Impact of a Small Woodlot on Biotic and Chemical Stream Quality

A Report of a Senior Study

By

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Abstract

The effects of forest fragments on stream health have been widely studied as more and more native riparian habitat is subjected to anthropogenic land-use change. No research has been performed on the impact that the Maryville College Woods exerts on Browns Creek as it flows from areas of high rural and agricultural land-use into a deciduous forest fragment. The goal of this study was to investigate the effects of The Maryville College Woods, a 60 hectare mixed deciduous forest fragment in Maryville, Tennessee, on the aquatic macroinvertebrate community structure. Macroinvertebrates were sampled at two sites, upstream and downstream, using two different methods. Dissolved oxygen, temperature, and flow rate were measured in-stream and water samples were collected for analysis in the lab. Stream depth and width were measured. Downstream sites showed improvement in many macroinvertebrate measurements including: evenness, richness, abundance, number of sensitive families, number of sensitive individuals, number of EPT taxa, and Family-level Biotic Index. Dissolved oxygen was improved and stream depth increased at downstream sites. The Maryville College Woods appears to induce certain habitat changes, which promote a shift in macroinvertebrate communities from more tolerant assemblages at upstream sites to more sensitive assemblages at downstream sites. Further research is needed to understand the exact mechanism of this action.

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CHAPTER 1

INTRODUCTION

The biggest threat to aquatic ecosystems is the effect of anthropogenic activities (Dynesius and Nilsson 1994; Wang et al. 2001). Human-influenced impacts include, but are not limited to: Habitat destruction or alteration (Bunn and Arthington 2002), pollutants (Cooper, 1993), introduction of invasive species (Nystrom et al. 2001), and overexploitation of resources (Humphries and Winemiller 2009). Lotic ecosystems are considered some of the most degraded on earth (Giller and Malmqvist 1998). Of the 5.2 million kilometers of stream habitat in the United States, less than 2% is of sufficient quality to be federally protected (Benke 1990).

The southern Appalachian area of the United States has been undergoing extensive land-use change for the past three centuries. Some of the heaviest impacts, however, occurred between 1880 and 1920, when the majority of the timber of the southern Appalachians of Tennessee, North Carolina, West Virginia, and Virginia was harvested (Benfield 1995). Afterward, most of the usable land was utilized for agriculture or development (Otto 1983). Since the 1950's, agricultural activities in most Appalachian counties have decreased by as much as 30% and large tracts of land have been protected as a National Park (Wear and Bolstad 1998). With the reduction of agriculture, many areas previously devoid of forest began to recover, a process which continues today (Clark and Pelton 1999; Unger et al. 2013). Currently, the Southern Appalachians are experiencing continued reforesting in many areas and an increase in urbanization in others (Wear and Bolstad 1998).

Land use changes induced by humans can have a huge impact on stream health and dynamics. Streams are intimately tied to the surrounding riparian habitat and many important processes depend on the riparian zone interactions (Gregory et al. 1991). The stream is dependant upon the surrounding terrestrial area for allochthonous energy inputs in the form of leaves and detritis (Wallace et al. 1997), for infiltration of rain water to recharge groundwater stores (Harbor 1994), for temperature, moisture, and light level control (Gregory et al. 1991), bank stabilization (Simon and Collison 2002), and for the physical filtration of water (Cooper et al. 1987). Therefore, alteration of the landscape of a watershed affects many important land-water interactions which can influence the physical, chemical, and biological composition of lotic ecosystems (Allan and Johnson, 1997).

Agriculture occupies the largest percentage of land use in many developed catchments (Allan 2004). Increased percentage of agricultural land in a catchment is associated with degraded water quality (Wang et al. 1997), habitat, and biological assemblages (Sponseller et al. 2001). Watersheds that drain agricultural and urban landscapes have been found to contain significantly increased levels of nitrogen and phosphorous(Tong and Chen 2002). Runoff from cultivated land and livestock trampling often result in a greater deposition of sediment on and within the streambed, which is a major factor in stream impairment (Waters 1995). Streams draining areas of agriculture often contain fewer species of environmentally sensitive insects and fish when compared to forested streams (Genito et al. 2002), and these changes in biotic communities can be long-lasting. Harding et al. (1998) found that reforested watersheds that had been used for agriculture within the past 50 years contained macroinvertebrate assemblages that

were more similar to agricultural land than to forested streams without any history of agriculture. This suggests that high impact, sustained anthropogenic activities, such as agriculture, may have the power to profoundly impact biotic communities, even years after the disturbance has been removed.

Urban land use often comprises a low percentage of catchment land use, yet it has a disproportionately large influence on stream health on a large spatial scale (Paul and Meyer 2001). Urbanization often creates increased areas of impervious surfaces in catchment basins which can alter the natural dynamics of a stream ecosystem. Asphalt, concrete, rooftops, compacted soil, and paved roads all create surfaces which decrease infiltration and increase surface runoff. The decrease of water infiltration can alter the hydrologic cycle by lowering water tables and reducing groundwater recharge which can result in decreased stream flow during dry periods (Harbor 1994). Urban stream water quality is affected by an increase in storm water runoff. This surface runoff flushes pollutants into the channel, accelerates channel erosion, alters the composition of the streambed, and changes the dynamics of the biotic community (Klein 1979). Roy et al. (2003) found that when greater than 15% of a catchment was classified as urban, a less diverse, more pollution tolerant aquatic macroinvertebrate community was observed. This was a result of increased transport of sediment, reduced streambed sediment size, and increased solutes.

Most streamside land use change requires the removal of riparian forests. When natural riparian forests are removed, the remaining stream is normally warmer in the summer and experiences fewer energy inputs as leaf litter (Quinn 2000). Riparian forest acts by absorbing some of the incoming solar radiation, thereby reducing the maximum

daily temperature of the stream and then acts as an insulator to offset heat loss of the stream at night. This helps to reduce overall fluctuations in stream water temperature. Streamside forests can determine the micro-climate of a stream by effecting evaporation, ground temperature, and water temperature (Rutherford et al. 1997). The changes in water temperature induced by the removal of streamside vegetation can alter riparian biological communities by increasing the density of tolerant organisms and excluding sensitive taxa (Quinn et al. 1994). Sedimentation, which often happens as a result of runoff or bank instability, increases sediment deposition in and on a stream and its banks (Waters 1995). Sedimentation, along with a decrease in woody debris deposition, results in a decrease in habitat heterogeneity for aquatic organisms (Platts et al. 1987; Smith 1976).

Water quality monitoring is vital to protect healthy ecosystems, to understand how to restore damaged waterways, and to predict how changes to a landscape might affect the health of a water system (Hirsch et al. 1988). There are a wide variety of methods for assessing the various aspects of stream health. Assessment may involve examining chemical constituents of the water (e.g nitrates, phosphates, dissolved oxygen, or pH) (Maher et al. 1999), or physical components of the stream (e.g. channel width, flow rate, and bank stability) (Maddock 1999). However, catchment heterogeneity and irregular areas of human impact can cause wide variation in the conditions of a stream over time (Ramirez et al. 2006).

Biological indicators such as specific species of macroinvertebrates, fish, and amphibians are useful as indicators of stream health and overall ecological integrity. The presence or absence, abundance, and community structure of these organisms allows

scientists to make inferences about the condition of a water system (Marchant 2007; Wright et al. 1993; Walsh 2005; Lear et al. 2009; Barbour et al. 1999). Because the presence of certain organisms is influenced by past and present conditions, biological monitoring can provide a broader temporal and spatial aspect than certain chemical and physical monitoring techniques (Hoang et al. 2001). Another advantage of biological monitoring is that it can be relatively inexpensive compared to chemical assessment (Ohio EPA 1987). The status of biological communities is also of direct interest to the public that more easily understands the concept of living organisms as indicators of a healthy ecosystem (Barbour et al. 1999).

Macroinvertebrates are the most common biological indicator utilized in determining stream health (Boothroyd and Stark 2000; Klemm et al. 2002). The benefits of using macroinvertebrates as biological indicators are many. Most stream macroinvertebrate species are not free-swimming, rather, they are benthic, meaning they are associated with surfaces of the channel bottom (Hauer and Lamberti 2007). Because this life history does not allow a migration of great distances, aquatic macroinvertebrates make good indicators of local conditions (Keup et al. 1966). In addition, the sampling of benthic macroinvertebrates allows insight into short-term environmental variations. Due to the macroinvertebrate life cycle, which is long enough so as to not produce a response to temporary relief from pollution, it is possible to gauge the conditions of a site over time (Klemm et al. 2002). Another valuable trait of the macroinvertebrate as a biological indicator, is the variation across groups to tolerance of diminished water quality and pollutants (Cairns and Dickson 1971). Aquatic organisms are excellent indicators of the

overall wellness of a lotic water system due to their sensitivity to variations in nutrient content, sunlight, temperature, and habitat heterogeneity of an aquatic system.

One of the most effective ways to protect and improve streams is through proper management and restoration of riparian areas (Naiman and Decamps 1997; Quinn 2005). A riparian zone is the integration of aquatic and terrestrial zones (Gregory et al. 1991) and can act as a buffer for lotic ecosystems to protect against bank erosion, introduction of pollutants, increased light and temperature levels, low levels of allocthonous input, and excessive in-stream primary production (Lyons et al. 2000; Peterjohn and Correll 1984). "The presence or absence of trees adjacent to stream channels may be the single most important factor altered by humans that affect the structure and function of stream macroinvertebrate communties." (Sweeney 1993)   Prior to human alteration, the dominant land cover of the Southern Appalachians was native forest (Wear and Bolstad 1998). This suggests that restoration of riparian zones to native forest may help offset anthropogenic impacts to streams.

Although it would be beneficial to restore whole catchments to native forest, it would not be economically feasible. The more cost effective method might be to restore fragmented riparian patches to native vegetation and to protect remaining patches of intact forest. However this approach can only be effective if the patches are of sufficient size to positively impact stream conditions (Scarsbrook and Halliday 1999). The necessary patch size will depend on which variable is being tested (Storey and Cowley 1997). Certain variables such as light levels, will be ameliorated relatively quickly. Other variables, such as nitrate levels, will require larger buffer zones (Scarsbrook and

Halliday 1999). In addition, larger order streams may require longer buffers to improve chemical, physical, and biological indices (Niyogi et al. 2007)

Many studies have focused on streamside forest buffers and the optimal width needed to mitigate the effects of human activities that occur streamside, such as logging and agriculture (Quinn 2005; Clinton 2011). These studies are helpful in understanding how riparian buffer zones can prevent deterioration of water quality. Often, the optimal buffer width depends on the characteristics of the particular landscape being studied. Clinton (2011) studied the optimal width of streamside forest buffer needed in southern Appalachian ecosystems to counteract the effects of logging. Buffer widths of 0 meters, 10 meters, and 30 meters were compared to a reference stream in a catchment that had never been logged. Stream water chemistry, temperature, and total suspended solids were used as indicators of water quality. Buffers of 10 meters and wider, prevented major deleterious effects to water quality. Peterjohn and Correll (1984) found that 90% of particulates being swept by runoff overland from agricultural areas was removed by riparian forest buffers of 19 meters. Robinson et al. (1996) found that the first 3 meters of riparian forest buffer removed 70% of sediment runoff in Iowa streams.

Other studies have chosen to investigate the effects that larger forest segments can exert on lotic water quality that has already been negatively impacted by upstream activities. Storey and Cowley (1997) investigated impaired streams that passed from agricultural land through forest remnants of up to 600 meters and compared those measurements with measurements taken from an undisturbed forest stream. Storey and Cowley determined that within 300 meters of entering a forest remnant, the temperature and dissolved oxygen of the stream had returned to forest-stream levels. Nitrate, nitrite,

phosphate, and suspended solid levels were variable, but there was some evidence of significant in-stream processing when the stream continued over 600 meters through a forest fragment. Over 600 meters, macroinvertebrate communities resembled the control stream communities and changed from a more pollution-tolerant fauna to a more pollution-sensitive fauna.

Scarsbrook and Halliday (1999) performed a similar study to assess the effects of late-succession native riparian forest on water quality, epilithon (biofilm on the surfaces of rocks and other substrates in aquatic habitats), channel morphology, and aquatic macroinvertebrates in three first-order streams draining pasture land. Sampling sites were located 50 meters and 300 meters into a forest remnant in each stream. It was found that within 300 meters, shade, channel width, and epilithon biomass were returned to conditions similar to the control site. Invertebrate community began to become more similar to the control stream community within 50 meters of entering a forest fragment, and was completely restored within 300 meters. Water chemistry and sediment levels responded more slowly than the other variables.

Harding et. al (2006) found no improvement in physical, chemical, or biological indices of water quality when two catchments with forest fragments were compared to undisturbed, forested streams. This study suggests that factors such as fragment size, vegetation type, and fragment location may play critical roles in enabling forests to mitigate the effects of agriculture. Other studies performed in a similar fashion, also found no improvement in water quality measurements, but did find improvements in macroinvertebrate communities (Chakona et al. 2009; Arnaiz et al. 2011; Suga and Tanaka 2013).

Further studies of the effects of forest fragments on stream water quality are extremely important to understand the various factors that may impact stream health (Harding et al. 2006). Preservation and restoration of streamside riparian forests is becoming increasingly urgent as more and more habitat is destroyed and altered in order to accommodate a growing population (Benke 1990). Understanding the impacts of forest fragments on stream health is vital to their preservation.

CHAPTER II

MATERIALS AND METHODS

Study Area

Brown's Creek is a small-order stream located in North-central Blount County, Maryville, Tennessee (Latitude 35.74954, Longitude -83.95319; Figure 1). It is within 50 kilometers of some of the most pristine waterways in the southern Appalachians, which are preserved within the Great Smoky Mountains National Park. Despite its proximity to these waterways, it is listed as an impaired stream with siltation/sedimentation being listed as the source of impairment (The 2012 State of Tennessee Stream Assessment, http://tnmap.tn.gov/wpc/ [accessed 25 November 2013]). A southeast branch of approximately 2.3 kilometers, merges with a northeast branch of 1.7 kilometers and then travels approximately 337 meters before entering the Maryville College Woods, a 57 hectare mixed deciduous forest fragment, first designated as a Stewardship Forest in 2000 (Crain 2012). Brown's Creek enters the southeast corner of the Maryville College Woods and runs northwest through the woods for a total of approximately 827 meters. At 512 meters from its southeast entrance it briefly exits and then re-enters the woods and begins to become impacted by bank deforestation and bank armoring on one side of the channel. It flows solely in the Maryville College Woods for 512 meters. Upstream sites were sampled within 40 meters of the entrance of the creek into the woods. Downstream sites began at 512 meters and extended upstream 40 meters, in order to avoid the possible confounding variables of the intersection with Duncans

Branch and the effects of land-use change after 512 meters.   No previous research has been done to assess the impacts of this forest fragment on the overall health of Brown's creek.

Aquatic Macroinvertebrate Sampling

In order to assess macroinvertebrate abundance and community structure, kick-net sampling was conducted on February 23 and again on April 1, 2014 using a 1x1 meter, 500-micron mesh kick-net (Forestry Suppliers 205 West Rankin St., P.O Box 8397, Jackson, MS 39284-8397). At each site (Appendix 1), approximately one square meter of upstream area was sampled for 1.5 minutes by 1-4 people. This area was sampled by kicking the substrate and turning over all possible rocks. On February 23, a total of 6 samples were collected. Three samples were collected from the entrance of Browns Creek into the woods and three samples were collected from the stream just above its connection with Duncan's Branch.   5 additional samples were collected at each site on April 1, 2014 for a total of 10 samples.

Kick-net samples were preserved with 70% ethanol the day of collection.   Due to a large amount of filamentous Green Algae, 24 hours later, samples were drained and preserved with fresh 70% ethanol. Samples were carefully sorted and all aquatic macroinvertebrates were removed and preserved in 70% ethanol.

As an additional measure of macroinvertebrate abundance and community structure, 20 artificial leaf packs were made and placed February 28, 2014 using 18.9"x 31.9" polypropylene mesh bags (https://www.onlinefabricstore.net). Leaves for each artificial leaf pack were gathered streamside at each leaf pack site to simulate the natural allochthonous input of each site. Leaf volume for each leaf pack was determined by

loosely packing a 1.36 kg bucket with leaves. Leaf packs were tied at each end with 3-ply jute twine. After being placed in the streambed, leaf packs were each anchored to the substrate using 4 7" aluminum tent stakes (Texsport 1628 Jefferson Avenue, Ridgewood, NY 11385).   Each leaf pack was then tied loosely to the terrestrial riparian substrate (tree, root, etc.) using 3-ply jute twine to ensure that high water did not dislodge the pack. Artificial leaf packs were evenly spaced, as allowed by streambed morphology, at each study area at 0, 10, 20, 30, and 40 m (Appendix 2). Coordinates were taken with a Garmin 72H GPS (Garmin International, Inc.1200 E. 151st St.   Olathe, KS 66062-3426). 2 leaf packs were placed every ten meters for a total of 10 leaf packs at each end of the stream. Leaf packs were collected on April 1, 2014 and placed in 14-gallon plastic trash bags for transport to the lab.   Samples were sorted within 48 hours using a sieve with graduated mesh sizes (Newark Wire Cloth Co. 160 Fornelius Ave., Clifton, NJ 07013) to separate smaller debris and macroinvertebrates from the larger leaf litter. The smaller debris and leaf litter were then emptied into a dissection tray and all macroinvertebrates were removed and preserved in glass specimen jars in 70% ethanol.

All aquatic macroinvertebrates were identified to family using a key by William L. Hilsenhoff in Ecology and Classification of North American Freshwater Invertebrates and an Accu-scope 3061 series stereo microscope (73 Mall Drive, Commack, NY 11725). All aquatic macroinvertebrates were classified according to tolerance value (Hilsenhoff 1988).

Due to departures from normality in the data non-parametric tests were used for data analysis. A Wilcoxon Signed-Ranks test was performed to compare the evenness of families between upstream and downstream sites. A Mann-Whitney U test was used to

compare macroinvertebrate abundance in each kick-net sample, number of intolerant individuals present per kick-net sample, richness of upstream and downstream kick-nets, difference in Family-level Biotic Index, and the number of pollution intolerant families present at each site and the number of EPT (*Ephemeroptera, Plecoptera, Trichoptera*) families present at upstream and downstream sites. The Orders *Ephemeroptera, Plecoptera*, and *Trichoptera* are generally pollution intolerant and are therefore commonly used as bio-indicators of water quality and stream health (Wallace et al. 1996).

A Family-level Biotic Index was calculated (Equation 1) (Hilsonhoff 1982). This is an average of tolerance values (Appendix 3) of all macroinvertebrate families in a sample and can be used to rapidly assess the health of a stream. In certain streams, pollutants are introduced into the channel in short-term events such as run-off caused by heavy rainfall or substrate disturbance or removal caused by flash flooding. These events are difficult to detect with many chemical and physical water assessment techniques, as they are short-lived. Aquatic macroinvertebrates can act as long-term indicators to short-term disturbances. The Family-level biotic index is calculated by multiplying the number in each family by the tolerance value for that family, then summing the products, and dividing by total insects in the sample.

Equation 1:
$$B.I. = \frac{\Sigma \, n_i \, n_a}{N}$$

n_i: number of insects in each family

n_a: tolerance value for the famiy

N: number of insects in the entire sample

Table 2: Water quality evaluation using the family-level biotic index (Hilsonhoff 1988)

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.00 - 3.75	Excellent	Organic pollution unlikely
3.76 - 4.25	Very good	Possible slight organic pollution
4.26 - 5.00	Good	Some organic pollution probable
5.01 - 5.75	Fair	Fairly substantial pollution likely
5.76 - 6.50	Fairly Poor	Substantial pollution likely
6.51 - 7.25	Poor	Very substantial pollution likely
7.26 - 10.0	Very Poor	Severe organic pollution likely

Chemical Water Assessment

Water samples were collected 3/22/14 and 4/6/14 in 250 mL bottles at each leaf pack site. A total of 40 samples were collected and immediately placed on ice in the field until they could be transported back to the lab for refrigeration. Chemical analysis was conducted 4/8/14 using Vernier LabQuest (13979 SW Milikan Way, Beaverton, OR 97005-2886). Water samples were tested in the lab for nitrates, sulfates, turbidity, conductivity, calcium, and ammonium using Vernier Probes. Dissolved oxygen was tested in-stream at each artificial leaf-pack site using Vernier LabQuest2 and a Vernier Optical DO Probe. The data from the samples collected on March 22, 2014 were not used as it was discovered in the process of testing that extended refrigeration causes a decrease in accuracy of the detection of the chemical constituents. All probes used in water samples collected on March 22, displayed a high level of drift, which caused inaccurate readings. Data from chemical water assessment was analyzed using a Mann-Whitney U-test.

CHAPTER III

RESULTS

Benthic Macroinvertebrate Sampling

A total of 23 families were caught and identified from the kick-net samples performed between February 23 and April 1, 2014. 3,447 aquatic macroinvertebrates were identified from upstream sites and a total of 7,484 individuals were identified from downstream sites (Table 1).

Mean family richness was found to differ significantly ($p < 0.05$) when comparing upstream and downstream kick-net sites (n=8), with upstream sites having a mean of 11.38 ± 0.92 families present and downstream sites having a mean of 14.25 ± 0.53 families present (Table 3). Mean aquatic macroinvertebrate abundance per kick-net sample also differed significantly ($p < 0.05$) between upstream and downstream (n=8). Upstream samples had a mean abundance of 430.88 ± 120.94 insects per sample and downstream samples had a mean abundance of 935.38 ± 173.28 insects per sample (Table 2).

Table 1: Total number of each family found in kick-net samples at upstream and downstream sites (n=8) and total number of aquatic macroinvertebrates found at upstream and downstream sites.

| Benthic Macroinvertebrate	Upstream Sites	Downstream Sites
Coleoptera Elmidae	135	345
Coleoptera Psephenidae	28	236
Diptera Chironomiidae	2810	4223
Diptera Empididae	42	42
Diptera Ephydridae	30	22
Diptera Simulidae	65	318
Diptera Tipulidae	14	22
Ephemeroptera Baetidae	2	48
Ephemeroptera Ephemerellidae	11	2
Ephemeroptera Heptageniidae	14	311
Ephemeropterea Isonychiidae	2	148
Lepidoptera Pyralidae	0	2
Odonata Aeshnidae	7	2
Odonata Calopterygidae	15	4
Odonata Coenagrionidae	2	0
Odonata Gomphidae	2	2
Plecoptera Taeniopterygidae	0	2
Trichoptera Hydropsychidae	215	613
Trichoptera Leptoceridae	0	1
Trichoptera Limnephilidae	1	1
Trichoptera Philopotamidae	14	1034
Trichoptera Rhyacophilidae	0	20
Trichoptera Uenoidae	38	86
Total	**3447**	**7484**

Mean family richness was found to differ significantly ($p < 0.05$) when comparing upstream and downstream kick-net sites (n=8), with upstream sites having a mean of 11.38 ± 0.92 families present and downstream sites having a mean of 14.25 ± 0.53 families present (Table 3). Mean aquatic macroinvertebrate abundance per kick-net sample also differed significantly ($p < 0.05$) between upstream and downstream (n=8). Upstream samples had a mean abundance of 430.88 ± 120.94 insects per sample and downstream samples had a mean abundance of 935.38 ± 173.28 insects per sample (Table 2).

Family-level Biotic Index numbers significantly differed between upstream and downstream sites ($p < 0.05$). The average upstream index of 5.50 is representative of a stream with fair water quality which is indicates that fairly substantial pollution is likely. The average downstream index of 4.94 indicates a stream with good water quality with some organic pollution probable (Hilsenhoff, 1988).

Table 2: Mean values for taxa richness ($p < 0.01$) and aquatic insect density ($p < 0.05$) (± SE), and Family-level Biotic Index for Kick-net samples ($p < 0.05$) (n=16).

Site	Mean Richness	Mean Abundance	FBI
Upstream Sites	11.38 ± 0.92	430.88 ± 120.94	5.50
Downstream Sites	14.25 ± 0.53	935.38 ± 173.28	4.94

The evenness of families between upstream and downstream kick-net samples was significantly different ($p < 0.005$). Downstream sites exhibited significantly more individuals per family than upstream sites (Figures 1 & 2).
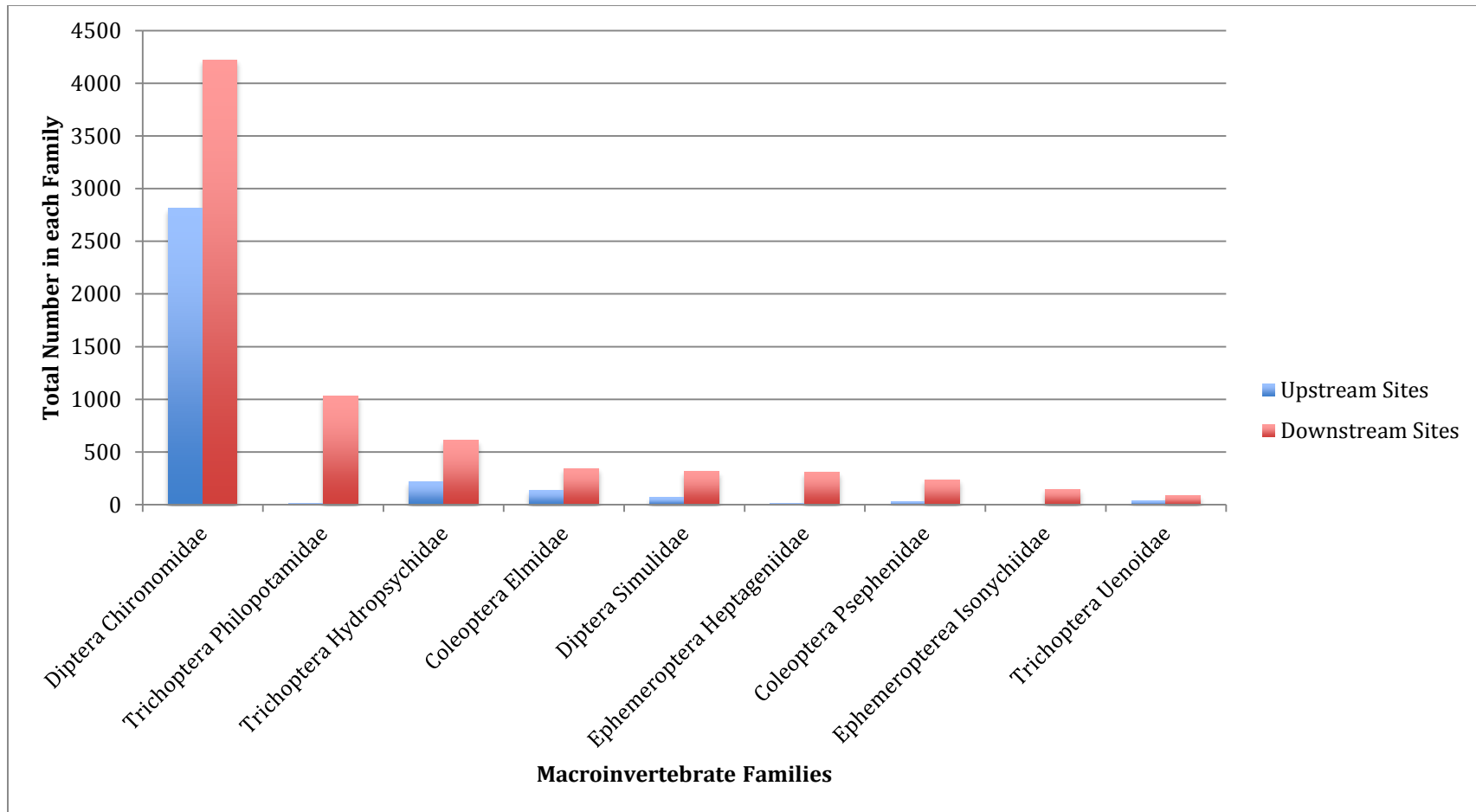
Figure 1: Evenness of upstream and downstream Sites (P <0.005) showing the 9 most common families of kick-net samples (n = 16).
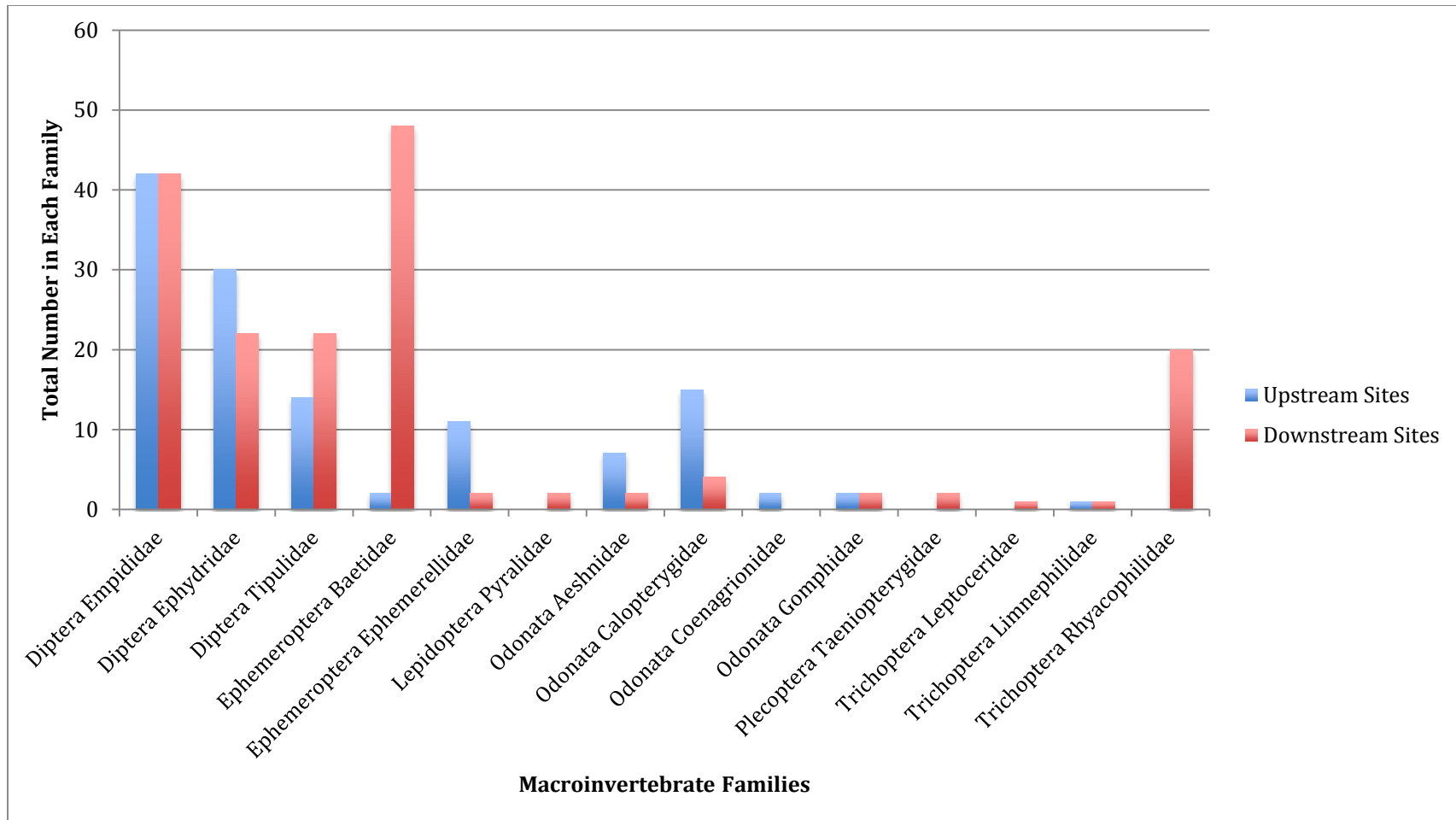
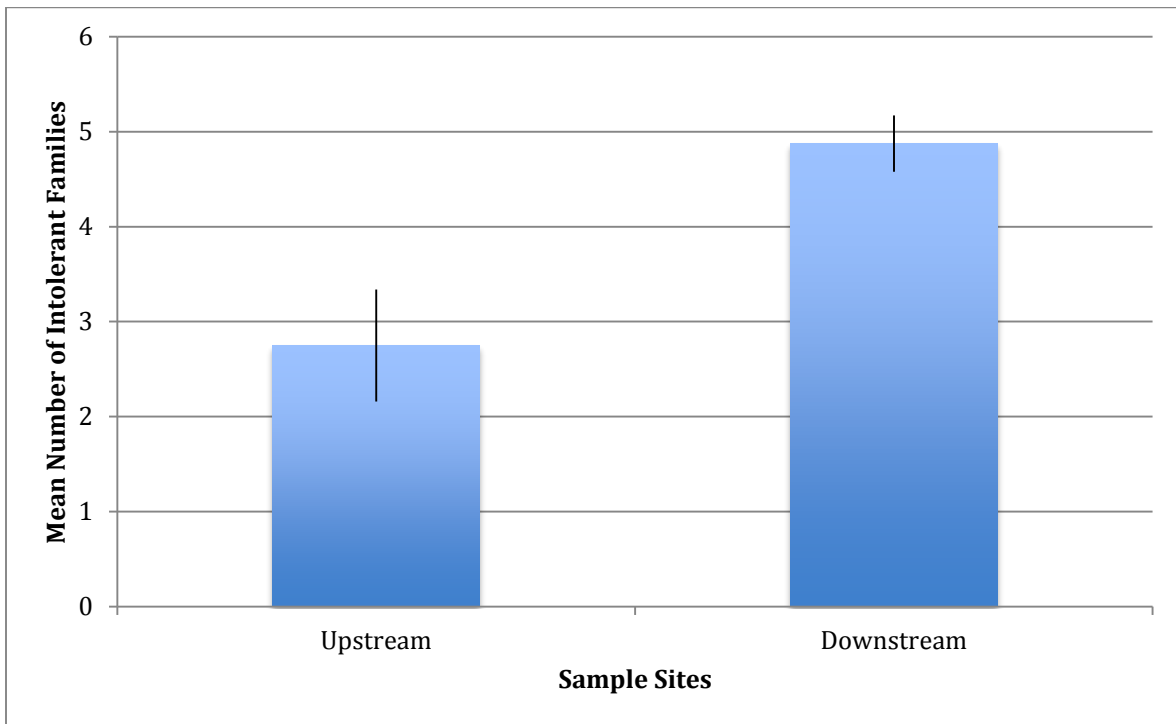**Figure 2:** Evenness of upstream and downstream sites (p < .005) showing 14 less common families of kick-net samples (n = 16).

The numbers of families from the Orders *Ephemeroptera, Plecoptera*, and

Trichoptera differed significantly between upstream and downstream sites (p = 0.0005).

Upstream sites had a mean of 3.5 ± 1.20 families and downstream sites had a mean of

6.88 ± 0.83 families (Figure 3).
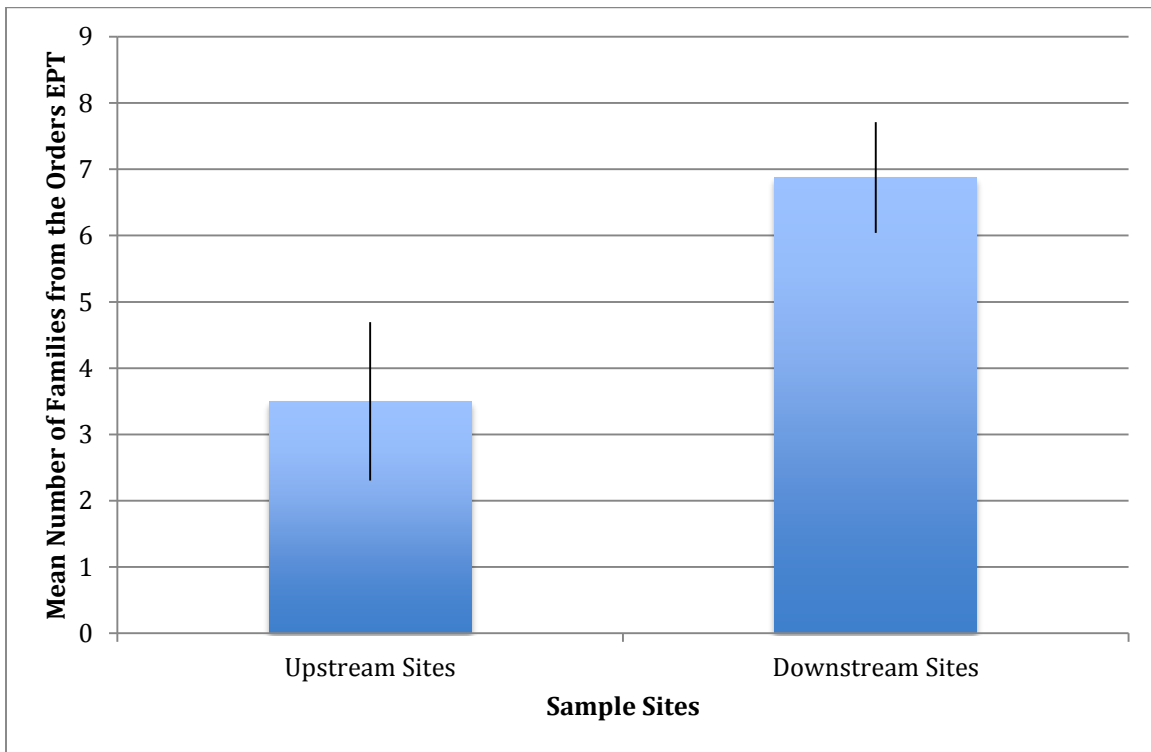
Figure 3: Mean number of families (±SD) (p = 0.0005) from the Orders of
Ephemeroptera, *Plecoptera*, and *Trichoptera* of upstream (3.5 ± 1.20) and downstream
(6.88 ± 0.83) sites identified from kick-net samples (n = 16) of Browns Creek 2/23/14
and 4/1/14.

The number of intolerant families differed between upstream and downstream kick-nets ($p < 0.01$). Mean number of families for upstream samples (±SD) was 2.75 ± 1.67 families. Mean number of families for downstream samples (±SD) was 4.88 ± 0.83 (Figure 4).

Figure 4: Mean number of intolerant (tolerance value of 3 or less) (Appendix 4) families (±SE) of upstream and downstream sites ($p < 0.01$) of kick-net samples (n = 16) of Browns Creek.

The number of intolerant individuals also differed between upstream and downstream kick-net sites (p = 0.0005). The mean number of individuals (±SE) in upstream kick-nets was 11 ± 5.10. The mean number of intolerant individuals (±SE) in downstream kick-net samples was 164.63 ± 74.76 (Figure 5).
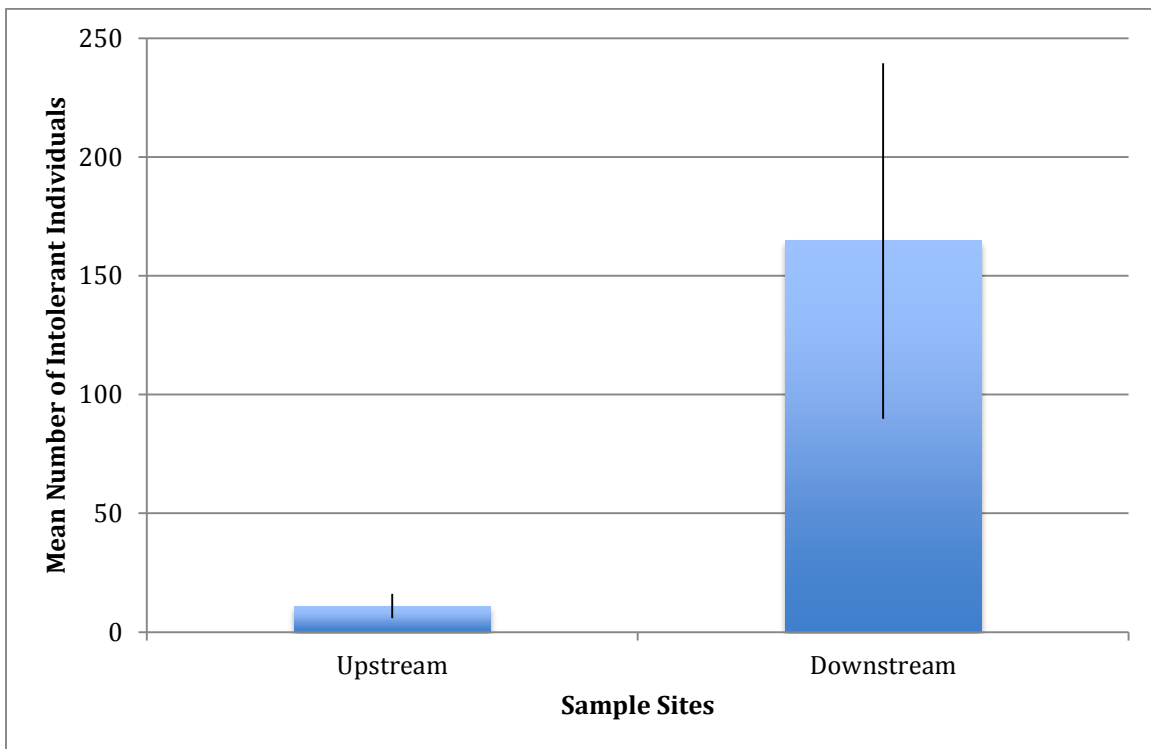

Figure 5: Mean number of intolerant (tolerance value 3 or less) (Appendix 4) individuals (±SE) found in kick-nets (n = 16) in upstream and downstream sites (p = 0.0005).

Artificial leaf-packs failed to capture large numbers of aquatic macroinvertebrates. A total of 1406 aquatic insects were identified vs. 10,931 insects identified in the kick-net samples. There was no statistical significance in evenness, abundance, or richness in upstream vs. downstream leaf-packs. Several different families were found in the artificial leaf-packs that were not found in the kick-nets including *Coleoptera Dytiscidae*, *Coleoptera Haliplidae*, *Diptera Ceratopogonidae*, and *Collembola Isotomidae*.

Chemical Water Assessment

Sulfate between upstream and downstream sites was found to differ significantly ($p < 0.05$). Upstream water samples contained a mean (±SE) of 1.56 ± 0.41 ppm while downstream sites contained a mean 3.53 ± 0.64 ppm.

The difference in dissolved oxygen levels between upstream and downstream sites was highly significant ($P < 0.001$) (Figure 5). The mean dissolved oxygen level (±SD) for upstream sites was 9.42 ± 0.03 ppm (91.2 - 93.1 - % saturation). The mean dissolved oxygen level (±SD) for downstream sites was 10.02 ppm (96.8 – 99.3% saturation). 100% oxygen saturation for water at 15.1 C and barometric pressure at 768.35 mmHg is between 10.15 and 10.29 ppm.
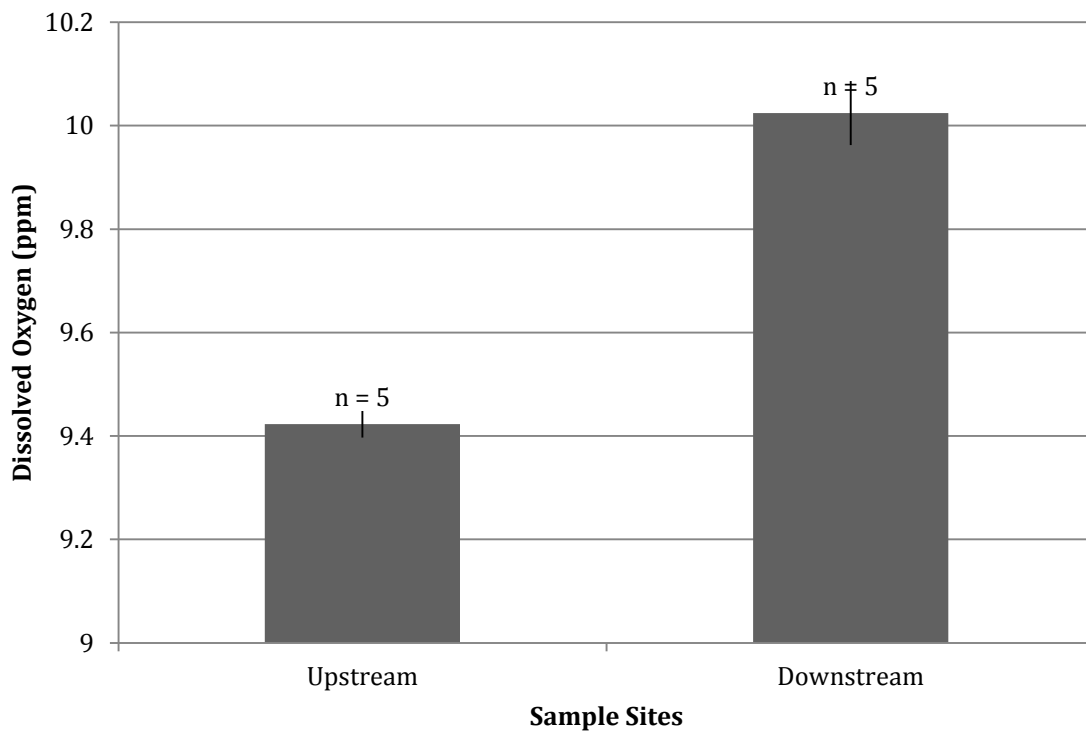
Figure 5: Dissolved oxygen levels (± SD) between upstream (9.42 ± 0.03 ppm) and downstream (10.02 ± 0.06 ppm) sites (p <0.0001).

No other chemical constituents of the water samples were found to differ

significantly (Table 4). Ammonium levels were too low to detect with the

equipment used.

Table 4: Average levels of water constituents of Browns creek. Samples collected
3/22/14 and 4/6/14. Samples analyzed 4/8/14.

Nitrate	pH	Turbidity	Calcium	Conductivity
37.76 ppm	7.08	16.64 NTU	40.38 ppm	352.92 µS/cm

Width of the channel between top and bottom sites did not differ significantly.

Mid-channel stream depth did differ significantly ($p < 0.05$). Mean upstream depth was

18.92 ± 2.22 cm. Mean downstream depth was 26.92 ± 9.82 cm.

CHAPTER IV

DISCUSSION

Family evenness, richness, abundance, and Family-Level Biotic Indices were significantly different between upstream and downstream sites. Many factors in a lotic water system can influence the assemblages of aquatic macroinvertebrate communities. Large-scale factors such as catchment size and channel width, as well as small-scale factors such as current velocity, substrate particle size, and habitat heterogeneity are capable of influencing the structure of macroinvertebrate communities (Graca et al. 2004).

Benthic macroinvertebrates are commonly utilized indicators of the overall health of a water system (Rosenberg and Resh, 1993). They make excellent indicators because they are ubiquitous and so are vulnerable to disturbances in many different habitats. They are also species rich and the abundant number of species produces a large range of responses to water system conditions. The sedentary mode of life of most benthic macroinvertebrates makes the assessment of spatial extent of a disturbance possible. In addition, they are long-lived which allows for changes in abundance and life stages to be observed. Macroinvertebrates are also vulnerable to temporary changes so they provide long-term evidence of conditions over time (Mandaville 2002).

The Family-level Biotic Indices (FBI) differed significantly between upstream and downstream sites. The upstream FBI of 5.50 is suggestive of an assemblage of macroinvertebrates that would typically be observed in a stream which experiences substantial pollution. The downstream FBI is improved, with a score of 4.94. This is

suggestive of an assemblage of macroinvertebrates that would typically be found in a stream with only some organic pollution (Hilsonhoff 1988). The FBI is an index that takes into account the tolerance values of all macroinvertebrate families in a sample in order to assess the health of a stream. Macroinvertebrate community structure has often been used as an indicator of the health of water systems due to the fact that aquatic macroinvertebrate families are differentially sensitive to many variables in their environment, both biotic and abiotic (Mandaville 2002). Biotic indices have been developed to give numerical scores to indicator organisms at a given taxonomic level that have certain specific requirements in terms of chemical water constituents and physical conditions. The presence or absence of such organisms could indicate that the conditions of a water system are outside of their required range (Rosenberg and Resh 1993). Alternatively, the presence of numerous highly tolerant organisms can indicate poor water quality (Hynes 1998).

The differences in macroinvertebrate family evenness, richness, and abundance, as well as a significant difference in the Family-level Biotic Index, suggests that there are differences in physical and/or chemical conditions between upstream and downstream sites. The only variables that this study observed to differ between upstream and downstream study sites were dissolved oxygen levels, sulfate levels, stream depth, and proximity to upstream areas of deforested stream banks, agricultural land, manicured lawn, and impervious surfaces such as roads and rooftops. These are all differences that have all been observed to impact macroinvertebrate communities (Graca 2004, Allan 2004)

Dissolved oxygen levels were significantly higher in downstream sites than in upstream sites. This may account for some variation in biotic assemblages between study sites. Oxygen levels were not particularly low at either site with a mean of 9.42 ± 0.03 ppm at upstream sites and a mean of 10.02 ± 0.06 ppm at downstream sites. When taking water temperature and barometric pressure into account, 100% oxygen saturation for water was between 10.15 and 10.29 ppm. This means that the water at upstream sites was between 91.2% and 93.1% saturated. The water at downstream sites was between 96.8 and 99.3% saturated.

Many factors can influence dissolved oxygen levels in a stream such as temperature, stream velocity, respiration and photosynthesis of plants, utilization of animals for respiration, chemical oxidation, and the decay of organic material (Ice 2003). Burton & Likens (1973) found that for a stream flowing through alternating sections of forested and deforested sections, water temperature on a sunny day rose and fell by 4-5 C over distances as short as 50 m. For this study, temperature was measured at the same time as dissolved oxygen and was not found to significantly differ between upstream and downstream sites. Measurements were performed on a cloudy, cool day. Further study could be conducted on the temperature regime of the stream when air temperatures are consistently warmer and on sunny days when the stream is subject to increased solar radiation. If temperature was found to fluctuate greatly between upstream and downstream sites, this may be a determining factor for dissolved oxygen levels and macroinvertebrate assemblages. Quinn et al. (1994) found that stoneflies and mayflies are less abundant in streams that reach temperatures of 19 C and 21 C, respectively.

Temperature may be a determining factor in the levels of dissolved oxygen in Browns Creek and a limiting in the assemblages of macroinvertebrate communities.

Dissolved oxygen levels can fluctuate due to respiration and photosynthesis of plants.   Often, levels of oxygen will fluctuate diurnally and drop off steeply at night, as sunlight can no longer be used in the oxygen generating cycles of the photosynthesis of plants. Future studies may investigate the diurnal patterns of dissolved oxygen for Browns Creek as well as the populations of macrophytes and aquatic plants in upstream and downstream sites. I performed an informal survey of in stream macrophyte cover after the study had concluded. I used a 1 m^2 quadrat to estimate the percentage of substrate covered with macrophytes. The five upstream sites contained a significantly ($p < 0.05$) lower percentage of macrophyte substrate cover than lower sites. Future studies into the relationship between macrophyte communities and macroinvertebrate communities could be particularly useful.

Streambed roughness can also influence dissolved oxygen levels. An informal study of riffle embeddedness as well as other physical variables (Appendices 5-9) was performed after the completion of this study. 5 sites, top and bottom, were rated on a scale of 1-4 on the extent of riffle embeddedness (Appendix 7).   There was found to be a significant difference ($p < 0.01$) between upstream and downstream riffle embeddedness, with upstream riffles suffering from a substantial amount of embeddedness. This suggests that the streambed substrate at the upstream sites has been covered in sediment. This embeddedness can decrease substrate heterogeneity and roughness, which in turn can decrease the capability of rough substrate to reaerate the water (Ice 2003).   Substrate embeddedness also inhibits interaction of the surface water with the hyporheos which can

impact dissolved oxygen levels (Wood and Armitage 1997). Further study into the

embeddedness of the substrate of Browns Creek would be very useful.

Sulfate levels were the only chemical water constituent found to differ

significantly ($p < 0.05$) between upstream and downstream sample areas of Browns

Creek. Water samples taken at upstream sites contained a mean of 1.56 ppm, whereas

water samples taken from downstream sites contained a mean of 3.53 ppm. There are

many possible sources of Sulfate input into lotic systems including sulfate salts found in

some soils, the decay of plant and animal tissue, burning of fossil fuels, and some

industrial wastes. It is possible that the increase in allochthonous inputs in the Maryville

College Woods in the form of plant matter is contributing to this increase in sulfate

downstream. There is also a wastewater line, which runs adjacent to the stream that may

be contributing to increased sulfate levels. Nutrient concentrations are often upheld as

major factors affecting macroinvertebrate communities (Quinn & Hickey, 1990b). This

study and others (Storey & Cowley 1997) have found that nutrient concentrations and

macroinvertebrate distributions and compositions are not well correlated. This study may

have failed to find a significant correlation between nutrient concentrations and

macroinvertebrate community structure because most pollution events in Browns Creek

are likely to be short-term disturbances resulting from storm-water runoff. These events

would be difficult to detect with the methods used in this study, but would likely impact

macroinvertebrate communities in a more long-term fashion, as most aquatic

macroinvertebrates have complex, sometimes multi-year life cycles.   In lotic ecosystems

where nutrient concentrations are sufficiently high enough to affect macroinvertebrate

communities, their removal within forest fragments may be an important part of stream recovery (Storey & Cowley 1997).

The difference in stream depth that was observed between upstream and downstream sites at mid-channel could indicate increased sedimentation in the streambed. Increased sediment supplies can lead to streambed aggradation, which may cause a decrease in stream depths as sediments fill the channel (Paul and Meyer 2001). The 2012 State of Tennessee Stream Assessment lists Browns Creek as an impaired stream due to sedimentation/siltation. This indicates that Browns Creek is vulnerable to the hydrology, morphology, and biological changes that are caused by a high sediment load. Sedimentation has the potential to increase turbidity, increase scouring and abrasion of substrate and aquatic vegetation, impair primary production causing bottom-up food web effects, fill interstitial habitat that is vital to invertebrates and gravel-spawning fishes, coat gills and respiratory surfaces of aquatic animals, and reduce stream depth (Allan 2004). All of these factors have the potential to substantially alter aquatic macroinvertebrate community structure. Multiple studies have shown that a forest fragment can help to remove excess sediment from stream waters (Storey & Cowley 1997, Harding et al. 1998). The informal, post-hoc analysis that I performed did find a significant amount of sedimentation in upstream sites when compared to downstream sites ($p < 0.05$). The potential for Maryville College Woods to remove sediment from Browns Creek is a potential topic for future study as well as a possible factor influencing the differences in macroinvertebrate community structure that was observed during this study.

There are many possible complex effects that the Maryville College Woods could be having on Browns Creek and the macroinvertebrate assemblages that inhabit the stream. Because macroinvertebrate communities are long-term indicators of short-term disturbances, it is possible that the macroinvertebrate assemblages that were observed in this study were impacted by factors not accounted for in the scope of the study.

It is well established that riparian vegetation plays a vital role in the food webs of small-order streams (Vannote et al. 1980, Quinn et al. 1993, Wallace et al. 1997). In Bear Brook, more than 98% of the organic matter was supplied by the surrounding forest (Fisher and Likens 1973). It was found that forests along a prairie stream in Kansas contribute significantly greater quantities of organic matter than do the grasslands in upstream areas (Gurtz et al 1988). Since energy flows downstream in lotic ecosystems, it seems likely that the biotic communities of downstream sites of Browns Creek in the Maryville College Woods would have access to greater quantities of organic matter than the biotic communities of the upstream sites that are in very close proximity to manicured lawns and agricultural land that has been subject to the removal of native riparian vegetation and has been shown to contribute smaller quantities of organic matter than native riparian forests. Macroinvertebrate communities are strongly influenced by leaf litter inputs (Wallace et al. 1997) and it is likely that the leaf litter inputs of The Maryville College Woods are positively impacting the aquatic macroinvertebrate community structure. The available organic material that is made available to the aquatic communities by the input of leaf litter from this riparian forest fragment is a possible factor in the differences observed between macroinvertebrate assemblages at upstream and downstream sites.

Browns Creek enters the woods after flowing through areas affected by agricultural fields, manicured lawns, and roads. All of these land-use types have the potential to decrease infiltration and increase surface runoff. This surface runoff often flushes pollutants into the channel, accelerates channel erosion, alters the composition of the streambed, and ultimately changes the dynamics of the biotic community (Klein 1979).

Rosgen (1985, 1994) developed a stream and river classification system that outlined the premise that stable stream channels naturally have a morphology that provides appropriate distribution of flow energy during high water events. He identifies 8 variables that affect the stability of channel morphology: channel width, channel depth, flow velocity, discharge, channel slope, roughness of channel materials, and sediment load. He argues that when one of these characteristics in a stream is altered, the stream loses some of its capability to dissipate flow energy. This results in accelerated rates of channel erosion. A few of the components of the habitat that will function to dissipate flow energy are: sinuosity, bed and bank roughness, and stream bank and riparian zone vegetation. As Browns Creek flows through urban and agricultural landscapes, it is subjected to removal of stream bank and riparian zone vegetation. It also suffers from an increased sediment load and is listed as an impaired stream due to siltation/sedimentation. Sedimentation also decreases the roughness of channel materials due to the scouring of substrate by suspended sediment. It is then likely, that upstream from the Maryville College Woods, the channel morphology of Browns Creek, as it enters the MC woods, would lack the ability to properly dissipate flow energy of high water events. In highly modified landscapes, destruction of riparian vegetation and alterations of the floodplains

in the headwaters results in increased water levels downstream during flooding events and therefore increased damage to the stream channel at the first unaltered stream reach (Sparks et al., 1990). It is then possible that the area where Browns Creek enters the Maryville College Woods would be particularly vulnerable to the effects of the anthropogenic landscape changes upstream.

Maryville College Woods marks the first location where Browns Creek flows relatively unaltered, with the riparian forest still intact and would therefore be susceptible to the effects of increased flow energy during storm events that the upstream reaches of Browns Creek would be incapable of dissipating. These effects include, but are not limited to: stream bank erosion, increased sediment deposition, scouring of the substrate, removal of the streambed substrate, removal of in stream organic mater, increased drift of macroinvertebrates, elimination of taxa if high flow events occur during sensitive life stages (Richards et al. 1996, Richards et al. 1997, Quinn 2000, Allan 2004). It is also possible that the natural structural components of the Maryville College Woods would begin to help dissipate some of this flow energy as it continues downstream. It has been shown that the presence of natural vegetation in riparian zones serves to improve stream hydrology and reduce sedimentation in disturbed watersheds (Harding et al. 1998). The Maryville College Woods may help to slow high flows and trap some of the suspended sediment. Trapping suspended sediment would result in an increase in roughness of bed and bank materials, which would in turn also help to dissipate energy from high flow events. These differences between the upstream and downstream areas of Browns Creek could help to explain the differences that were observed in the macroinvertebrate communities. In fact, an informal follow-up analysis of the physical characteristics of

Browns Creek (Appendix 6 & 7) did find significant differences ($p < 0.05$) in bank stability and substrate embeddedness, which, if verified in future studies, could confirm the fact that the Maryville College Woods does help to improve the hydrology and sediment load of Browns Creek, which would in turn alter aquatic macroinvertebrate communities.

Although this study has inspired many intriguing questions best answered by future study of Browns Creek, this study has shown that the Maryville College Woods has the ability to positively impact aquatic macroinvertebrate communities, shifting from more tolerant taxa upstream to more sensitive taxa downstream. This study has also shown that the forest fragment in question can provide effective refuges for aquatic macroinvertebrates in Browns Creek through the alteration of certain habitat characteristics such as dissolved oxygen levels and channel depth. These refuges are important for the conservation of a diverse biological community. It is likely that the changes in habitat that this forest fragment provides are critical to the diverse taxa observed in this particular catchment of mixed land-use.

APPENDICES

Appendix 1: map of study site.

Appendix 2: Coordinates for kick-net samples of
Browns Creek performed on 3/22/14 and 4/1/14.

Site	GPS Coordinates	
Upstream 1	17S 0233019	3960095
Upstream 2	17S 0233026	3960096
Upstream 3	17S 0233015	3960097
Upstream 4	17S 0232858	3960436
Upstream 5	17S 0232850	3960428
Upstream 6	17S 0232867	3960406
Upstream 7	17S 0232868	3960409
Upstream 8	17S 0232876	3960400
Downstream 1	17S 0232857	3960439
Downstream 2	17S 0232863	3960429
Downstream 3	17S 0232899	3960409
Downstream 4	17S 0232998	3960125
Downstream 5	17S 0233002	3960115
Downstream 6	17S 0233002	3960107
Downstream 7	17S 0233002	3960092
Downstream 8	17S 0233026	3960078

Appendix 3: Artificial leaf-pack coordinates placed in Browns Creek from 2/28/14 -4/1/14.   These coordinates were also the site of dissolved oxygen sampling, measurements of width and depth, water samples, and physical assessment.

Site	GPS Coordinates	
Upstream 1&2	17S 0232867	3960406
Upstream 3&4	17S 0233016	3960101
Upstream 5&6	17S 0233005	3960115
Upstream 7&8	17S 0233009	3960127
Upstream 9&10	17S 0232999	3960131
Downstream 1&2	17S 0232872	3960388
Downstream 3&4	17S 0232868	3960399
Downstream 5&6	17S 0232866	3960411
Downstream 7&8	17S 0232856	3960425
Downstream 9&10	17S 0232846	3960427

Appendix 4: Table of macroinvertebrate families, functional feeding group, and tolerance value as defined by Hilsonhoff for the Family-level Biotic Index, 1988.

Benthic Macroinvertebrate	Functional Feeding Group	Tolerance Value
Coleoptera Elmidae	Scrapers	5 (Moderate)
Coleoptera Psephenidae	Scrapers	4 (Moderate)
Diptera Chironomiidae	Collectors/Gatherers	6 (Moderate)
Diptera Empididae	Predators	6 (Moderate)
Diptera Ephydridae	Collectors/Gatherers	6 (Moderate)
Diptera Simulidae	Collectors/Filterers	6 (Moderate)
Diptera Tipulidae	Shredders	3 (Low)
Ephemeroptera Baetidae	Collectors/Gatherers, Scrapers	4 (Moderate)
Ephemeroptera Ephemerellidae	Collectors/Gatheres	1 (Low)
Ephemeroptera Heptageniidae	Scrapers	4 (Moderate)
Ephemeropterea Isonychiidae	Collectors/Filterers	2 (Low)
Lepidoptera Pyralidae	Shredders	5 (Moderate)
Odonata Aeshnidae	Predators	3 (Low)
Odonata Calopterygidae	Predators	5 (Moderate)
Odonata Coenagrionidae	Predators	9 (High)
Odonata Gomphidae	Predators	1 (Low)
Plecoptera Taeniopterygidae	Shredders (some Scrapers)	2 (Low)
Trichoptera Hydropsychidae	Collectors/Filterers	4 (Moderate)
Trichoptera Leptoceridae	Collectors/Gatherers, Shredders	4 (Moderate)
Trichoptera Limnephilidae	Shredders	4 (Moderate)
Trichoptera Philopotamidae	Collectors/Filterers	3 (Low)
Trichoptera Rhyacophilidae	Predators	0 (Low)
Trichoptera Uenoidae	Scrapers	3 (Low)

Appendix 5: Channel Condition. (Little River Watershed Association 2002)

Value	Description
4	Natural channel; no structures. No evidence of erosion
3	Evidence of past channel alteration but with significant recovery of channel and banks. Any dikes or levies set back to provide access to an adequate flood plain.
2	Altered channel; <50% of the reach with riprap and/or channelization. Braided channel. Excess sediment accumulation in the channel. Dikes or levies restrict flood plain width
1	Channel is actively eroding. >50% of reach with riprap or channelization. Dikes or levies prevent access to flood plain.

Appendix 6: Bank Stability. (Little River Watershed Association 2002)

Value	Description
4	Banks are stable and low; 33% or more of bank area in outside bends is protected by roots.
3	Banks are moderately stable and low; less than 33% of bank area in outside bends is protected by roots
2	Banks are moderately unstable and typically high (but may be low); outside bends are actively eroding, and there are signs of slope failures like fallen streamside trees or chunks of banks that have collapsed
1	Banks are unstable and typically high; some straight reaches and inside edges of bends are actively eroding as well as outside bends; numerous signs of slope failures.

Appendix 7: Riffle Embededness. (Little River Watershed Association 2002)

Value	Description
4	Gravel or cobble particles are <20% embedded
3	Gravel or cobble particles are 20-30% embedded
2	Gravel or cobble particles are 30-40% embedded
1	Gravel or cobble particles are >40% embedded

Appendix 8: Pools. (Little River Watershed Association 2002)

Value	Description
4	Deep and shallow pools abundant; pools >5 ft deep
3	Pools present but not abundant; pools >3 ft deep
2	Pools present but not shallow; pools <3 ft deep.
1	Pools are absent.

Appendix 9: Insect/Invertebrate Habitat. (Little River Watershed Association 2002)

Value	Description
4	>5 types of habitat available; woody debris and logs not freshly fallen
3	3-4 types of habitat; some potential habitat such as overhanging trees may provide habitat haven't yet entered stream.
2	1-2 types of habitat; substrate is disturbed, covered or removed by high stream velocities and scour, or by sediment deposition.
1	0-1 types of habitat available.

Cover types: Fine woody debris, submerged logs, leaf packs, undercut banks, cobbles, boulders, coarse gravel.

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