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# Using Macroinvertebrates to Assess the Effects of Nutrient Input Between the Nolichucky and Pigeon Rivers

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# Using Macroinvertebrates to Assess the Effects of Nutrient Input Between the Nolichucky and Pigeon Rivers

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by

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ABSTRACT

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Previous work found significant differences in growth rates of native mussels at locations downstream from the regulated Walter's Dam and the out-of-service, free-flowing Davy Crockett Dam. The purpose of this study is to investigate differences within the macroinvertebrate communities related to factors driving the differences in mussel growth between rivers. Macroinvertebrate samples were collected following the Tennessee Department of Environment and Conservation protocol for SQKICK collection and analyzed using the Tennessee Macroinvertebrate Index (TMI). There were no significant differences in TMI scores between the downstream sites of the rivers, but there were significant increases in chlorophyll$_a$, dissolved oxygen, and specific conductance downstream compared to upstream in both rivers. This suggests that these indices are suitable to identify pollution changes, but potentially not the productivity differences that impacted mussel growth.

ACKNOWLEDGEMENTS

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# CHAPTER 1. INTRODUCTION

## *Background*

The Pigeon River runs 113 kilometers from Haywood County, North Carolina, to the western half of Cocke County, Tennessee. The Pigeon River watershed encompasses an area of 1823 $km^2$. Although a large portion of the watershed is forested on the steep slopes, the valleys are heavily used for agriculture and urban development (USDA 2004). For decades the river was polluted due to a papermill located approximately 65 kilometers from the Tennessee state line in Canton, North Carolina. From the time the mill opened in 1908 until the U.S. Environmental Protection Agency took over the permits in 1988, toxic chlorinated wastewater flowed into the river and into eastern Tennessee (Bartlett 1995). Despite improvement in water quality, the river remains 303(d) listed for biological impairment due to pollution from irrigated crop production and alteration by upstream impoundments (TDEC 2017). The Pigeon River watershed is displayed in Figure 1.

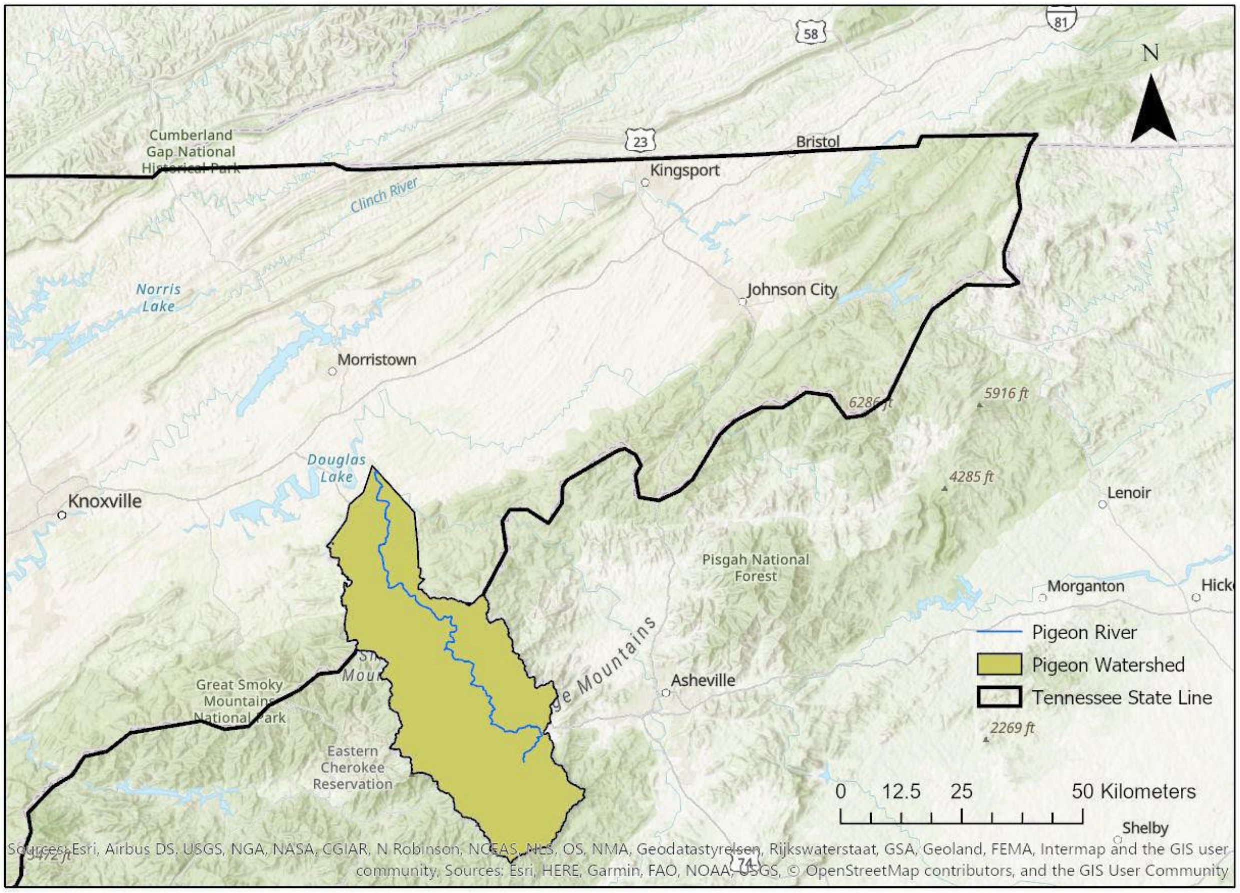


Figure 1. Map of Pigeon River watershed

The primary impoundment on the Pigeon River is Walters Dam which was completed in 1930. Walters Dam is unique in that the brick powerhouse stands 10 kilometers downstream from the dam itself. The hydrostation is used to generate nearly 112 MW for Duke Energy annually (Duke Energy 2020). Between the months of April and September, the flow of the river is regulated to generate power, giving the river times of high and low flow (USDA 2004).

The Nolichucky River originates in Mitchell County, North Carolina. The Nolichucky River watershed has an area of approximately 2,922 km$^2$. The river spans 185 kilometers from its origin to its confluence with the French Broad River in East Tennessee. The Nolichucky River watershed is displayed in Figure 2.

The confluence of the Nolichucky and French Broad River occurs upstream from the Douglas Lake impoundment created by Douglas Dam. Douglas Reservoir extends 14.4 kilometers up the Nolichucky River from its confluence with the French Broad River. Located 74 kilometers from the confluence with the French Broad near Greenville, TN, are the Nolichucky Dam and the 154.9-hectare Davy Crockett Reservoir which extends 12 kilometers from the Nolichucky Dam (National Park Service 1980; TDEC 2006). The Nolichucky Dam was built by Tennessee Eastern Electric Company (TEEC) in 1913 as a hydroelectric dam and was taken out of service in 1972 due to siltation of the reservoir (TDEC 2006). It is now used primarily for flood control and recreation through the creation of Davy Crockett Reservoir. The Nolichucky River free flows over top of the dam leaving the flow regime uninterrupted.

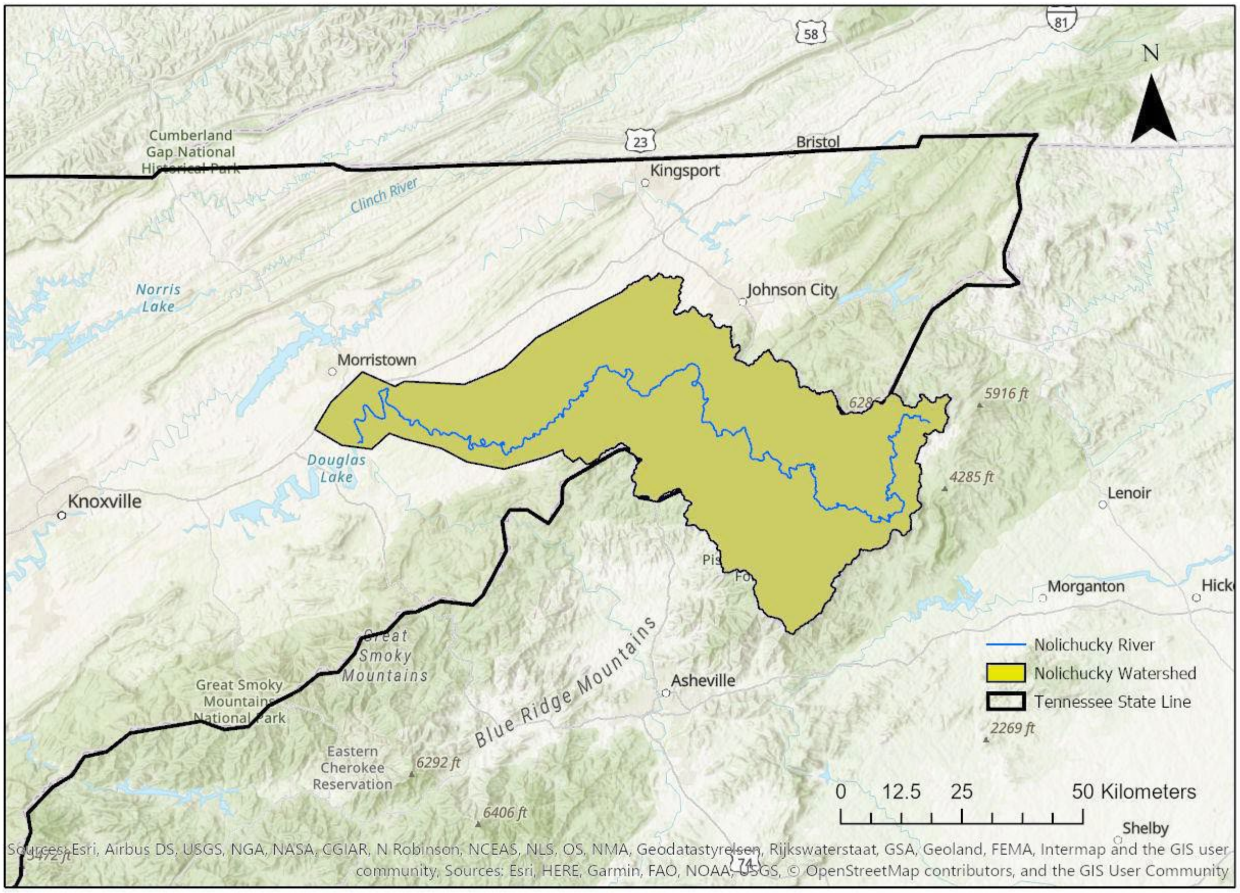


Figure 2. Map of Nolichucky River watershed

As might be expected, damming of rivers is associated with significant flow disruption that can impact downstream aquatic biota. Biodiversity and movement of organisms suffer due to changes in thermal regime, physio-chemical properties, and sedimentation caused by interrupting the flow of the river (Gierszewski et al. 2019). For example, Quist et al. (2005) found a greater number of exotic fish species above an impoundment of the Great Plains River as compared to below where native species dominated. Native species were reduced or lost in the reservoir system and introduced species were highly successful because of the relatively stable lentic habitat and limited competition with other species (Quist et al. 2005). Boeckman and Bidwell (2008) found no live freshwater mussels immediately downstream from an impoundment in

Oklahoma, although numbers of mussels increased with downstream distance from the impoundment.

In 2019, an unpublished study by Bidwell and Roden investigated seston quality and its relationship to freshwater mussel health in the regulated Pigeon River and free-flowing Nolichucky River. The sites were chosen based on instream habitat, hydrologic features, and watershed land use. They found growth and glycogen content of juvenile pocketbook mussels (*Lampsilis ovata*) were significantly lower downstream from the dams in the Pigeon River as compared to those in the Nolichucky River. There were no differences in mortality between the two rivers which suggests that flow regulation may have been the primary driver for the growth differences rather than some acute stressor that resulted in lethality. This previous study raises questions regarding ecological effects of impoundments and the ramifications of ceasing operation of hydroelectric dams on the benthic macroinvertebrate community. Specifically, investigating if any structural differences in the benthic macroinvertebrate community existed between the sites that could help further explain the growth differences in native mussels that was previously observed in an unpublished study completed by Bidwell and Roden (2019).

Benthic macroinvertebrate community surveys have been used previously to compare habitat differences between sites.

*Using Macroinvertebrates as Indicators of Stream Health*

The Tennessee Department of Environment and Conservation (TDEC) investigates the severity of stream impairment using macroinvertebrates as indicators for habitat conditions for fish and other aquatic life. These surveys use biological criteria (biocriteria) to present a very detailed description of the condition of the stream as a whole (TDEC 2017). Biocriteria are the quantitative measures that describe the biological conditions of aquatic communities inhabiting bodies of water intended to support aquatic life.

Benthic macroinvertebrates are easily targeted when assessing the biological condition of a body of water. Benthic macroinvertebrates are organisms that dwell on the bottom of bodies of water that can be collected using a 500-μm sieve. These organisms are visible without the aid of a microscope and do not have a backbone. These most often include groups of aquatic and semi- aquatic insects, crustaceans, and molluscs. These organisms are frequently used to evaluate stream condition since they often rely on a stable habitat and are sensitive to a range of anthropogenic water quality impacts and pollutants (TDEC 2017).

Macroinvertebrates often exhibit a range of sensitivity to changing water quality. For example, insects in the orders Ephemeroptera, Plecoptera, and Trichoptera (mayflies, stoneflies, and caddisflies), collectively referred to as EPT, are typically more sensitive to changes than other orders. In contrast, the most tolerant species are in the subclass Oligochaeta and order Diptera (earthworms and true flies). The abundance and diversity within the macroinvertebrate community can provide insight to the type and severity of the habitat disturbance within a stream system (Kellogg 1994).

Benthic macroinvertebrate community surveys have been used previously to compare habitat differences between sites. For example, Ortiz et al. (2006), examined how community measures and composition of macroinvertebrates were related to habitat parameters in the La Tordera River. The macroinvertebrates were collected upstream and downstream from a point source input of treated wastewater. They found density and biomass of macroinvertebrates were positively correlated with resources such as benthic organic matter, chlorophyll, and vascular plants observed. The taxa richness was negatively correlated with conventional habitat parameters such as velocity and depth of the water at collection site. The study also found that the number of significant correlations between the macroinvertebrates and habitat variables at the upstream sites were increased due to the upstream sites having higher taxa richness.

Water flow affects morphology of streams, sedimentation, water chemistry, and the biology of organisms inhabiting the system (Wetzel 2015). White et al. (2017) investigated the response of macroinvertebrates across temperature gradients for regulated rivers compared to non-regulated rivers. Community abundances, functional traits of the organisms, and biomonitoring indices were determined. The regulated sites exhibited reduced low-flow variability and peak flow discharge greater that of the non-regulated site. This study found that macroinvertebrate functional groups were particularly sensitive to flow changes and indices based on functional groups improved the ecological discrimination between the rivers.

Both structural and functional attributes of the benthic macroinvertebrate community have been used to assess stream health (Carlisle and Clements 2003). Structural metrics are used to encompass the occurrence, distribution, and population size of single species which are the infrastructure of communities (Shlacher et al. 2014). Often these simple metrics can be used to explain complex relations within benthic ecology (Tonkin 2014). In a study conducted by Lynch (2013), structural metrics were used to evaluate the effect of a drought on the benthic community by assessing the macroinvertebrate assemblage within the riffles of Ozark highlands streams.

The Tennessee Macroinvertebrate Index (TMI) is an example of a structurally based multimetric indicator used by the TDEC. The TMI uses seven taxonomic biometrics in a single habitat assessment to evaluate the biological conditions of the habitat. The TMI scores range from 0-42. Depending on the ecoregion in which samples are collected, a score of 31 or lower would indicate poor stream health. This is the case in the ecoregion where this study was conducted.

Functional metrics go beyond the traditional taxonomic approach to assess stream health. These metrics are related to the ecosystem functionality and stability in such a way that they can give insight to individual, population, and ecosystem level processes (Laini et al. 2019). For example, populations that persist in chronically impaired conditions may exhibit increased metabolic rates which could result in lower total biomass (Carlisle and Clements 2003).

Community metabolism is an example of a functional property that is more indicative of stressors than other structural properties such as species composition alone (Odum 1985).

The rate at which the community of benthic macroinvertebrates use energy and accumulate mass over time is called secondary productivity. Oxygen consumption is commonly used to estimate the metabolic rate of an organism (Nelson 2004). Oxygen is the electron acceptor during the process of synthesizing Adenosine triphosphate (ATP), therefore, oxygen consumption is an indicator of how much energy is being used to keep an organism in stable condition. Biotic and abiotic conditions can lead to increased oxygen consumption or respiration. Odum (1985) describes a few trends that can be expected of both the energetics of the components of the community and the community itself. Trends expected of energetics are that the community respiration increases whether it's the "pumping out" the disorder (Odum 1967) or "dissipative structure" (Prigogine et al. 1972), the production:respiration ratio is unbalanced (< or > 1), and the mantinance:biomass ratio becomes increased. Trends expected of the macroinvertebrate community are the increase in proportion of r-strategists, decrease in size of organisms, decrease in food web size, and decrease in species diversity.

*Objectives and Hypotheses*

The objective of this study is to further compare four sampling sites on the Nolichucky and Pigeon Rivers to determine structural differences in the benthic macroinvertebrate community that could help further explain the growth differences in native mussels previously observed in an unpublished study done by Bidwell and Roden (2019). Benthic macroinvertebrate community surveys have been used previously to compare habitat differences between sites. Findings of the study conducted by Bidwell and Roden in 2019 raise questions concerning whether the Pigeon River is a more stressful environment for macroinvertebrate communities compared to the Nolichucky River based on lower growth rates of transplanted freshwater mussels in the former as compared to the latter. Regulated flow regime and potential contaminants can alter growth rates and energy stores within the community. Investigating the assemblages of macroinvertebrates between sites of the two rivers will give an indication of the effects of flow on the entirety of the macroinvertebrate community. Further, investigation will determine whether differences in levels of productivity observed in the study with transplanted mussels extends to the broader macroinvertebrate community. Evaluating oxygen consumption of the macroinvertebrate community gives insight to how the macroinvertebrates are using energy because it can be influenced by physical and chemical properties of the habitat, supply and quality of food sources, and activity levels (Rostgaard and Jacobsen 2005). In order to compare the effects of flow regime on the macroinvertebrate communities within the rivers, samples must be collected upstream and downstream of point source inputs.

The TMI is predicted to be higher for the Nolichucky River than the Pigeon River due to lack of flow regulation and lower long-term contamination. A higher TMI score is indicative of a higher diversity of taxa along a gradient of pollution sensitive to pollution tolerant macroinvertebrates. Oxygen consumption levels are predicted to be higher in the Pigeon River than the Nolichucky River due to more stressful conditions.

# CHAPTER 2. METHODOLOGY

## *Site Selection*

A total of four sites (two per river) were selected based in part on the locations used in the study by Roden and Bidwell (2019). On the Pigeon River, a site was located at approximately river mile 11.9 at the confluence of Cosby Creek and the Pigeon River (35° 52' 12.3168" N, -83° 11' 34.8324" W). This site was selected because it had been used to monitor mussel growth in the study by Roden and Bidwell (2019) and because it had previously been used as a site to reintroduce freshwater mussels by the Tennessee Wildlife Resources Agency (TWRA). The second site on the Pigeon River was located at approximate river mile 3.5 below the Newport wastewater treatment facility (35° 59' 2.8968" N, -83° 11' 55.7484" W). This site was selected based on the proximity to the wastewater treatment plant and the potential effects of effluent from the plant on the macroinvertebrate assemblage. Both sites on the Pigeon River are highlighted in Figure 3.


Figure 3. Map of sites: Pigeon River

On the Nolichucky River, one site was located immediately upstream of the Davy Crockett impoundment at the Route 107 bridge in Greeneville, TN at river mile 55.4 (36° 9' 14.9646"N, -82° 43' 25.2624"W). The second site on this river was located at West Allens Bridge in Greeneville, TN at river mile 34.8 (36° 3' 33.501"N, -82° 54' 30.438"W). This site was also included in the study by Roden and Bidwell (2019) to investigate freshwater mussel growth. It is located downstream from the Davy Crockett impoundment and was selected to evaluate the influence of primary productivity in the impoundment on the macroinvertebrate assemblage. Figure 4 highlights both sites on the Nolichucky River.


Figure 4. Map of sites: Nolichucky River

*Collection*

Collection of macroinvertebrates followed the semi-quantitative riffle kick (SQKICK) method described by TDEC (2011). Samples were collected using a two-person kick net measuring 1 $m^2$ with 500-µm mesh. Starting at the end of a cobble riffle, the net was placed into the stream and an area of one square meter was disturbed so debris and organisms within the substrate flowed into the net. Larger rocks and debris were scrubbed by hand to dislodge invertebrates. Once the kick was complete, the net was placed into a 500-micron sieve bucket and rinsed for the organisms and debris to be collected. The goal of the SQKICK was to collect a minimum of 200 organisms. If that target number was not met on the first kick, a second kick must be done to collect the 200 organisms required. The sample was then transferred to a wide mouth plastic container and preserved in 80% ethanol. If it appears more than 25% of the sample is organic material, then 95% ethanol is used to preserve the sample. The approach for this study deviates from the TDEC protocol in that four samples were collected at each site as opposed to two.

Water quality data were collected before each riffle kick occured. Using a calibrated YSI ProDSS Multiparameter Water Quality Meter (YSI Inc., Yellow Springs, OH), the temperature, pH, dissolved oxygen, specific conductivity, and chlorophyll-a were measured and recorded a total of four times at each visit per site.

After the collection of macroinvertebrate samples and water quality data, the samples were transported to East Tennessee State University to be sorted. Macroinvertebrates were separated from debris and identified down to genus using Merritt et al. (2008). The taxonomic data collected were used to determine the Tennessee Macroinvertebrate Index (TMI).

*Tennessee Macroinvertebrate Index*

The organisms were sorted and identified to the genus level. The TMI was calculated using seven metrics in four categories according to TDEC's Probablistic Monitoring of Wadeable streams in Tennessee (2007). Metrics are described in Table 1.

Table 1. Metrics Used to Measure Tennessee Macroinvertebrate Index (TDEC, 2007)

| **Category** | **Metric** | **Definition** | **Predicted response to increased disturbance** |
|---|---|---|---|
| Richness Metrics | Total Number of Taxa | Measures the overall variety of the macroinvertebrate assemblage. | Decrease |
| | Total Number of EPT Taxa | Number of taxa in the insect orders Ephemeroptera, Plecoptera, and Trichoptera(mayflies, stoneflies, and caddisflies) | Decrease |
| Composition Metrics | % EPT | Percentage of mayfly, stonefly, and caddisfly larvae out of the total number of individuals. | Decrease |
| | %OC | Percentage of oligochaetes and chironomids out of the total number of individuals. | Decrease |
| Pollution Tolerance Metrics | NCBI | North Carolina Biotic Index uses tolerance values to weight abundance in an estimate of overall pollution (Lenat, 1993). | Increase |
| | % Nutrient Tolerant | The percentage of the 14 nutrient tolerant taxa out of the total number of individuals (Brumley et al, 2003). | Increase |
| Habitat Metrics | % Clingers | Percentage of individuals with adaptations for attachments to surfaces in flowing water. | Decrease |

*Oxygen Consumption*

At each collection site, a wood framed box filled with cement measuring 91.5 x 15 x 5 cm was filled with four 10 x 10 x 6 cm plastic trays and buried in the stream bed for three weeks, so the macroinvertebrate communities had adequate time to colonize the trays (Clements 1989). A 1-inch-wide nylon strap was stapled across the top to secure the plastic trays in the wood boxes while anchored to the stream bed.

Metabolic chambers were made of acrylic tubing 10 centimeters deep so the colonized trays could be taken from the wood boxes and placed inside. The chambers were designed to have an airtight lid with an opening to secure a dissolved oxygen probe. The chamber layout is displayed in Figure 5. The oxygen consumption readings take place using a YSI Pro-BOD probe within the stream to avoid change in the temperature of the water.

To account for photosynthetic organisms in the water, half of the chambers were placed in a black tub within the river and covered to stay in complete darkness throughout the trial. Oxygen consumption was measured in both a light and a dark environment without the colonized trays present, referred to as "blanks." The difference in light and dark conditions between the chambers can account for primary productivity.

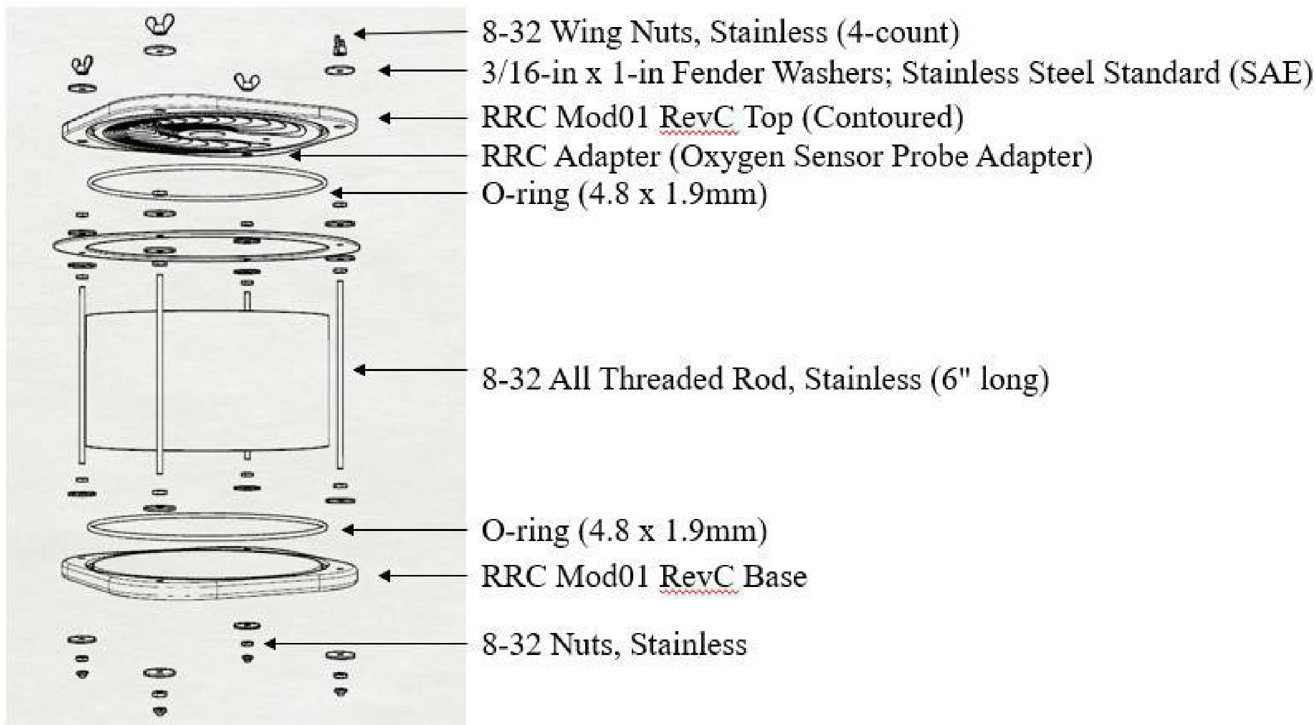


Figure 5. Diagram of oxygen consumption chamber

*Data Analysis*

The data were collected, analyzed, and pooled according to season. Summer collection was taken in July, fall collection was taken in early December, Winter collection was taken in early March, and Spring collection was taken in May.

After testing for normality and equal variances using a one-sample Kolmogorov-Smirnov test, the TMI scores were analyzed using a two-way analysis of variance (ANOVA) with an alpha level of $\alpha=0.05$. The fixed factors of the ANOVA were the rivers (Pigeon and Nolichucky Rivers) and the site location (upstream and downstream).

After testing for normality and equal variances by using a Shapiro-Wilk test, the water quality data collected were analyzed using a multivariate analysis of variance (MANOVA) with an alpha level of $\alpha=0.05$. The fixed factors for the MANOVA were also the rivers, and the site locations within the rivers.

# CHAPTER 3. RESULTS

*Tennessee Macroinvertebrate Index*

On a scale of 0 to 42, the TMI scores over the course of the project ranged from 28 to 39. There were significant interactions between season and site within the individual rivers. For the Pigeon River, there was a difference between the upstream and downstream sites with upstream being significantly higher during the summer season ($p<0.001$) The average score for the site above the nutrient input site for the Pigeon River was 36 and the downstream site average was 31. There were no significant differences during the fall, spring, or winter seasons. There was also a significant difference between the upstream and downstream sites during the summer season in the Nolichucky River with the upstream site having a significantly higher average ($p=0.025$). The average TMI score at the site upstream from the nutrient input site was 36 and the average score for the site downstream from the nutrient input site was 32. There were no significant differences during the fall, spring, or winter seasons.

There was no significant difference between the downstream sites between rivers during any season which is shown in Table 2. The pairwise comparisons in Table 3 determined the difference between season and placement for each river. There was no significant difference between upstream sites between rivers during any season as well.

Table 2. Results of the Two-Way ANOVA Analyzing the TMI Scores

**Tests of Between-Subjects Effects**

Dependent Variable: TMI Score

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 349.984[a] | 15 | 23.332 | 5.085 | <.001 |
| Intercept | 70158.766 | 1 | 70158.766 | 15289.992 | <.001 |
| Placement | 31.641 | 1 | 31.641 | 6.896 | .012 |
| Season | 202.547 | 3 | 67.516 | 14.714 | <.001 |
| River | 1.266 | 1 | 1.266 | .276 | .602 |
| Placement * Season | 63.672 | 3 | 21.224 | 4.625 | .006 |
| Placement * River | 1.891 | 1 | 1.891 | .412 | .524 |
| Season * River | 22.547 | 3 | 7.516 | 1.638 | .193 |
| Placement * Season * River | 26.422 | 3 | 8.807 | 1.919 | .139 |
| Error | 220.250 | 48 | 4.589 | | |
| Total | 70729.000 | 64 | | | |
| Corrected Total | 570.234 | 63 | | | |

a. R Squared = .614 (Adjusted R Squared = .493)

Table 3. The Pairwise Comparisons of the TMI Scores at the Upstream and Downstream Sites in Both the Pigeon and Nolichucky Rivers

**Pairwise Comparisons**

Dependent Variable: TMI Score

| Season | River | (I) Placement | (J) Placement | Mean Difference (I-J) | Std. Erro | Sig.[b] | 95% Confidence Interval for Difference[b] Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|---|---|
| Fall | Nolichucky | downstream | upstream | -1.500 | 1.515 | .327 | -4.545 | 1.545 |
| | | upstream | downstream | 1.500 | 1.515 | .327 | -1.545 | 4.545 |
| | Pigeon | downstream | upstream | 2.500 | 1.515 | .105 | -.545 | 5.545 |
| | | upstream | downstream | -2.500 | 1.515 | .105 | -5.545 | .545 |
| Spring | Nolichucky | downstream | upstream | -2.000 | 1.515 | .193 | -5.045 | 1.045 |
| | | upstream | downstream | 2.000 | 1.515 | .193 | -1.045 | 5.045 |
| | Pigeon | downstream | upstream | .250 | 1.515 | .870 | -2.795 | 3.295 |
| | | upstream | downstream | -.250 | 1.515 | .870 | -3.295 | 2.795 |
| Summer | Nolichucky | downstream | upstream | -3.500* | 1.515 | .025 | -6.545 | -.455 |
| | | upstream | downstream | 3.500* | 1.515 | .025 | .455 | 6.545 |
| | Pigeon | downstream | upstream | -6.000* | 1.515 | <.001 | -9.045 | -2.955 |
| | | upstream | downstream | 6.000* | 1.515 | <.001 | 2.955 | 9.045 |
| Winter | Nolichucky | downstream | upstream | 7.772E-16 | 1.515 | 1.000 | -3.045 | 3.045 |
| | | upstream | downstream | -7.772E-16 | 1.515 | 1.000 | -3.045 | 3.045 |
| | Pigeon | downstream | upstream | -1.000 | 1.515 | .512 | -4.045 | 2.045 |
| | | upstream | downstream | 1.000 | 1.515 | .512 | -2.045 | 4.045 |

Based on estimated marginal means

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

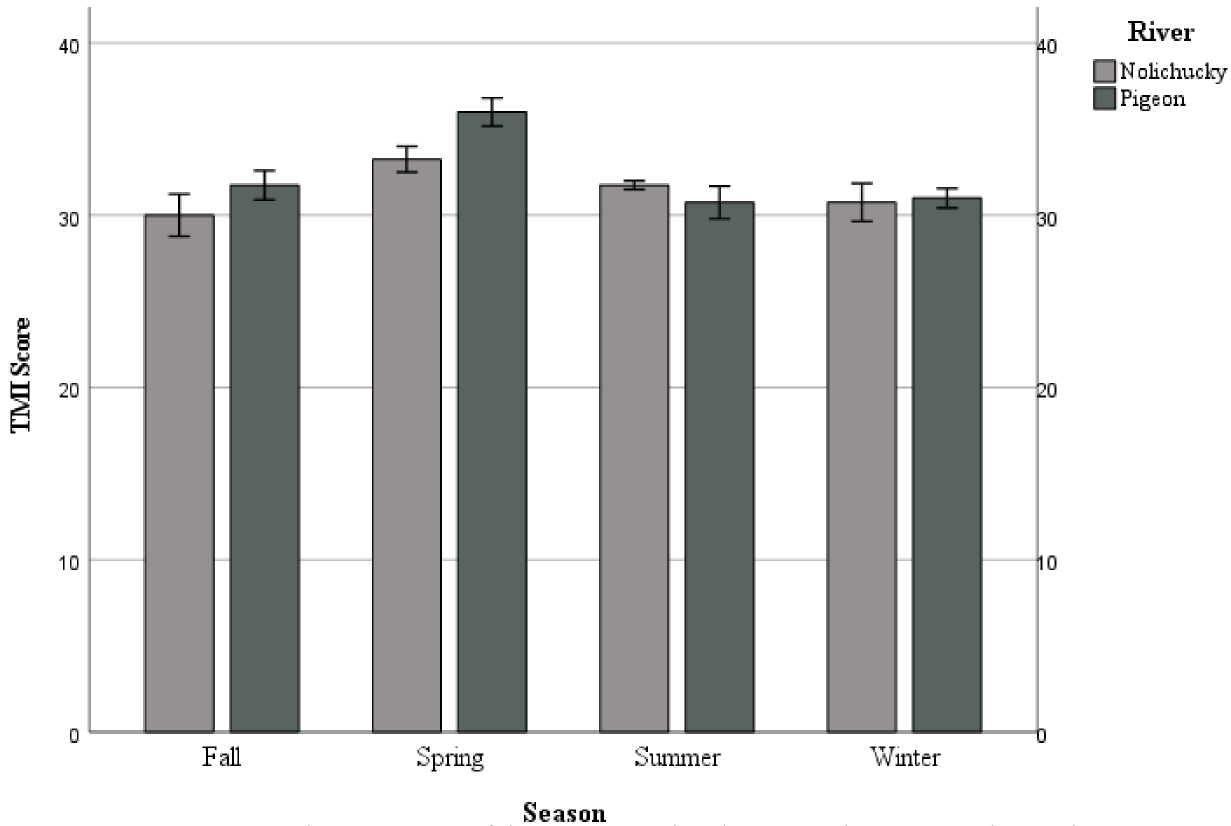

Figure 6. Average seasonal TMI scores of downstream sites between rivers. Error bars +/- 1 standard error

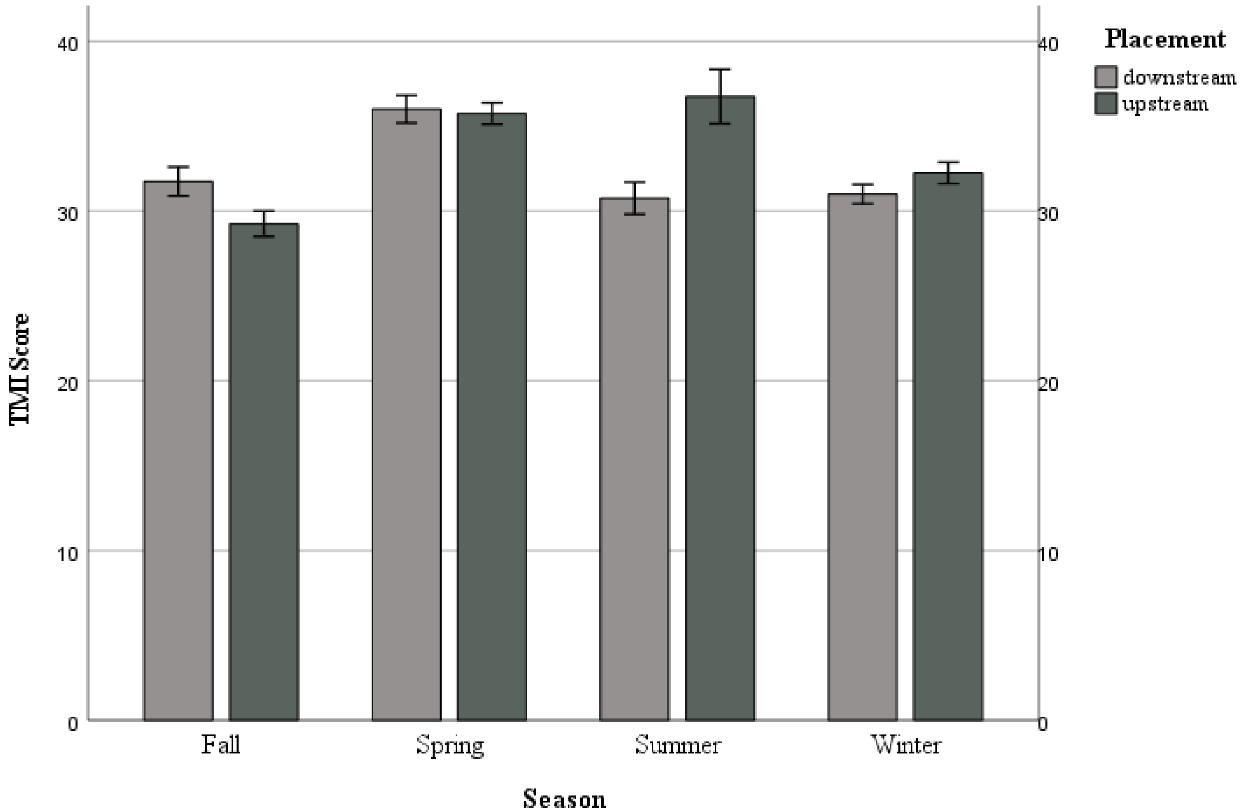


Figure 7. Average seasonal TMI scores between site location in the Pigeon River. Season*Site interaction was significant ($p<0.001$) for the summer season. Error bars +/- 1 standard error

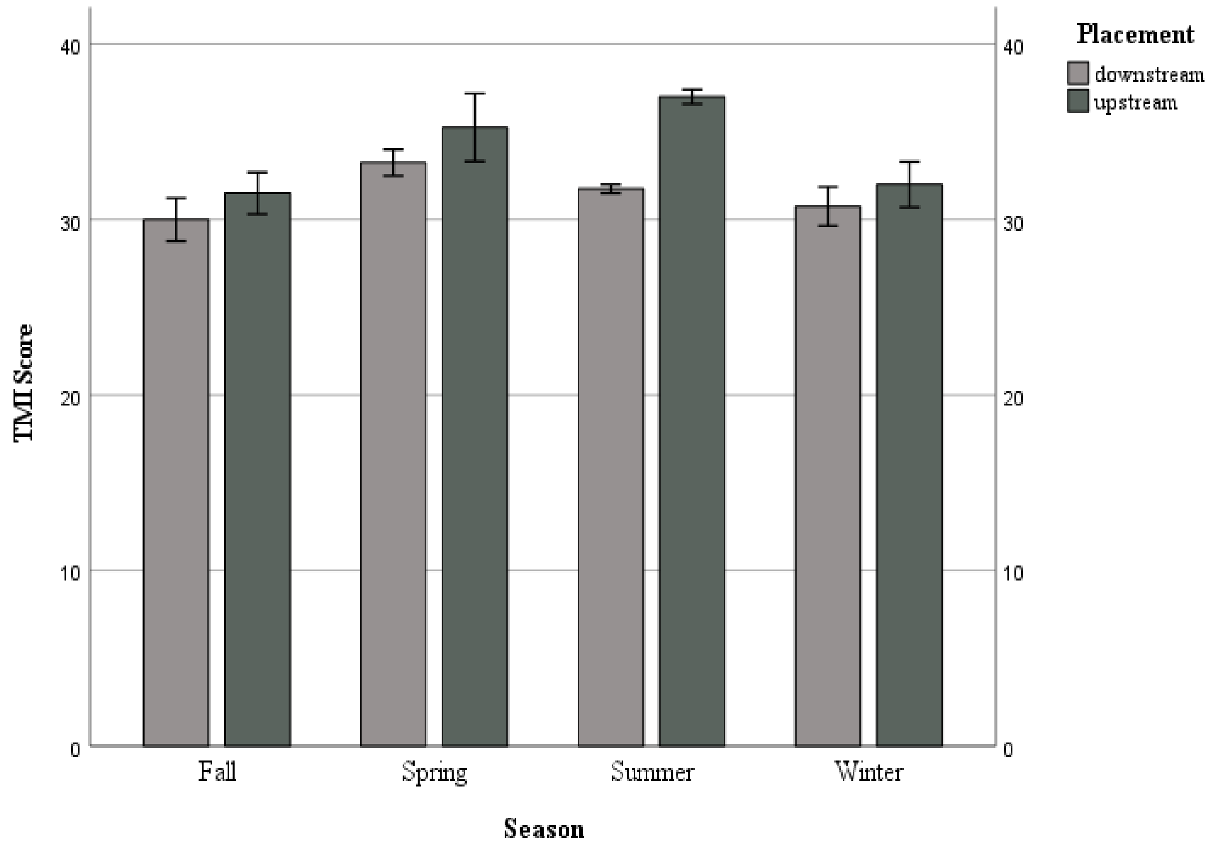


Figure 8. Average seasonal TMI scores between site locations in the Nolichucky River. The Season*Site interaction was significant (p=0.025) for the summer season. Error bars +/- 1 standard error

*Water Quality*

The water quality parameters tested were dissolved oxygen, SPC, pH, and chlorophyll. Each of these were tested between upstream and downstream sites to compare the downstream sites between both the Nolichucky and Pigeon Rivers. Although sites downstream from the nutrient input sources were being compared initially, the upstream sites had to be monitored to evaluate the potential changes in water quality caused by the input.

A multivariate analysis of variance (MANOVA) was conducted to analyze the water quality data collected. Dissolved oxygen was significantly higher in the downstream sites in the Pigeon River during Fall ($p<0.001$) with the site upstream from the point source wastewater treatment input measuring 11.39 mg/L and downstream measuring 12.0875 mg/L. Dissolved oxygen was significantly higher in the site downstream from the wastewater treatment plant than that of the upstream site during Spring ($p<0.001$). The upstream site measured an average of 8.9 mg/L, and the downstream site measured an average of 10.63 mg/L. The downstream site measured significantly higher during the summer season as well ($p<0.001$) with the site upstream from the nutrient input site measuring an average of 8.1 mg/L and downstream measuring an average of 9.1325 mg/L. There was no significant difference during the Winter season. This is illustrated in Figure 9.

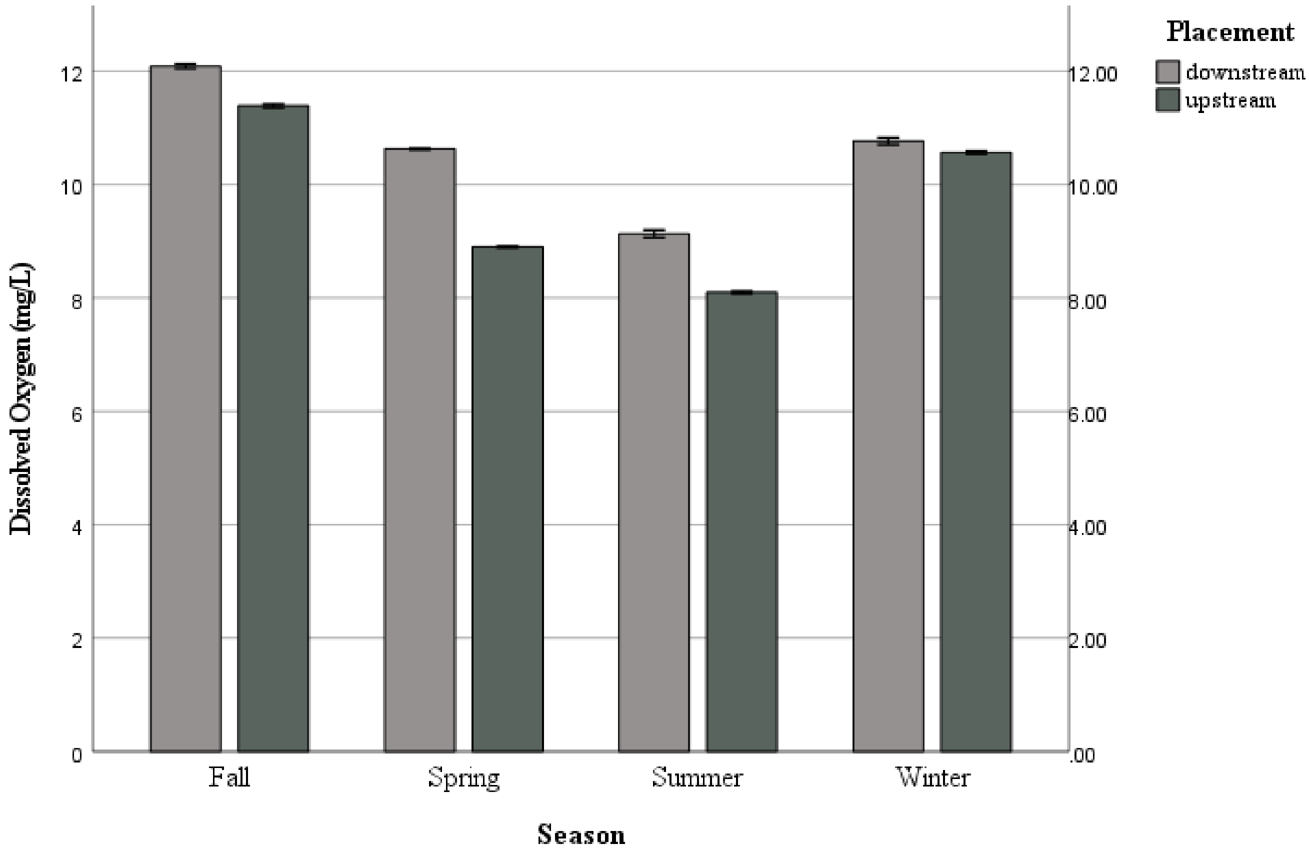


Figure 9. Average dissolved oxygen between upstream and downstream sites for each season in the Pigeon River. The sites were significantly different in Fall (p<0.001), Spring (p<0.001), and Summer (p<0.001). Error bars +/- 1 standard error

Figure 10 shows the dissolved oxygen levels for the Nolichucky River across all seasons. Dissolved oxygen was significantly higher at the downstream site in the Nolichucky River during the Fall season (p<0.001) with upstream measuring an average of 12.0725 mg/L and downstream measuring 12.73 mg/L. Summer was significantly different with the downstream site measuring higher than the upstream site (p<0.001). Upstream averaged 7.9725 mg/L and downstream averaged 8.39 mg/L.

There was a significant difference between the upstream and downstream sites during the winter ($p<0.001$) season with downstream measuring higher than upstream. The upstream site had an average of 11.0925 mg/L and the downstream site measured an average of 11.625 mg/L. There was no significant difference between sites during the spring season.

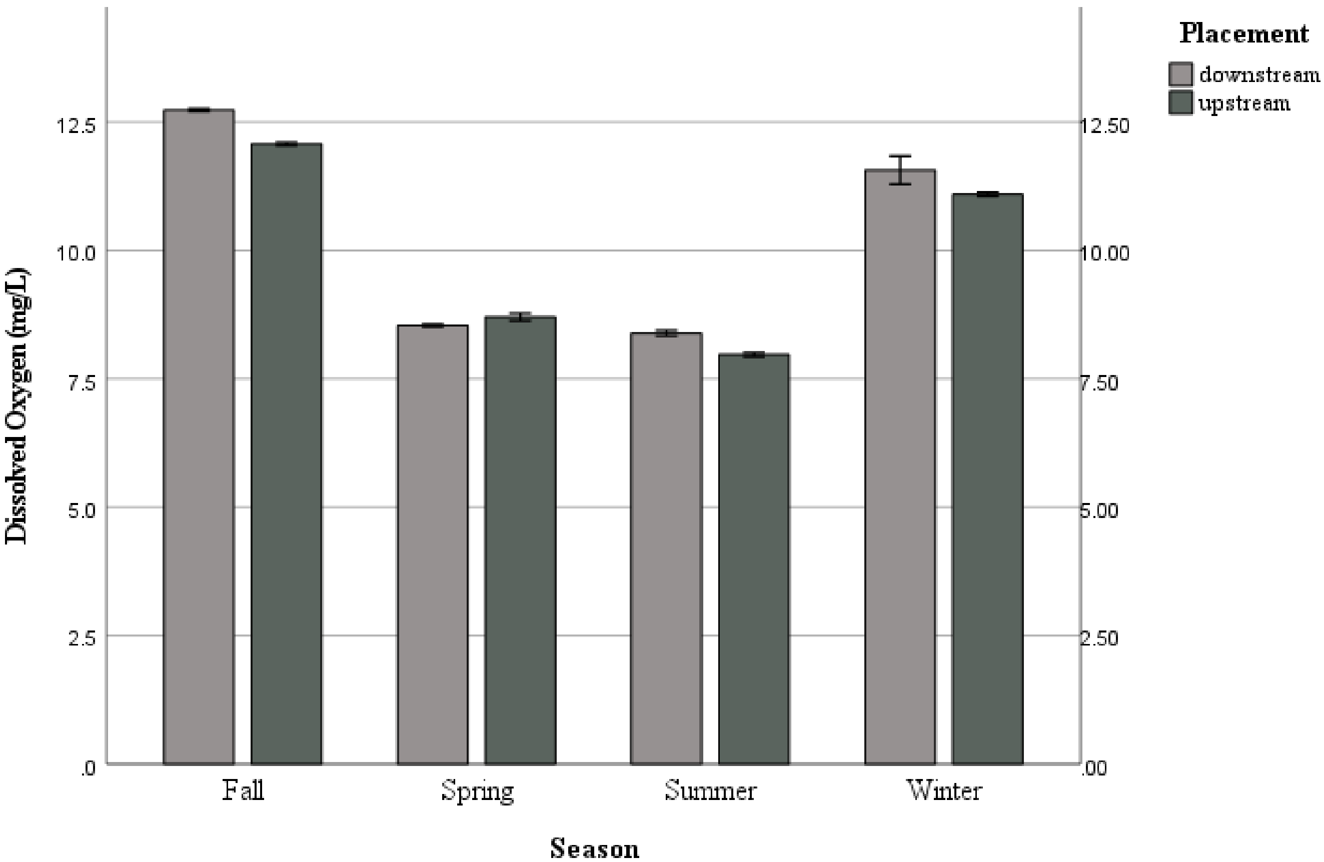

Figure 10. Average dissolved oxygen between upstream and downstream sites within the Nolichucky River. The sites were significantly different in Fall (p<0.001), Summer (p<0.001), and Winter (p<0.001). Error bars +/- 1 standard error

In the Pigeon River average specific conductivity (SPC) was significantly higher in the downstream sites across all seasons (p<0.001) (Figure 11). In the Pigeon River, during the fall season the SPC measured 138 µs/cm upstream and 150.05 µs/cm downstream. During the spring season, the upstream site had an SPC of 153.725 µs/cm and downstream read 169.075 µs/cm. In the Summer season the upstream in the Pigeon River measured 85.775 µs/cm and downstream measured 152.125 µs/cm. During the winter season the upstream site measured 113.35 µs/cm and downstream measured 121.9 µs/cm.

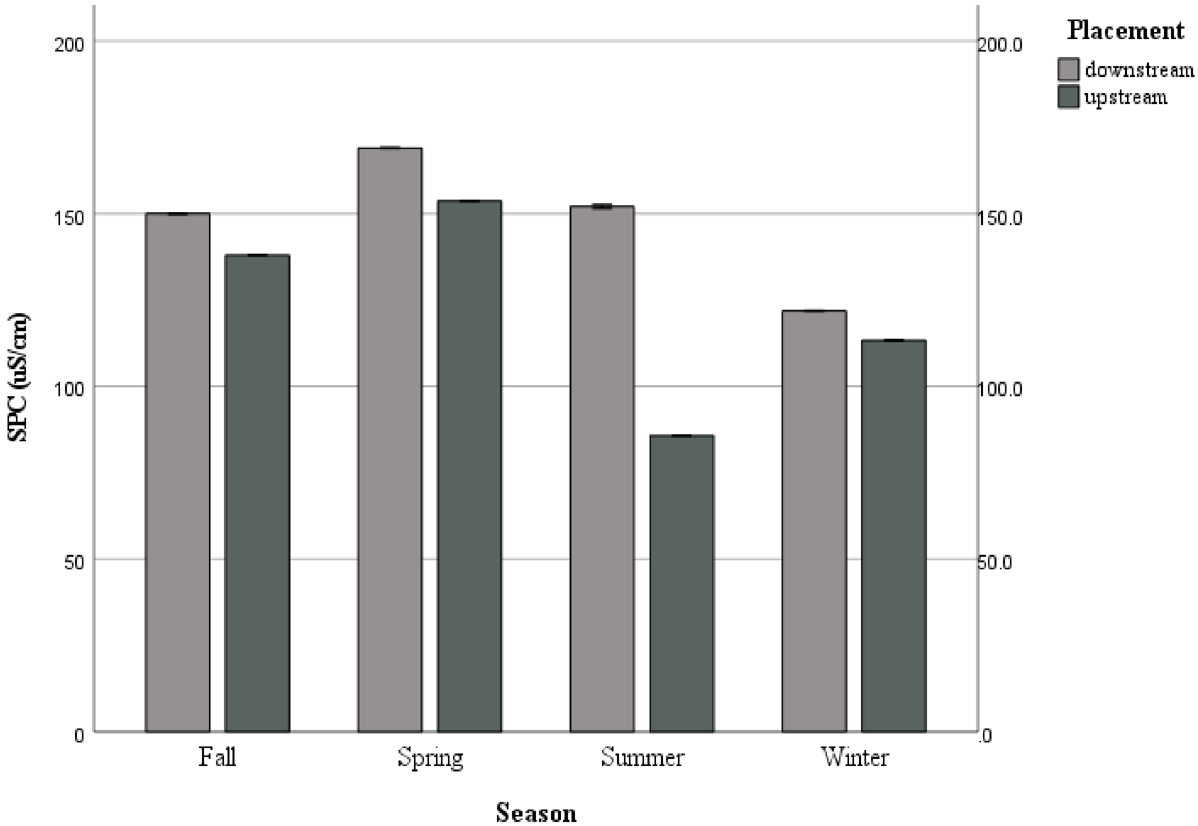


Figure 11. Average specific conductivity between upstream and downstream sites in the Pigeon River. There was a significant difference between sites across all seasons ($p<0.001$). Error bars +/- 1 standard error

Figure 12 shows the average SPC for the upstream and downstream sites within the Nolichucky River. The SPC readings were significantly higher in the downstream site across all seasons ($p<0.001$). During the fall season, the upstream sites had an average of 113.3 µs/cm and downstream averaged 127.05 µs/cm. The Spring season averaged 146.95 µs/cm above the out-of-service dam and 166.475 µs/cm downstream from the dam. The summer season averaged 128.325 µs/cm upstream from the nutrient input site and 153.725 µs/cm downstream.

During the winter season, upstream average 146.2 µs/cm which was significantly lower than downstream that averaged 163.12 µs/cm.

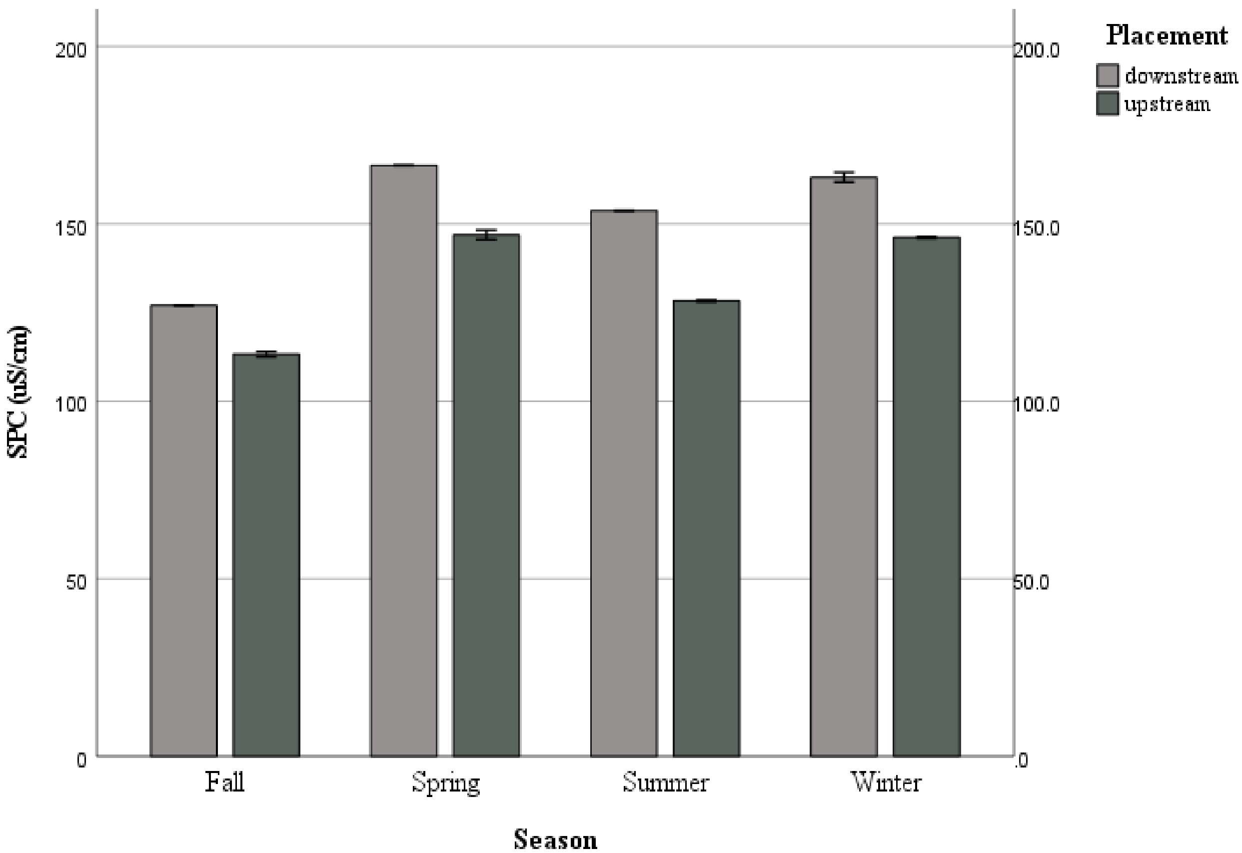


Figure 12. Average specific conductivity between upstream and downstream sites in the Nolichucky River. There was a significant difference between sites across all seasons ($p<0.001$). Error bars +/- 1 standard error

Figure 13 shows the average chlorophyll readings between the upstream and downstream sites in the Pigeon River. There was a significant difference between sites during the Spring season ($p<0.001$). Above the wastewater treatment plant chlorophyll averaged 0.5925 µg/l and downstream from the wastewater treatment plant averaged 1.1275 µg/l.

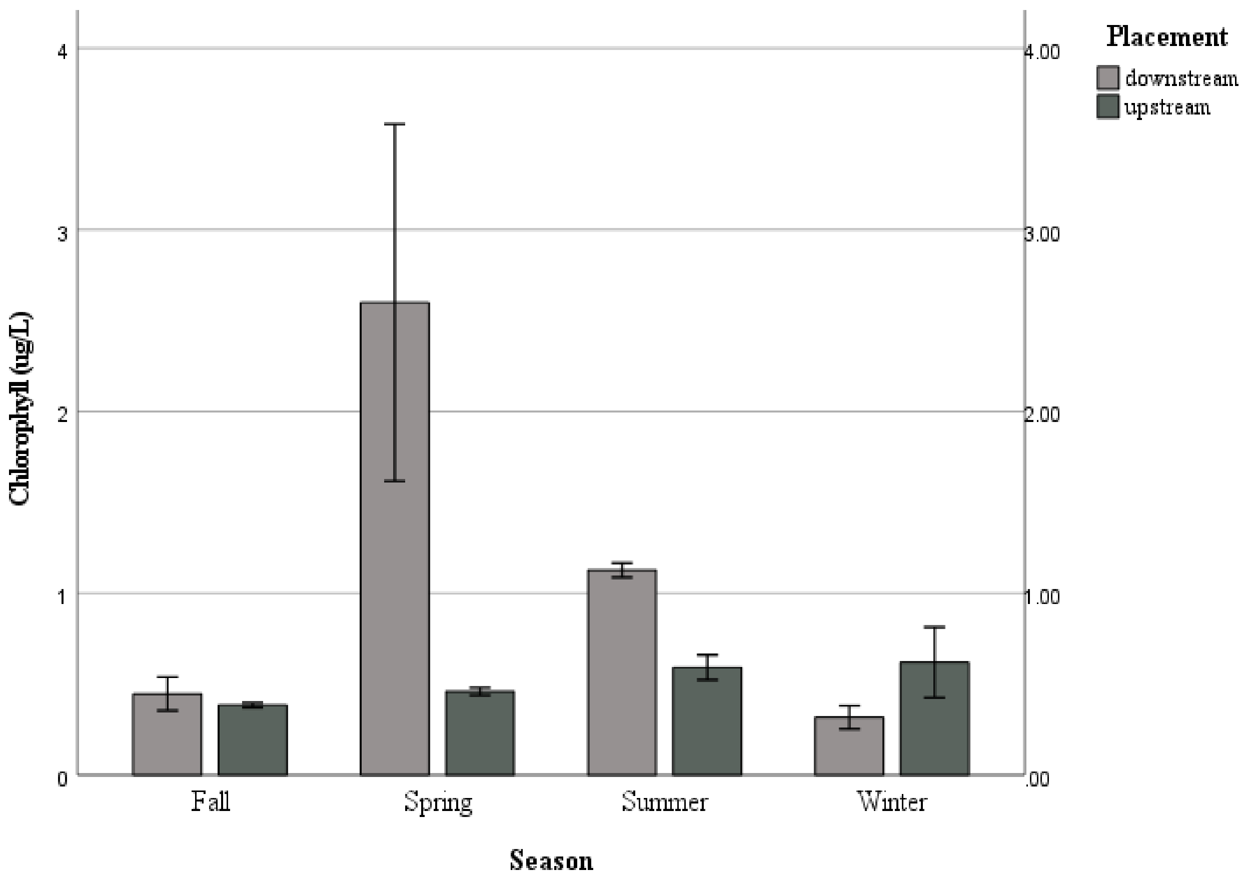


Figure 13. Average chlorophyll between upstream and downstream sites for each season in the Pigeon River. There was a significant difference between sites during the spring season ($p<0.001$). Error bars +/- 1 standard error

For the Nolichucky River, as shown in Figure 14, chlorophyll was significantly higher in the downstream site during the Summer ($p=0.034$) and Winter ($p=0.003$) seasons. During the summer season above the nutrient input site the chlorophyll averaged 0.5925 µg/l and downstream averaged 1.62 µg/l. The upstream site was significantly lower than the downstream site during the winter season. The upstream site averaged 0.0525 µg/l and the downstream site averaged 1.6475 µg/l.

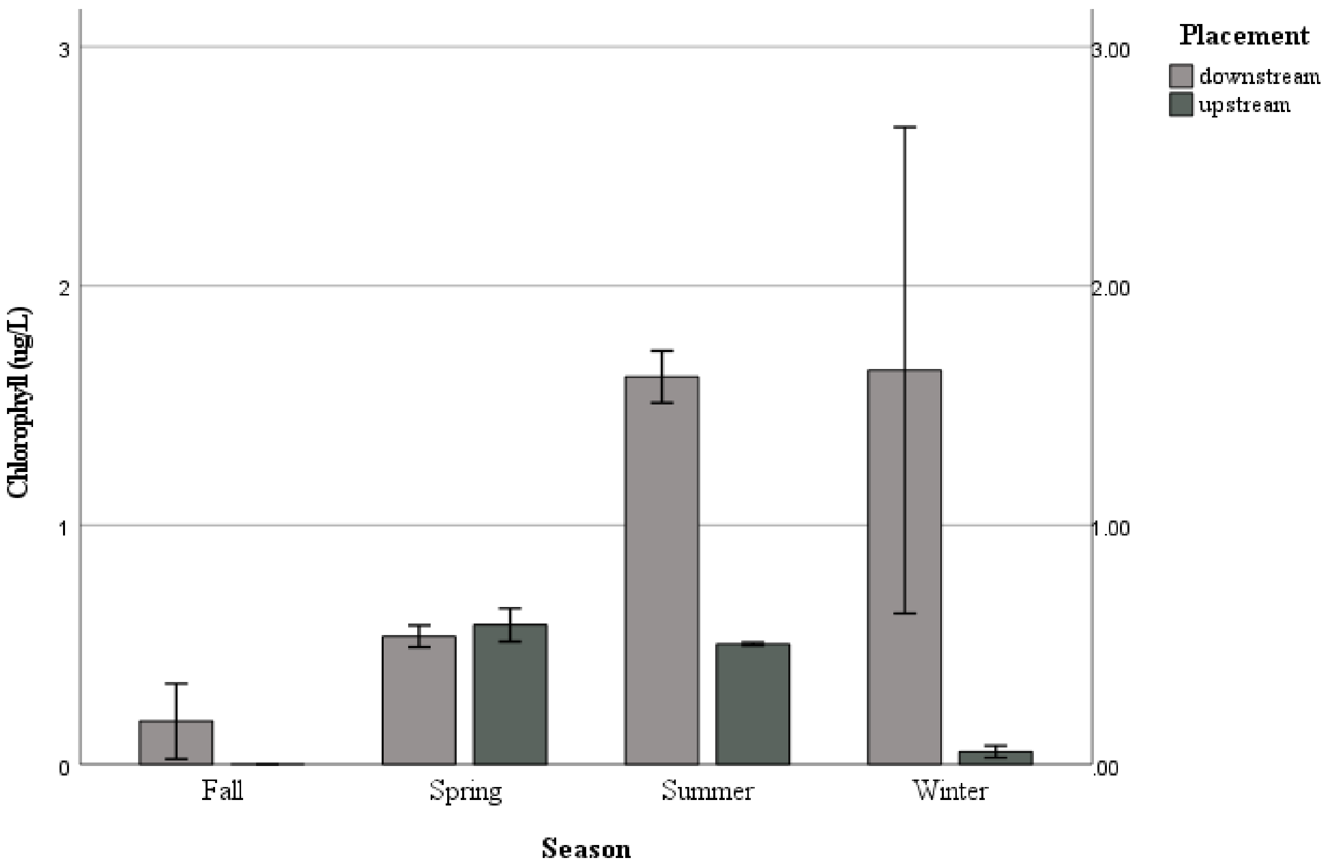


Figure 14. Average chlorophyll between sites for each season within the Nolichucky River. There was a significant difference between the sites during the Summer (p=0.034) and Winter (p=0.003) seasons. Error bars +/- 1 standard error

# CHAPTER 4. DISCUSSION

*Background*

Bidwell and Roden (2019) found that native freshwater mussels transplanted in the free-flowing, unregulated Nolichucky River exhibited significantly higher growth and glycogen levels than those in the Pigeon River, although survivorship of mussels between the two rivers was comparable. The differences in growth and glycogen were attributed to higher chlorophyll in the Nolichucky River site as compared to the Pigeon River site. It was further hypothesized that this difference in chlorophyll may have been due to the changing flow regime in the Pigeon River which washed out algae during periods of high discharge. Organic material in the Nolichucky River may also have been significantly subsidized from the upstream Davy Crockett impoundment which could have provided better conditions for elevated primary productivity than what occurs in faster flowing river water. Roden and Bidwell (2019) also concluded that the comparable survival of mussels between the two rivers indicated there was no acute lethal stressor in the Pigeon, even though it had experienced a greater level of historic pollution than the Nolichucky River.

The objective of the current study was to further compare the sites on the Nolichucky and Pigeon Rivers that had been the focus of the study by Bidwell and Roden (2019). Specifically, the goal was to determine if any structural differences in the benthic macroinvertebrate community existed between the sites that could help further explain the growth differences in native mussels that was previously observed.

A previous study conducted by White et al. (2017), was completed using 6 sites across three rivers over the course of eight years. We found that there were no significant differences in macroinvertebrate assemblages between the regulated Pigeon River and non-regulated Nolichucky River using the Tennessee Macroinvertebrate Index (TMI). The current study was conducted using only four sites over the course of four seasons. White et al. (2017) did find significant differences in the macroinvertebrate assemblages based on flow regime differences in regulated and non-regulated rivers. This suggests that although the current study can tell us that the river systems are not negatively impacted at the time of investigation, not enough data was collected to draw any major conclusions about the ongoing functionality and potential productivity differences of the macroinvertebrate communities in the regulated Pigeon River and free-flowing Nolichucky River.

Gafner and Robinson (2007) determined that nutrient enrichment changes the response of macroinvertebrate communities because it changes the community structure of primary consumers. Work done by Sterling and Rosemond (2016) found that watershed urbanization negatively impacted macroinvertebrate biomass and community structure. These variables were directly correlated to the percentage of impervious surface cover. Ultimately, though, macroinvertebrate community response is determined by the type of stressor they are exposed to. Elevated nutrients in some streams can increase secondary production, but lower taxa richness due to increased associated conductivity (Johnson et al. 2013). On the other hand, some contaminants reduce both secondary production and taxa richness. A lot of times, especially in areas more disturbed by humans, multiple stressors can uniquely affect a response variable (Sterling and Rosemond 2016). The current study found no significant difference between the TMI scores calculated based on the macroinvertebrate assemblages of the rivers regardless of the nutrient input differences, flow regime differences, contamination history differences, and water quality differences- apart from the summer season. This suggests there needs to be a deeper investigation of impact of temperature change throughout seasons. In a previous study conducted by Bidwell and Roden (2019) chlorophyll differences within the rivers were significantly correlated with flow and temperature. Wassenaar et al. (2010) found that nutrient input sources often have a stimulating effect on aquatic photosynthesis and community respiration during the spring and summer months. This further solidifies the importance of continuing efforts to measure metabolic response to seasonal temperature changes via oxygen consumption as well as comparing mass measurements of the samples.

*Tennessee Macroinvertebrate Index*

Biological indices are used worldwide as tools for assessing ecological integrity of water and are calculated based on taxonomic composition and abundance information as well as environmental sensitivity values assigned to each taxon (Mao et al. 2019).

The Tennessee Macroinvertebrate Index (TMI) scores were predicted to be higher for the Nolichucky River than the Pigeon River due to there being no flow regulation and lower long-term contamination. A higher TMI score is indicative of a higher diversity of taxa along a gradient of pollution sensitive to pollution tolerant macroinvertebrates (TDEC 2017). In the current study no difference in TMI scores was identified between downstream sites of the regulated Pigeon River or the free-flowing Nolichucky River.

Although there was no significant difference between the TMI scores for the downstream sites of the rivers, the TMI scores were different within each river during the summer season. The upstream sites had higher TMI scores than downstream sites in both the Pigeon and Nolichucky Rivers during the summer season.

TMI scores are calculated to assess the biological integrity of streams. This is the ability to support and maintain a balanced and integrated adaptive assemblage of organisms having species composition, diversity, and functional organization comparable to that of natural habitat of the region (Karr and Dudley 1981). Changes of the environmental condition resulting from human activities cause a decline in biological integrity and can make the environment uninhabitable for appropriate organisms (Karr et al. 1986). The TMI is focused mostly on pollution tolerance of the macroinvertebrate community but has been used to evaluate biological impact of differences in flow. A study conducted by Elkin et al. (2013) saw differences in TMI scores when comparing sites above and below a golf course water withdrawal. The downstream site had a much lower TMI score than the upstream site when the flow was significantly decreased. When flow was decreased in the Pigeon River, it was only a short amount of time before a dramatic influx of resources replenished the downstream sites whereas in the Elkin et al. (2013) study, resources were consistently being withdrawn without replenishment. White et al. 2017 found that macroinvertebrate community abundances alone exhibit a weak response to flow alteration. These results do agree with those of the current study because there were no significant differences in TMI score between the free-flowing, unregulated Nolichucky River and the regulated Pigeon River at either upstream or downstream sites. The only differences seen were within each river during the summer months. Being said, the differences seen between Elkin et al. (2013) and the current study can likely be attributed to the resources that are readily available to the macroinvertebrate communities of the rivers. The purpose of the Tennessee Macroinvertebrate Index is for interpretation of narrative biological criteria (Arnwine and Denton 2001), but may not be apt to detect the productivity differences that were impacting the mussel growth in the study completed by Roden and Bidwell (2019). This is because there were no significant differences in the TMI scores between rivers where there were significant differences in mussel growth.

The TMI scores showed no differences between rivers or within rivers, other than during the summer months, with there being a significantly higher score at the upstream compared to downstream sites of both rivers. This suggests that increased temperature could be a factor that negatively alters the macroinvertebrate community organization. A study conducted by Almeida et al. (2009) examined longitudinal discontinuity in small rivers during the wet and dry seasons in Brazil. They found that the dam was inducing negative changes in the macroinvertebrate community during the hot dry season. The biological integrity was reestablished further downstream and matched that of the site above the dam. This suggests that adding an additional site further downstream from the current downstream sites could isolate the specific areas that are potentially negatively impacted by changes in flow regime during the warmest months.

*Water Quality*

Physiochemical and habitat variables explain further variation in macroinvertebrate assemblage throughout seasons better than hydrological variables alone (Helms et al. 2009). Elevated chlorophyll levels were observed at the downstream sites when compared to the upstream sites in both the Pigeon River and Nolichucky River. In the previous study done by Roden and Bidwell (2019), a significant correlation between flow and seston measures was observed. Seston includes the total amount of all photosynthetic organisms, organic material, and mineral content. Chlorophyll readings were much lower during times of low flow and in the colder months and increased when flow increased. Average chlorophyll concentration generally increased downstream. The increased levels at the downstream site on the Pigeon River could be related to the proximity of the site to the Newport wastewater treatment plant which discharged a nutrient rich effluent. For the Nolichucky River, elevated chlorophyll levels could be related to the proximity of the site to the Davy Crockett impoundment. The impoundment serves as a pool with a large retention time which gives way to conditions for primary production to take place and according to Westhorpe et al. (2015) can disrupt the water quality pattern along the downstream continuum. This is a potential factor as to why the TMI was lower downstream from the wastewater treatment plant on the Pigeon River as well as the Davy Crockett reservoir on the Nolichucky River during the summer months.

A study conducted by Muholland et al. (2005), at Fort Benning found that the daily amplitude of dissolved oxygen deficit was directly correlated with the daily rate of gross primary production, and the daily maximum of dissolved oxygen deficit was directly correlated with the daily rate of ecosystem respiration. In the current study, significantly higher levels of dissolved oxygen were measured at the downstream sites compared to the upstream sites in both the Pigeon River and the Nolichucky River. Dissolved oxygen levels can be influenced by primary productivity at the sites below the Davy Crockett Reservoir and Newport wastewater treatment plant. Mulholland et al. (2005) found that daily dissolved oxygen deficit is a useful indicator of stream metabolism and the effect of catchment-scale disturbance. Gross primary production and total ecosystem respiration were determined for the streams by using single station diurnal dissolved change method. This suggests that the time of day that we collected data, which was between 10:00 A.M.- 2:00 P.M., could have influenced the dissolved oxygen readings.

Reservoirs affect physical, chemical, and biological characteristics of streams (Ignatius and

Rasmussen 2016). Ignatius and Rasmussen (2016) found the proportion of developed land cover within the watershed showed positive correlations with reservoir specific conductivity (SPC) values. High SPC indicates high dissolved solids. The way in which a dam releases water has an influence on the water quality. Overflow, or top-releasing, dams show higher dissolved oxygen and pH than bottom-releasing dams (Ignatius and Rasmussen 2016). Pond et al. (2014) investigated macroinvertebrate communities downstream from Appalachian mountaintop coal mining and valley fills. They found that after 30 years, nearly 90% of the streams investigated below the mining sites showed signs of biological impairment. Not only were the functional feeding groups altered, but there were significant biological correlations to the specific conductance levels which reduced the taxa identified. It is important to discuss this study because Pond et al. (2014) found no significant difference between the sites below impoundments and sites that were not below impoundments. This study showed similarities in taxa richness and diversity that can be expected out of streams in the region; and that the flow-regime had a much lower impact on the macroinvertebrate community than the water quality of the habitat. There were significantly elevated SPC levels downstream from the nutrient input sites in both the Pigeon River and Nolichucky River in all four seasons that data were collected. With the summer season being the only season that we saw a significant difference in macroinvertebrate indices calculated, the difference may not be related specifically to the SPC levels or the way in which the water is released from the regulated dam on the Pigeon River and the out-of-service free-flowing dam on the Nolichucky River. Based on these findings, a long-term, comparative study investigating the macroinvertebrate assemblage and water quality of the Pigeon and Nolichucky Rivers is warranted.

*Oxygen Consumption*

Oxygen consumption levels were predicted to be higher in the Pigeon River than the Nolichucky River due to more stressful conditions caused by long-term contamination. Oxygen consumption trials could not take place due to COVID-19 time constraints that delayed construction of the respirometry chambers as well as vandalism of the macroinvertebrate colonization baskets when they were deployed in the rivers. There is potential utility of measuring oxygen consumption in an assemblage of organisms such as macroinvertebrates.

Oxygen consumption by invertebrates can be used as a biomarker for early indication and long-term detection of polluted and harmful environments (Martins et al. 2009). Using *Daphnia magna,* Martins et al. (2009) found significant changes in oxygen consumption after contaminating the environment with different common pollutants. Changes to the environment such as temperature fluctuations and anthropogenic functions have also been found to influence oxygen consumption levels which influence mussel responses such as survival, growth, and reproduction (Ganser 2015). Although pollutants play a role in physiological responses such as oxygen consumption and growth rate, food consumption has a stronger relationship with growth rate.

Community level oxygen consumption is useful information for managing ecological response to river discharge. Wassenaar et al. (2010) investigated the community aquatic metabolic response to point-source wastewater input in studies conducted across the Bow River and South Saskatchewan River. The study investigated the oxygen cycles downstream from wastewater input sites compared to upstream control sites. They found that wastewater had a significant stimulating effect on aquatic photosynthesis, as well as there being a two-to-threefold increase in in photosynthesis and community metabolic responses during the spring and summer months.

Considering the macroinvertebrate responses in the current study were significant during the warmest season, as well as the increase in chlorophyll, dissolved oxygen, and SPC levels at the downstream sites between rivers across seasons further investigation into the community metabolic response is needed.

Moving forward, completing a study using the respiration chambers that were built would give more insight to what the macroinvertebrate communities are experiencing both upstream and downstream from the nutrient sites on the Pigeon and Nolichucky Rivers. Along with investigating the impact of flow regime on nutrient input and quality and how this influences macroinvertebrate community respiration, biomass of the macroinvertebrate community should be measured.

# CHAPTER 5. CONCLUSION

In this study, the macroinvertebrate communities of the Pigeon and Nolichucky Rivers were examined with the goal of further explaining the differences in growth rate exhibited by native freshwater mussels in the rivers in a previous study conducted by Bidwell and Roden (2019). The previous study found that mussels transplanted to the Pigeon River grew at a slower rate that than the same species in the Nolichucky River, but no more lethal. It was hypothesized that the differences in growth rates were from elevated chlorophyll levels in the Nolichucky River as well as the steady, natural flow of the river compared to the regulated flow of the Pigeon River. It was also noted that the chlorophyll was highly subsidized by the Davy Crockett reservoir above the out-of-service dam that the Nolichucky freely flows over. A wastewater treatment input on the Pigeon River was used as a comparative nutrient input site, and sites above and below the nutrient sites were compared. The rivers differ in flow regime, recreational use, and historical use; but we determined there were no apparent differences between the communities of the rivers in any season so calculated by the Tennessee Macroinvertebrate Index (TMI). During the summer season, the TMI score was significantly lower at the downstream sites as compared to the sites above the nutrient input sites. Water quality parameters measured at the time of collection were significantly increased downstream from the nutrient input sites across many seasons. This is consistent with the movement of water due to flow, as well as an increase in photosynthetic autotrophs (chlorophyll) from nutrient input sites. Future studies should incorporate more community response parameters such as oxygen consumption and biomass.

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