveyors were able to run upstream approximately 7km to the southernmost mussel site (37) to begin sampling, with the last transect approximately 2km downstream of Riverview Park. The sampling gear consisted of a 16 ft. Smith-Root Inc. aluminum boat (SR-16EB) with a Smith-Root 5.0 GPP and two boom-mounted Smith-Root SAA-6 anode arrays delivering 60 pulses/sec DC current at 4-5amps output. One individual operated the boat while two others netted stunned fish from the bow and transferred them to a holding tank on board. Sampling was initiated from upstream moving down and focused on course woody debris and other habitat types in the vicinity such as gravel bars, side channels, bridge pilings, and rock outcrops. A transect was considered complete after approximately 1000 seconds of sampling effort (which was consistent with the wadeable fish protocol). Due to high numbers of shad (both *Dorosoma petenense* and *D. cepedianum*), in the Neosho River, surveyors collected approximately 15-20 individuals from each transect and then focused on the other fish species in the river.

For both fish sampling protocols, fish were measured for total length and identified using keys by Robison and Buchanan (1992), Pflieger (1997), and Miller and Robison (2004), and subsequently released. Any fish not identified in the field were preserved in 10% buffered formalin and brought back to the laboratory for further identification.

STABLE ISOTOPES

At selected sites within each river, collections of material from multiple trophic levels were made for stable isotope (^{15}N and ^{13}C) analysis. Sediment was collected from the river benthos and placed in screw-cap vials for transport back to the laboratory. River seston was collected with a 64μm mesh zooplankton net, held in the water column for 5 minutes. Collected seston was then transferred to 125-mL polyethylene bottles and held on ice for transport back to the laboratory. Three replicate benthic invertebrate samples were also collected using the D-frame kicknet methodology described above. These samples were also placed on ice and the invertebrates were separated from substrate material within 24hr. of collection. Live invertebrates were placed in moderately hard reconstituted water (USEPA, 2002) and allowed 24 hours to depurate gut contents. Organisms dead at time of separation from substrate materials were immediately dried. Collected invertebrates were identified and separated by genus in order to combine tissue of individuals within the same genus. Native mussel foot tissue was collected by carefully prying open the valves and clipping a small portion of the foot tissue with scissors. The tissue was placed in screw-cap vials and held on ice for transport to the laboratory. Fish collections for stable isotope analysis were also made during the fish sampling. Dorsal muscle tissue was collected from each species for analysis. Small species were collected whole while larger individuals had a small section of dorsal muscle removed using scalpel and scissors, and then returned to the river. For species with high abundances, 5 specimens were collected and their tissue pooled for the analysis.

All collected material was dried at 80˚C for 24hr. and ground using a mortar and pestle. Appropriate amounts of material (specifications provided by U.C Davis Stable Isotope Facility) were then packaged into 4x6mm tin capsules, sealed and shipped to the Stable Isotope Facility and University of California-Davis. Samples were analyzed for ^{15}N and ^{13}C using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire UK) and results expressed in delta notation.

GEOGRAPHIC INFORMATION SYSTEMS (GIS)

County level National Agricultural Imagery Program (NAIP) images of northeastern Oklahoma were downloaded from The Geocommunity, Oklahoma Center for Geospatial Information (OCGI), and the Center for Spatial Analysis (see literature cited section). Oklahoma rivers and reservoirs layers were downloaded from the Oklahoma Water Resources Board and a county roads layer from the OCGI was added to the compilation. GPS coordinates taken at each sampling location were converted to Universal Transverse Mercator (UTM) coordinates using DNRGarmin 5.4.1 (available from the Minnesota Department of Natural Resources) and added to the NAIP and rivers layers. A database table was created in Microsoft Excel detailing the mussel species found at each site and their abundances. The kicknet invertebrate metrics, (discussed below), were also added to the database table and it was joined to the GPS locations layer in ArcMap.

Spatial analyses, used to determine site proximity to public access areas, were accomplished by applying various buffers around the sampling locations and querying for sites that intersected a county road. Buffers ranged from 50m to 250m around each sampling location. To identify stream segments crossed by a bridge, which often provide public access, a query was run searching for stream segments intersected by a county road. Additionally, during sampling it was noticed that a county road often ran parallel with the river, yet never actually crossed it. To identify these areas, which also often provide public access, a 50m buffer was applied to each stream and another query was run, highlighting the stream segments intersected by a county road with the buffer applied.

STATISTICAL ANALYSES

Mussels

For each river, summary statistics for timed abundance and richness of mussel taxa were calculated for each site sampled. Mussel incidence or number of sites at which a particular species was encountered were also calculated. Length distributions for the 4 most common mussel species were generated for each river. One way ANOVA's were used to compare the length distributions of mussels common to all three rivers. Prior to each ANOVA, data were tested for normality using the Shapiro-Wilk method and the Levene Median test for heterogeneity of variance. If data did not meet normality or

heterogeneity of variance assumptions, statistical transformations were attempted such as log, natural log, square root etc. If transformation did not normalize the data, an ANOVA on ranks was conducted with Dunn's method multiple pairwise comparison tests if ANOVA on ranks revealed significant differences. Additionally, to compare mussel timed abundance and richness among the three rivers, a single factor Analysis of Variance (ANOVA) was employed, treating the sample locations as replicates. If significant differences existed within normal data, all multiple pairwise comparisons were further evaluated using the Holm-Sidak procedure.

In the Verdigris River, to assess the potential impact of zebra mussels on native mussel community composition, species richness and abundance were estimated separately for sites above the lake, which contained no zebra mussels, and those below the lake where zebra mussels were present. Similar analyses were not conducted for the Caney and Neosho Rivers as widespread zebra mussel presence was not detected in either river.

Within each river, in order to compare mussel survey results of the present study with those compiled by Vaughn (1998), a 2-sample paired t-test for means was used evaluating both mussel timed abundance and richness at each site between the two surveys. If significant differences existed, further analysis was initiated beginning with a t-test to examine time spent searching for mussels. Additionally, log-transformed abundance vs. richness curves were generated and resulting regression coefficients and elevations from the best fit lines were used to further assess significant differences between surveys. All analyses were performed at the $\alpha = 0.05$ level using SigmaStat version 3.1 (Systat Software Inc. San Jose, CA.).

Invertebrate Surveys

For the invertebrates collected in the kicknet samples, taxa lists were compiled for each river, indicating abundance of each species collected at each site. Additionally, invertebrate richness, % Ephemeroptera/Plecoptera/Trichoptera (%EPT) taxa, Shannon index, and a biotic index were calculated for each site within each river. The biotic index values were taken from Missouri Department of Natural Resources standard operating procedures document MDNR-WQMS-209 (2005). MDNR lists Lenat

(1993), Huggins and Moffett (1988), Hilsenhoff (1987), and Bode et al. (1996) as sources for these values. Summary tables including the mean and standard error of the invertebrate metrics listed above were generated for each river. Jaccard similarity index was also calculated to determine the relative similarity of the invertebrate community between each river. Values can range from 0 to 1 with 0 being completely different and 1 indicating identical communities. Additionally, to compare the metrics generated among the three rivers, a single factor ANOVA was used, treating the sample locations as replicates. Normality and heterogeneity of variance and non-normal data transformations were tested and treated as described above. If significant differences were detected, all multiple pairwise comparisons were further evaluated using the Holm-Sidak procedure or Dunn's method for data not meeting normality assumptions.

To determine if sites that supported a rich invertebrate community, also supported greater mussel abundance and richness, a Pearson product moment correlation (Zar, 1999) was conducted between each of the invertebrate metrics and mussel timed abundance and richness estimates. A positive correlation coefficient indicates the two variables increase or decrease together while a negative correlation indicates one variable increases while the other decreases. This analysis was conducted for each river, treating the sample sites as replicates, and summary tables were generated for each river.

Fish Surveys

For the fish surveys, taxa lists were compiled indicating abundance of each species collected at each sample location within each river. Additionally, fishes that serve as known mussel glochidia hosts were also indicated on these tables. Summary statistics with total abundance, richness and catch per unit effort (CPUE) were generated for each sample site. Additionally, to compare the summary statistics generated among the three rivers, a single factor ANOVA was used, treating the sample locations as replicates. Normality and heterogeneity of variance and non-normal data transformations were tested and treated as described above. If significant differences were detected, all multiple pairwise comparisons were further evaluated using the Holm-Sidak procedure or Dunn's method for data not meeting normality assumptions.

V. RESULTS AND DISCUSSION

RIVER WATER CHEMISTRY

Basic water chemistry was collected at each of the mussel sampling locations and is summarized in Table 1. Water chemistry for each site is reported in Appendix 1 with the individual site characteristics. Seasonal differences accounted for much of the variability within each river. For instance, in the Verdigris River, sampling was initiated in July and concluded in early October. Higher water temperatures and lower dissolved oxygen concentrations were recorded in July and vice versa for October. Seasonal effects also accounted for much of the variability between rivers. For example, the Neosho River (spring sampling) had significantly lower water temperature, and greater conductivity, alkalinity and hardness than the Verdigris, Caney and Neosho River (summer sampling) ($P<0.05$ for all comparisons). Additionally, in the Neosho River spring sampling, dissolved oxygen and pH were significantly greater than the Verdigris and Caney Rivers ($P<0.05$ for all comparisons).

Overall, the Caney River had significantly lower dissolved oxygen concentrations than the Verdigris or Neosho Rivers (both spring and summer samplings) and the Neosho River (both spring and summer samplings) had greater pH than the Verdigris and Caney Rivers ($P<0.05$ for all comparisons). Low dissolved oxygen in the Caney River may partially explain the relatively higher invertebrate biotic index value in the Caney when compared with the Verdigris and Neosho Rivers (see invertebrate section below).

MUSSEL SURVEYS

Verdigris River

Mussel survey sites were selected to correspond to a previous survey (Figure 1a-b) (Vaughn, 1998). Individual site characteristics for all three rivers are listed in Appendix I. Thirty-one sites were surveyed in the Verdigris River between July and October 2006 and 17 species of native mussels were found (Table 2a-c). Of the mussels identified, *Cyprogenia aberti* (western fanshell) and *Quadrula cylindrica* (rabbitsfoot) are listed as Tier I species by Oklahoma's Comprehensive Wildlife Conservation

Strategy (OCWCS, ODWC, 2005). Additionally, *Lampsilis cardium* (plain pocketbook) and *Quadrula nodulata* (wartyback) are listed as Tier II species, with *Amblema plicata* (3-ridge) *Megalonaias nervosa* (washboard), *Potamilus purpuratus* (bleufer), and *Quadrula metanevra* (monkeyface) all Tier III (ODWC, 2005).

Total abundance for mussels collected on the Verdigris River was calculated separately for sites occurring upstream and downstream from Oologah reservoir (Figure 2). *Q. metanevra* was the most abundant mussel found regardless of location relative to the reservoir. *A. plicata, Obliquaria reflexa (*three-horned wartyback*), P. purpuratus,* and *Tritogonia verrucosa* (pistolgrip) were common mussels found above Oologah reservoir. In addition to monkeyface mussels, *O. reflexa, Q. cylindrica* and *Quadrula pustulosa* (pimpleback) were found in high numbers below Oologah dam. *C. aberti, Lasmigona complanta*, and *Lampsilis teres* were only found upstream from Oologah Lake, while *M. nervosa, Q. cylindrica* and *Q. nodulata* were only found below. Generally, abundant species were also widespread, with *Q. metanevra* found at 26 different sites. However, *Q. cylindrica* was the 6th most abundant species yet it was only found at 3 different sites in a short section of river (Figure 3). Lee et al. (1998) and Berg et al. (2007) suggest host-fish vagility may explain unionid distribution patterns, with mussels that utilize fish hosts with greater home ranges typically showing greater abundance and distribution. The known fish hosts for *Q. cylindrica* include several species of *Notropis* (Yeager and Neves, 1986; Fobian, 2007), which have relatively small home ranges (Goforth and Foltz, 1998). This characteristic of its fish host may explain the concentrated distribution of *Q. cylindrica* in the Verdigris.

Length distributions were developed for the four most abundant species; *Q. metanevra, T. verrucosa, O. reflexa,* and *A. plicata* (Figure 4). The distributions for all four species were negatively skewed indicating fewer small individuals than might be predicted given a normal population distribution, with kurtosis values generally positive, except for *O. reflexa* with a value of -0.74, indicating a slightly more uniform distribution. Some care should be taken in interpreting the population length distributions from each river since timed snorkel searches may bias against encountering very small or particularly cryptic individuals (Hornbach and Deneka, 1996; Obermeyer, 1998; Metcalfe-Smith et al., 2000).

Furthermore, juveniles frequently bury completely in the substrate, making detection of these cohorts difficult when using snorkel searches (Neves and Widlak, 1987; Amyot and Downing, 1991; Yeager et al., 1994; Sparks and Strayer, 1998). In spite of these issues, timed snorkel search techniques are commonly used for determining mussel richness and locating rare species (Metcalfe-Smith et al., 2000; Vaughn and Spooner, 2004) and are more cost effective when surveys of large areas are needed as compared with quadrat methods.

Live mussels were found at all but two sites. Timed abundance estimates for sites with live mussels ranged from 3 to 156 mussels per hour (MPH) with a mean of 38 for all 31 sites (Table 6). Taxa richness ranged from 2 to 10 species per site with a mean of 5.2 species for all 31 sites combined. No evidence of zebra mussels (*Dreissena polymorpha*) was found at any site above Oologah Lake, however zebra mussels and byssal threads (structures produced by zebra mussels that allow attachment to firm structure) were found on substrate and unionid shells at every site below the lake. While many native mussels collected below the lake had byssal threads on the valves, few had live zebra mussels attached. Low abundance of zebra mussels may have resulted from a significant die-off that occurred within the lake beginning in June 2006. Sites below Oologah were surveyed in September and October 2006, after adult zebra mussel densities had been reduced from 40,000/m^2 to 0/m^2 (Boeckman and Bidwell, unpublished data). Since late 2006, zebra mussels in Oologah Lake have remained in low abundances; however, steady annual increases in veliger densities are occurring.

In order to assess the potential impact of zebra mussels on native mussel community composition, species richness and abundance were estimated separately for sites above and below the lake. Sample locations above the lake had a mean richness of 5.6 species per site, and an abundance of 39.3 MPH (Table 6). Sites below Oologah Lake had a mean richness of 4.4 species per site with an abundance of 35.7 MPH (Table 6). There was no significant difference in overall mussel species richness or abundance between upstream and downstream sites. While no differences in mussel richness and abundance were apparent when sites were combined within upstream and downstream sections, a downstream longitudinal gradient in these parameters was apparent. Both mussel abundance ($r^2 = 0.697$, $P = 0.0014$) and species

richness ($r^2 = 0.539$, $P = 0.0138$) were significantly positively associated with distance from the dam (Figure 5). However, these analyses may be influenced by two downstream sites that had the greatest abundance of any of the sample locations. When these two sites are removed from the analyses, the longitudinal relationship is no longer significant (MPH $r^2 = 0.31$, $P = 0.120$; richness $r^2 = 0.38$, $P = 0.077$).

The effects of impoundments on native mussel communities have been well established (Bogan, 1993; Vaughn and Taylor, 1999; Bednarek, 2001; and Sethi et al., 2004). For example, Vaughn and Taylor (1999) described a reduction in native mussel populations below an impoundment and found a distance of 20 km was needed for these populations to recover to pre-impoundment levels. In our survey, downstream sites less than 20 km from the dam generally had poor or less than average abundance with two sites immediately downstream from the dam having no live mussels at all. Species richness was also affected by the dam, although less distance was needed to "recover" as compared with abundance.

In the spring of 2007, significant flooding caused an oil spill from a refinery located near the Verdigris River in southeastern Kansas. In order to assess the potential impact on the native mussel community from the high water and contamination from the refinery, seven sites located between the OK-KS border and Oologah Lake were resurveyed in the fall of 2007. Among the seven sites resampled, there was no significant difference in mussel timed abundance or richness between the 2006 and 2007 sampling dates ($P = 0.093$ and $P = 0.055$, respectively). While effects on the benthic community due to the oil spill were not readily apparent, significant erosion at sites near the OK-KS border was noticed during the resurvey. Sediment deposits at a few sites in this area may pose a threat to unionid mussel habitat.

Caney River

Twenty-nine sites were surveyed for mussels in the Caney River between August and October 2007 and 11 species were found (Table 3a-c). While no Tier I species were identified, two Tier II (*L. cardium* and *Q. nodulata*) and two Tier III species (*A. plicata* and *P. purpuratus*) were collected.

T. verrucosa was the most often encountered species (Figure 6) with *Leptodea fragilis* (fragile papershell), *O. reflexa*, and *Q. pustulosa* the next 3 most abundant mussels (Figure 6). Mussel incidence largely followed mussel abundance, with *T. verrucosa* found at 15 sites, *Q. pustulosa* at 11, *L. fragilis* at 10 sites (Figure 7). Length distributions for the four most common species are presented in Figure 8. Kurtosis and skewness values were near 0 except for *Q. pustulosa*, which was positively skewed (3.1) indicating more small individuals in the population than might be expected and had high kurtosis at 10.6 indicative of many individuals concentrated around the mean length (71.1 mm).

Live mussels were found at 21 of the 29 sites surveyed. At sites containing live mussels, timed abundance estimates ranged from 4 to 264 mussels per hour (mean = 32.5 mph) with species richness ranging from 1 to 6 species per site (mean = 2.1) (Table 6). Unlike the Verdigris River, there was no evidence of zebra mussel colonization at any of the 29 sites sampled.

Neosho River

Seventeen sites were surveyed in the Neosho River in March 2009 and again in August 2009. A total of 10 species were found between the two sampling events, four in March and an additional six in August (Tables 4a-b and 5a-b). While no live Tier I species were observed, a freshly dead, juvenile *Lampsilis rafinesqueana* (Neosho mucket) was found with some soft tissue still remaining at site 31, just downstream from Stepps Ford Bridge. *L. cardium* was the only Tier II species encountered, with *A. plicata*, *M. nervosa*, *P. purpuratus*, and *Q. metanevra* representing Tier III species.

During the March sampling (spring) *P. purpuratus* was the most abundant species with 11 individuals collected among the 17 sites. *Truncilla donaciformis* (fawnsfoot) was the least abundant with only one individual found (Figure 9). *P. purpuratus* and *Q. metanevra* were the most often encountered mussels in the spring and were found at four sites overall. During the August sampling (summer), *Q. metanevra* was the most abundant mussel with 35 individuals. *A. plicata*, *L. cardium*, and *M. nervosa* were the least common at two individuals each (Figure 9). *P. purpuratus* was the most often encountered mussel in the summer, found at 7 sites followed by *O. reflexa* and *Q. metanevra* at 6 sites and *T. verrucosa* at 5 sites (Figure 9).

Length distributions were generated for the four species collected in the spring 2009 (Figure 10). *P. purpuratus* and *Q. metanevra* both exhibited negative kurtosis (uniform distributions) and only slightly positive skewness. *T. verrucosa* had a positive kurtosis (peaked around the mean) with a negative skewness indicative of fewer small individuals in the population than what might be expected. Length distributions for the four most common species detected in the summer survey are presented in Figure 11. Generally, kurtosis and skewness calculations were near zero except for *T. verrucosa* which appeared to have a more uniform distribution than in the spring. The lower water levels and better sampling conditions in August may have allowed surveyors to find smaller mussels than what was possible in March. Additionally, detection of small mussels in March was difficult with the neoprene gloves needed during that survey. Warmer water temperatures in August allowed surveyors to use bare hands to grub through substrate, and may have increased detection of small mussels.

During the spring survey, live mussels were found at 8 of 17 sites with mussel timed abundance ranging from 2 to 12 mussels per hour at sites with live mussels (mean = 2.8). Species richness ranged between one and two species per site (mean = 0.65) (Table 6). During the August survey, live mussels were found at 11 of 14 sites. Mussel timed abundance estimates ranged from 2 to 21 mussels per hour with a mean of 8.4 for all 14 sites. Species richness ranged between 1 and 6 species per site with an average of 2.6 species (Table 6). Difference in river conditions likely explain the variability between the spring and summer surveys. During March 2009 the Neosho was flowing near 2000 cubic feet per second (cfs), while in August the flow had decreased to near 800 cfs, making areas of better habitat more visible and easier to sample.

During both the spring and summer mussel surveys, there were no signs of zebra mussels or their byssal threads. However, in a kicknet invertebrate sample collected in association with the mussel surveys (discussed below), one dreissenid mussel (4 mm total length) was collected at site 27 (just upstream from Stepps Ford Bridge) during the spring survey. Subsequent surveys of this area have not revealed any other invasive mussels.

River Comparison

Several species of mussels were common in two or three of the rivers surveyed which facilitates a comparison of length distributions. For example, Caney River *T. verrucosa* were significantly larger than individuals found in the Verdigris and Neosho rivers ($P < 0.05$). In contrast, there was no difference in the length distributions of *O. reflexa* between the three rivers. *Q. metanevra* was found in the Verdigris and Neosho rivers, but absent from the Caney River and those individuals in the Neosho were significantly larger than those from the Verdigris ($P < 0.05$).

Mussel timed abundance was also significantly different between the rivers ($p = 0.013$), with the Verdigris River having greater abundance than the Neosho summer survey ($p<0.05$) (the Neosho spring survey was not included in these analyses due to low mussel recovery). There was no difference in timed abundance between the Verdigris and Caney Rivers, or the Caney and Neosho summer survey. Similarly, mussel richness was also significantly different between rivers ($P = 0.001$) with the Verdigris having greater richness than both the Caney and Neosho Rivers ($P < 0.05$). There was no difference in species richness between the Caney and Neosho Rivers.

Comparison with Vaughn (1998) Survey

In the Verdigris River, Vaughn (1998) reported a mean abundance for all 31 sites of 14.4 MPH and a mean richness of 3.3 species per site. Current abundance and richness across all 31 sites was 38.0 MPH and 5.2 species per site, which are significantly greater than the values reported by Vaughn ($P = 0.002$, $P = 0.001$, respectively). Mean sampling time between the two surveys was not significantly different ($P = 0.65$) with 46.8 min in this survey versus 49.8 min in Vaughn (1998). In order to evaluate the extent to which the increase in richness resulted from locating more mussels overall, abundance vs. richness curves were prepared from both studies (Figure 12). These curves were linearized by log-transforming abundance to facilitate statistical analysis. There was no significant difference in the regression coefficient (36.7 for the present study versus 65.7 for the Vaughn study,) or elevation derived from the best fit lines from these data, indicating that mussel abundance explained a similar degree of variation in taxa richness in both studies. Thus the greater taxa richness observed in the present study appears to have been driven by our finding a greater number of mussels during the timed searches.

In the Caney River, Vaughn (1998) reported a mean abundance for all 29 sites of 12.6 MPH and a mean richness of 2.1 species per site. Current abundance and richness across all 29 sites was 32.5 MPH and 2.1 species per site. There was no significant difference in either timed abundance or richness between the two surveys ($P = 0.203$ and $P = 1.00$, respectively). There was, however, a significant difference in mean time spent searching, with 56.4 minutes for Vaughn (1998) and 22.4 minutes in the current survey ($P < 0.001$). Given sampling time was less in the current survey, our timed abundance data may be overestimating the actual mussel density as compared to Vaughn's survey. Therefore, the actual number of live mussels encountered was compared between the two surveys, irrespective of the time spent searching. There was no difference in mean number of live mussels encountered (9.79 vs. 11.45, $P = 0.891$). Therefore, it appears the current survey was able to produce a similar abundance and richness of mussels with significantly less time spent searching than in the previous survey.

In the Neosho River, there was a significant difference in mussel timed abundance between the spring and summer sampling sessions ($P < 0.05$), however there was no difference between either the spring or summer surveys and the Vaughn survey. Similarly, richness was not significantly different between our surveys and Vaughn (1998), however there was a significant difference in mussel richness between the spring and summer sessions in our survey ($P < 0.05$). Vaughn (1998) searched for a longer time than our spring survey, however, there was no difference in search time when compared with our summer survey. Finally, when search time is removed, the number of live mussels encountered was not significantly different between our surveys and Vaughn (1998), however there was a significant difference between our spring and summer sampling sessions ($P < 0.05$).

Mussel Summary

Our survey indicates native mussels appear to be increasing the Verdigris River, although, given the semi-quantitative nature of the sampling technique employed in both ours and previous surveys (Vaughn, 1998), care should be taken when interpreting these data. Strayer (1999) found low statistical power for detecting population declines when using presence/absence techniques; therefore these results should at a minimum support the need for more quantitative techniques throughout the study area. Miller

and Obermeyer (1997) and Miller and Lynott (2006) reported an increase in 10 different species in the Kansas portion of the Verdigris River as compared to 1991 levels using quadrat methods. They attribute this increase to improvements in habitat quality, namely reduction in pollution, increase in fish hosts, and lack of severe drought. These same factors may be working to improve native mussel populations in the Verdigris River of Oklahoma.

Two species of special interest were discovered in the Verdigris River, *C. aberti* and *Q. cylindrica*. *C. aberti* was previously thought extirpated from Oklahoma and while densities remain low, *C. aberti* is increasing in Kansas (Miller and Lynott, 2006) and downstream drift or host fish movement (logperch) should help improve abundance of this species in the Oklahoma portion of the Verdigris River. *Q. cylindrica* is a candidate for listing as a federally endangered species and was previously thought extirpated from the Verdigris River. In subsequent surveys of the sites containing *Q. cylindrica*, over 60 individuals were found in a two hour search, indicating the area supports one of the most dense assemblages of *Q. cylindrica* in Oklahoma, Missouri and Kansas (Todd Fobian, Missouri State University, personal communication). *Q. cylindrica* should also be carefully monitored since these sites occur downstream from Oologah Lake which has historically been infested with zebra mussels.

Caney River mussel populations were similar to previous surveys (Vaughn, 1998) although our survey needed less time spent searching to produce similar abundance and richness of mussels. No Tier I species were detected, but the Caney river supports a large number of *Q. nodulata* which has been given a Tier II ranking by the OCWCS. Several sites within the Bartlesville, OK city limits support a rich assemblage of native mussels.

The Neosho River mussel abundance and richness also does not appear to have changed significantly since the previous survey (Vaughn, 1998). No live Tier I species were recovered, however a freshly dead juvenile *L. rafinesqueana* with soft tissue remaining was noted at site 31 in the August survey. This individual may have been one of those released during recent propagation and reintroduction efforts by the Peoria Nation and other organizations (Meredith Garvin, Tribal Environmental Management Services, Miami OK. personal communication). As with the *Q. cylindrica*

population in the Verdigris River, zebra mussels have recently been found in Marion Reservoir in Kansas. This reservoir ultimately discharges into the Neosho River and will be a source of zebra mussels to this area. One dreissenid mussel was noted in our macroinvertebrate surveys at a site approximately 2km upstream from where the relic *L. rafinesqueana* shell was recovered. While zebra mussels do not appear to be currently well established in the Neosho River, if the population in Marion Reservoir increases, native mussels in the Neosho River may be impacted.

MACROINVERTEBRATES

At each mussel site, three replicate kick-net samples were collected and the invertebrates identified and enumerated. Fifty different taxa were collected in the Verdigris River (Table 7a-c, Table 11), which was the most diverse of the three rivers surveyed. Total abundance ranged from 41 to 842 individuals per site with taxa richness varying between six and 18 taxa per site (Figure 13 a&b). A summary metric that combines the abundance of invertebrates in the Ephemeroptera, Plecoptera, and Trichoptera (EPT) orders is commonly used in macroinvertebrate surveys since these groups tend to include taxa that are more sensitive to habitat degradation (Maxted et al., 2000; Merritt et al., 2008). The EPT comprised between 29 and 87 percent at any given site on the Verdigris (Figure 14a), while the Shannon diversity index ranged from 1.3 to 2.3 (Figure 14b). The average biotic index for sites on the Verdigris River ranged from 4.8 to 7.4 (Figure 15).

Thirty-eight different taxa were identified from 29 sites on the Caney River (Table 8a-c). Average invertebrate total abundance ranged from 15 to 1,142 individuals per site, with taxa richness between 3.3 and 14 taxa per site (Figure 16 a&b). Contributions from EPT orders ranged from 0 to 88% and Shannon diversity index ranged from 0.6 to 1.9 (Figure 17 a&b). Finally, the average biotic index ranged from 5.2 to 7.7 (Figure 18).

The Neosho River was sampled twice, once in March and once in August 2009. In March, 28 different invertebrate taxa were collected (Table 9a-b), while in August that number increased to 32 different taxa (Table 10a-b). In the spring, average total abundance ranged from 2.7 to 259 individuals (Figure 19a) and richness ranged from two to 14.6 taxa per site (Figure 19b). Invertebrates from the EPT

orders ranged from 1.2 to 53.8 percent with average Shannon diversity index varying from 0.5 to 1.7 (Figure 20 a&b). Finally, average biotic index for the spring survey ranged from 3.7 to 7.4 (Figure 21).

Generally, invertebrate metrics increased during the August 2009 survey. Average total abundance for the summer survey ranged from 12.3 to 250.3 individuals per site, with taxa richness ranging between 6.3 and 12.3 taxa per site (Figure 22). EPT orders contributed between 6.4 and 79.1 percent with average Shannon diversity index ranging from 0.5 to 1.9 (Figure 23). The average biotic index ranged from 4.5 and 7.5 (Figure 24).

Invertebrate summary metrics, averaged across all sites, were compared for all three rivers. The Verdigris and Caney rivers had significantly greater total abundance than the Neosho spring sampling ($P < 0.05$) but not the Neosho summer survey. Taxa richness in the Verdigris river was significantly greater than in the Caney, Neosho spring, and Neosho summer surveys ($P < 0.001$, $P < 0.001$, and $P = 0.004$, respectively). Additionally, taxa richness for the Caney River and Neosho summer was greater than that determined for the Neosho spring survey ($P = 0.019$, $P = 0.014$, respectively).

Percent EPT taxa in the Verdigris River was significantly greater than both the Neosho spring and summer surveys ($P < 0.05$ for both) and the Caney EPT contribution was also significantly greater than the Neosho spring survey ($P < 0.05$). The Shannon index was significantly greater in the Verdigris River as compared to the both the Neosho spring and summer surveys as well as the Caney River ($P < 0.05$ for all comparisons). The biotic index did not differ significantly among any of the three rivers or sample seasons ($P = 0.053$).

Overall, 22 of the 67 taxa were found in all three rivers (32.8%). The Verdigris River contained 14 taxa that were not found in the Caney or Neosho Rivers. Similarly, the Neosho had 10 unique taxa between the two sampling dates, and the Caney River contributed another seven unique taxa. The Verdigris and Caney Rivers were most similar in macroinvertebrate community composition with a Jaccard's Similarity index value of 0.52. The Verdigris and Neosho macroinvertebrate communities were the most dissimilar with a similarity index of 0.41 and the Caney-Neosho community comparison scored 0.47. In summary, the Verdigris River had the most abundant, diverse, and sensitive macroinvertebrate

community of the three rivers. Aside from invertebrate total abundance, summary metrics between the Caney and Neosho Rivers were relatively similar.

While the Neosho River generally scored the lowest in these summary metrics, it is important to note that the habitat characteristics of each river are quite different. The Verdigris River, (stream order value of 6, USGS, 2002), contained many riffle areas with a mixture of substrate sizes from consolidated sand-clay mixture to gravel to cobble. Twenty-six of the 31 sites surveyed (84%) were riffle areas in the Verdigris River. The Caney River, (stream order value of 5, USGS, 2002) also had a diversity of habitat types with clays, silt and exposed bedrock below the impoundment and large amounts of woody debris, to riffle areas comprised of gravel and cobble from the city of Bartlesville and south. Twenty-one of the 27 sites surveyed (78%) were riffle areas in the Caney River. The Neosho River (stream order value of 5, USGS, 2002), on the other hand did have a few gravel riffle areas (five of the 17 sites or 29%); however sampling sites were largely located on large gravel bars with no riffle. These bars were largely unconsolidated and very loose, which generally do not provide as good a habitat for these benthic invertebrates as do the more consolidated substrates with good flow (Death and Winterbourn, 1995; Jowett, 2003). Also, where there were riffle areas in the Neosho, invertebrates were very abundant. For example, Neosho sites 27 and 30 are both riffle areas that supported a high abundance, richness, % EPT, Shannon and the lowest biotic index values for the river (Figures 19-21).

Pearson Correlations between the mussel and invertebrate metrics were generated to determine if sampling locations that supported a diverse invertebrate community also supported a diverse mussel assemblage. The results of these correlation analyses are presented for each river in Tables 12-15. All significant correlations are noted, however not necessarily meaningful. For example, in Table 12 as invertebrate total abundance increases, so does invertebrate richness (0.734, $P<0.001$), this relationship would be expected, as the greater the number of invertebrates collected, the higher the probability that each subsequent individual will be of a different taxon. Perhaps of greater interest for the present study are the significant correlations between the invertebrate metrics and mussel metrics. For example, in Table 12 as invertebrate total abundance increases so does mussel total abundance (0.645, $P<0.001$).

Similarly in the Verdigris River, invertebrate total abundance was correlated with greater mussel richness (Table 12, P=0.015). Invertebrate richness, in the Verdigris River, was positively correlated with mussel total abundance (P=0.041).

Pearson correlations between invertebrate and mussel metrics in the Caney River are presented in Table 13. Invertebrate richness and % EPT was positively correlated with mussel richness (P=0.025 and P<0.001 respectively). Shannon diversity index was positively correlated with both mussel total abundance and richness (P=0.02 and P<0.001, respectively). The Caney River invertebrate biotic index was negatively correlated with both mussel total abundance and richness (P=0.013 and P<0.001, respectively). The negative correlation results from the lower BI values indicating more sensitive taxa (ie. the lower the BI value the "better" the community). These correlations further support the findings of Spooner and Vaughn (2006) and Vaughn et al. (2008) which demonstrate that native freshwater mussels provide nutrition and habitat amenities that enhance richness and abundance of other benthic macroinvertebrates. Additionally, sites in the Caney River with high mussel richness and total abundance also supported sensitive macroinvertebrates. Good water quality at these locations may allow for the proliferation of these species. Conversely, there were no significant correlations between invertebrate and mussel metrics in the Neosho River for either the spring or summer sampling periods (Tables 14 and 15). This may have resulted from the relatively low abundance of both mussels and macroinvertebrates in the Neosho River. Increased sampling effort in the Neosho River may increase macroinvertebrate and mussel metric correlations.

FISH

Lists of fish species collected in each river are presented in Tables 16-18. Thirty-nine species of fish were identified among the three rivers with 11 (28%) of these present in all three rivers. The Verdigris and Caney Rivers were most similar in species composition with a Jaccard similarity of 0.53, although the Caney and Neosho communities scored 0.5. The Verdigris and Neosho communities were less similar with a Jaccard value of 0.37. In the Neosho River, 1 blue sucker (*Cycleptus elongates*), a species with Tier II ranking on the OCWCS (ODWC, 2005), was identified at site 29-30.

Twenty-three fish species were identified in the Verdigris River, with gizzard shad (*Dorosoma cepedium),* longear sunfish (*Lepomis megalotis*), and red shiner (*Notropis lutrensis*) the three most abundant. The Caney River contained the most species with 26 overall. Among those, channel catfish (*Ictalurus punctatus*), red shiner, and freckled madtoms (*Noturus nocturnes*) were the three most abundant. Gizzard shad, threadfin shad (*Dorosoma petenense*) and red shiners were most common among the 25 species collected in the Neosho River. It appears that longnose (*Lepisosteus osseus),* shortnose (*L. platostomus*) and spotted gar (*L. oculatus*) as well as flathead catfish (*Pylodictis olivaris*) largely represent the piscivorus feeding guild in this section of the Neosho River. Hybrid striped bass (*Morone chrysops X saxatilis*), white crappie (*Pomoxis annularis*), and largemouth bass (*Micropterus salmoides*) were also present in small numbers.

In the Verdigris and Caney Rivers, all fish stunned were collected, measured and identified. Due to excessive numbers of shad (both gizzard and threadfin) in the Neosho River, at sites 38-42 the objective was to obtain representatives of every species observed rather than collecting abundance and length data. As such, total abundance and catch per unit effort (CPUE) are most likely underestimates (Table 19). When comparing summary statistics between rivers, the Verdigris River had greater abundance and CPUE than the Neosho ($P = 0.004$ and $P = 0.003$, respectively). As discussed above, this difference likely resulted from our focus on a qualitative estimate of species richness, not abundance in the Neosho River. There was no difference in taxa richness among the three rivers ($P = 0.56$).

Fish are important components of native mussel life history cycles as the mussel glochidia are briefly parasitic on fish before they metamorphose into a juvenile stage that occurs in the river benthos. Additionally, during this parasitic phase, fish serve as vectors for dispersal of native mussels to new areas (Watters, 1992). The degree of host fish specificity varies among mussel taxa, with some mussels having only one known fish host, while other mussel taxa are able to parasitize many common fishes (Isom and Hudson, 1984; Vaughn, 1997). Table 20 lists the mussel species found during these surveys, with the associated fishes that serve as hosts for the glochidia. Host fish species were collected for every mussel taxa found in each river. The fish host for *Obliquaria reflexa* is unknown, with some references

suggesting metamorphosis can occur without parasitism, (Oesch, 1995), however this species was widespread and abundant in all three rivers.

As mentioned above, fishes also serve as dispersal vectors for mussels during the parasitic phase of their lifecycle. As such fishes that have large home ranges may help disperse mussel species over wider areas. Conversely, distributions of mussel species using fish hosts with small home ranges may be more concentrated with dispersal outside this localized patch occurring more slowly (Lee et al., 1998; Berg et al., 2007). As an example, successful reintroduction of *Cyprogenia aberti* from Kansas into sites in the Oklahoma portion of Verdigris River will depend upon logperch (*Percina caprodes*) as it is the only fish host present in this portion of the river. Fortunately, logperch have high dispersal rates when compared with other darter species (Turner, 2001), which should help facilitate the reintroduction in this area.

STABLE ISOTOPES

Rivers

Analyses of stable isotopes in the biota collected from the Verdigris River indicated four discrete trophic levels (Figure 25). Net seston comprised the first trophic level, followed by chironomids, then invertebrates such as caddisflies (Trichoptera), beetles (Coleoptera) and mayflies (Ephemeroptera) as well as native mussels. Lastly, fish were most enriched in ^{15}N and ^{13}C positioning them as the top predators. Invertebrate predators such as stoneflies and hellgrammites (*Neoperla* and *Corydalus*) occupied trophic positions between herbivore invertebrates and fish based on ^{15}N concentrations (Figure 25). They were however, more depleted in ^{13}C than fish, which may indicate they rely more heavily on invertebrates within riffle zones as these areas are more depleted in ^{13}C than pools (Finlay et al., 2002).

The ^{15}N signatures of the organisms collected in the Caney River indicate three trophic levels (Figure 26). The base of the food web was represented by seston with riffle beetle adults (*Stenelmis*) and hellgrammites (*Corydalus*) composing the second trophic level. The third trophic level was composed of fish such as longear sunfish (*Lepomis megalotis*) and red shiner (*Notropis lutrensis*). Interestingly, the stonefly *Neoperla* had an enriched ^{15}N signature (Figure 26) which is consistent with it being a predator

(Merritt et al., 2008). Gizzard shad (*Dorosoma cepedianum*) and threadfin shad (*Dorosoma petenense*) as well as madtoms (*Noturus nocturnes*) were more depleted in ^{13}C than other fish species, indicating they are feeding on organisms also depleted in ^{13}C. These might include filter feeding organisms that utilize a more terrestrially-derived carbon source or invertebrates that are restricted to riffle areas as these habitats tend to have lower ^{13}C values (Finlay et al., 2002). Given their planktivorous feeding strategy and their having been shown to ingest particulate organic matter and sediments (Robinson and Buchanan, 1992), the depleted signatures of gizzard and threadfin shad are not surprising. In contrast, logperch (*Percina caprodes*) and longear sunfish are more enriched in ^{13}C indicating they are utilizing more autochthonous food resources. ^{13}C values for green sunfish (*Lepomis cyanellus*), indicate a more omnivorous feeding strategy, utilizing both depleted and enriched resources. Robinson and Buchanan (1992) suggest logperch feed primarily on aquatic insects and list both longear and green sunfish as omnivorous, consistent with the stable isotope results.

In the Caney River, unionids such as the fragile papershell (*Leptodea fragilis*), 3 horn pimpleback (*Obliquaria reflexa*) and pistolgrip (*Tritogonia verrucosa*) occupied the second trophic level. *Corbicula fluminea* were also positioned at this level. All of these organisms are filter-feeders which appear to be utilizing a carbon resource depleted in ^{13}C, such as terrestrially-derived materials.

While seston and chironomids had similar ^{15}N and ^{13}C concentrations between rivers, mussels and fish in the Caney River were reduced by one trophic level as compared with those in the Verdigris River. This may reflect a difference in river productivity, as smaller, less productive rivers tend to have lower ^{13}C concentrations (Finlay, 2001). Differences in ^{15}N may also be explained by the degree of human influence on the rivers as discharges from wastewater treatment facilities are known to have high ^{15}N concentrations (Cabana and Rasmussen, 1996). The Verdigris River contains 16 permitted outfalls vs. 6 for the Caney River (USEPA, 2010), in congruence with the elevated ^{15}N results in the Verdigris River.

Due to extended high water levels in the Neosho River, samples for stable isotope analyses were not collected until the fall of 2009. These samples are currently at the University of California, Davis,

Stable Isotope Facility awaiting analysis. We expect to receive those results in late January 2010 and will submit an addendum to this report, detailing those analyses as soon as they are received.

Sooner Lake

Since zebra mussels were not present in any river during stable isotope sampling, collections of zebra mussels for stable isotope analysis were made in Sooner Lake, OK. This reservoir contains an abundant population of zebra mussels, possibly introduced from the Arkansas River. Collections of various materials were made to determine zebra mussel trophic position in that system. Sooner Lake covers 5400 acres and is located in Noble and Pawnee counties in north-central Oklahoma. It also serves as a cooling reservoir for a coal-fired power plant, and consequently receives a warm water discharge from the plant. Weirs have been constructed in the lake to direct water flow and ensure intake water used for cooling has returned to ambient temperature. Therefore, a thermal gradient exists within the lake.

Mussels collected from the west boat ramp (warm site) had significantly greater concentrations of ^{15}N versus those collected from the east boat ramp and intake buoy ($P = 0.02$ and 0.01, respectively). Additionally, west boat ramp mussels also had greater ^{13}C concentrations ($P < 0.001$) as compared with mussels from the east boat ramp and intake buoys (Figure 27). While zebra mussels collected from the east boat ramp were significantly smaller than the intake buoy ($P < 0.001$) and the west boat ramp ($P < 0.001$), there was no difference in total length between mussels from the west boat ramp and intake buoy ($P = 0.41$); therefore it does not appear zebra mussel length was responsible for the difference in isotope concentrations. However, the increase in ^{15}N concentrations among zebra mussels collected from the west boat ramp (0.7‰), while statistically different from mussels collected from the other sites, is not great enough to constitute a significant change in trophic positioning. A 2-5‰ increase in ^{15}N is generally noted between successive trophic levels (Gannes et al., 1998; Post, 2002).

A more likely explanation for the variability in isotope composition among Sooner Lake zebra mussels is a change in the composition of resources between the sites. Sediment was used as a baseline for isotope composition among the sites and was most enriched in ^{13}C at the warmest site, similar to zebra mussel enrichment patterns. Additionally, *Corbicula* were also most enriched in ^{15}N within the discharge

channel. Therefore, the enrichment of zebra mussel tissue appears to be correlated to the resources available within individual sites at Sooner Lake. Studies of zebra mussel isotope composition in large riverine systems have also demonstrated site specific patterns. Fry and Allen (2003) were able to distinguish between isotope signatures of zebra mussels collected from multiple sites along the Mississippi River based on specific watershed inputs.

Of more importance to the current study is the close association in isotope composition between zebra mussels and *Corbicula* (Figure 28, red circle). At both the end discharge and intake channel locations, zebra mussels and *Corbicula* had very similar isotope compositions, indicating a high degree of overlap between their food resources. Given the current cosmopolitan distribution of *Corbicula*, (they were noted at nearly every mussel survey site) it may be possible to use *Corbicula* as a surrogate to determine where zebra mussels would be positioned in the food web, should they colonize an area. While this topic is largely unexplored in the scientific literature, the reported isotope concentrations for both zebra mussels and *Corbicula* generally fall between 8-10‰ ^{15}N and -26 to -24‰ ^{13}C for both species, (Mitchell et al., 1996; Doi et al., 2005) in accordance with the current study.

Corbicula in the Caney River exhibited some overlap in ^{15}N with native mussels (Figure 26) although there were differences in ^{13}C concentrations between these organisms. Given that *Corbicula*, zebra mussels, and native mussels are all filter feeders, some degree of overlap in their stable isotope concentrations may be expected. Additionally, other invertebrates such as Hydropsychid caddisflies had similar isotope concentrations to native mussels (Figure 25). These organisms may also be negatively impacted by zebra mussel infestations due to competition for food resources. On the other hand, the presence of zebra mussels has also been shown to increase certain invertebrate taxa such as deposit feeders, small gastropods, trichopterans and ephemeropterans, particularly in soft substrate habitat (Mayer et al., 2002; Beekey et al., 2004), but also in areas with hard substrate (Ricciardi et al., 1997). These increases are largely due to increased habitat complexity and deposition of additional food resources from the water column to the benthos.

Regardless, zebra mussels are known to reduce native mussel populations (Schloesser and Nalepa, 1994), which are already undergoing a significant reduction worldwide (Lydeard et al., 2004). While zebra mussel druses and shells may provide refuge for some invertebrate taxa, they also compete for food resources with these same taxa. Overall, stable isotope results indicate invertebrates in the Coleoptera, Ephemeroptera, Trichoptera, and Diptera orders are likely to compete with zebra mussels should they be introduced into these systems.

GEOGRAPHIC INFORMATION SYSTEMS

Our objectives for the GIS component of this project were to map sampling locations on the Verdigris, Caney, and Neosho River (Figure 29) and also show areas of zebra mussel infestation. Additionally, we wanted to identify sites that may be at risk of future zebra mussel invasion. Hard copies of some the figures generated to address these objectives have been included here to facilitate discussion (Figures 29-32). An electronic copy of the entire GIS database is also included with this final report.

Sites that contained mussel species with a Tier I, II, or III ranking in the state OCWCS (ODWC, 2005) document are presented in Figure 30. The Verdigris River was the only one of the three rivers that had live Tier I species. These are the western fanshell (*Cyprogenia aberti*) and the rabbitsfoot mussel (*Quadrula cylindrica*). The Caney River had numerous sites supporting Tier II species, largely the wartyback mussel (*Quadrula nodulata*) however one plain pocketbook (*Lampsilis cardium*) was also located. Tier II species located in the Neosho River consist of the plain pocketbook as well. Finally, the Verdigris and Neosho Rivers also support a number of Tier III species, namely the monkeyface (*Quadrula metanevra*) and bleufer mussels (*Potamilus purpuratus*). Also in Figure 30, we identified areas that are known to contain zebra mussels, which are shown in orange cross-hatched notation.

Additionally, we wanted to determine which sites occurred near roads or bridges as these areas often facilitate public access. These areas may then be targeted for higher priority monitoring to help prevent human-induced disturbance to mussel beds or the introduction of invasive species. After adding the county road layer, a buffer of 250 meters was applied to all sites containing Tier I, II or III mussels to identify those that occurred within 250 m of a county road (Figure 31).

As mentioned above, bridges often provided areas of public access to these rivers. Figure 32 identifies stream segments, rather than specific sites, that may be visited by the public. Once on the river a canoe or small boat could provide transportation to nearby mussel beds. Additionally, it was noticed during the mussel surveys that county roads may run parallel with the river for a short distance yet never cross it. These locations may also serve as access areas. To locate these areas, a 50 meter buffer was applied to each river and areas where county roads overlapped the rivers were highlighted (Figure 32). Large sections of the Caney River were highlighted as areas that are fairly accessible to the public and this was evident during mussel surveys as bridges offered good access to the river. The Neosho River is also indicated as relatively accessible, however this was not as evident during the surveys. Aside from a short stretch of road running parallel with the river north of Stepps Ford Bridge and the bridge itself, access to the upper Neosho was more limited than the Caney. The city of Miami, OK also provided good access to a number of locations along the Neosho.

Based on the areas highlighted with the GIS software, several conservation management implications may be indicated. First, the Tier I species in the Verdigris River should have first priority for conservation efforts, such as continued monitoring, periodic defouling if zebra mussel densities return to high levels, or propagation and reintroduction of mussels to restore both the western fanshell and the rabbitsfoot to their historic distributions. Reintroduction of the rabbitsfoot mussel to the Verdigris River upstream from Oologah Lake would not only help restore the historic distribution of this species but would also provide another population that might be less prone to zebra mussel fouling since no zebra mussels were detected at any site above the lake. The western fanshell was found in low numbers (2 individuals) from sites near the Oklahoma-Kansas border, and since this species is increasing in abundance in portions of the Verdigris River in Kansas (Miller and Lynott, 2006), continued downstream dispersal may facilitate population increases in Oklahoma as well.

Second, areas that are highly visited by humans may also be targeted as sites to monitor to help prevent human induced disturbance to mussel beds or the introduction of invasive species. The Caney River should be the focus of these efforts, given the high accessibility to the river, and the large number

of sites that contain Tier II mussel species. Additionally, it has been shown that zebra mussels may require an upstream source for continued proliferation in some riverine habitats (Horvath et al., 1996; Bobeldyk et al., 2005). Therefore, Hulah and Copan Lakes should also be monitored for zebra mussel introductions, considering a population in either of these lakes may spread zebra mussels throughout the river and into the city of Bartlesville where they can have detrimental effects on both the riverine biota and industries that draw water from the Caney River.

Lastly, in the Neosho River, the Stepps Ford Bridge was highlighted as an area that provided access to the river. During sampling, a 4mm dreissenid mussel was collected in one kicknet sample from just upstream of the bridge. This was the only dreissenid mussel collected in the entire river, however it may indicate that dreissenid mussels are in the area. Subsequent sampling in August did not reveal any additional invasive mussels. There was some debate about whether this dreissenid was a zebra (*Dreissena polymorpha*) or Quagga mussel (*Dreissena bugensis*). Genetic analyses proved unsuccessful as the soft tissue had degraded sufficiently prior to analyses. Regardless, the ecological effects are similar between the two species (Mills et al., 1996), and their effects on native unionids are well documented (Burlakova et al., 2000; Martel et al., 2001; Schloesser et al., 2006). Marion Reservoir in Kansas was recently found to harbor a population of zebra mussels (Jason Goeckler, Kansas Department of Wildlife and Parks, personal communication). This lake is upstream from the Oklahoma portion of the Neosho River, which provides an aquatically-linked source of zebra mussels. John Redmond Reservoir, which lies between Marion and the Oklahoma-Neosho River, would provide a more immediate source of the invasive. Consequently, this area should also be the focus of continued monitoring efforts.

VI. SIGNIFICANT DEVIATIONS

As mussel and macroinvertebrate sampling was delayed in the Neosho River due to continued high water levels, stable isotope analyses of representative biota in this system are currently being analyzed by the Stable Isotope Facility at the University of California-Davis. We anticipate these data

arriving in late January or early February. We will submit an addendum to this report with a full analysis of the stable isotope data for the Neosho River, as soon as those data are received.

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