

**Composition of Benthic Aquatic Insects Among Reference-Condition Streams of the
Northern Cross Timbers Ecoregion of Oklahoma**

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Honors Thesis

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Abstract

In this study, I investigated the composition and distribution of benthic aquatic insects, a group of benthic macroinvertebrates in the streams throughout the Level IV Northern Cross Timbers Ecoregion of Oklahoma. Fifteen study sites, each with a sampling reach, were established to collect benthic aquatic insects and associated stream habitat data. Major environmental variables that can affect the composition and distribution of benthic aquatic insect assemblages were identified and determined in this study. Benthic aquatic insect samples were collected from the fifteen study sites in the summers of 2019 and 2020.

In total, 1286 individuals belonging to 93 unique benthic macroinvertebrate taxa were collected across all samples in the summer of 2019. Similarly, a total of 1572 individuals belonging to 104 unique taxa were collected across all sites in the summer of 2020. Insects belonging to the genus *Cheumatopsyche sp.* showed the most dominant abundance followed by *Corydalus cornulus* among the study sites in the summer of 2019. For the summer of 2020, *Tricorythodes sp.*, *Cheumatopsyche sp.*, and *Caenis sp.* were the dominant taxa in all study sites combined. The average score for total habitat for woody substrate type study sites was 165.9 and 167.3 for the rocky substrate type study sites. In addition, the Hilsenhoff Biotic Index was calculated with the following results: 5.31 for woody study sites and 5.22 for rocky study sites. Spearman correlations analysis was conducted to evaluate the relationships between environmental variables and benthic aquatic insects as well as between the aquatic benthic insects metrics itself. A statically significant negative correlation was reported between the average EPT (orders Ephemeroptera, Plecoptera, and Trichoptera) index and average Hilsenhoff Biotic Index (HBI), which suggested that an increase in one biological metric contributes to the decrease in other biological metrics. A statistically significant negative correlation was reported

between EPT Richness and Nitrogen concentrations (mg/L) suggested the degradation of a stream if excess amount of nutrients is available in the streams.

Introduction

Biological monitoring, which is the study of biological organisms and their responses to the environment, is often used to evaluate and determine environmental conditions (Onyena, 2019). One type of biological monitoring is known as the biological survey (bio-survey), which involves collecting, processing, and analyzing aquatic organisms to determine the health of the biological community in a stream (EPA, 2012). Bio-surveys can indicate when the stream ecosystem is impaired by pollution or some sort of habitat loss (Herbst et al, 2001).

Monitoring stream health using benthic macroinvertebrates is an effective tool for determining reference condition streams (Li et al, 2010). Benthic macroinvertebrates are used for the determination of a stream's water quality. Aquatic insects constitute the majority of a benthic macroinvertebrate community in streams and are used as bioindicators as was done in this study (Arimoro and Ikomi, 2009). Aquatic benthic insects lack a backbone (invertebrate), are large enough (macro) to be seen with the naked eye, inhabit all types of streams, and include insects in their larval or nymph forms. Several parameters such as land use, water flow and depth, and field parameters influences water quality (Patang et al., 2018). Macroinvertebrate diversity has been a topic of interest for research as it is one of the most effective and inexpensive ways to study and determine the quality of water (Patang et al., 2018). Analysis of the physical and chemical properties of water is often used for determining the water quality of streams; however, more evaluation, such as biological evaluation, is needed for establishing a proper picture of a stream's water quality (Patang et al., 2018). This study specifically collected, identified, and studied aquatic insects, a type of benthic macroinvertebrates, during the summers of 2019 and 2020 to

evaluate the composition of benthic aquatic insects and how they can indicate a stream's water quality.

Many aquatic organisms such as mayflies (order Ephemeroptera), caddisflies (order Trichoptera), and stoneflies (order Plecoptera) spend part or most of their early life cycles attached to submerged rocks, logs, and vegetation. Stream quality is better indicated by benthic aquatic insects because they are unable to escape exposure to pollution and are critical to stream health since they are the base of the food web (EPA, 2012; Jun et al, 2016). These organisms show the effects of short- and long-term pollution events. Some organisms are very intolerant to pollution, and they are affected by the physical, chemical, and biological conditions of the stream, and they are relatively easy to sample and identify (EPA, 2012; Jun et al, 2016). A stream with a higher diversity of aquatic life is healthier, and benthic aquatic insect diversity and abundance are significant community features that can be controlled by a variety of mechanisms at different spatial levels (EPA, 2012; Minshall et al, 1985).

Several studies have investigated and identified: (1) how benthic aquatic insect assemblages respond to environmental factors, (2) relationships among benthic aquatic insect assemblages and chemical variables, and (3) which factors effectively explain benthic aquatic insect distribution and abundance (Onyena, 2019; Jun et al, 2016; Li et al, 2010; Patang et al, 2018). Habitat-related physical factors like flow and current velocity, elevation, stream size, and substrate vegetation have been that were widely demonstrated as primary contributors to the stream health (Jun et al, 2016). It is crucial to have studies on the spatial distribution patterns of benthic aquatic insect assemblages that are based on the relationship of the assemblages to environmental factors. The results provide the readers valuable information on “conservation and restoration of biodiversity against anthropogenic disturbances” (Jun et al, 2016).

The objectives of this study were: (I) to identify the dominant benthic aquatic insect taxa in each type of sampled habitat, (II) to evaluate if there a difference in selected benthic aquatic insect metrics between the type of stream substrates sampled, and (III) to identify the environmental variables that impact selected biological metrics of benthic aquatic insects.

Methodology

Study sites:

Fifteen study sites were chosen in the Northern Cross Timbers Ecoregion of Oklahoma to survey for benthic macroinvertebrates and stream habitat (Figure 1 and table 1). The following criteria were used for the selection of the candidate stream sites: (1) the State of Oklahoma's 303(d) list, a state's list of impaired and threatened water bodies, currently does not have the stream segment along with the candidate site, (2) the stream segment as well as the candidate site did not have a significant regulation of flow, (3) the stream segment upstream of the candidate site does not have any point-source discharged, (4) the candidate site must be more than 50 meters from upstream and/or 300 meters downstream of a stream confluence, (5) the stream segment including the candidate site holds a riparian buffer that is greater than 30 meters in width from each bank side, and (6) each candidate site has land cover in the upstream watershed that is less than 20 percent urbanized.

Benthic aquatic insects were collected from fifteen study sites in the Northern Cross Timbers Ecoregion of Oklahoma (Figure 1 and table 1) for both years of study; however, we only subsampled fourteen study sites in the summer of 2019 because a sample from one of the sites was unintentionally disturbed (was accidentally spilled in the floor and there was not any time remaining to return to the site and sample it again). As shown by figure 1, the Northern Cross

Timbers of Ecoregion of Oklahoma (level IV) that is roughly 16,000 km², is the setting for this research study (<https://www.epa.gov/eco-research/regions>, accessed on 12 Nov 2021). This ecoregion consists of an oak savannah that is dominated by eastern red cedar, scrubby oak forests, and tall grass prairie (Hoagland et al, 1999). Most of the streams in the Northern Cross Timbers Ecoregion have sand beds. Bedrock, boulders, and cobbles dominated the stream channels that are in the far northern portion of the Northern Cross Timbers Ecoregion.

Table 1: List of Streams, their locations, and their watershed area (Km²).

Site ID	Stream Name	Y Coordinate	X Coordinate	Watershed Area (Km ²)
27	Rock Creek	34.49676	-96.98013	112.325
54	Rock Creek	35.08595	-96.24396	34.391
45	Graves Creek	35.17514	-96.27079	77.016
106	Montezuma Creek	35.54996	-95.92839	77.016
108	Coon Creek	36.99725	-96.22616	17.29
111	Travertine Creek	34.50122	-96.96203	8.774
101B	Mahala Creek	36.42674	-96.3183	43.602
52B	Pond Creek	36.89377	-96.31390	62.649
41	Salt Creek	35.08595	-96.78899	365.379
46	Beaver Creek	35.34751	-96.47085	2.48
76	Spring Brook Creek	34.78263	-96.75392	152.409
89	Sand Creek	35.35104	-96.49359	26.289
104	Sandy Creek	35.02986	-96.67029	22.912
105	Pond Creek	35.01605	-97.14753	76.128
113	Guy Sandy Creek	34.50401	-97.03018	77.016

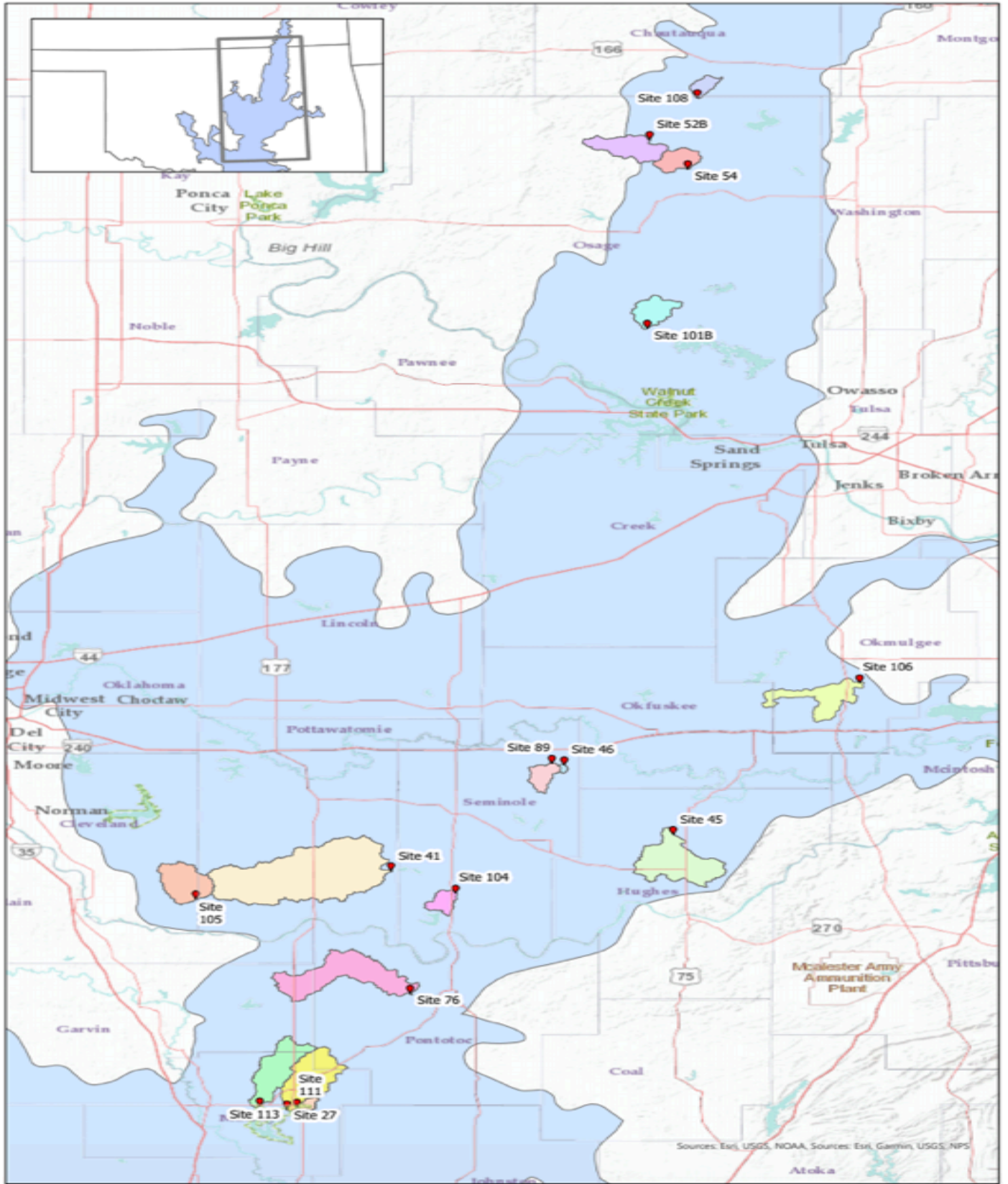


Figure 1. Fifteen study locations within the Northern Cross Timbers Ecoregion of Oklahoma and their contributing watershed were selected

Selected environmental factors were considered for the study locations to define their effects on benthic macroinvertebrates assemblages. Watershed-scale regional variables and reach-scale water quality variables were the categories of environmental variables that were used in this study.

Land-use and riparian factors were considered as regional variables for watershed characteristics in determining reference-conditions streams. A geospatial geographic information system (GIS) was combined with non-geospatial data from the Public Land Survey System (PLSS dataset) to format and standardize a 140-acre spatial grid for the study area. Candidate streams that intersected each 140-acre cell and met the aforementioned candidate screening criteria were selected for further consideration. Initially 71 sites were selected, and 42 of these were chosen for on-site reconnaissance. After on-site reconnaissance, sites were selected if they fit all the following criteria: (1) wadable, (2) perennial, (3) readily accessible, and (4) the landowner could be contacted for permission to sample. A final set of fifteen sites was selected.

A Manta +30 multi-probe portable meter was used to measure and record water quality variables (i.e., pH, water temperature (°C), specific conductance ($\mu\text{s}/\text{cm}^2$), and dissolved oxygen (mg/L) were measured in the centroid of flow in the channel at the upstream boundary of each study site. One water sample was collected from the centroid of flow and placed in clean sterilized plastic bottles at each site. Water samples were kept on ice and transported to a laboratory in Ada, Oklahoma, to be analyzed. The samples were analyzed for nitrogen as nitrate

+ nitrite (mg/L) and total phosphorus (mg/L) by Environmental Resources Technologies, Incorporated.

Sampling of Benthic Macroinvertebrates

An aquatic sampling net composed of 500 μm mesh was used to collect benthic aquatic insects. Samplings were collected in flowing riffle or run habitats within a 400-meter sampling reach. Following the protocols, a total of five minutes were spent collecting from woody/vegetative substrate type streams for a benthic macroinvertebrates sampling. Similarly, a total of three minutes was spent collecting from rocky substrate type streams for the sampling of benthos. Samples were cleaned as much as possible by removing entrained organic and inorganic debris at the study site using stream water, stored, and labeled together in a plastic bottle with 70 percent ethanol for preservation. The samples were then taken back to the laboratory at East Central University for further processing.

Once back in the laboratory, all samples were subsampled for benthic aquatic insects by hand-sorting the captured organisms from any inorganic matter and organic detritus, and stored in ethanol. The following steps, which align with the protocols provided by the Quality Assurance Project Plan for the KDEP Surface Water Monitoring Program (2018), were used in cleaning and subsampling of the benthos:

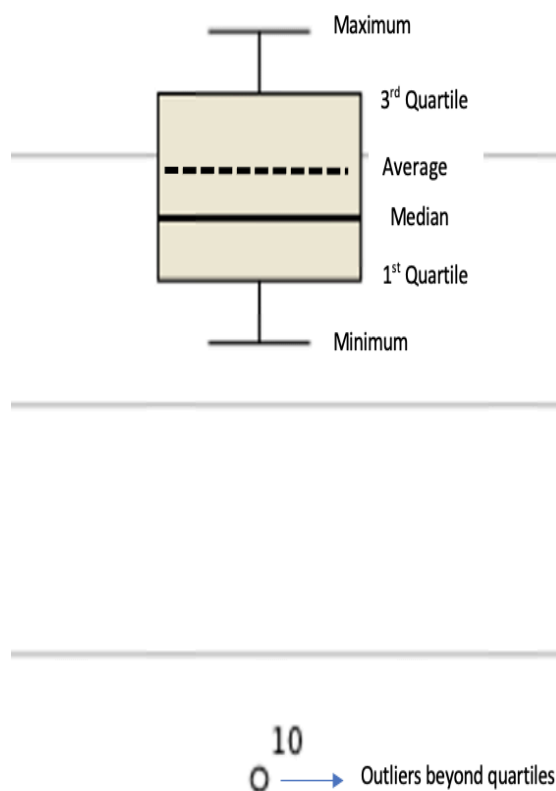
1. Place a 500 μm sieve on top of a large plastic tray or bucket.
2. Pour contents of the sample bottle in a 500 μm sieve.
3. Capture the preservative in a jar in the tray/bucket to return to the sample jar later.
4. Place the sieve over an empty tray/bucket to continue processing.
5. Rinse the sample jar thoroughly with tap water and pour the contents into the sieve.

6. Place the sieve in a large plastic tray filled with tap water.
7. Partially (half) submerge the sieve and evenly distribute the sample in the sieve.
8. Using forceps, gently remove any large debris such as leaves, twigs, and gravel.
9. Rinse each piece of debris over the sieve with tap water using a squirt bottle to dislodge any organisms.

Benthic aquatic insects were subsampled and stored in a vial with 70 percent ethanol. Once half-submerged in the tray, the organisms were randomly distributed throughout the tray. Using a cell randomization sheet produced by Excel®, organisms were randomly selected from the tray and placed in a vial with 70 percent ethanol for preservation. Subsampling was completed once 100-150 organisms were collected. Subsampled organisms from all the sites were then shipped to EcoAnalysts in Moscow, Idaho for identification, enumeration, and metric calculations. Each benthic aquatic insect was identified to the lowest possible taxonomic level such as genus or species. Some taxa that could only be identified at the family level provided limited information. All individuals in each subsampled were identified and accounted for.

Data Analysis and Management

Data were input and managed using Excel. For analysis, data were exported to and analyzed in IBM Statistical Package for the Social Sciences (SPSS) software (Arkkelin, 2014). The following statistical analyses were used in this study: Mann-Whitney U (MWU) nonparametric test, and Spearman correlations. The selected metrics including: EPT index, Hilsenhoff Biotic Index, Shannon-Wiener diversity index, and composite habitat score were determined for each site. The following sample boxplot provides a legend for the boxplots analyses (EPT index, Species Richness, HBI, and Shannon-Wiener diversity) used in this study:



Interpretation:

-Minimum: the lowest value in the dataset found at the bottom of the graph.

-Maximum: the highest value in the dataset found at the top of the graph.

Quartiles: values are distributed in a range from lowest values to highest and divided (quartered) into four relatively equal sections.

-1st Quartile: the lowest 25% (quarter) of values

-Median: the 50% value with two quartiles above and two quartiles below.

-Average: mean value of the dataset

-3rd Quartile: 75% of the values fall between the minimum and this quartile

Outliers: data points that fall outside of the quartiles

Results

A total of 93 unique taxa were identified from fourteen streams sampled during the summer of 2019. Similarly, a total of 104 unique taxa were identified from the fifteen streams sampled during the summer of 2020. Table 2 shows the most dominant taxa reported for the summer of 2019 (*Cheumatopsyche sp.*, and *Corydalus cornulus*) for all study sites. Similarly, table 3 indicates that *Tricorythodes sp.*, *Cheumatopsyche sp.*, and *Caenis sp.* were the dominant taxa, in that order, for all study sites combined for summer 2020. Most organism were aquatic insects in their larval or nymphal form.

	Site Name	1st Dominant	2nd Dominant
Rock Substrate	Rock Creek #27	<i>Heliopsyche sp.</i>	<i>Corydalus cornutus</i>
	Rock Creek #54	<i>Cheumatopsyche sp.</i>	<i>Chimarra sp.</i>
	Graves Creek	<i>Stenelmis sp.</i>	<i>Stenacron interpunctatum</i>
	Pond Creek #52B	<i>Cheumatopsyche sp.</i>	<i>Stenelmis sp.</i>
	Mahala Creek	<i>Cheumatopsyche sp.</i>	<i>Polypedilum flavum</i>
	Montezuma Creek	<i>Hydropsyche sp.</i>	<i>Cheumatopsyche sp.</i>
	Coon Creek	<i>Cheumatopsyche sp.</i>	<i>Polypedilum illinoense gr.</i>
	Travertine Creek	<i>Thienemannimyia gr. sp.</i>	<i>Argia sp.</i>
Wood Substrate	Salt Creek	<i>Polypedilum illinoense gr.</i>	<i>Tricorythodes sp.</i>
	Beaver Creek	<i>Caenis latipennis</i>	<i>Hyaella sp.</i>
	Pond Creek #105	<i>Caenis latipennis</i>	<i>Paracloeodes sp.</i>
	Guy Sandy Creek	<i>Fallceon sp.</i>	<i>Cheumatopsyche sp.</i>
	Spring Brook Creek	<i>Cheumatopsyche sp.</i>	<i>Maccaffertium terminatum</i>
	Sand Creek	<i>Cheumatopsyche sp.</i>	<i>Corydalus cornutus</i>

Table 2. 1st and 2nd Dominant Taxon found within the two substrate type study sites for the summer of 2019.

	Site Name	1st Dominant	2nd Dominant
Rock Substrate	Rock Creek #27	<i>Tricorythodes sp.</i>	<i>Hydropsyche scalaris</i>
	Rock Creek #54	<i>Hyaella sp.</i>	<i>Cheumatopsyche sp.</i>
	Graves Creek	<i>Cheumatopsyche sp.</i>	<i>Stenelmis sp.</i>
	Pond Creek #52B	<i>Polypedilum flavum</i>	<i>Stenelmis sp.</i>
	Mahala Creek	<i>Chimarra sp.</i>	<i>Corydalus sp.</i>
	Montezuma Creek	<i>Cheumatopsyche sp.</i>	<i>Baetis intercalaris</i>
	Coon Creek	<i>Stenonema femoratum</i>	<i>Polypedilum flavum</i>
	Travertine Creek	<i>Camelobaetidius variabilis</i>	<i>Tricorythodes sp.</i>
Wood Substrate	Sandy Creek	<i>Tricorythodes sp.</i>	<i>Cheumatopsyche sp.</i>
	Salt Creek	<i>Tricorythodes sp.</i>	<i>Corydalus sp.</i>
	Beaver Creek	<i>Caenis sp.</i>	<i>Procladius sp.</i>
	Pond Creek #105	<i>Caenis sp.</i>	<i>Argia sp.</i>
	Guy Sandy Creek	<i>Maccaffertium terminatum</i>	<i>Cheumatopsyche sp.</i>
	Spring Brook Creek	<i>Cheumatopsyche sp.</i>	<i>Tricorythodes sp.</i>
	Sand Creek	<i>Caenis sp.</i>	<i>Polypedilum illinoense gr.</i>

Table 3. 1st and 2nd Dominant Taxon found within the two substrate type study sites for the summer of 2020.

For both summers, a combined 205 mayflies (Order Ephemeroptera), stoneflies (order Plecoptera) and caddisflies (Order Trichoptera) combined for both years of survey was reported (135 mayflies and 70 caddisflies) as shown in Figure 2. Order Plecoptera was not recorded more

than once in this study because they are not common in this ecoregion. They are more common in the areas to the east in the Ozark Plateau as well as northern parts of Oklahoma.

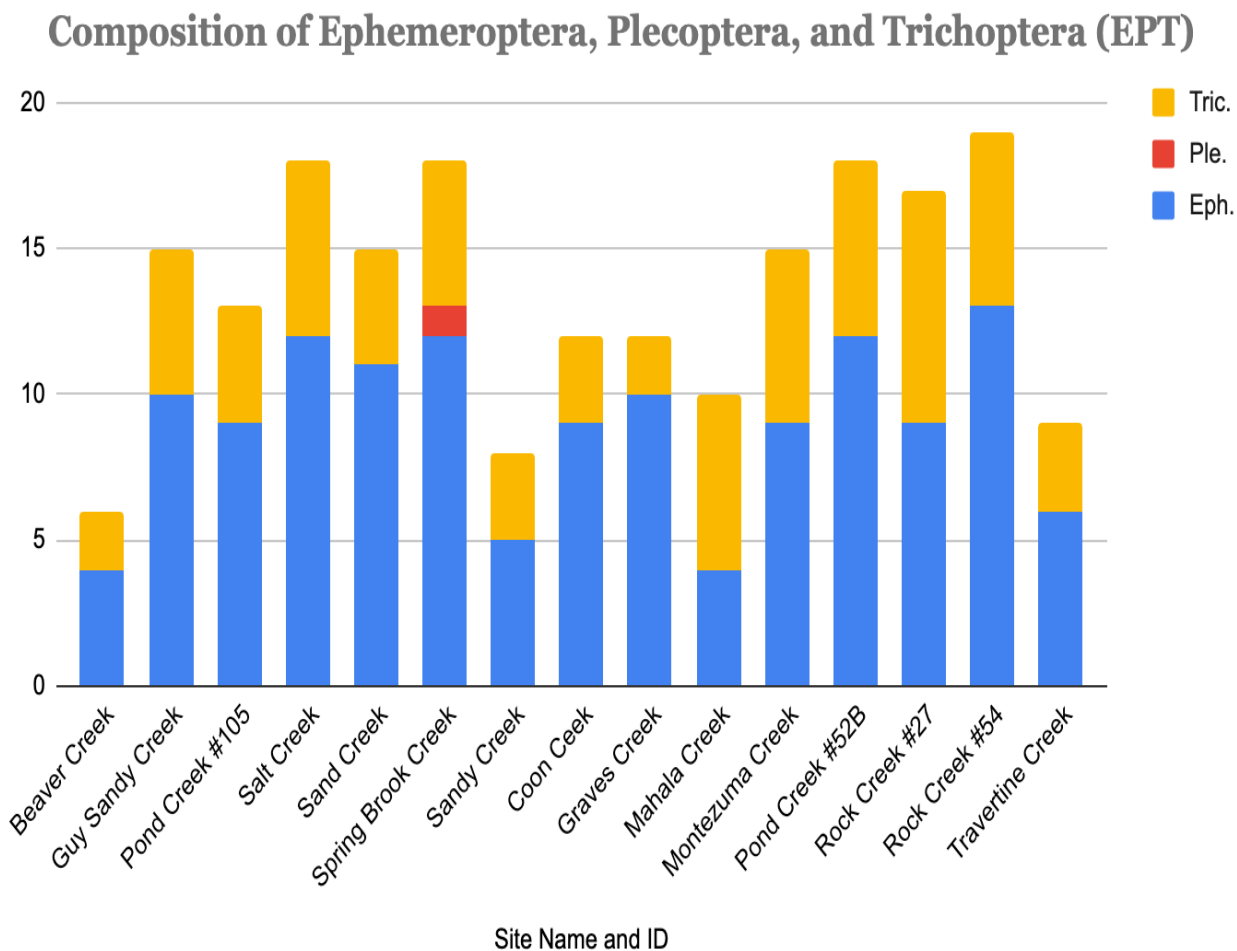


Figure 2. Stacked graph of EPT (Order Ephemeroptera, Order Plecoptera, and Order Trichoptera) composition among the fifteen study sites. The first seven sampling sites represent the wood substrate sampling sites while the last eight sampling sites represent the rock substrate sampling sites.

The number of mayfly (order Ephemeroptera), stonefly (Order Plecoptera), and caddisfly (Order Trichoptera) (EPT index) taxa combined had a higher (figure 3) average for the rock

substrate (7.0 EPT taxa) as compared to the wood substrate (7.2 EPT taxa). However, it was noted that the median EPT index was higher for the wood substrate when compared with rock substrate type study locations (Figure 3). There was not a statistically significant difference (MWU = 31, $p = 0.779$) for EPT between rock and wood substrates.

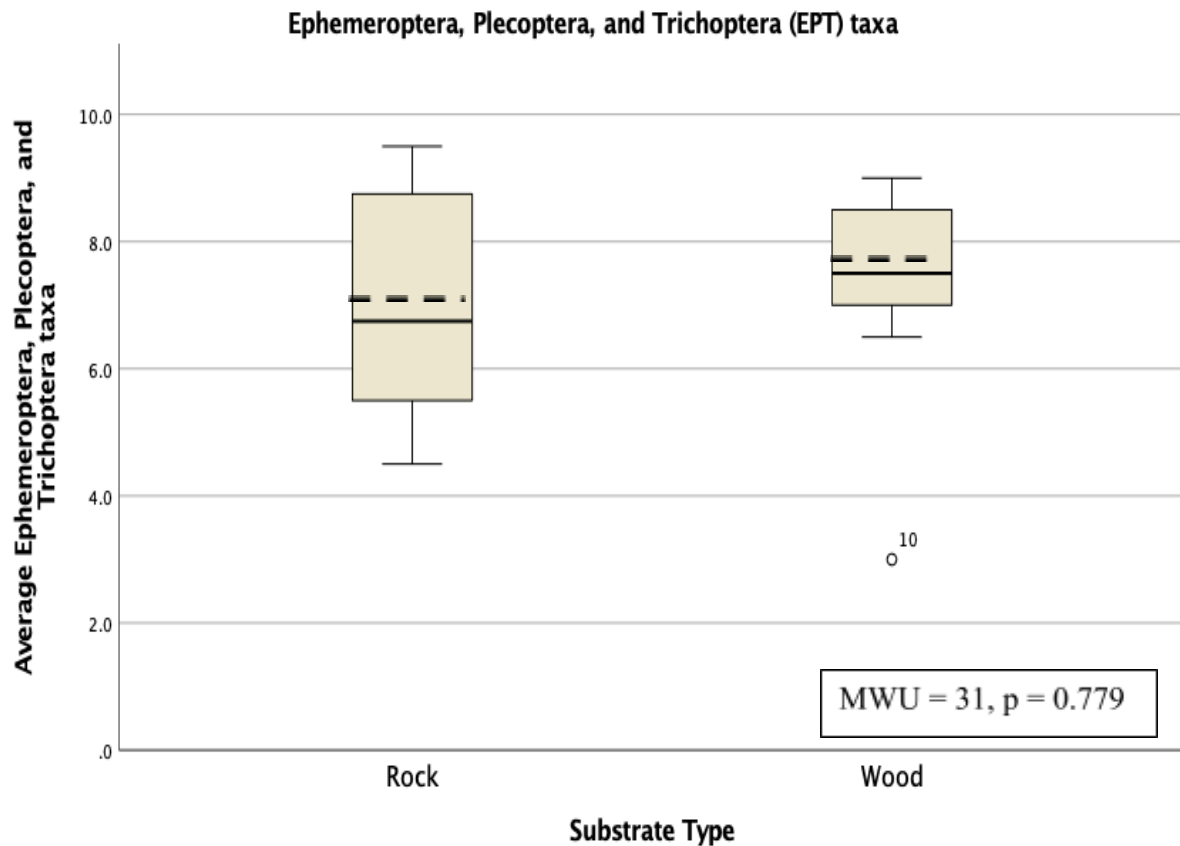


Figure 3. Comparison of the average Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPT) Index score from all fifteen sites for the two substrate types focused for this study.

Considering only aquatic insects for 2019 and 2020, the average species richness, of aquatic insects alone, for the rock substrate type was 17.5 while for the wood substrate type was 22.4 for 2019 and 2020 (Figure 4). Species richness was statistically significant different (MWU = 52.5, $p = 0.002$) between substrate types. Species richness is defined as the number of species within a community and/or sample (Hassan et al, 2005).

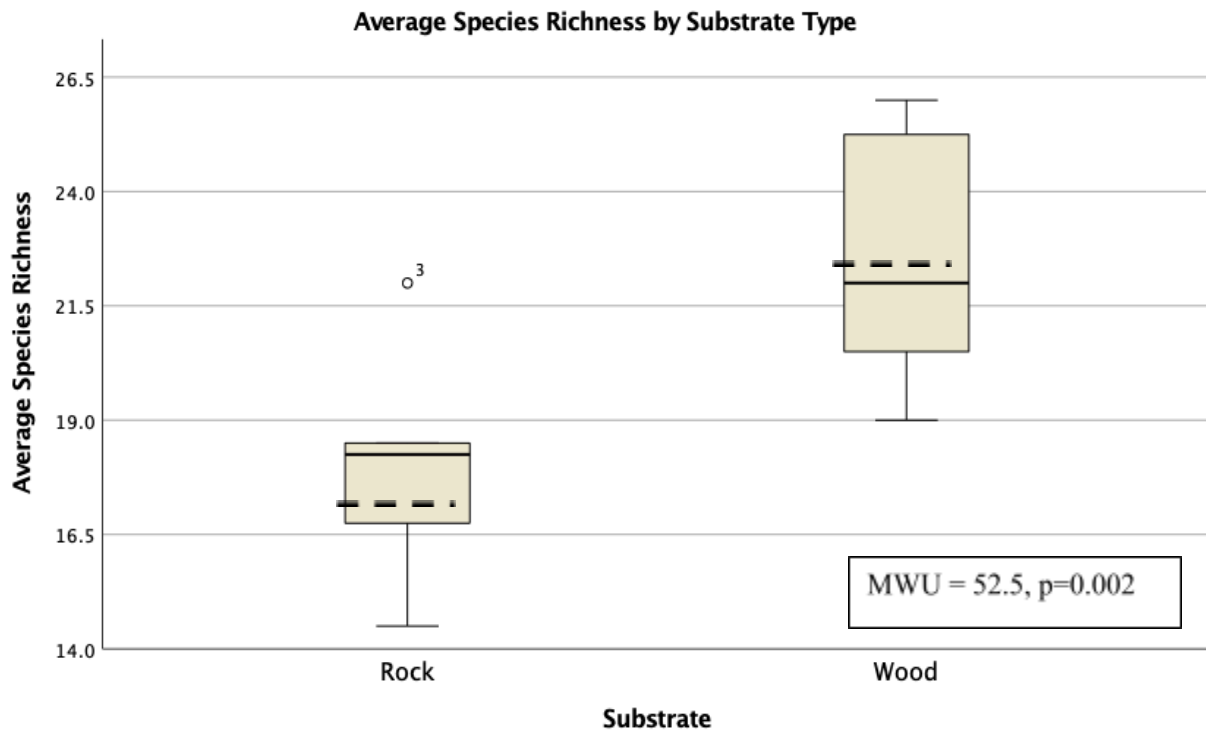


Figure 4. Comparison of the average number of species richness from all fifteen study sites for the two substrate types focused for this study.

The average Hilsenhoff Biotic Index (HBI) for the two summers was not statistically significantly different (Mann-Whitney U test = 24; $p = 0.694$) between rock and wood substrates (Figure 5). The Hilsenhoff Biotic Index is used as a community metric in the evaluation of overall stream health and its water quality by incorporating characteristics of taxonomic diversity with “tolerance values of individual taxa” (USGS, 2002). Hilsenhoff Biotic Index scores estimate the overall tolerance of the community in a sampled area that is further weighted by the relative abundance of each taxonomic group. The Hilsenhoff Biotic Index ranges from tolerance values of 0 to 10, which indicates the group’s known sensitivity to organic pollutants in the streams; 0 is

most sensitive to pollutants while 10 is most tolerance to pollutants (Hilsenhoff, 1987). The woody study sites had an average HBI of 5.31 while the rocky study sites had an average HBI of 5.22 (figure 5). This statistic was calculated by tallying up the Hilsenhoff Biotic Index value for the sampling site, in each habitat (wood or rock), for both years of study. The tallied-up sum was then divided by the number of study sites that were categorized into the habitat types (7 wood and 8 rock substrate study sites) to get the average Hilsenhoff Biotic index.

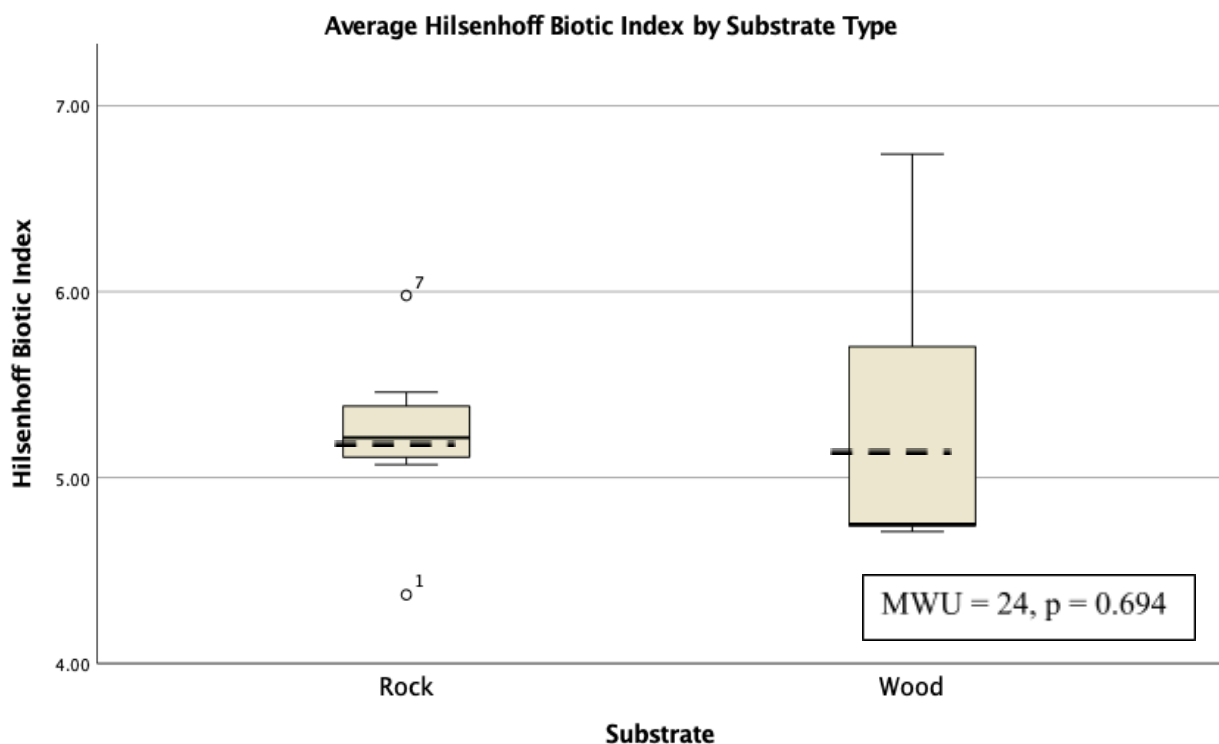


Figure 5. Comparison of the average Hilsenhoff Biotic Index from all fifteen study sites for the two substrate types used for this study

The Mann-Whitney U (MWU) nonparametric test (LaMorte, 2017) was used to compare differences between selected biometrics for the two substrate types. This non-parametric test was

used because the data used for all comparisons were found to be non-normally distributed, and the MWU test is a good substitute for a parametric test for paired comparisons. The null hypothesis that the distribution of average EPT is the same across categories of substrate was not rejected. However, the null hypothesis that the distribution of average species richness is the same across the categories of substrate is rejected by the test. Similarly, the null hypothesis that the distribution of Shannon-Wiener (\log_{10}) is the same across the categories of substrate was rejected. The average Shannon-Wiener Index for the two summers was statistically significantly different (Mann-Whitney U test = 51; $p = 0.006$) between rock and wood substrates (Figure 6). Shannon-Wiener Index is a diversity measure that is based on combining species richness and their relative abundance. As shown by Figure 6, the study locations dominated by a woody substrate had a larger range of Shannon-Wiener Index suggesting that it is a higher value of the two different comparisons. Wood substrate study sites had a greater median value as well as a higher maximum and minimum value than rocky substrate locations.

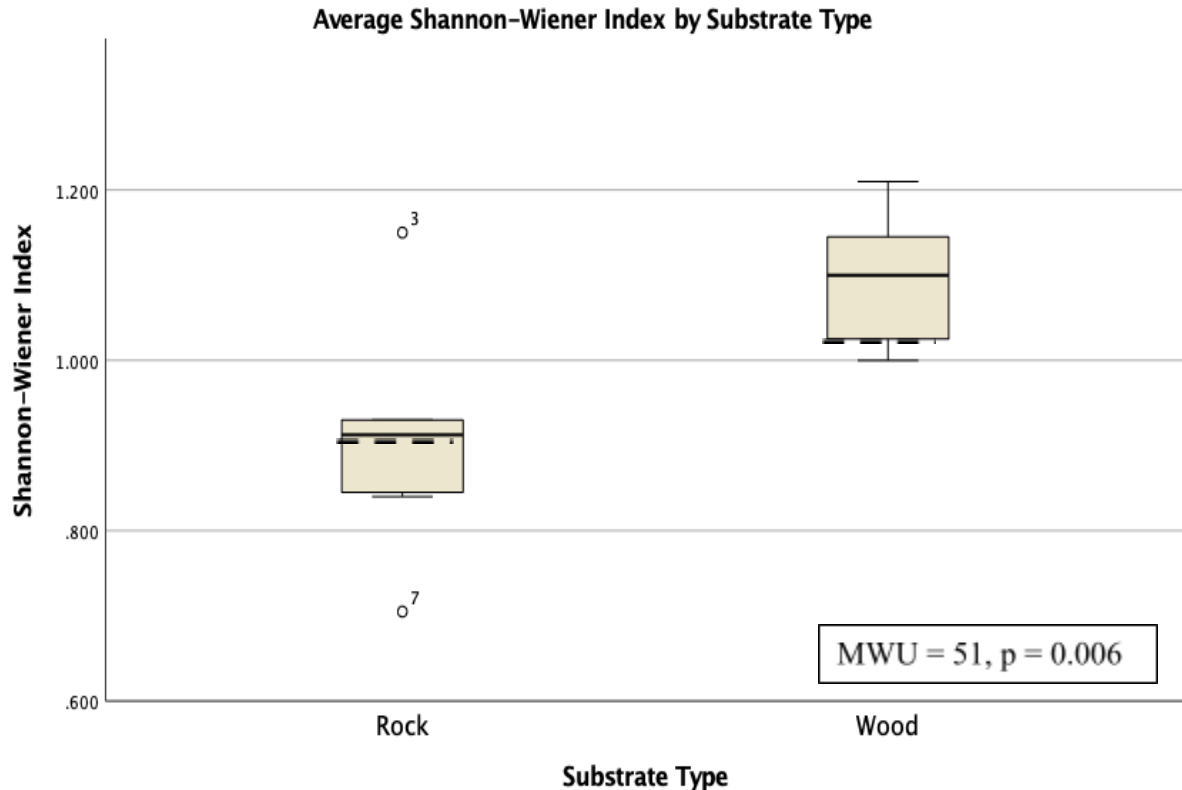


Figure 6. Comparison of the average Shannon-Wiener Index from all fifteen study sites for the two substrate types focused for this study.

There was a statistically significant positive correlation ($R^2=0.679$, $p<0.01$) between the watershed area, measured in Km^2 , and the average phosphorus concentrations, measured in mg/L (Figure 7). Figure 8 indicates a statistically significant negative correlation was found between the average EPT Richness and average Hilsenhoff Biotic Index ($R^2 = -0.677$, $p<0.01$). Additionally, a statistically significant negative correlation ($R^2 = -0.629$, $p<0.01$) was recorded between the average EPT Richness and average Nitrate-Nitrite (mg/L) concentrations (Figure 9).

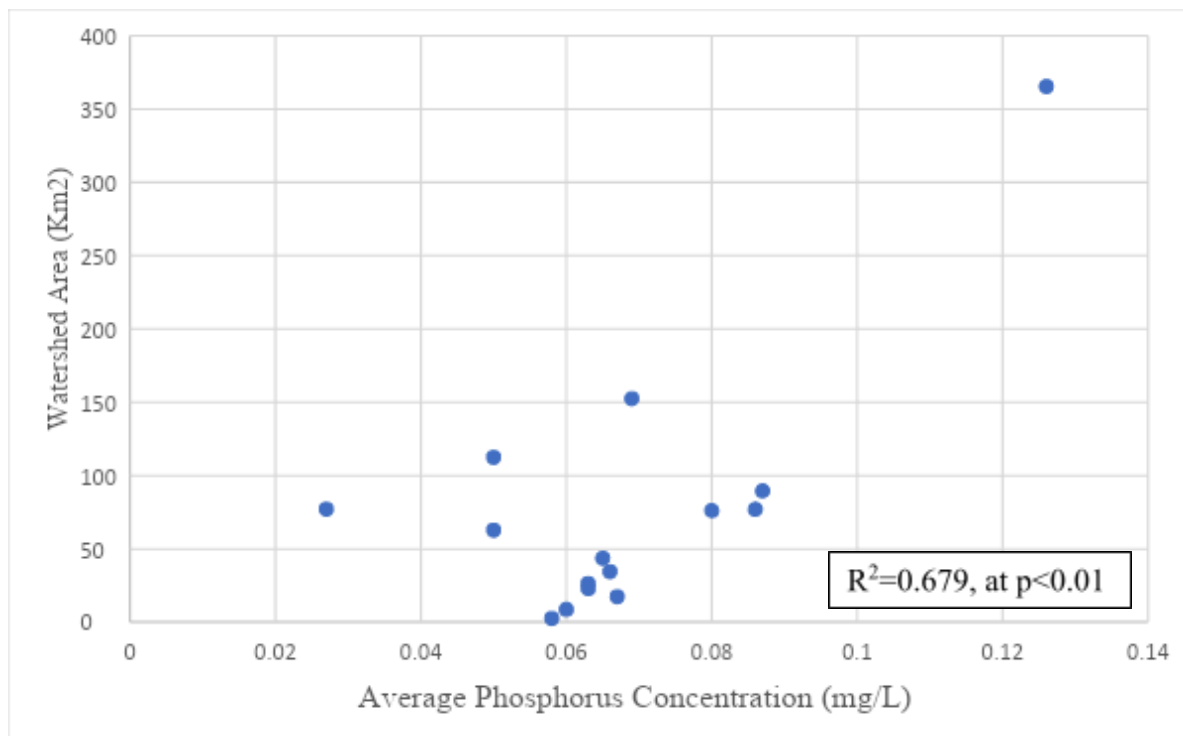


Figure 7. A statistically significant positive correlation between average watershed Area ((Km²) and average phosphorus concentration (mg/L).

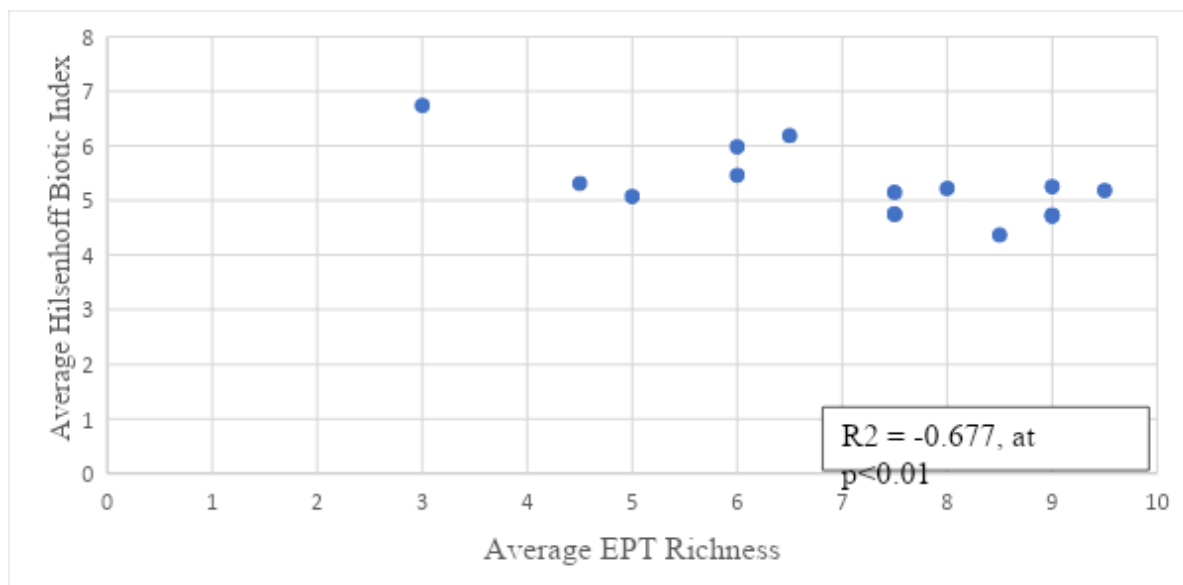


Figure 8. A statistically significant negative correlation between average EPT Richness and average Hilsenhoff Biotic Index.

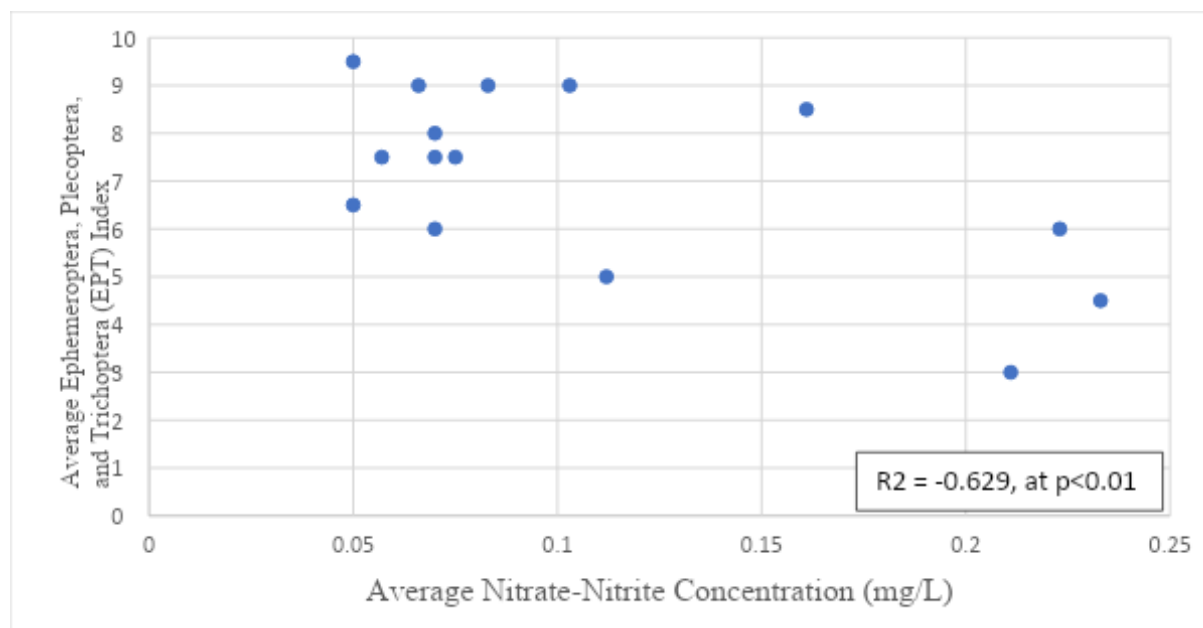


Figure 9. A statistically significant negative correlation between average EPT Richness Index and average Nitrate-Nitrite concentration (mg/L).

The average composite habitat score for study sites dominated by the woody substrate was 165.9 and 167.3 for sites dominated by rocky substrate. This was calculated by taking the mean habitat score for all sites within one substrate type for both years of the ecological habitat survey. The habitat score was determined for each 400-meter study reach following a habitat assessment for low gradient streams assessment procedures that is detailed in the Quality Assurance Plan for the KDEP's Surface Water Monitoring Program (KDEP, 2018). Ten individual habitat measures including in-channel and riparian zone measures were utilized in calculating the composite habitat score. A score range of 0 to 20 was used for each measurement; this included the scale that was subdivided into four condition categories ranging from optimal to poor. A stream that had a composite score of ≥ 160 was considered an optimal habitat score (KDEP, 2018).

Discussion

Clean water is vital to healthy aquatic habitats. The impact of increased development in many different areas can be observed in the water quality of aquatic ecosystems. The benthic macroinvertebrates, particularly benthic aquatic insects, reveal important information about the streams that make up their habitat. Benthic aquatic insects are valuable bioindicators in freshwater streams and rivers as they live along the stream bottom, are affected by the quality of water in their habitat, and are large enough to be seen with the naked eye. In other words, they are optimal organisms for studying the health of freshwater ecosystems, especially when one understands their role in the environment, and how they interact with and are affected by their environment.

In the summer of 2019, insects belonging to the taxon *Cheumatopsyche sp* showed the most dominant abundance, followed by *Corydalis cornulus*. In the summer of 2020, three taxa showed dominance (*Tricorythodes sp.*, *Cheumatopsyche sp.*, and *Caenis sp.*). Larvae of *Cheumatopsyche sp.* was the most common dominant taxon found in both years of sample collection.

Cheumatopsyche is a genus of net-spinning caddisflies, belonging in the order *Trichoptera* and family *Hydropsychidae*. *Cheumatopsyche* are important in nutrient cycling and energy flow in the aquatic ecosystems food webs. Over 40 species are included in the genus *Cheumatopsyche* in North America, and they are widely distributed in most types of freshwater habitats including spring streams, rivers, lakes, and more (Merritt and Cummins, 1996). Species and genera belonging to the family *Hydropsychidae* are often sedentary organisms that “construct fixed retreats” with a net at one end so they can sieve food from currents in water (Merritt and Cummins, 1996). Currents are an important aspect to these organisms’ survival as they are dependent on the water to carry food to their retreats. *Tricorythodes* and *Caenis* are both in the

order Ephemeroptera. *Tricorythodes sp.* belongs to the *Leptohyphidae* family and are widely distributed throughout Oklahoma. Both *Tricorythodes* and *Caenis* are gatherers, but *Caenis* are scrapers as well (Merritt and Cummins, 1996). *Caenis* belongs to the family *Caenidae* and are widely distributed. These organisms typically live-in habitats that are lotic (depositional) and lentic (sediments) (Merritt and Cummins, 1996). These organisms function as the dominant taxa found in the sampled sites indicating that because there were a higher number of sensitive taxa in the streams, the streams were of good quality.

A total of 205 *Ephemeroptera*, *Plecoptera*, and *Trichoptera* (*EPT Index*) individuals were collected in both years of sampling. The EPT index is often utilized by many researchers as an indicator of the ecological health of streams (Moring, 2002; Jun et al, 2016; & Hamid and Rawi, 2017). The EPT index constitutes very intolerant taxa that has low tolerance for water pollutants (Hamid and Rawi, 2017). Member of the order Plecoptera were collected in only one sampling site (Spring Brook Creek) because they are uncommon in this ecoregion. Figure 2 indicates that more taxa in the order Ephemeroptera were identified than order Trichoptera (except for Mahala Creek) in samples from both rock and wood substrates. The average EPT index for both substrate types were not significantly different, indicating that the streams were related in terms of their tolerance taxa. The averages (7.0 for rock substrate and 7.2 for wood substrate) were both near the tolerance level, indicating that the water quality was in a good condition. Similarly, the Hilsenhoff Biotic Index did not report statistically significant different averages among the two sampling types. As specified by figure 6, the HBI values were only slightly different (difference of 0.09). The average HBI values for both rock and wood sampling locations were around 5, which shows that the streams contained an equal number of sensitive as well as tolerant species. In addition, the average difference in species richness (aquatic insects only) was statistically

significantly different between the wood substrate (22.4) and the rock substrate (17.4), implying that a larger number of different species lived in the habitat that was dominated by the wood substrate type. Overall, the woody habitat had a greater diversity of aquatic insects ecosystem.

Multiple relationships (between environmental variables and biological variables) were evaluated by analyzing the correlations between them. Nutrients, such as nitrate-nitrite and phosphorus (measured in mg/L) were collected and analyzed as the environmental variables to see how they influence selected aquatic insect metrics. A statistically significant positive correlation was found between the watershed (Km²) and the phosphorus concentration (mg/L) (figure 7). Higher phosphorus is typically associated with eutrophication of a body of water, which can be caused by an excess amount of nutrients (nitrogen and phosphorus) causing a dense growth of plant life and death of animal life forms due to lack of oxygen (USGS, “Nutrients in Streams”; Munn et al., 2018). Due to the positive correlation relationship between the phosphorus concentration (mg/L) and the watershed area (Km²), it can be concluded that as watershed area increases, the phosphorus concentration in the streams also increases. As the stream watershed area gets larger, greater the chances of eutrophication occurring.

A statistically significant negative correlation was found between the average EPT Richness and the average Hilsenhoff Biotic Index. The negative correlation among the two different categories indicates that these variables increase/decrease in the opposite direction to one another. As the average EPT index increase, the average HBI slightly decreased. EPT index is the number of taxa within the orders (Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), which are sensitive to pollution (Reif, 2003). HBI is found by evaluating an organism’s relative sensitivity to stream-quality conditions (Jun et a., 2016). If the EPT index number is higher, this indicates that there are more intolerable taxa in the water.

Similarly, a higher HBI number also indicates more tolerant taxa to water pollutants. In this study, the EPT index average was 7.0 for rock substrate sampling type and 7.22 for wood substrate sampling type, indicating that there were more tolerant species in comparison to the sensitive species. In contrast, the HBI value was approximately around 5.0 (5.22 for wood substrate type; 5.31 for rock substrate type), which indicates that the level of pollution in the streams was in the middle range. Low HBI values show more abundance of sensitive groups, which suggests a lower level of pollution. So, the negative correlation between EPT index and HBI shows that as one tolerant factor increases, the other factor becomes intolerant of the pollution. High HBI value indicates a high tolerant taxon while a low HBI value reflects a higher abundance of sensitive groups, which indicates a lower level of pollution in the water (Hilsenhoff, 1987). If the EPT index is high, then the HBI number is expected to be low because a higher Hilsenhoff Biotic Index implies that more tolerant taxa are present.

A statistically significant negative correlation was noted between the average EPT index and the average Nitrate and Nitrite concentrations (mg/L). Nitrate and nitrite are forms of nitrogen, and are important plant nutrients necessary for growth (U.S. EPA, 2012; Munn et al, 2018). However, if an excess amount of nitrate-nitrite is found in streams, it can cause water quality problems such as algal blooms (Ward, 2008). As with phosphorus, nitrate-nitrite can contribute to the eutrophication of a body of water. Thus, if there is a higher nitrate-nitrite concentration in the streams, then the number of sensitive species (EPT Index) in the streams will decrease because nitrate-nitrite will contribute to the pollution in the streams (figure 9).

Conclusion

The composition of benthic aquatic insect assemblages in the Northern Cross Timbers Ecoregion (level IV) of Oklahoma may be explained by a few strong environmental and

biological variables. It is critical to understand the relationships between environmental factors and aquatic insects communities as well as the relationship within the aquatic insects communities; understanding these relationships allows better evaluation of the health of a body of water. Following benthic aquatic insects dominate species that function as important indicators of stream health and water quality were recorded for both years of study:

Cheumatopsyche sp., *Tricorythodes sp.*, and *Caenis sp.* These are all species belonging to biological orders that are sensitive to pollutants. Based on the EPT index and HBI values, this study determined that both substrate (rock and wood) types sampled contained approximately the same amount of pollution according to the number of sensitive as well tolerant species present. In addition, the negative correlation between the nitrogen (nitrate-nitrite) concentration and the EPT index suggests the availability of an excess amount of nutrients in a stream contributes to a decrease in the number of sensitive (to pollution/harmful variables) organisms recorded. Overall, the distribution of benthic aquatic was consistent throughout the Northern Cross Timbers Ecoregion of Oklahoma as many aquatic families were identified repeatedly in the many streams sampled in this study.

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References

- Ab Hamid, S., & Md Rawi, C.S. (2017). Application of Aquatic Insects (Ephemeroptera, Plecoptera, and Trichoptera) In Water Quality Assessment of Malaysian Headwater. *Tropical life sciences research*, 28 (2), 143-162.
- Arkkelin, Daniel, "Using SPSS to Understand Research and Data Analysis" (2014). *Psychology Curricular Materials*. 1.
- Arimoro, F.O., & Ikomi, R.B (2009). Ecological integrity of upper Warri River, Niger Delta using aquatic insects as bioindicators. *Ecological Indicators*, 9(3), 455-461.
- Comparison of dissolved oxygen and aquatic biota ... - USGS*. U.S.G.S Science for a changing world. (2002, August). Retrieved November 14, 2021, from https://pubs.usgs.gov/fs/fs06302/pdf/FS_063-02.pdf
- Environmental Protection Agency. (2012, March 6). *Chapter 4 Macroinvertebrates and Habitat | Monitoring & Assessment | US EPA*. United States Environmental Protection Agency. Retrieved October 28, 2021.
- Hassan R, Scholes R, Ash N (eds) (2005) Millenium Ecosystem Assessment: Ecosystems and Human Wellbeing, Volume 1, Current State and Trends. Island Press, Washington.
- Herbst, D.B., et al (2001). The California streamside biosurvey. *Sacramento: California State Water Resources Control Board*.
- Hilsenhoffm W, L (1987) An improved Biotic Index of Organic Stream Pollution. *Michigan Entomology Society*, 20(11):9-13

Hoagland, B.W (1999) “Ecology and Vegetation of the cross timbers in Kansas, Oklahoma, and Texas” in the Savannas, Barrens, and Rock outcrop Plant Communities of North America. *Cambridge University Press*.

Kickapoo Department of Environmental Programs. (2018). Quality Assurance Project Plan for the KDEP’s Surface Water Monitoring Program.

LaMorte, W. W. (2017, May 4). *Nonparametric tests*. Mann Whitney U Test (Wilcoxon Rank Sum Test). Retrieved November 14, 2021.

Li, Li, et al (2010). Biomonitoring and bioindicators used for river Ecosystems: Definitions, approaches, and trends. *Procedia environmental sciences*, 2, 1510-152

Malakane, k., et al (2020) Benthic Macroinvertebrates as Bioindicators of Water Quality in the Blyde River of the Olifants River Systems South Africa. *Applied Ecology & Environmental Research*, 18(1), 1621-1635

Merrit, R.W., & Cummins, K.W. (1996). *An introduction to the aquatic insects of North America*. 3rd ed. Dubuque, Iowa: Kendall/Hunt Pub. Co

Minshall, G.W., et al (1985). Species richness in streams of different size from the same drainage basin. *The American Naturalist*, 125(1), 16-38.

Moring, J.B. (2002). Baseline assessment of instream and riparian-zone biological resources on the Rio Grande in and near Big Bend National Park, Texas. *Water-Resources Investigations Report*, 02-4106.

Munn, M.D., et al (2018) Understanding the influence of nutrients on stream ecosystems in agricultural landscapes. *U.S. Geological Survey Circular 1437*, 80 p.

Nutrients in Streams. Major issues and findings--nutrients in streams. (2016, November 13).

Retrieved November 14, 2021, from <https://pubs.usgs.gov/circ/circ1171/html/nutrients.htm>.

- Onyena, A. P. (2019). Composition, distribution, and diversity of benthic macroinvertebrate from creeks around Lagos Lagoon, Nigeria. *Journal of Applied Sciences and Environmental Management*, 23(5), 857-863
- Patang, F., Soegianto, A., & Hariyanto, S. (2018). Benthic Macroinvertebrates diversity as bioindicator of water quality of some rivers in East Kalimantan, Indonesia. *International Journal of Ecology*, 2018
- Reif, A. G. (2002). Assessment of stream quality using biological indices at selected sites in the Red Clay and White Clay Creek Basins, Chester County, Pennsylvania, 1981-97. *US Department of the Interior, US Geological Survey*.
- Ward, M. H. (2009). Too much of a good thing? Nitrate from nitrogen fertilizers and cancer. *Reviews on Environmental Health*, 24(4), 257-363.
- Yung-Chul Jun, et al (2016). Spatial Distribution of Benthic Macroinvertebrate Assemblages in Relation to Environmental Variables in Korean Nationwide Streams. *Water (20734441)*, 8(1), 27.