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Comparing Macroinvertebrate Total Abundance and Total Biomass on Five Substrate Types from Upstream to Downstream on the North Branch of the Au Sable River

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Comparing Macroinvertebrate Total Abundance and Total Biomass on Five Substrate Types from Upstream to Downstream on the North Branch of the Au Sable River

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Abstract

The North Branch of the Au Sable River is located in the northern lower peninsula of Michigan and is known for prolific hatches of Ephemeroptera (mayflies), Trichoptera (caddisflies), and Plecoptera (stoneflies). Macroinvertebrates play an important role in processing and recycling organic material in rivers and are a valuable food source for trout. In 2018, anglers were reporting catching fewer numbers of brook and brown trout (*Salmo trutta* and *Salvelinus fontinalis*). The Michigan Department of Natural Resources (MDNR) determined significantly lower abundance than the historical average. We hypothesized that trout abundances were lower due to a lack of prey availability. Quantitative sampling techniques were used to assess each available substrate and respective aquatic macroinvertebrates throughout the river. Our estimates indicate that coarse woody debris substrates supported the highest abundances with the most individuals/m^2. Originally, we hypothesized gravel substrates to have the highest abundances from past literature. We determined community composition and total abundance of macroinvertebrates. We determined that there was a shift in the community composition from mostly Ephemeroptera, to a 1:1 ratio of Ephemeroptera and Trichoptera. Chironomidae was the most abundant taxa of macroinvertebrates on every substrate. Our results suggest that the current macroinvertebrate composition and population would support the current trout population.

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Abbreviations

ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
CPOM	Course Particulate Organic Matter
CWD	Coarse Woody Debris
DO	Dissolved Oxygen
EGLE	Environment, Great Lakes and Energy
EPT	Ephemeroptera, Plecoptera, Trichoptera
FPOM	Fine Particulate Organic Matter
MDNR	Michigan Department of Natural Resources
MNFI	Michigan Natural Features Inventory
NMDS	Non-metric Multidimensional Scaling

Chapter 1

Introduction

Many factors influence the structure of a river such as geology, hydrogeology, flow, gradient, watershed features, etc. (Newson & Newson, 2000; Vannote et al., 1980), and most rivers are unique with unique combinations of these factors. Ultimately, the combination of factors that shape a river's characteristics will influence the flora and fauna of that river. In turn, the flora and fauna play functional roles influencing the ecology of streams.

Natural flow regimes are essential to ecological integrity (Poff et al., 1997). Altered stream flows can decrease species richness and abundances (Bunn & Arthington, 2002; Poff & Allan, 1995; Zorn et al., 1997). Anthropogenic activity such as dams, culverts, and destruction of riparian zones all lead to habitat and ecosystem degradation (Poff et al., 1997). Predictable flow regimes form and determine which species and habitats are present.

Macroinvertebrates are a key group of organisms that play a critical role in the ecology of a river. For example, they are central to the process of energy transfer between trophic levels and aid in nutrient cycling (Cummins & Klug, 1979). Because of their importance and known ecological requirements, macroinvertebrates are used in many cases for determining water quality. Percentages of sensitive taxa are compared typically throughout the available substrates and a few other key factors. Macroinvertebrates are sensitive to chemical pollutants, sediment, and flow (Lenat, 1988; Gaufin, 1973). Environment, Great Lakes, and Energy uses a protocol called P-51, which looks at percent abundance of EPT and is then used to determine water quality (MDEQ, 1990). USEPA also has a similar quantitative sampling method which is used again to determine the overall quality of the river (Barbour, 1999). EPT are intolerant macroinvertebrates that when present give an indication of water quality.

Macroinvertebrates can be assigned to functional feeding groups that feed on an array of foods. There are four functional feeding groups that are comprised of predators, grazers, shredders, and collectors (Cummins & Klug, 1979). These macroinvertebrate groups feed on the CPOM and cycle nutrients within the river (Cummins & Klug, 1979). Vannote et al. (1980) hypothesized that rivers are a continuum that can cycle nutrients from upstream to the downstream due to the actions of macroinvertebrate functional feeding groups. The smaller order streams are generally more shaded with high inputs of coarse particulate organic matter (CPOM). In this section of the river shredders and collectors are gathering and breaking down CPOM to fine particulate organic matter (FPOM). Further down larger order streams have more sunlight and an increase in algal growth. Because of inefficiencies in processing material, filtering collectors will process the FPOM from upstream. Grazers will feed on the algae attached to rocks moving downstream as food becomes scarce. Lastly, the largest order of rivers are generally comprised of filtering collectors processing FPOM. Predators can be found in every area of the river feeding on these benthic macroinvertebrates functional feeding groups (Vannote et al., 1980). Macroinvertebrates need stable substrates such as gravel beds and well oxygenated riffles (Brown & Brown, 1984; Duan et al., 2009). Drifting macroinvertebrates are a typical food source for trout throughout the year. As food becomes scarce macroinvertebrates will drift in the water column making them vulnerable to trout (Waters, 1972). Hatching macroinvertebrates are an important food source for trout during spring, summer, and fall. Trout will feed on the surface for these emergent macroinvertebrates, and will subsidize their diet with terrestrial macroinvertebrates (Erős et al., 2012; Flecker, 1992; Wilson et al., 2014).

The modern Great Lakes were formed by the Wisconsin glaciers that began melting and retreating approximately 13,000 years ago (Zorn & Sendek, 2001; Krist, & Lusch, 2004). These glacial retreats would form the Great Lakes and the rivers that would flow into them. The glaciers melted and carved their way into the landscapes, leaving behind outwash sediments of gravel and sand (Zorn & Sendek, 2001). These sediments are the groundwork for Michigan rivers, how they were formed and what they are today.

In Northern lower Michigan, the glaciers deposited a deep layer of sand and gravel, forming a large dome. The highest elevations are near Otsego and Crawford Counties, which then slopes downward toward the Great Lakes. Many of Michigan's best trout streams are associated with the glacial dome, due to their relatively stable flows and temperature regimes, which, support diverse and complex communities.

The North Branch of the Au Sable River located in the Northern portion of the lower peninsula is a stream that was formed by the outflow from Otsego Lake and connects to the main stem of the Au Sable River. The upper reaches of the North Branch are supplied by warmwater outflow from Otsego Lake. Whereas the lower portions (Ford Road to confluence) are fed by groundwater and are considered "cold water" because of the groundwater discharge. The Au Sable River and its tributaries were used heavily during the mid to late 1800's for logging (Zorn & Sendek, 2001). Large woody structures were removed to aid in the transportation of large logs (Zorn & Sendek, 2001). The construction of dams was used heavily on these high gradient tributaries such as the North Branch of the Au Sable. They utilized dams for logging, and hydropower at the turn of the century (Zorn & Sendek, 2001). Anthropogenic activity described above lead to the extirpation of the native arctic grayling (*Thymallus acrticus*) in the early 1900's (Zorn & Sendek, 2001). Grayling were overfished, lost most of their habitat, and were outcompeted by other introduced fish species. Brook (*Salvelinus fontinalis*) and brown trout

(*Salmo trutta*) were introduced in the early 1900's and the final reason why grayling was extirpated in this region (Zorn & Sendek, 2001).

The North Branch for example is a trout stream that provides habitat for brook (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) to forage and grow. Brook and brown trout have similar feeding behaviors, foraging for macroinvertebrates during peak drift times or during nightly hatches in the spring and summer (Bishop, 1969; Elliott, 1970; Flecker, 1992).

On the North Branch the abundance of trout is declining based on data most recently collected in 2018 for unknown reasons. Research conducted by the Michigan department of EGLE have determined that this is not due to chemical pollution, extreme weather events, or predation (Godby, 2019). Rapid bioassessment (P-51) at multiple sites determined that the water quality was "excellent". There was no indication that there were shifts in macroinvertebrate community composition to suggest that water quality was declining. This led to the hypothesis that macroinvertebrate abundance and biomass had declined, causing a decline in fish abundance.

Purpose

The purpose of this study was to quantify the abundance of macroinvertebrates on the dominate substrates found in the North Branch across five segments of the river. After quantifying the total abundance of macroinvertebrates, we used head capsule-weight relationships to estimate biomass of macroinvertebrates on each substrate throughout these segments (Benke et al., 1999; Smock, 1980). With this information we hope to determine if the available macroinvertebrate biomass is a potential limiting factor for trout populations.

Scope

This study will focus on the North Branch of the Au Sable a third order stream located in the northern portion of the lower peninsula of Michigan. The North Branch is a coldwater river that has supported strong populations of brook (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*), but current trout populations have significantly lower abundances than previous years. This study considered the hypothesis that the trout may be limited by the total numbers of macroinvertebrates. Although this study focused on the North Branch Au Sable River, these data may have broad application to other rivers that originate in the same geological region

Assumptions

When we estimated macroinvertebrate abundance and biomass for the five sample sites, we are assuming that this is representative of macroinvertebrate populations across the entire river. This is important to understand when looking at the overall estimates of biomass. We also assumed that our estimate of abundance and biomass are an accurate reflection of current macroinvertebrate communities. We also assume that we sampled in the same manner over the five sample sites and five dominant substrate types which is central to the previous assumption. We sampled within a two-week period to reduce the impact of time. In doing this we are assuming that we did not lose species or biomass due to macroinvertebrate emigration.

Hypothesis

We hypothesized that the total biomass of macroinvertebrates was limiting trout populations. Previous literature shows that macroinvertebrates are an important resource needed to help sustain trout in these rivers. Although trout can change their feeding behavior for drifting or emergent macroinvertebrates (Waters, 1972; Flecker, 1992), it may not be sufficient to compensate for lower invertebrate numbers. If macroinvertebrates are limiting in this river than we should observe a similar relationship with trout.

Because there is a diversity of available substrates in the North Branch Au Sable River, we determined which substrates supported the greatest abundance of macroinvertebrates at the five sample sites. Previous literature suggests riffles with coarse sediments such as gravel have the highest abundance and diversity (Duan et al., 2009; Brown & Brown, 1984). We also hypothesized that gravel substrates in the North Branch would have the greatest abundance and diversity of macroinvertebrates amongst the other substrates.

Significance

Trout populations on the North Branch of the Au Sable have declined significantly in recent years. Concern over this trend has led to speculation about potential causes. My study is significant because: 1) It will provide baseline information for the future management of this river. 2) We quantify the abundance of macroinvertebrates on multiple substrates across five sample sites of the North Branch Au Sable River, and 3) It provides estimates of the forage base available to support trout populations.

Definitions

For the estimations of biomass that are referred to in Chapter II of this thesis we calculated using the length width relationships of head capsules. Benke et al. (1999) summarizes past studies that have investigated the relationship of body length and head capsule length. There is a linear and exponential relationship between the two estimates. We used the estimates of head capsule width to determine the overall weight. We took an average of the head capsule width and then plugged them into a predictive equation:

$W = a\,L^b$

W is the estimated weight that is calculated by taking the constants of a and b which and multiplying them by the head capsule width. The constants a and b are found by using the estimated weight and the dry weight of the macroinvertebrate. This was done by the genus level of identification, and each constant is different for the different macroinvertebrates present.

We also used a method used originally by Cooper and Testa (2001) to estimate the surface area of a piece of CWD. The equation is a trinomial predictive equation, that takes the displacement of water and calculates the surface area of the irregular shape. The equation is:

$$y = 19.495x^3 - 194.82x^2 + 867.57x$$

For each of our samples in CWD we were able to plug the displacement of water into the x in this equation which would then give us the surface area in cm^2 which we then converted to m^2 to be consistent with the rest of the study.

Shannon's Diversity index was used to calculate the diversity of the substrates from upstream to downstream in the North Branch of the Au Sable River. The formula we used was described by Guerold (2000). The formula is:

$$H = - \Sigma [(p^i) * log_{10}(p^i)]$$

Σ is the summation of $p^i *log_{10}(p^i)$.

Where p^i is the individual taxa divided by the total taxa for that sample.

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Chapter 2

Comparing Macroinvertebrate Total Abundance and Total Biomass on Five Substrate Types from Upstream to Downstream on the North Branch of the Au Sable River

Abstract

The North Branch of the Au Sable River is located in the northern lower peninsula of Michigan known for its prolific hatches of Ephemeroptera (mayflies), Trichoptera (caddisflies), and Plecoptera (stoneflies). Macroinvertebrates play an important role in processing and recycling organic material in rivers. Lastly, macroinvertebrates make up a large portion of trout diets and are a valuable food resource. We hypothesized that macroinvertebrate densities would vary among substrates and along an upstream to downstream gradient. Quantitative sampling techniques were used to assess invertebrate communities on each available substrate from upstream to the downstream. Our estimates indicate that coarse woody debris (CWD) supported the highest abundances with the most individuals/m^2. Gravel, soft sediment, and vegetation had similar invertebrate abundances, which were somewhat higher than sand. We determined course woody debris to be a crucial substrate in the North Branch for macroinvertebrate production. We observed a shift in community composition from mostly Ephemeroptera, to a 1:1 ratio of Ephemeroptera and Trichoptera. Chironomidae was the most abundant group of macroinvertebrates on every substrate. Our results suggest that a diverse array of substrate types is critical to maintaining macroinvertebrate diversity and that increasing CWD would enhance macroinvertebrate abundance.

Introduction

Streams are important landscape features that are the product of local geological and hydrological conditions. As streams flow from upstream to downstream they create a sequence of ecologically diverse conditions (Cummins, 1974). These conditions provide microhabitats such as riffles, runs, and pools within stream segments (Robson & Chester, 1999). Stream segments vary greatly in water temperature from upstream to downstream. Forested headwater streams are typically narrow, shallow, and spring fed (MacDonald & Coe, 2007). These conditions make for a cool water habitat rich in allochthonous material. Further downstream segments are wider, deeper, generally with less shading. Less shading and more surface area of water typically makes for warmer conditions (MacDonald & Coe, 2007). Factors such as water temperature, flow regime, and food availability ultimately determine species composition. These factors support vital and complex biological communities that play crucial roles within streams. For example, aquatic macroinvertebrates are essential to the process of breaking down organic material (allochthonous or autochthonous) and cycling nutrients from upstream to downstream (Cummins & Klug, 1979). Often upstream communities influence downstream communities due to the inefficiencies of processing organic material (Vannote et al., 1980). These inefficiencies provide diverse communities of macroinvertebrates throughout the stream.

Aquatic macroinvertebrates are an important element in the structure and function of aquatic ecosystems (Vannote et al., 1980). Macroinvertebrate distribution is influenced by flow regime, substrate size, temperature, and food availability (Allan, 2004; Brasil et al., 2020). The downstream movement of macroinvertebrates is a well understood process (Palmer et al., 1996) Upstream migration is however, only facilitated by the upstream aerial movement of adult macroinvertebrates (Griffith et al., 1998). Adult macroinvertebrates will travel upstream to lay their eggs. These eggs hatch into larvae and as they mature, they move downstream processing organic material (Griffith et al., 1998). Substrate size is crucial in determining where and how macroinvertebrates colonize streams. Macroinvertebrates such as Ephemeroptera, Plecoptera, and Trichoptera are adapted to stable substrates (gravel and cobble) and predictable flow regimes (Biggs et al., 2005; Buendia et al., 2014; Brown & Brown, 1984; Duan et al., 2009). Abundant food on gravel and oxygen rich flows have led to adaptations such as external gills and ventrally compressed bodies (Flowers & Hilsenhoff, 1975). Other macroinvertebrates such as Chironomidae and Oligochaeta can be found in less stable substrates like sand or silt. They survive due to their resilience to pollutants and low oxygen levels. Macroinvertebrates are generally found across a wide variety of substrates and are not limited to the previously mentioned substrates (Silva et al., 2014).

Macroinvertebrate distribution from upstream to downstream and across substrates is influenced by several factors. Vannote et al. (1980) hypothesized that fluvial geomorphology, should influence species composition based on functional feeding groups. The physical shape of the river and its hydrologic cycle determine how functional feeding groups process organic material from an upstream to downstream manner. Based on these factors' macroinvertebrates capitalize on the inefficiencies from upstream to downstream. Vannote et al. (1980) and Cummins and Klug (1979) characterized these functional feeding groups as shredders, grazers, predators, and collectors/gatherers. Each group has a specific function in processing organic material along the stream gradient. In forested headwater streams, inputs of organic material colonized by microbial communities are utilized by shredders that reduce coarse particulate organic matter (CPOM) to smaller size fractions (Baldy et al., 1995; Cummins et al., 1989). Grazers feed on attached algae by scraping the algal material from rocks and other surfaces.

Further downstream, the collectors/gatherers and filter feeders consume fine particulate organic matter (FPOM) that results from shredders reducing CPOM. Lastly, predatory macroinvertebrates feed on other macroinvertebrates or vertebrates including fish and amphibians. Along the gradient of the stream organic matter is processed as it moves downstream feeding each of the functional feeding groups.

Macroinvertebrates feed on organic material breaking it down from CPOM to FPOM as it travels further downstream (Vannote et al. 1980). Macroinvertebrates travel downstream behaviorally (due to scarce resources) or because of a disturbance event (catastrophic drift) that has dislodged them from the substrate (Gibbins et al., 2007). Behavioral drift is done under the cover of darkness as to avoid predation from fish, such as trout (Flecker, 1992). Synchronous emergence, unlike drift, can happen at various times of day (Baxter et al., 2005). Aquatic macroinvertebrates are most vulnerable during these drifting and emergence events. Emerging macroinvertebrates are not only energy for trout but also other predators, including bats, birds, amphibians, reptiles, and spiders (Baxter et al., 2005; Gray, 1993). Macroinvertebrates emerge at certain times of the year, generally in the spring and summer. During winter months macroinvertebrates will not hatch. In early spring and summer, trout feed selectively on these synchronous emergence events. They also incorporate varying amounts of terrestrial insects in their diets (Erős et al., 2012; Wilson et al., 2014). Warmer water temperatures increase trout metabolism requiring more energy to sustain them (Elliott, 1975a). Elliott (1975) determined that reduced rations of macroinvertebrates would cause *S. trutta* growth rates to decrease leading to skinnier fish. Therefore, macroinvertebrates are an important input of energy for trout, without which they would not thrive.

The North Branch of the Au Sable River (henceforth referred to as the North Branch) has been considered one of the most iconic trout streams in Michigan. Historic hatches of macroinvertebrates were said to cover the entire river which, brought anglers from near and far. Recent declines in trout population have led to a series of questions about possible causes. Reduced amounts of macroinvertebrates have been shown to reduce trout growth and abundance (Elliott, 1975b). Reports of diminished macroinvertebrate hatches and loss of biodiversity could be one factor influencing the decline of trout populations. Our study focused on five segments of the North Branch. From upstream to downstream we sampled five sites and five substrates (triplicates at each substrate) to determine macroinvertebrate abundance and composition. The purpose of this study was to gain a more complete understanding of aquatic macroinvertebrate community composition, total biomass, and abundance in the North Branch.

Methodology

We conducted this study on the North Branch of the Au Sable River a second order stream in Michigan's northern lower peninsula (Fig. 2.1). The region is dominated by glacial moraines and outwash sediments (Zorn and Sendek, 2001) which accounts for the relatively stable flow regime (Zorn and Sendek, 2001). Summer temperatures do not normally exceed 21°C, however, warmer temperatures have been observed (USGS 04135800). Dissolved oxygen content is approximately 7 mg/L. The river is generally comprised of run and rifle sequences (Zorn and Sendek, 2001). Stream banks and pools are mostly composed of sand, while riffles and runs are generally made up of gravel and cobble. The gradient of the North Branch generally ranges from 0.95-3.03 m/km (5.01 – 16.0 ft/mile) with an average of 1.34 m/km (7.1 ft/mile) (Zorn and Sendek 2001). There are four dams located above Ford Road (Fig. 1.1), historically there were dams in the downstream segment, but they have been removed in recent years.

We selected five sample sites on the North Branch. From upstream to downstream the North Branch has relatively consistent gradient and substrate types, so study sections were selected partly based on access and partly on the availability of historic data. Invertebrate sampling sites were located at upstream boundary of each segment. The most upstream sample site was at Ford Road (Fig. 1.1; Table 2.1), which is approximately 24 miles upstream from the confluence, is considered to be the upstream limit of cool/cold water and was identified as Top-Quality Coldwater by Zorn and Sendek (Zorn and Sendek, 2001). The length of the Ford Road section is approximately 6.5 miles (Table 2.1). The second study segment was located 6.5 miles downstream at Twin Bridge Road (Fig. 1.1; Table 2.1). This section is relatively wide and shallow with some deeper pools and well oxygenated riffles. The Twin Bridge segment extended about 1.5 miles downstream and has a gradient of approximately 0.88 m/km (4.67 feet/mile) (Table 2.2). The Copper Fisherman site (Fig. 1.1; Table 2.1) was approximately 0.53 mile upstream of Lovell's Bridge the nearest road crossing. This site is shallow and wide (Table 2.1) with sand and gravel beds. The entire segment was approximately 5.8 miles long. The next downstream site, Dam 4 (Fig. 1.1; Table 2.1), is shallow with some deeper holes and with well-defined gravel beds. Stream width varies at the Dam 4 site between 85 feet and 120 feet, and the entire reach length was approximately 6.2 mile (Table 2.1). The site farthest downstream was located at Kellogg's Bridge (Fig. 1.1; Table 2.1). This site is much deeper and wider than the other sites, with mostly sand. From Kellogg's Bridge to the confluence with the Main Branch Au Sable River is approximately 6 miles.

Quantitative Sample Collection

We quantitatively sampled the dominate substrates, including coarse substrates (rocks), fine sediment (sand), soft sediment or silt, coarse woody debris (CWD), and macrophyte beds at each of our sample locations. Triplicates samples were collected from each substrate. The samples were preserved in 70% ethanol in the field. Macroinvertebrates were removed from each sample and identified in the lab using Merritt, Cummins, & Berg (2019). A Nikon model C-FMBN dissecting scope was used for sorting and identification to the lowest taxonomic rank practical. Macroinvertebrates were generally identified to the genus level. With the exemption of Chironomidae, Oligochaeta, Bivalvia, Gastropoda, Asellidae, and Gammaridae were brought to Family or Order. Shannon's diversity index was calculated for each substrate at each site. Replicates of each substrate were averaged, and all substrates were averaged (replicates included) for a mean index of quality at each site.

Sand and Gravel

We used a Hess sampler to collect quantitative samples from sand and gravel. Working from downstream to upstream we selected suitable patches of gravel or sand. The sampler was placed in the substrate and the sediment was disturbed using approximately equal effort at each sample. Macroinvertebrates were removed from the sampler and preserved in 70% ethanol for later sorting and identification in the lab.

Coarse Woody Debris (CWD)

Quantifying CWD surface area is difficult due to its irregularities in shape and size. We carefully selected three naturally occurring structures at each site and removed small limbs from each. The Diameter of cut limbs ranged from 0.5 to 2 centimeters. Caution was used to prevent macroinvertebrates from falling. Macroinvertebrates were removed from each piece of CWD in the field and preserved in 70% ethanol. Surface area of each piece of CWD was calculated using volume displacement and trinomial conversion equation described in Cooper and Testa (2001). The volume of each piece of CWD was estimated using the displacement of water. A plastic beaker filled with a known volume of water was used to measure the displacement of the cut piece of CWD. Volume was measured in the field to reduce the loss of organic material from decay (Harmon et al., 1986).

Macrophytes

Macrophyte beds were sampled using a modified protocol described originally by Alonso and Camargo (2010). The Hess sampler was placed in the substrate over macrophytes with a D-net downstream of the plant. The entire macrophyte was plucked from the substrate and placed in 70% ethanol with attached macroinvertebrates. Macroinvertebrates were hand picked off preserved macrophytes and identified later in the lab. Surface area of macrophytes was calculated by drawing polygons utilizing the program NIS Elements D 3.2 64 Bit with a dissecting scope (Nikon SMZ1500) and attached camera (DS-Fi1).

Soft Sediment

An Ekman Dredge with a pole attachment was used to sample shallow soft sediment for macroinvertebrates (Hudson, 1970). Sediments were sampled to a depth of approximately 7 centimeters. Sediment samples were sieved (354 µm) in the field and the remaining sample was preserved in 70% ethanol. Macroinvertebrates were sorted from the sediment and identified in the lab.

Biomass Estimations

Biomass of macroinvertebrate taxa was estimated using head capsule size to biomass relationships (Smock, 1980; Benke et al., 1999). We calculated dry weight associated with each substrate at each site using the formula:

Biomass/m^2 of substrate = total abundance of a taxon/m^2 of substrate X biomass/ind.

The total biomass for each river segment was calculated by summing the estimated biomass of invertebrates associated with each substrate which was calculated as:

Total Biomass = Biomass of all taxa/m^2 of a substrate X total area of that substrate

Qualitative Assessment

In addition, we calculated a qualitative index of water quality using our macroinvertebrate community data. We used the macroinvertebrate metrics used by the Michigan Department of Environment, Great Lakes, and Energy (EGLE). The protocol (P-51) is used to assess the quality of a stream using 9 macroinvertebrate metrics based on using family level identification. We randomly selected one replicate from each substrate sample set for each sample site. Generally, P-51 is performed in the field focusing on all available habitats for macroinvertebrates with an assessment of physical habitat quality. We calculated the nine metrics on our quantitative data but did not perform a habitat assessment. We then compared our water quality assessment to previous assessments on the North Branch.

Statistical Analysis

Non-metric Multidimensional Scaling (NMDS) was used to determine similarities and dissimilarities among substrates within sites based on total abundances of macroinvertebrates (Rstudio, packages: Vegan, MASS, and dplyr). An NMDS was also used to determine similarities and dissimilarities of sites across substrates. With significant results an Analysis of Similarity (ANOSIM) was used to determine the similarities of substrates among sites. Similarities of percentages (SIMPER) post hoc test determined which taxa were dominant on which substrate. Differences in total abundance of macroinvertebrates were tested among substrates and sites using a two-way ANOVA (Rstudio, packages: tidyverse, broom, AICcmodavg, car, and lsr). We used a two-way ANOVA to examine, percent composition of dominant taxa, and total abundance of dominant taxa among sample sites. Taxa were deemed dominate if >5% on all substrates. Using these criteria, we found that only Chironomidae met these assumptions. Another two-way ANOVA tested the next most abundant taxa on the substrates gravel and CWD. The taxa *Drunella* and *Ephemerella* were the next most abundant taxa on these two substrates. We selected and combined these genera because they were >5% on at least two substrates (gravel and CWD). We ran two-way ANOVAs on total abundance of Trichoptera and Diptera, relative abundance of Diptera, and total biomass of Trichoptera. With significant results from a parametric test (for two-way) a pairwise *t* test with Holm correction was run to assess differences and similarities between sites and substrates. Prior to statistical analysis, data were tested for normality (Shapiro-wilk test) and equal variance (Levene's test). Total abundance data were log10 transformed prior to statistical analysis. A non-parametric Kruskal-Wallis test was run on total abundance of Ephemeroptera, relative abundance of Ephemeroptera and Trichoptera, and total biomass of Ephemeroptera and Diptera. Another Kruskal-Wallis was used to determine differences of total biomass across sites and substrates. If significant results were determined a pairwise Wilcoxon test was run. All statistical tests were run using version 1.2.5033 of Rstudio (Rstudio Team, 2019).

Results

Total Macroinvertebrate Abundances

We summed estimates of macroinvertebrates/m^2 for all substrates together to calculate total abundance for each site giving us an estimate of total macroinvertebrate/5 m^2. Total abundance of macroinvertebrates combined across all substrates generally increased from upstream to downstream with lowest densities at Ford Road and highest at Dam 4, although abundance decreased at Kellogg's Bridge (Fig. 2.2). Total combined abundances at sites varied from 47,869/5 m^2 (9,573/m^2) at Ford Road to 159,366/5 m^2 (31,873/m^2) at Dam 4 (Fig. 2.2; Table 2.2) with abundances among sites not significantly different (Table 2.3). In addition, total macroinvertebrate abundance varied significantly among the five substrate types (Fig. 2.2; Fig. 2.3; Table 2.2; Table 2.3). Total macroinvertebrate abundance was significantly higher on CWD (Table 2.3), and the highest abundance on CWD was observed at Dam 4 reaching 113,207/m^2 (Fig. 2.3; Table 2.2). In contrast, sand supported the lowest densities of macroinvertebrates (Fig. 2.2; Fig. 2.3; Table 2.2; Table 2.3). Abundances on vegetation, soft sediment, and vegetation were also not significantly different (Fig. 2.3). The interaction term between site and substrate was not significant (Table 2.3).

Ephemeroptera, Trichoptera, and Diptera had the highest total abundances across all sites. Diptera was the most abundant group, and generally increased from upstream to downstream, however numbers decreased at Kellogg's Bridge (Fig. 2.4). Diptera total abundance was significantly different across sites (Table 2.3), reaching peak density at Dam 4, however, a multiple comparisons test could not distinguish among sites. Diptera total abundances also were significantly different across substrates (Table 2.3). CWD, soft sediment, and vegetation supported the highest densities of Diptera, whereas gravel and sand supported lower densities, however a multiple comparisons test of Diptera total abundance could not determine clear differences among substrates (Fig. 2.5). In addition, the interaction term was also significant (Table 2.3) Similarly, the most abundant family of macroinvertebrates found at all sites and on each substrate was Chironomidae. Chironomidae total abundance was significantly different across the sample sites following the same pattern as Diptera from upstream to downstream (Table 2.3). The multiple comparisons test could not determine differences among sites. Chironomidae total abundance (Fig. 2.6) was significantly different across substrates as well

(Table 2.3). Generally, densities were highest on CWD and soft sediment with lower densities on gravel, sand, and vegetation (Fig. 2.6).

Trichoptera abundance across sites was also relatively similarly and were not significantly different (Table 2.3). The densities of Trichoptera across substrates were significantly different with highest densities occurring on CWD (Fig. 2.7; Table 2.3). Trichoptera abundances were similar on gravel and vegetation, which were higher than on sand and soft sediment (Fig. 2.7).

Ephemeroptera abundances were relatively similar among sites (Fig. 2.4) and were not significantly different (Table 2.4). However, densities did vary significantly among substrates (Table 2.4). Densities were significantly higher on CWD, with second highest densities on gravel. Densities were relatively low on sand, soft sediment, and vegetation (Fig. 2.8).

Macroinvertebrate Biomass

As with total abundance, we summed the biomass estimates (kg/m^2) for the five substrates to estimate total biomass at each site (macroinvertebrate biomass kg/5 m^2). Estimates of macroinvertebrate biomass (kg/5 m^2) indicate that sites supported varying amounts of invertebrate biomass (Fig. 2.9). Biomass was lowest at Ford Road at 0.032 kg/5 m^2, (0.0064 kg/m^2) increased slightly to 0.053 kg/5 m^2 (0.0106 kg/m^2) in the middle reach (Copper Fisherman), then decreased to Kellogg's Bridge (Fig. 2.9). Differences in biomass were not significantly different among sites (Table 2.5). Biomass on individual substrates did vary significantly (Fig. 2.9; Table 2.5). In general, invertebrate biomass was highest on CWD from upstream to downstream sites (Fig 2.9; Fig 2.10; Table 2.5). In contrast, invertebrate biomass on macrophytes and gravel increased from Ford Road to Copper Fisherman then declined downstream (Fig. 2.9).

Comparing the biomass of individual taxonomic groups among sites and substrates identified several significant differences. For example, Diptera were numerically dominant at most sites, they contributed relatively less to total biomass than the other primary groups (Fig. 2.10: Fig. 2.11; Table 2.5). Ephemeroptera had higher biomass on CWD and gravel when compared to the other three substrates (Fig. 2.12; Table 2.5). In addition, Trichoptera biomass was not significantly different among sites (Table 2.5), but it did account for significantly more biomass on CWD and vegetation than on other substrate types (Fig. 2.10; Fig. 2.13; Table 2.6). There was also a significant interaction between site and substrate.

Total biomass estimates for each study reach (Table.1) determined that the reach from Copper Fisherman to Dam 4, had the highest biomass for the study area (Fig 2.1) with approximately 4000 kg of macroinvertebrate biomass. Ford Road and Dam 4 had the lowest biomass estimates with approximately 1200 kg. Kellogg's Bridge had slightly higher biomass at approximately 1900 kg. When we account for the variation in length of segment, we found that biomass dynamics changed. We estimated that biomass within the Copper Fisherman section was approximately 451.6 kg/km whereas the Twin Bridge section had 583.3 kg/km. The biomass estimate for Ford Road was 111.1 kg/km and Dam 4 was approximately 127.6 kg/km. And, at Kellogg's Bridge, the biomass estimate was 195.9 kg/km.

Relative Abundance of Major Taxonomic Groups

Ephemeroptera, Trichoptera, and Diptera accounted for the greatest proportion of organisms in our samples. In general, Ephemeroptera and Trichoptera made up between 40-50% of abundance at each site except at Dam 4, where Diptera were dominate (Fig. 2.15). Ephemeroptera and Trichoptera individually accounted for approximately 15-25% of relative abundance at each site. From upstream to downstream, Diptera became relatively more abundant and Ephemeroptera and Trichoptera became relatively less abundant (Fig. 2.15). However, relative abundances of the major groups were fairly similar at Kellogg's Bridge.

Although the relative abundance of Ephemeroptera varied across sites differences were not significant (Table 2.7; Appendix B) whereas the relative abundance of Trichoptera was significantly different across sites (Table 2.7; Appendix B). In contrast, relative abundances of mayflies were significantly different among substrates (Table 2.7; Appendix B) with CWD and gravel supporting higher relative abundances (Fig. 2.16). Relative abundance of Trichoptera was significantly different across substrates (Table 2.7; Appendix B) although all substrates had similar relative abundances except soft sediment (Fig. 2.17).

The relative abundance of Diptera was generally similar at the three upstream sites, increased at Dam 4, then decreased at Kellogg's Bridges, however differences were not significant across sample sites (Table 2.8; Appendix B). The other orders including Coleoptera, Oligochaeta, Mollusca, Sphaeriida, and other (combination of Odonata, terrestrial invertebrates, Isopoda, Amphipoda, and Hydracarina) accounted for between 10-20% of relative abundance at various sites.

Relative abundance of Diptera (Fig. 2.18) was significantly different across substrates (Table 2.8; Appendix B), however multiple comparisons test was unable to clearly separate groups (Fig. 2.18). Chironomids accounted for a substantial portion of the relative abundance of Diptera (Fig. 2.19: Table 2.8; Appendix B). Chironomidae relative abundances followed a pattern that was very similar to Diptera except on vegetation.

Community Composition and Diversity

A total of 19,010 individual macroinvertebrates were found in the 75 samples collected. There were 102 different taxa identified across the five sites representing 16 different orders, 50 different families, and 64 genera of unique macroinvertebrates. Ephemeroptera, and Trichoptera were represented by the greatest number of identified taxa with a total of 12 different genera of Ephemeroptera, and 21 Trichoptera genera were identified (Appendix A). We found only 4 genera of Plecoptera (Appendix A).

Comparing macroinvertebrate communities among sites using NMDS showed a considerable degree of overlap (Fig. 2.20). Post hoc ANOSIM (R-stat: 0.02232, p-value: 0.172) determined that sites were not significantly different. The 95% confidence intervals of group centroids indicated a significant overlap. In contrast to the taxonomic analysis for sites, NMDS of macroinvertebrate communities by substrate did separate (Fig. 2.21) in ordination space. Plotting the 95% confidence intervals of the centroids confirmed separation of groups by taxa abundances on each substrate. A post hoc ANOSIM using total abundances of taxa on each substrate was significantly different (R-stat: 0.725, P-value: 0.001). We also conducted a SIMPER test of determine which taxa contributed most to differences among substrate types. Because habitat differences were generally dominated by chironomids, we excluded then from the analysis. Comparing gravel to other substrates, simulids, mayflies, and caddisflies generally contributed to the dissimilarity among substrate community composition (Table 2.9). The comparison between gravel and soft sediment was the one exception where Asellidae accounted for 22% of the differences. The SIMPER test found that differences among sand communities and those on other substrates were driven by simulids, mayflies, Asellidae, and oligochaetes (Table 2.9). Comparing CWD with vegetation and soft sediment, and vegetation with soft sediment, simulids accounted for the greatest variation, and mayflies and caddisflies were also important.

The most common taxa on CWD (Appendix A) included *Hydropsyche*, *Brachycentrus*, and *Micrasema* (Trichoptera), *Ephemerella* and *Baetis* (Ephemeroptera), and *Simulium* and Chironomidae (Diptera). Macrophytes beds were dominated by *Brachycentrus* and *Hydropsyche* (Trichoptera), and *Simulium* and Chironomidae (Diptera) (Appendix A). Soft sediment depositional zones supported a diverse community with *Myastacides* (Trichoptera), Sphaeriidae (Bivalvia), Hydrobiidae, Planorbidae, Viviparidae, Physidae, and Tateidae (Gastropoda), Asellidae (Isopoda), Gammaridae (Amphipoda), Chironomidae (Diptera), and Oligochaeta (Appendix A). On gravel substrates we found (Appendix A) primarily *Drunella* and *Ephemerella* (Ephemeroptera), *Helicopsyche* (Trichoptera), *Macronychus* (Coleoptera), and Chironomidae (Diptera). Lastly, sand substrates supported a relatively modest community (Appendix A) including Sphaeriidae (Bivalvia), Hydrobiidae, Planorbidae, Viviparidae, Physidae, and Tateidae (Gastropoda), Chironomidae (Diptera), and Oligochaeta.

Shannon's diversity index indicated that the highest diversity on gravel substrates (Table 2.10). Gravel substrates were consistently diverse across the five sample sites generally scoring 0.9 on the index. CWD and sand were the next most diverse ranging from 0.7 to 0.6 (Table 2.10). Diversity was lowest on macrophytes and soft sediment with scores near 0.4 and 0.3 (Table 2.10). Generally, macrophytes and soft sediment were consistently low at all sites. Mean scores calculated for each site were consistently around 0.6 from upstream to downstream (Table 2.10).

Our P-51 analysis determined that overall water quality of the North Branch of the Au Sable River is tending towards excellent. Twin Bridges scored the highest water quality score with a 6 (highest score is 8). The lowest score we calculated was at Dam 4 Road with a score of 1. From the 9 metrics we calculated we did not see any site within the poor water quality scoring.

Water quality across all sites (according to EGLE protocol) would suggest excellent water quality on the North Branch (Table 2.11).

Discussion

Aquatic macroinvertebrates are an essential component of stream ecosystems. They play a critical role as functional groups and in the transfer of energy from lower to higher trophic levels (Vannote et al. 1980). This study was motivated by reports of declines in major aquatic insect hatches. Because macroinvertebrates are critical to energy transfer, it is logical that declining macroinvertebrate densities may partly account for declining numbers of trout in the North Branch Au Sable River. Thus, our goal was to quantify the distribution and abundance of macroinvertebrates in the North Branch and provide a baseline for future studies.

Our data show that the total abundance of macroinvertebrates did exhibit variation with a single site supporting a substantially high density compared to other sites. However, the abundance of macroinvertebrates across sites was relatively constant from upstream to downstream sample sites. It is possible that the length of our study area compared to other studies, relatively homogenous substrate and channel form from upstream to downstream, and the consistent flow regime contributes to the relatively constant macroinvertebrate densities. Other studies have outlined the importance of habitat, which serves as a template for spatial variation in macroinvertebrate communities. Environmental factors such as substrate heterogeneity, temperature, flow, etc. influence community composition and macroinvertebrate densities (Poff & Ward, 1990; Townsend & Hildrew, 1994). Poff and Ward (1990) hypothesized that streams with greater habitat heterogeneity will support macroinvertebrate communities that are more resistant to disturbance. Matthews et al. (1991) observed spatial differences of macroinvertebrate communities in a second order stream over a much shorter distance, but with greater habitat variation. Macroinvertebrate densities changed from upstream to downstream. A study on the Maple River in Michigan determined variation of Hydropsychidae from upstream to downstream. In the Maple River, Fairchild & Holomuzki, (2002) determined that there was variation in species abundances from upstream to downstream because of habitat factors such as substrate and temperature. Although previous studies have determined differences in macroinvertebrate abundance from upstream to downstream on both a large scale and small scale, our research did not suggest statistically significant differences in macroinvertebrate abundances from upstream to downstream even though one site had substantially higher invertebrate abundance. This suggests that the North Branch may be more spatially homogenous throughout our sample sites.

As expected, macroinvertebrate abundance was relatively low on sand. More surprising was the fact that the highest densities were generally found on CWD and not on gravel as we had expected. Although gravel substrates did support good macroinvertebrate abundances, CWD supported a much more robust invertebrate community. In addition, macrophyte beds and soft sediment had densities (individuals/m^2) that compare to gravel. Previous studies have suggested that gravel may be the most important habitat for benthic macroinvertebrates (Duan et al., 2009; Brown & Brown 1984). In this study, there was a large difference in macroinvertebrate abundances between CWD and gravel, which highlights the fact that CWD may be overlooked as an important habitat for macroinvertebrates that may contribute to stream ecosystem function. Currently, CWD covers about 10% of stream surface area, whereas historic estimates put CWD at about 30%. During the logging era, most naturally occurring CWD was removed to facilitate the transport of large logs downstream (Zorn & Sendek, 2001). Subsequently, the Civilian Conservation Corp (CCC) installed a substantial number of wood structures in the Au Sable during the 1930s to help improve habitat for fish stocking programs. In the 1960-1970's conservation groups began inputting woody structures back into the North Branch. Anecdotal reports from the 1930s and quantitative fish data from the late 1950s through the early 2000s suggest that trout populations were more robust than current population estimates. Based on our results and the historic patterns, the addition of wood structure to the channel may enhance invertebrate abundance and subsequently support fish populations by providing cover and serving as substrate for macroinvertebrates. For example, Wallace at al. (1995) placed large logs perpendicular to flow in a river and found positive correlation with most macroinvertebrate functional feeding groups. A similar study by Entrekin et al. (2009) determined that random placement of CWD did not negatively impact macroinvertebrate communities. The study determined that CWD was an important habitat component and source of food resources for many macroinvertebrate functional feeding groups (Entrekin et al., 2009). Finally, during our study, CWD had the highest macroinvertebrate abundances of all substrates with over 23,000/m^2. Benke et al. (1984) conducted a study in Georgia on the importance of CWD in the Satilla River. The upper Satilla River had approximately 30,000/m^2 and the lower Satilla had 26,000/m^2 (Benke et al., 1984). Although the North Branch Au Sable River and the lower Satilla River are very different systems, our data are comparable to the lower section of the Satilla River which suggests that CWD may be an important macroinvertebrate habitat across a range of river types.

Mean biomass for the North Branch had little variation from upstream to downstream. The highest mean biomass with combined substrates was at Copper Fisherman with 0.01 kg/m^2. Mean biomass on soft sediment was 0.0002 kg/m^2, for macrophytes 0.0018 kg/m^2, and CWD 0.006 kg/m^2. Krynak (2012) performed a similar study on Cedar Creek a sandy Michigan stream and found mean biomass in pools (depositional zones or soft sediment) was 0.004 kg/m^2, on macrophytes 0.01 kg/m^2, and on CWD 0.006 kg/m^2. Between our two studies our CWD substrates were very similar in mean biomass but differed in gravel and pool mean biomass. Both study sites were sandy bottom rivers and considered 3rd order streams. Similar physical habitats and water quality could be an indication of why our CWD mean biomass were so similar. It is possible that Cedar Creek has a dependence on macrophytes as the mean biomass was more than double what we observed on the North Branch.

P-51 assessment used determined that water quality was tending towards excellent at all five sites. The presence of sensitive macroinvertebrates at each of these sites leads us to believe that there are no issues changing the community composition. Excellent water quality is a good indicator that there are no chemical or physical changes occurring in the North Branch. This would lead us to believe that there are no serious issues with chemical runoff, pollutants or any major physical changes occurring. EGLE performed two water quality assessment on the North Branch sampling our Ford Road and Twin Bridge sites in 2018. At Ford Road EGLE scored water quality as a six, where we scored ours at a four. At Twin Bridges EGLE scored this site as a seven, and we scored ours at a six. EGLE was physically in the field performing this rapid bioassessment, where we used our quantitative data to assess these metrics. Possible differences could be due to our data having three replicates which could reduce any potential sampling bias. Generally, both sets of scores indicate that the water quality of the North Branch is in excellent water quality. The fact that macroinvertebrate metrics based on qualitative and quantitative sampling are similar is reassuring however, the additional information provided by quantitative sampling provides much greater insights into the long-term dynamics of macroinvertebrate populations and significance of each substrate type.

There are no previous studies on macroinvertebrate biomass and few studies of macroinvertebrate abundances on the North Branch. This makes it difficult to draw conclusions on temporal changes in abundances. Consequently, we cannot link macroinvertebrate densities with other biological elements, particularly fish abundances. However, our data does clearly identify the fact that CWD is an extremely important habitat element in the North Branch Au Sable River. We suggest that the addition of CWD to the North Branch would enhance macroinvertebrate densities and would very likely support higher trout populations.

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Figures

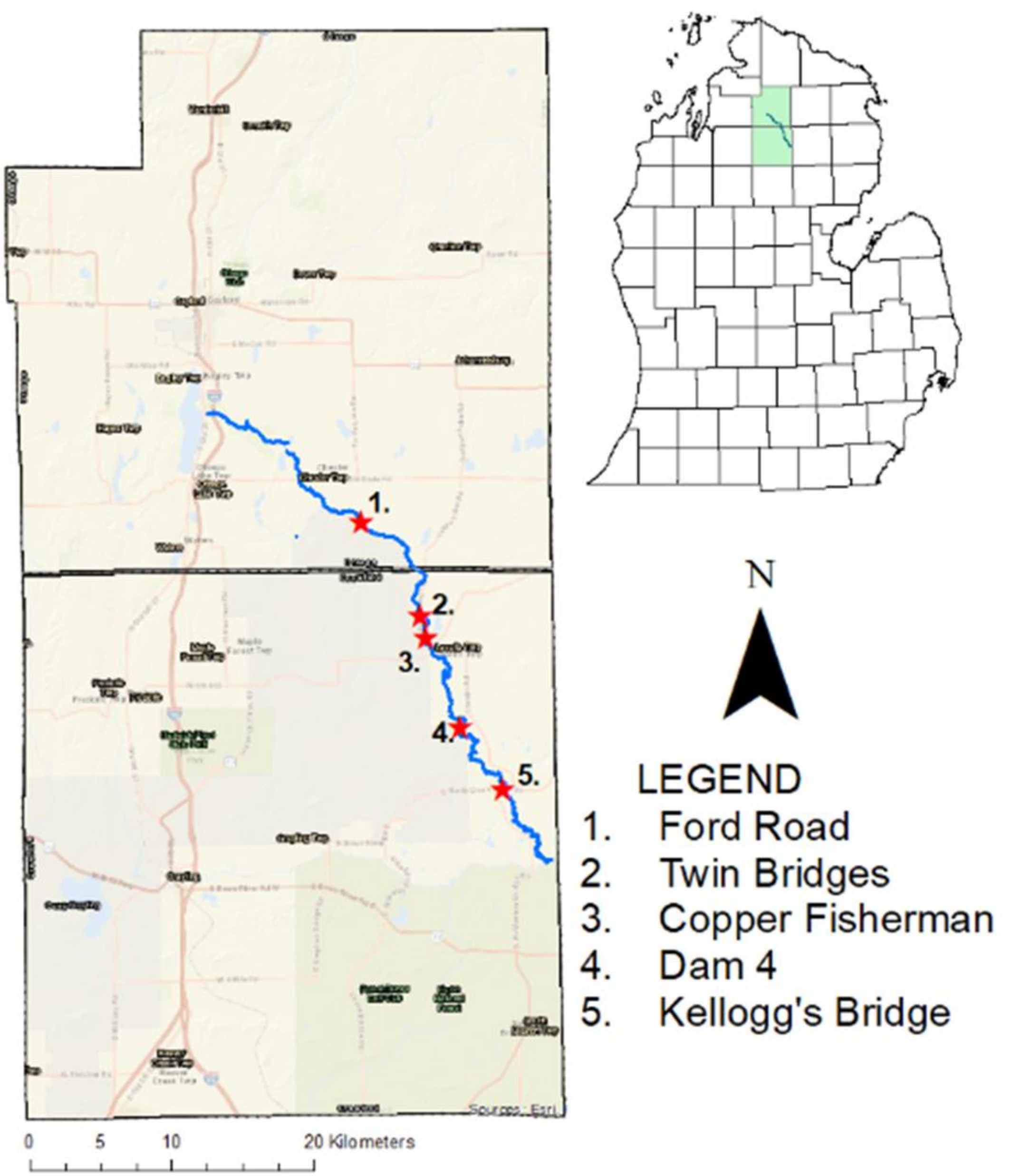

Figure 2.1: The State of Michigan and the location of the North Branch of the Au Sable River with sample site locations.

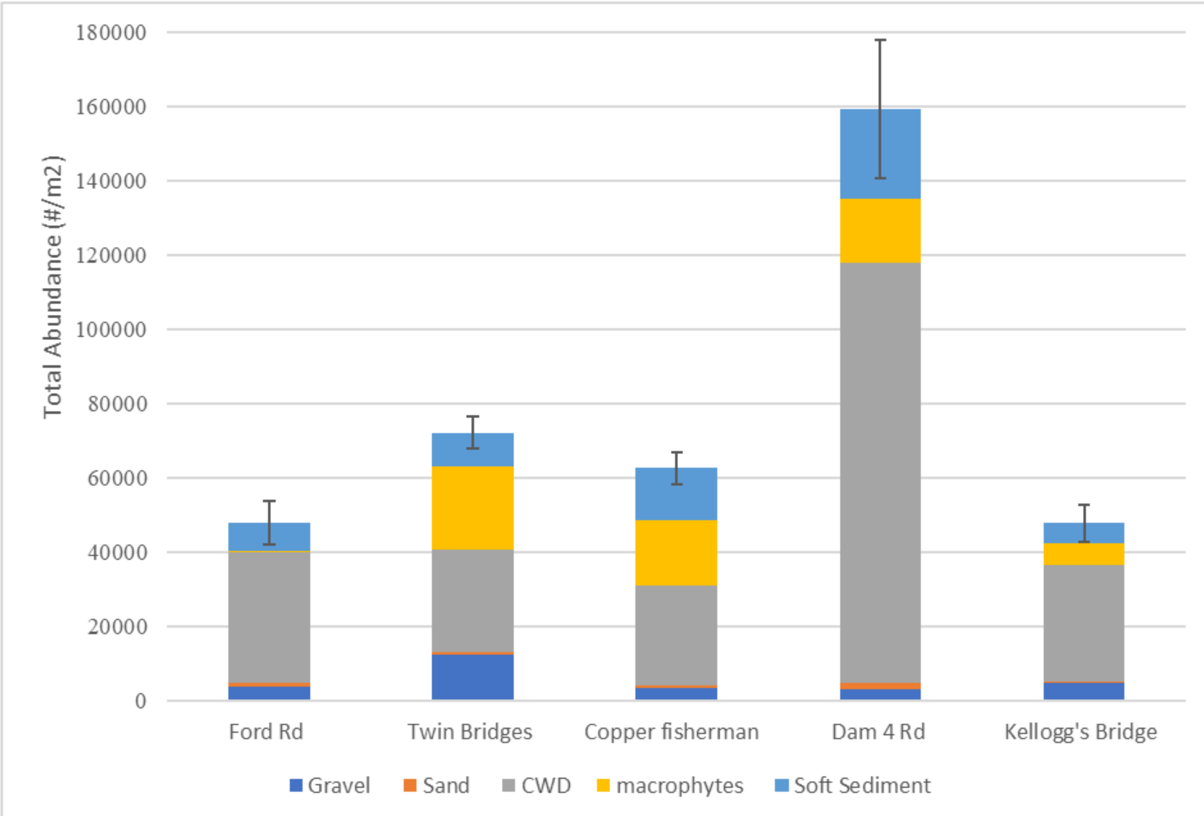

Figure 2.2. Stacked bar histogram of summed mean (1 ± SE) total abundances of individuals/m^2 at each site and on each substrate type sampled in the North Branch Au Sable River during summer 2020. Total abundance represents 5 m^2 of substrate.

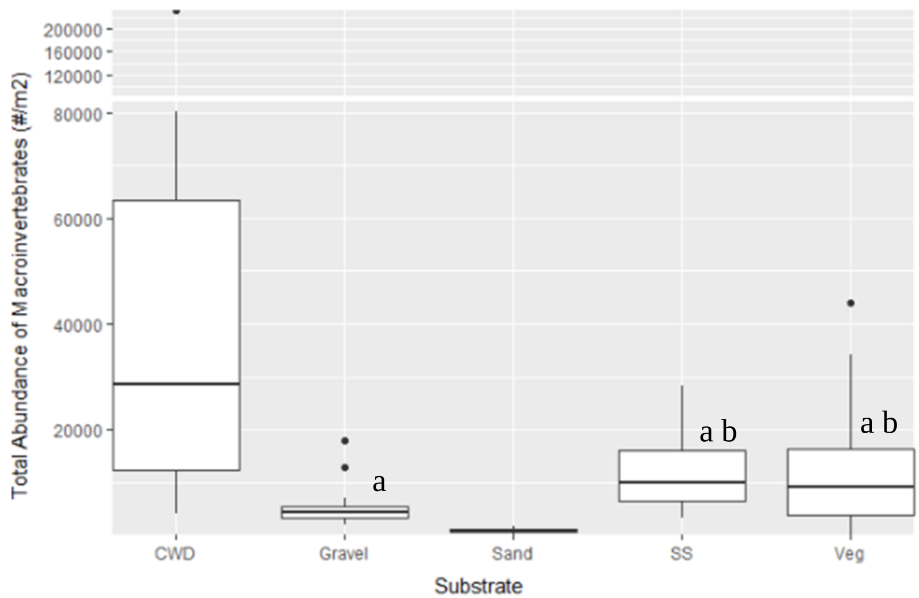

Figure 2.3. Mean total abundance of macroinvertebrates on five substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

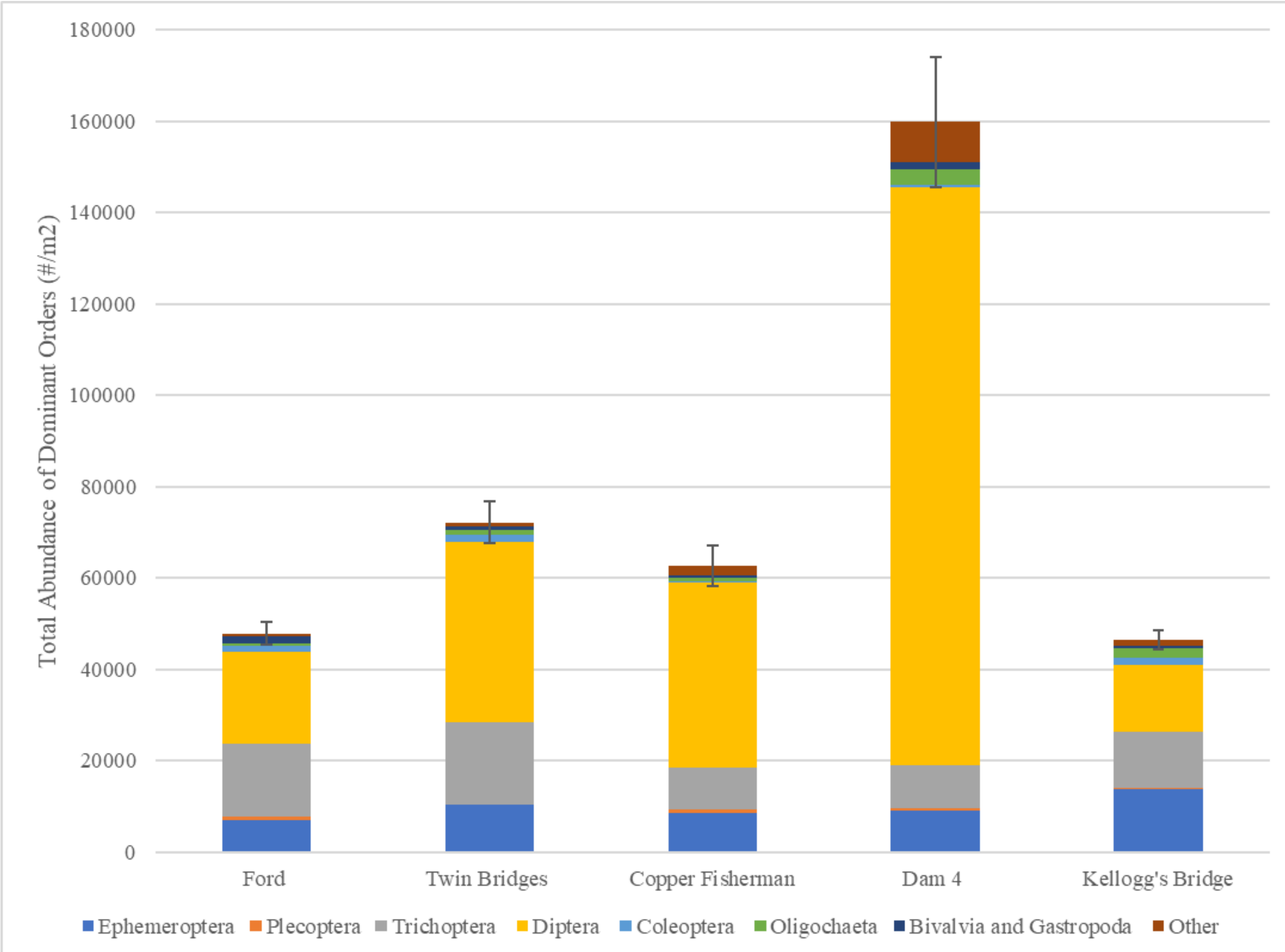

Figure 2.4. Stacked bar histogram of summed mean (1 ± SE) total abundances of Orders (individuals/m^2) at each site sampled in the North Branch Au Sable River during summer 2020. Total abundance represents 5 m^2 of substrate.

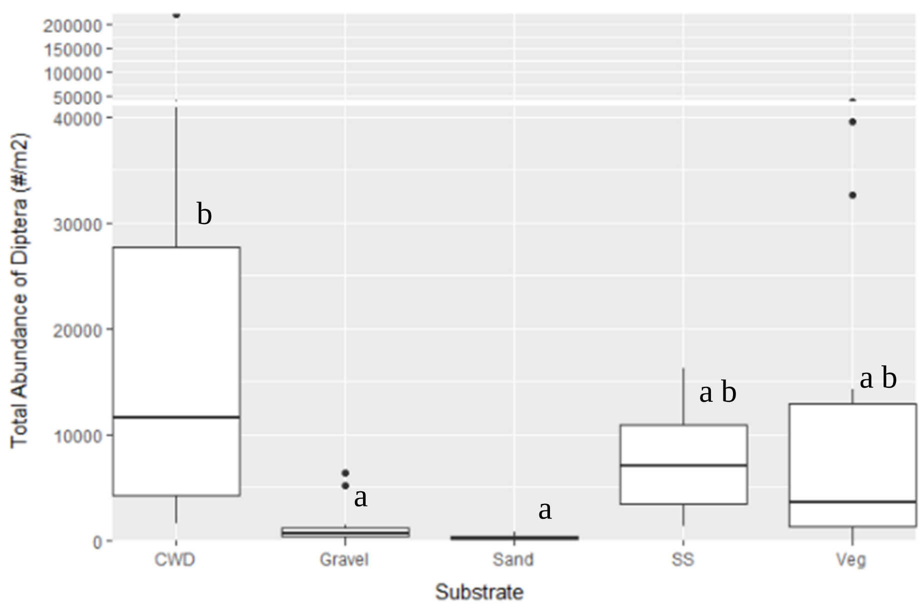

Figure 2.5. Mean total abundance of Diptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

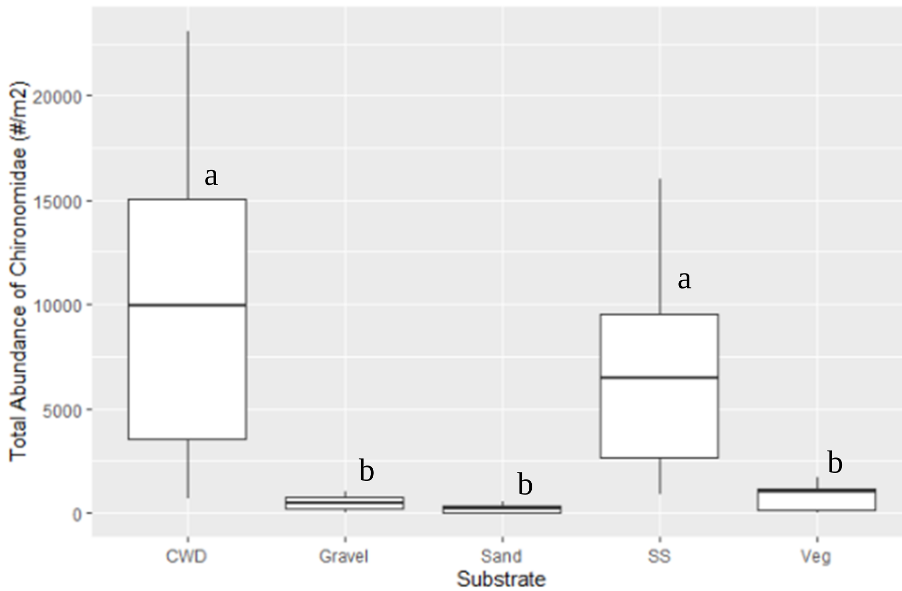

Figure 2.6. Mean total abundance of Chironomidae on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

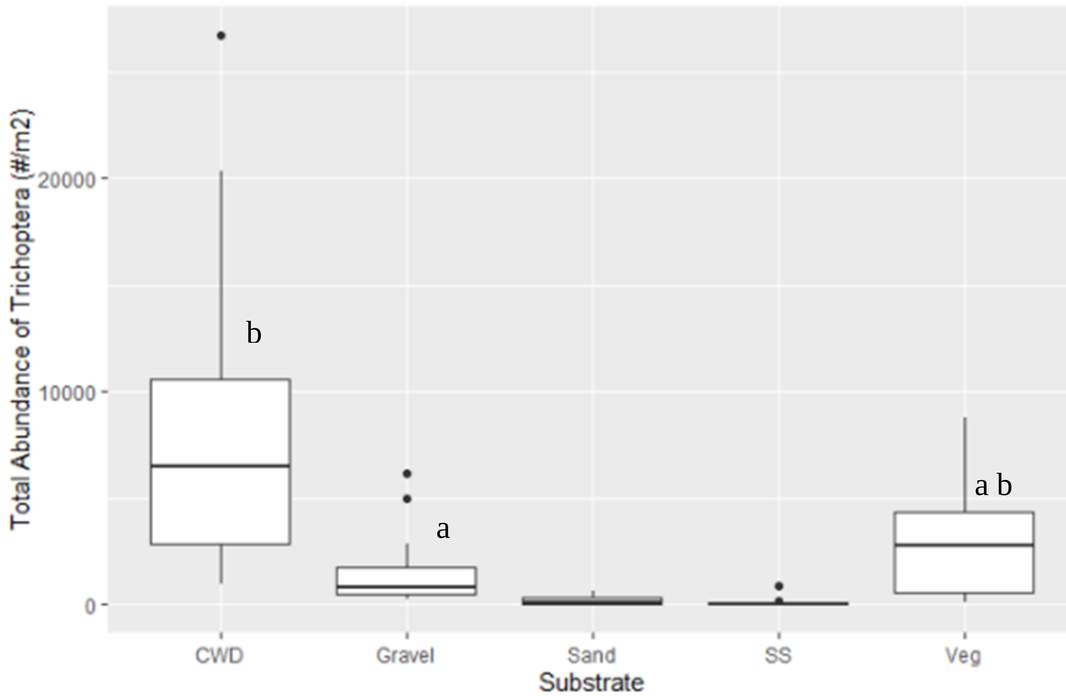

Figure 2.7. Mean total abundance of Trichoptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

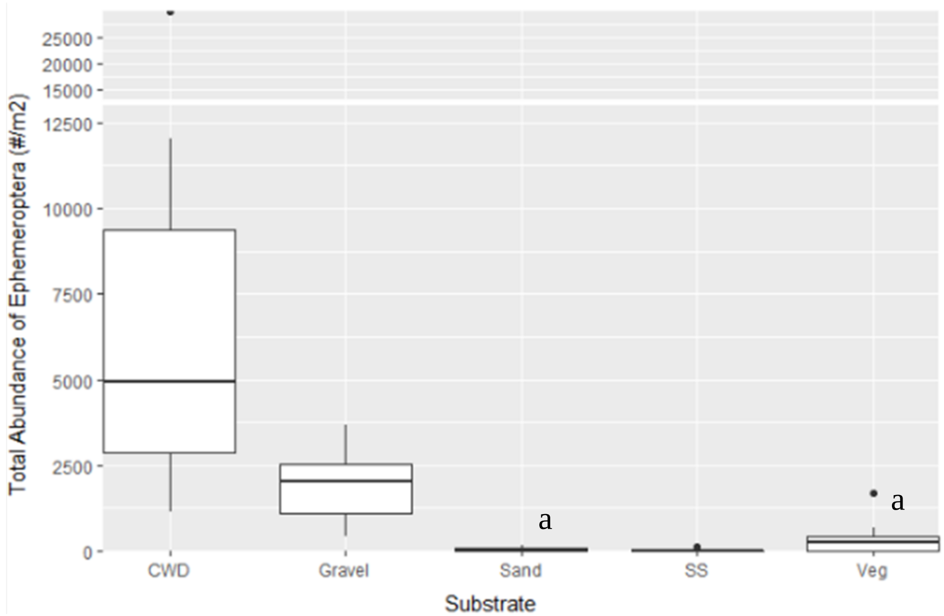

Figure 2.8. Mean total abundance of Ephemeroptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

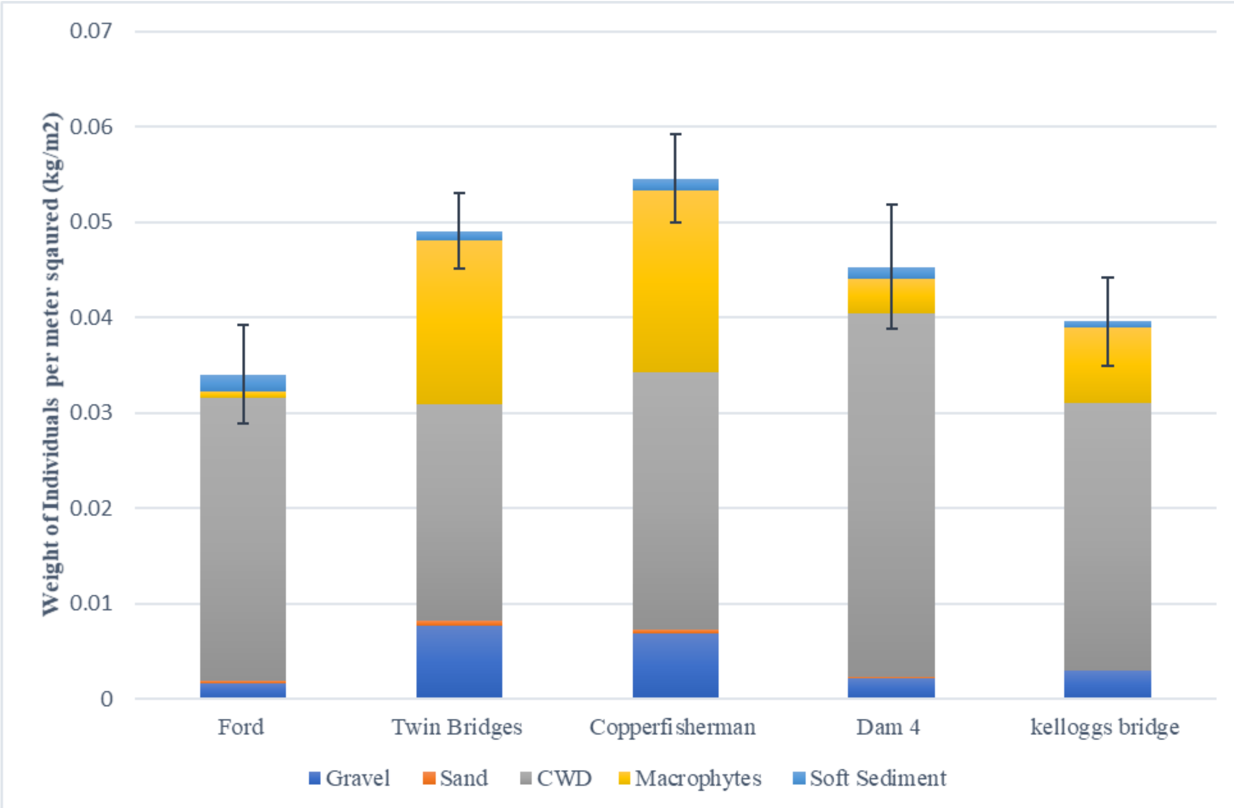

Figure 2.9. Stacked bar histogram of mean (1 ± SE) biomass of macroinvertebrates (kg/m^2) at each site and on each substrate sampled in the North Branch Au Sable River during summer 2020. Total abundance represents 5 m^2 of substrate.

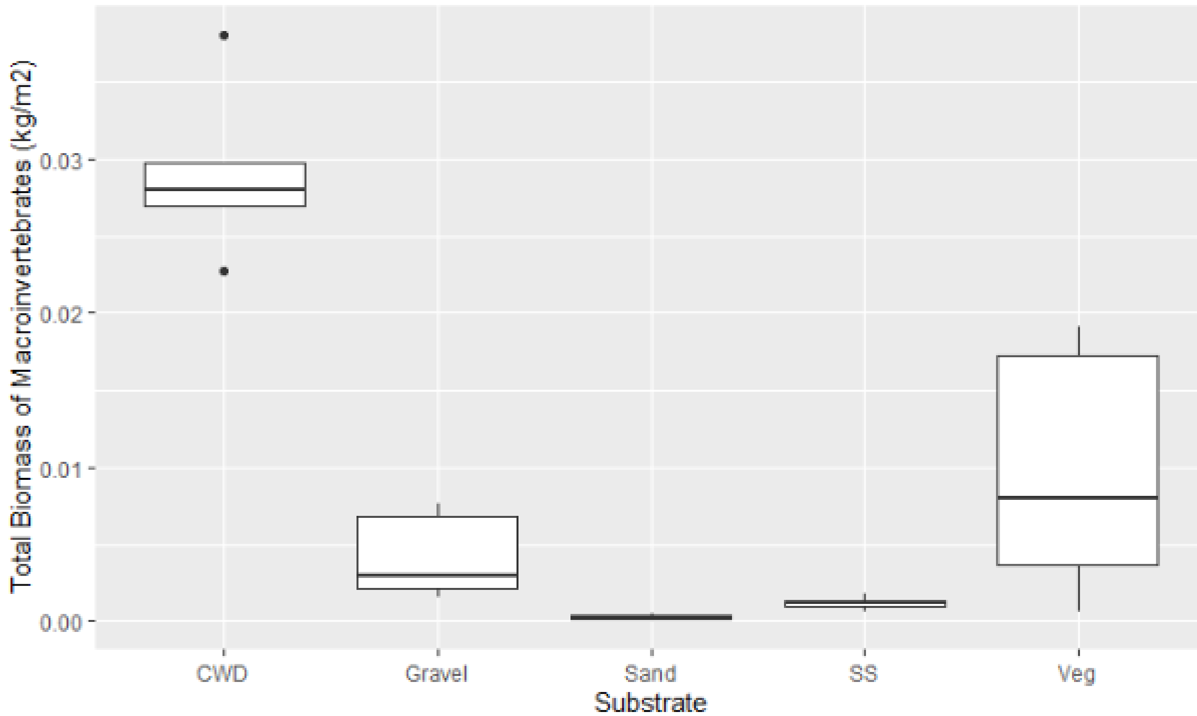

Figure 2.10. Mean biomass of macroinvertebrates (kg/m^2) on five substrates sampled in the North Branch Au Sable River during summer 2020.

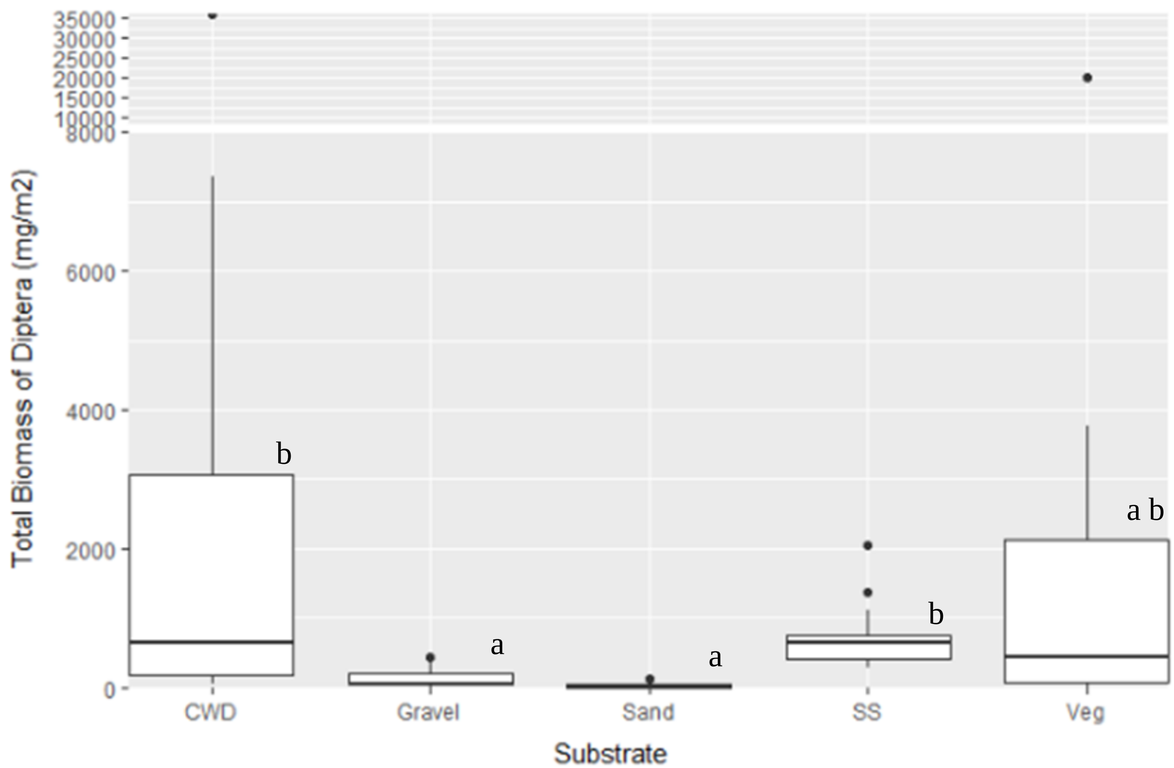

Figure 2.11. Mean total biomass (mg/m^2) of Diptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

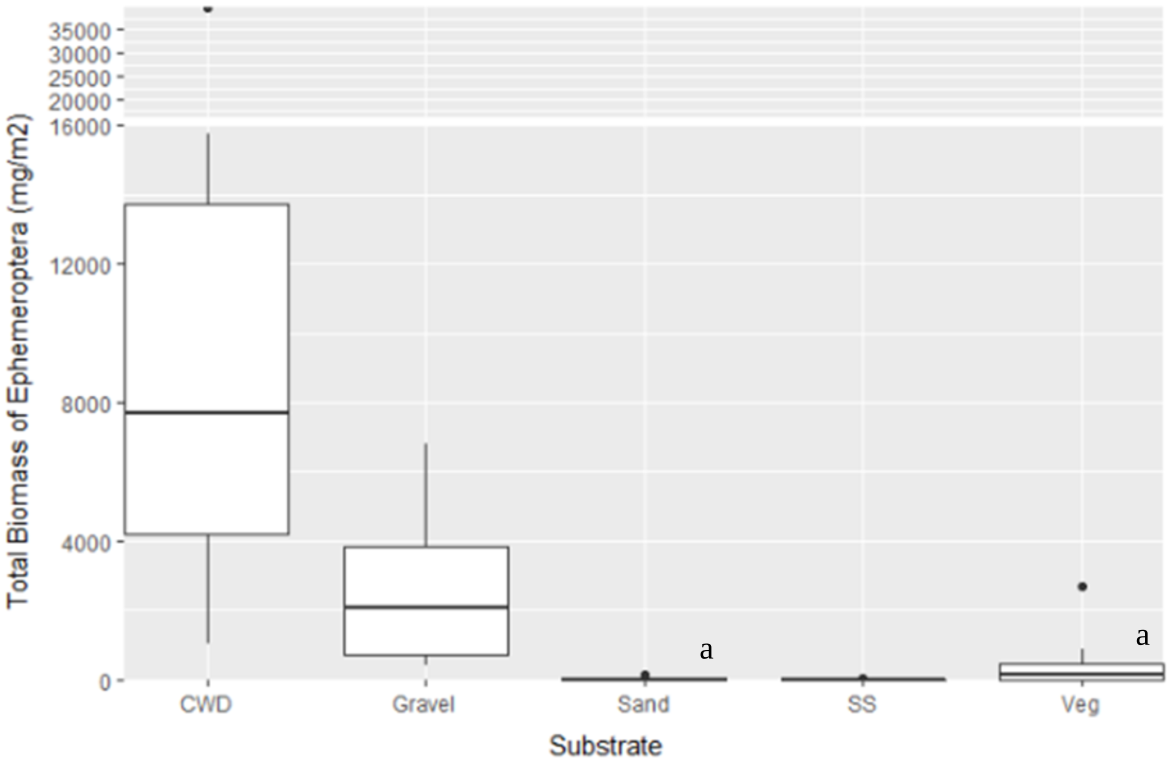

Figure 2.12. Mean total biomass (mg/m^2) of Ephemeroptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

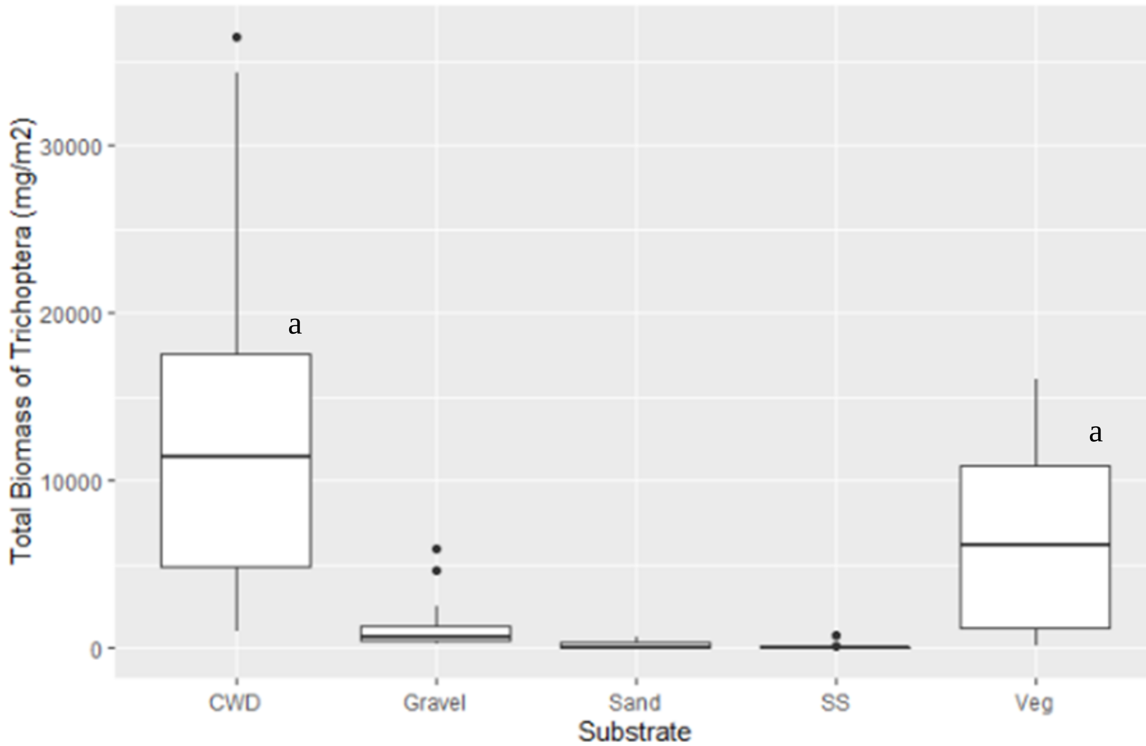

Figure 2.13. Mean total biomass (mg/m^2) of Trichoptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

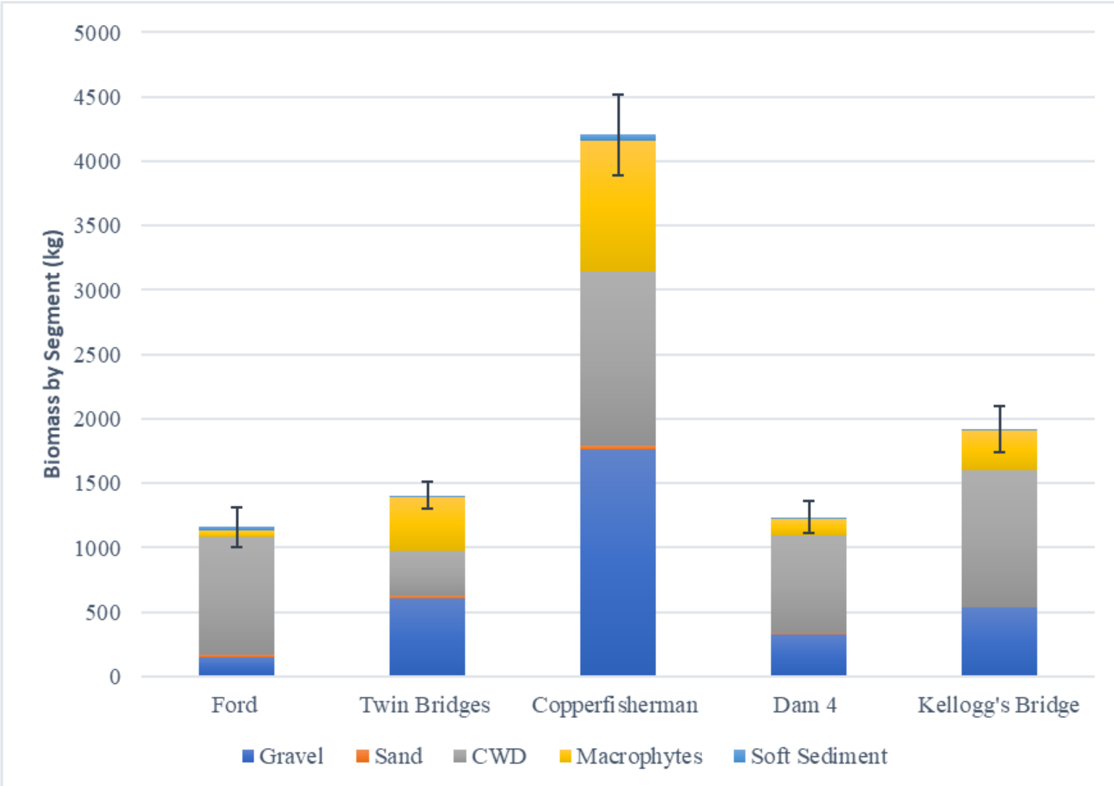

Figure 2.14. Stacked bar histogram of mean (1 ± SE) total macroinvertebrate biomass within each segment (kg) and on each substrate type sampled in the North Branch Au Sable River during summer 2020.

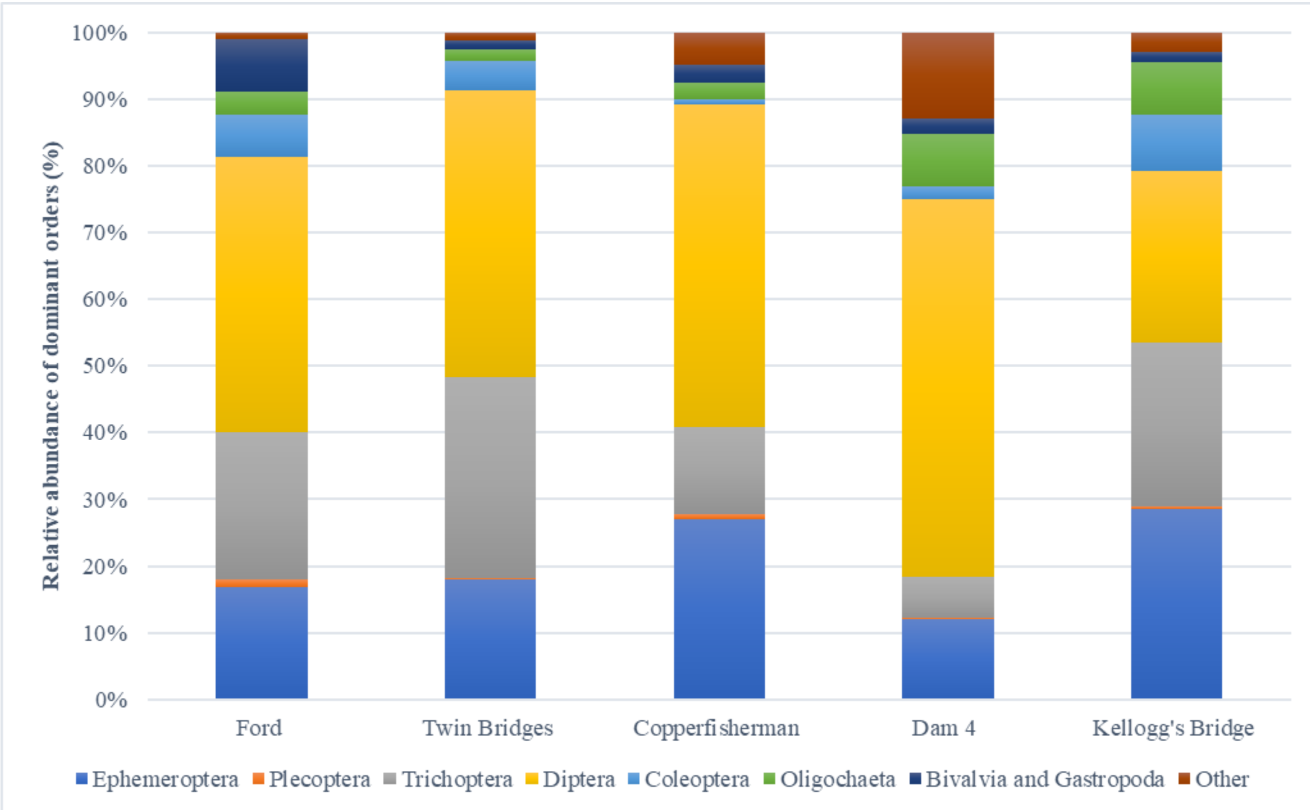

Figure 2.15. Relative abundance of most abundant orders of macroinvertebrates at five sites on the North Branch Au Sable River sampled during summer 2020.

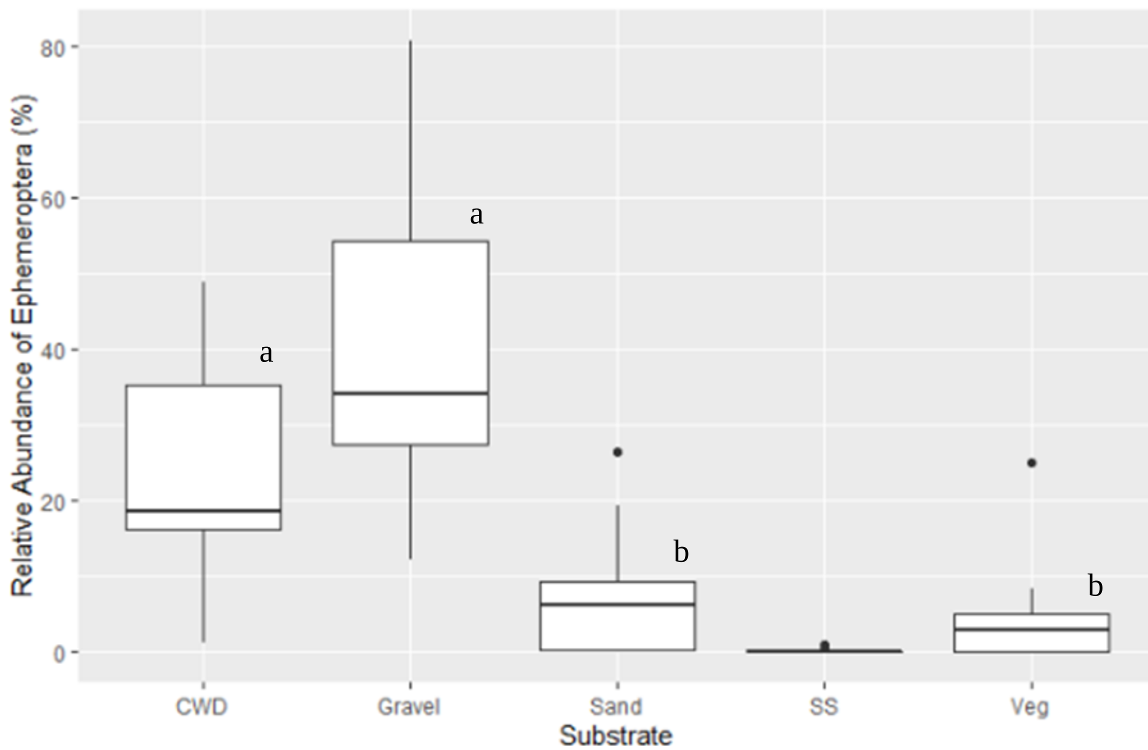

Figure 2.16. Mean relative abundance of Ephemeroptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

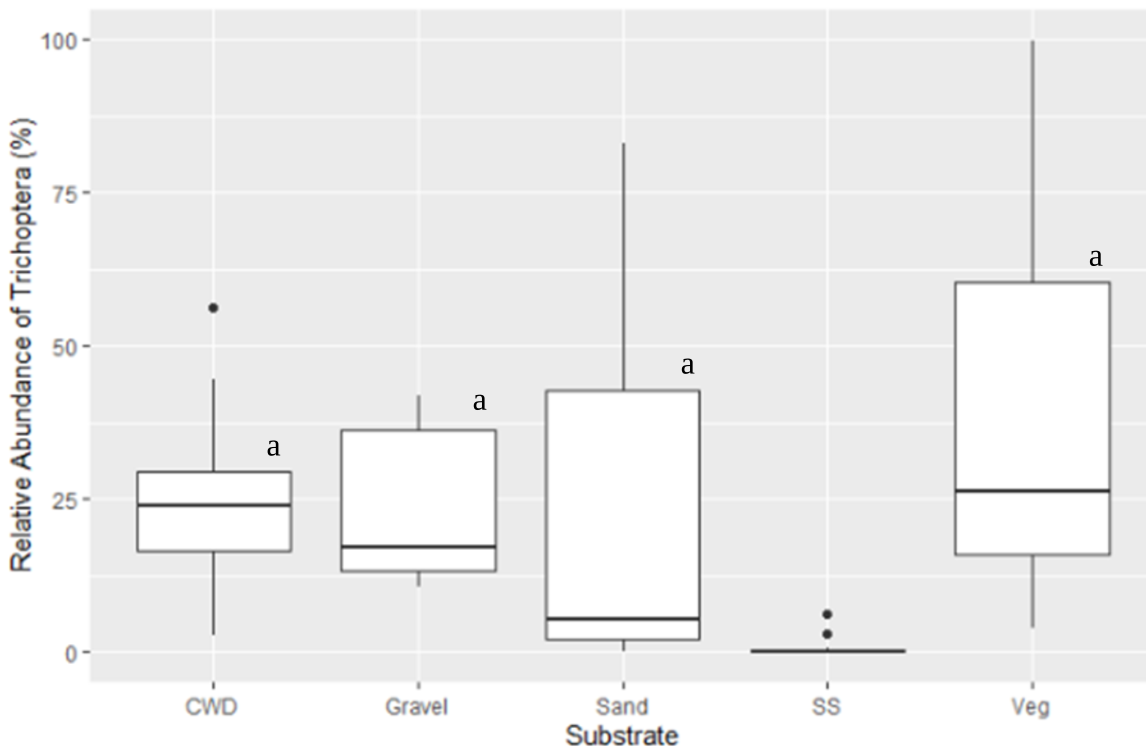

Figure 2.17. Mean relative abundance of Trichoptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

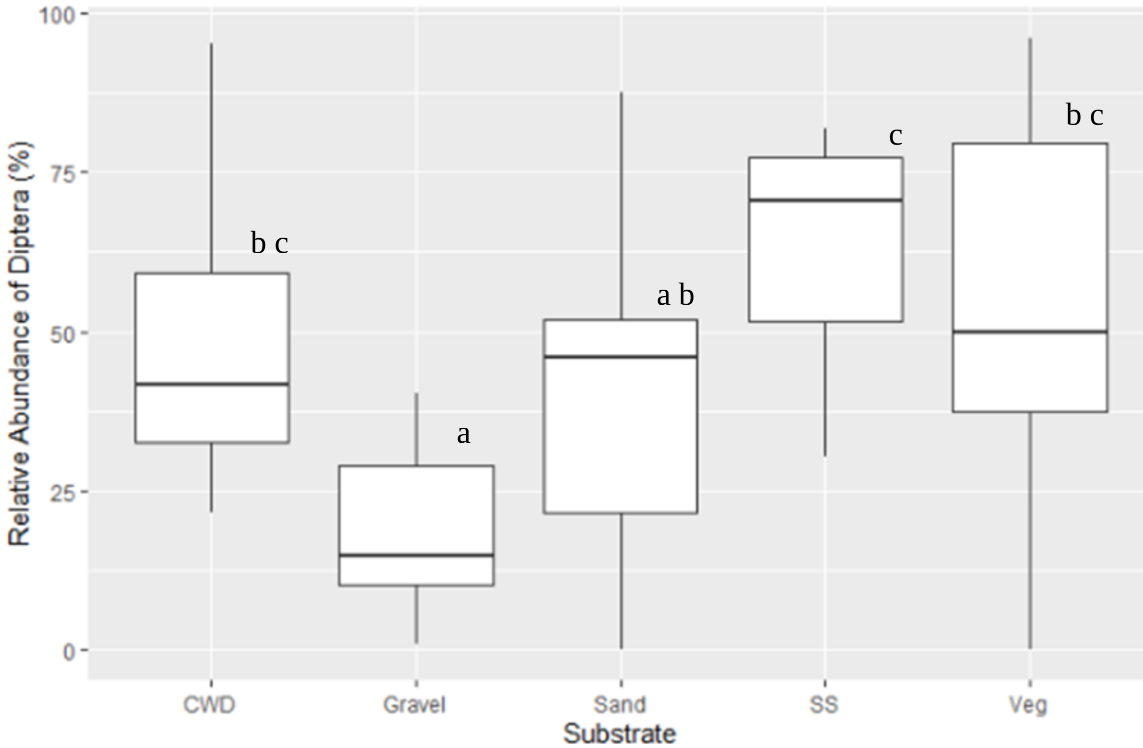

Figure 2.18. Mean relative abundance of Diptera on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

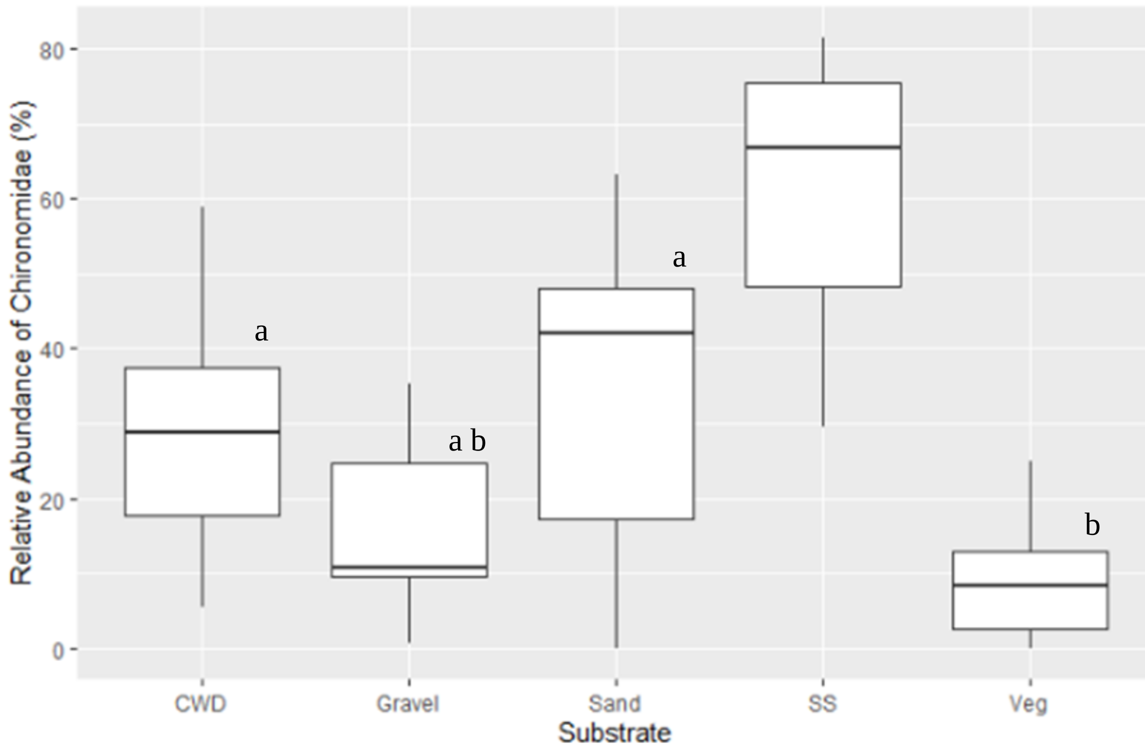

Figure 2.19. Mean relative abundance of Chironomidae on 5 substrates sampled in the North Branch Au Sable River during summer 2020. Substrate abbreviations are CWD = coarse woody, SS = soft sediment, Veg = macrophytes. The same lowercase letter indicates non-significant result for a pairwise *t* test (p-value = 0.05).

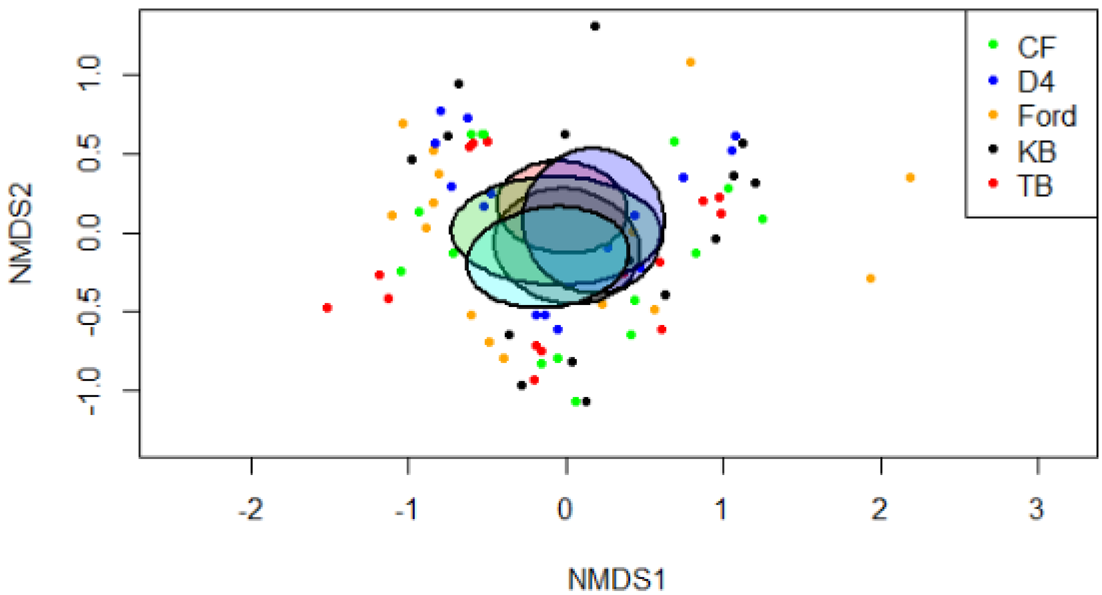

Figure 2.20. Non-metric Multidimensional Scaling (NMDS) of total abundance of macroinvertebrates communities at five sites on five substrates sampled in the North Branch Au Sable River during summer 2020. Individual dots represent an individual substrate. CF = Copper Fisherman, D4 = Dam 4 Road, Ford = Ford Road, KB = Kellogg's Bridge, and TB = Twin Bridges. Polygons with solid line and shaded areas are 95% confidence interval calculated on the centroid of each substrate.

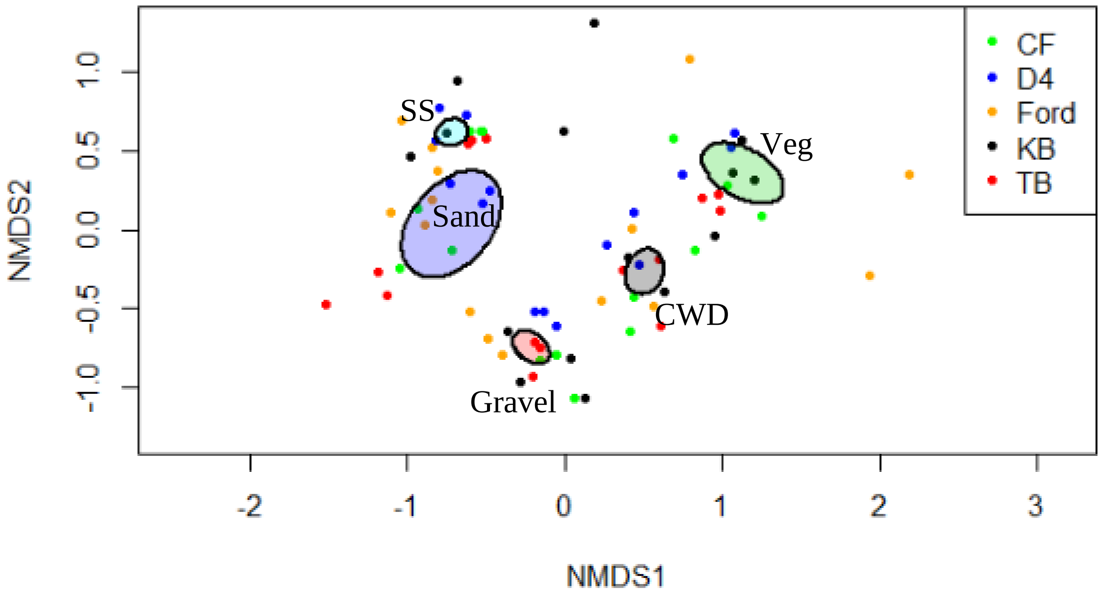

Figure 2.21. Non-metric Multidimensional Scaling (NMDS) of total abundances of macroinvertebrate taxa on five substrates at five sites sampled in the North Branch Au Sable River during summer 2020. CF = Copper Fisherman, D4 = Dam 4 Road, Ford = Ford Road, KB = Kellogg's Bridge, and TB = Twin Bridges. Polygons with solid line and circular shape are a result of the 95% confidence interval calculated on the centroid of each substrate.

Tables

Table 2.1. Physical characteristics of each stream reach sampled on the North Branch during summer 2020.

	Site				
Substrate	Ford	Twin Bridge	Copper Fisherman	Dam 4	Kellogg's Bridge
Latitude Longitude	44.887688N -84.542611W	44.827540N -84.490322W	44.809166N -84.485355W	44.756288N -84.457753W	44.716666N -84.419963W
Gravel (m^2)	94833	78735	257296	154848	182239
Sand (m^2)	67846	36942	70526	44309	60459
CWD (m^2)	30847	15452	50041	19776	38003
Macrophytes (m^2)	64461	24432	53636	35580	38118
Soft Sediment (m^2)	17494	11372	33089	10573	21661
Total Area (m^2)	275483	166935	464590	265088	340481
Total Area (mi^2)	0.106	0.0645	0.179	0.102	0.131
Total Length of Section (mile and km)	6.7 miles 10.8 km	1.5 miles 2.4 km	5.8 miles 9.3 km	6.1 miles 9.8 km	6.05 miles 9.7 km
Gradient (ft/mile and m/km)	8 ft/mile 1.5 m/km	4.67 ft/mile 0.88 m/km	8.5 ft/mile 1.6 m/km	8.0 ft/mile 1.5 m/km	8.4 ft/mile 1.59 m/km

Table 2.2. Mean total abundances of individuals/m^2 at each site separated by substrates.

	Site				
Substrate	Ford Road	Twin Bridges	Copper Fisherman	Dam 4 Road	Kellogg's Bridge
Gravel	3771	12640	3671	3302	4791
Sand	1000	605	671	1512	322
CWD	35368	27357	26838	113207	31673
Macrophytes	425	22536	17512	17206	5615
Soft Sediment	7306	9014	13972	24139	5514

Table 2.3. Two-way Analysis of variance of total macroinvertebrate density, density of Diptera, Trichoptera, and Chironomidae to determine differences due to site and substrate.

Source of Variation	**d.f.**	**F**	***p***
Site			
Total Density	4	2.21	0.09
Diptera	4	4.63	0.0029
Trichoptera	4	1.078	0.162
Chironomidae	4	3.032	0.026
Substrate			
Total Density	4	8.05	<0.001
Diptera	4	34.179	<0.001
Trichoptera	4	16.031	<0.001
Chironomidae	4	31.757	<0.001
Site x Substrate			
Total Density	16	1.35	0.204
Diptera	16	5.122	<0.001
Trichoptera	16	3.594	<0.001
Chironomidae	16	3.419	<0.001

Table 2.4. Kruskal-Wallis test of Ephemeroptera densities to determine differences due to site and substrate.

Source of Variation	**d.f.**	X^2	***p***
Site	4	0.542	0.969
Substrate	4	60.342	<0.001

Table 2.5. Kruskal-Wallis test of Diptera and Ephemeroptera biomass to determine differences due to site and substrate.

Source of Variation	**d.f.**	X^2	***p***
Total Biomass			
Site	4	0.546	0.969
Substrate	4	20.80	<0.001
Diptera			
Site	4	4.79	0.309
Substrate	4	37.9	<0.001
Ephemeroptera			
Site	4	1.182	0.8811
Substrate	4	58.207	<0.001

Table 2.6. Two-way Analysis of variance of biomass of Trichoptera, and Chironomidae to determine differences due to site and substrate.

Source of Variation	**d.f.**	**F**	***p***
Site			
Trichoptera	4	1.495	0.217
Chironomidae	4	3.032	0.0258
Substrate			
Trichoptera	4	87.142	<0.001
Chironomidae	4	31.757	<0.001
Site x Substrate			
Trichoptera	16	3.33	<0.001
Chironomidae	16	3.419	<0.001

Table 2.7. Kruskel-Wallis test of % Ephemeroptera and % Trichoptera to determine differences due to site and substrate.

Source of Variation	**d.f.**	X^2	***p***
Ephemeroptera			
Site	4	1.14	0.88
Substrate	4	54.03	<0.001
Trichoptera			
Site	4	9.76	0.04
Substrate	4	32.22	<0.001

Table 2.8. Two-way of % Diptera and Chironomidae

Source of Variation	**d.f.**	**F**	***p***
Site			
Diptera	4	2.38	0.06
Chironomidae	4	0.323	0.86
Substrate			
Diptera	4	16.03	<0.001
Chironomidae	4	34.25	<0.001
Site x Substrate			
Diptera	16	4.595	<0.001
Chironomidae	16	8.22	<0.001

Table 2.9. Pairwise comparison of individual habitat types using invertebrate taxa abundances in a similarity percentage (SIMPER) analysis.

Gravel vs sand		Gravel vs CWD		Gravel vs Veg		Gravel vs SS	
Taxa	%	Taxa	%	Taxa	%	Taxa	%
Drunella	20.3	Simuliidae	19.1	Simuliidae	36.8	Asellidae	22.2
Ephemerella	20.1	*Ephemerella*	13.4	*Brachycentrus*	16.5	*Ephemerella*	13.3
Helicopsyche	17.2	*Hydropsyche*	12.2	*Helicopsyche*	8.8	*Drunella*	13.2
Macronychus	6.7	*Micrasema*	5.4	*Ephemerella*	8.7	*Helicopsuche*	12.9
Oligochaeta	5.8	*Brachycentrus*	5.2	*Drunella*	8.2	Oligochaeta	11.2
Stenonema	2.2	*Helicopsyche*	4.9	*Macronychus*	3.2	*Macronychus*	4.5
Micrasema	2.1	*Drunella*	3.8	*Micrasema*	2.5	Gammaridae	2
Hydropsyche	1.8	*Macronychus*	2.7	*Oligochaeta*	2.2	*Bezzia*	1.6
Teloganopsis	1.7	*Teloganopsis*	2.5	*Hydropsyche*	1.2	*Micrasema*	1.5
Ordobrevia	1.5	*Baetis*	2.4	*Stenonema*	0.9	*Stenonema*	1.3
Protoptila	1.5	*Neophyax*	1.5	*Teloganopsis*	0.7	*Mystacides*	1.1
Baetis	1.3	*Oecetis*	1.4	*Protoptila*	0.7	*Hydropsyche*	1.0

Table 2.9. Continued

Sand vs CWD		Sand vs Veg		Sand vs SS	
Taxa	%	Taxa	%	Taxa	%
Ephemerella	22.5	Simuliidae	49.3	Asellidae	40.5
Simuliidae	21.7	*Brachycentrus*	26.3	Oligochaeta	26.6
Hydropsyche	15.2	*Helicopsyche*	5.5	*Helicopsyche*	6.9
Micrasema	7.1	Oligochaeta	5	Gammaridae	5.3
Brachycentrus	6.8	*Micrasema*	4.8	*Bezzia*	4.4
Drunella	4	*Hydropsyche*	2	*Mystacides*	3.6
Baetis	3.5	*Ephemerella*	1.6	Simuliidae	0.7
Macronychus	3.4	*Drunella*	1.4	*Drunella*	0.5
Teloganopsis	3.3	*Stenonema*	1.2	*Baetis*	0.5
Neophylax	1.9	*Baetis*	0.5	*Macronychus*	0.4
Oecetis	1.6	Gammaridae	0.3	*Ephemera*	0.3
Oligochaeta	1.3	*Ephemera*	0.3	*Teloganopsis*	0.3

Table 2.9. Continued

CWD vs Veg		CWD vs SS		Veg vs SS	
Taxa	%	Taxa	%	Taxa	%
Simuliidae	29.5	Simuliidae	19.6	Simuliidae	39.2
Ephemerella	13.8	*Ephemerella*	18.4	*Brachycentrus*	18.5
Hydropsyche	10.3	*Hydropsyche*	13.1	*Asellidae*	18.5
Brachycentrys	8.7	*Asellidae*	10	Oligochaeta	11.4
Micrasema	4.5	*Micrasema*	6	*Micrasema*	2.9
Drunella	2.6	*Brachycentrus*	5.6	Gammaridae	2
Macronychus	2.3	Oligochaeta	5.4	*Bezzia*	1.5
Teloganopsis	2.2	*Drunella*	3.4	*Ephemerella*	1.3
Baetis	2.1	*Macronychus*	2.9	*Mystacides*	1.2
Oecetis	1.3	*Teloganopsis*	2.8	*Hydropsyche*	1
Neophylax	1.2	*Baetis*	2.8	*Drunella*	0.9
Stenonema	0.8	*Neophylax*	1.6	*Helicopsyche*	0.5

Table 2.10. Shannon's Diversity index calculated for all substrates across all five sample sites.

	Sites				
Substrates	Ford	Twin Bridge	Copper Fisherman	Dam 4	Kellogg's Bridge
Gravel	1.15	0.92	0.72	0.89	0.89
Sand	0.63	0.37	0.64	0.58	0.62
CWD	0.78	0.91	0.76	0.52	0.82
Macrophytes	0.40	0.42	0.42	0.31	0.52
Soft Sediment	0.46	0.36	0.33	0.54	0.54
Total	0.68	0.60	0.57	0.57	0.68

Table 2.11. Calculated Procedure-51 scores metric values for five sites on the North Branch Au Sable River sampled during summer 2020. Calculations followed methods used by the Michigan Department of Environment, Great Lakes, and Energy. A score >5 is excellent, 4 to 1 is tending towards excellent, 0 is neutral, -1 to -4 is tending towards poor, and <-5 is poor water quality. Numbers in paratheses are the score for that metric.

Site	Total Taxa	Maylfly Taxa	Caddisfly Taxa	Stonefly Taxa	% Mayfly	%Caddisfly	%Dominant	%Isopod, Snail, Leech	% Surface dominant	Score
Ford Road	>22 (1)	10 (1)	14 (1)	2 (1)	16.47 (0)	19.97 (0)	30.61 (0)	2.46 (-1)	0 (1)	4
Twin Bridges	>22 (1)	6 (1)	14 (1)	2 (1)	13.81 (0)	29.94 (0)	32.05 (0)	0.84 (1)	0 (1)	6
Copper fisherman	>22 (1)	8 (1)	9 (1)	2 (1)	24.25 (0)	13.63 (0)	34.06 (0)	3.24 (-1)	0 (1)	4
Dam 4 Road	>22 (1)	6 (1)	8 (1)	1 (0)	11.37 (0)	3.97 (-1)	46.42 (-1)	6.03 (-1)	0 1)	1
Kellogg's Bridge	>22 (1)	7 (1)	11 (1)	2 (1)	20.29 (0)	25 (0)	30.94 (0)	1.12 (0)	0 (1)	5

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Chapter 3

Extended Review of Literature

Introduction

Currently the North Branch of the Au Sable River has experienced low trout abundance. We believe that sedimentation and a lower abundance of macroinvertebrates are possible factors that are limiting factors to trout abundance. Sediment limits the amount of available habitat for fish and benthic macroinvertebrates. This literature review will focus on the effects of sediment on fish and benthic macroinvertebrates and the ways to sample and assess it. Sedimentation comes from many different sources, outlined in this literature review.

Macroinvertebrate Ecology

Headwater streams, have high densities of collectors, grazers, and shredders, with few predators. Larger rivers have high densities of collectors due to an increase in fine particulate organic matter. Predator abundance will increase in this area because of the abundance of these collectors. Headwater streams are mostly shaded allowing some light penetration in a stream causing respiration rates to be higher than photosynthesis. In larger streams, there is less shading from the riparian zone which increases the photosynthetic rate and decreases the respiration rate. (Vannote et. al., 1980). This model demonstrates the inefficiencies of macroinvertebrates from upstream to downstream. This model only focuses on the longitudinal aspect of macroinvertebrate distribution. The North Branch has an open canopy with wide channels and well-defined riparian zone. What occurs on the North Branch are high respiration rates greater than the overall photosynthetic rate.

The applications for this model that Vannote et. al (1980) proposed, can help us better understand how a river functions. Brown and Brown (1984) observed and analyzed the distribution of insects within a river. Requirements for macroinvertebrate distribution were particle size, current velocity, food availability, dissolved oxygen, aquatic vegetation, light source, depth, and temperature (Brown & Brown, 1984). Brown and Brown found a preference for the beginning of riffles. Due to high abundance of prey, predators were found in high abundance in this area (Brown, & Brown, 1984).

Macroinvertebrates are a sensitive organisms used universally in stream ecology to determine water quality. Michigan's procedure 51, is a rapid bioassessment of quality (MDEQ, 1990). The procedure calls for a timed sampling of macroinvertebrates using a D-net. Time and effort are focused on the dominant substrate present. However, this sampling can be subjective considering the more sensitive macroinvertebrates are found in the larger more stable substrates (Duan et. al., 2009). After sampling has occurred, macroinvertebrates are identified to the family level. Nine metrics (percent abundance of EPT and number of taxa) are used to score water quality. The sensitive macroinvertebrates are mayflies, (*Ephemeroptera*) stoneflies, (*Plecoptera*) and caddisflies (*Trichoptera*). This sampling is a rapid assessment of quality and should be used for an initial observation. Further quantitative sampling should take place to further understand the river.

The United States Environmental Protective Agency (USEPA) have many methods for rapidly assessing benthic macroinvertebrates. For single habitats they suggest sampling at least a 100-meter stretch using a one-meter kick net. Chemical and physical data is taken concurrently to ensure no chemical pollutants are present (Barbour, 1999). In comparison to USEPA's protocol, Brua et. al. (2011) compared quantitatively if there were similarities between U nets and kick nets. The consensus was that U nets and kick nets were similar, but a larger sample size was needed in order to pool quantitative data (Brua et. al., 2011). Sampling multiple substrates can be done with a Dip net (D-net). Substrates like cobble, sand, banks, snags, and vegetation (Barbour, 1999). Other standard sampling techniques and other samplers are specified by USEPA. They explain how to use a Hess Sampler, Surber Sampler, and rectangular d-net (Barbour, 1999). Both protocols outlined are similar and useful for sampling multiple habitats and substrates.

Hess and Surber samplers are used for larger substrate like gravel. A Surber sampler is a square sampler that is put into the substrate with a catchment net attached to the back. These samplers were considered biased because of the opened front allowing larger benthic macroinvertebrates to immigrate out of the sampler (Canton & Chadwick, 1984). The sampler did not stop accidental drift of macroinvertebrates. Hess samplers are the preferred method due to its round shape which could be pushed into the substrate with relative ease. This sampler also was completely encased so that macroinvertebrates could not crawl out of the front (Canton & Chadwick, 1984).

Another important form of sampling for macroinvertebrates is using drift nets for accidental drifting macroinvertebrates. Drift nets are placed in the water and macroinvertebrates are calculated by grams per hour (Waters, 1965). Drift is conducted over a 24-hour period which shows key hours of drift. Migration at night protects the macroinvertebrates from predation from fish (Leung et al., 2009). Drift occurs in two forms, catastrophic drift, and normal behavioral drift. Catastrophic drift is characterized as large amounts of discharge displacing benthic macroinvertebrates (Gibbins et al., 2007). Behavioral drift occurs when a food source is limiting to benthic macroinvertebrates will suspend themselves in the water column in the cover of darkness (Gibbins et. al., 2007).

The above-mentioned samplers are commonly used for quantitative sampling in benthic ecology. Czerniawska-Kusza (2004) found that the use of artificial substrate sampler could determine preference of substrates. Artificial substrate samplers allow for drifting macroinvertebrates either catastrophic or behavioral to land on the different substrates. Rosenberg and Resh (1982) found that artificial substrate samplers were biased towards stoneflies. Letovsky et al. (2012) wanted to determine if Hester-Dendy samplers could be used to pool benthic data. Overall, pooling data could result in the loss of patch dynamics in a river (Letovsky et al., 2012). Artificial substrate samplers are important for understanding how benthic macroinvertebrates colonize the river. However, they should not be included in other quantitative data due to the loss of importance of substrate preference.

Trout Ecology

Brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) have specific habitat requirements. *S. fontinalis* and *S. trutta* are considered a coldwater species with specific thermal regimes. Optimal temperatures, according to the Forest Service, is approximately 13°C to 16°C, however they can be found in temperatures of 20°C to 24°C (Chadwick & McCormick, 2017; Belica, 2007; Ficke, 2009; Ficke, Peterson, & Janowsky, 2009). This higher range is very stressful to the fish and can lead to high mortality. *S. fontinalis* needs deep pools, woody debris, and tree coverage from riparian zone to thrive (Alexander & Hansen, 1986; Valerie & Daniels, 2021).

Cover is important because it provides habitat for warmer days in the summer, and provides protection from other predators (Raleigh 1982, Raleigh et al. 1984). Cover is classified as brush, tree cover, undercut banks, or CWD. The presence of deep pools and areas of coverage for *S. fontinalis* are crucial for their survival. Without this habitat present, *S. fontinalis* is easily preyed upon by *S. trutta*. It is documented that small *S. fontinalis* make up the diet of large *S. trutta* (Alexander, 1977). This is especially true since this was written about the North Branch back in 1977 when large predatory *S. trutta* were more prevalent. Both trout species are considered opportunistic or generalist feeders. Typically, trout will feed on drifting macroinvertebrates, terrestrial invertebrates, and small fish (Belica 2007; Fiske, 2009; Sweka & Hartman, 2008).

Sediment Sources

The Intermediate Disturbance Hypothesis states that disturbances like drought or flooding (in intermediate amounts) displaces flora and fauna which can improve an ecosystem (Lake, 2000; Resh et.al., 1988). Natural disturbance is good for a system because it creates new areas for colonization. Lake (2000) looked at the different disturbances of flow regimes (low and high). Lake (2000) mentioned that disturbance can be difficult to quantify for comparison. Townsend and Hildrew (1994) say that the smallest amount of displacement can be considered disturbance. Lake (2000) quantified disturbance for a more universally comparison. The hypothesis states that at intermediate levels of disturbance are beneficial to a river (Lake, 2000; Resh et. al., 1988; Townsend & Hildrew, 1994). Large amounts of flow with sediment are not beneficial in any stream. There are many forms of sedimentation that occur, and various sources in ecosystems.

One possible source of sedimentation is the American beaver (*Castor canadensis*). In the 1800's beaver pelts were important to the fur trade and the population was decimated (Zorn & Sendek, 2001). However, management of *C. canadensis* have helped the population recover. Gurnell writes that *C. canadensis* can colonize rapidly if resources are abundant. Resources like trees, woody vegetation, and the lack of predators. *C. canadensis* have also been known to reside in ponds, wetlands, large and small rivers. The North Branch is a wide and shallow stream with a well-defined riparian zone. A food resource and a great place for *C. canadensis* to burrow and create dams. *C. canadensis* prefers sand and soft substrate over a gravel substrate (Gurnell, 1998). Beaver dams can trap sediment and other allochthonous input. When populations are eliminated dams may fail leading to large sediment slugs, causing extreme damage and excessive sedimentation (Butler & Malanson, 2005). Alternatively, *C. canadensis* may burrow into the banks of the river. Increased activity could be a possible cause of sedimentation (Gurnell, 1998).

The banks of the North Branch are well developed by large tree species, which help trap and stabilize the sediment. Presence of these large tree species is important to the river health and function. *C. canadensis* has shown to increase the diversity of the riparian zone, establishing a better food source (Wright & Flecker, 2002).

Sources of sediment come from anthropogenic activity surrounding the river. Sedimentation can occur from construction sites not following guidelines limiting erosion. Lenat et. al. (1981) documented the variation in streams from construction runoff and their effects on stream benthos. They determined high velocity streams; macroinvertebrates need stable substrates. Periods of high flow in mostly sandy bottom streams led to no colonization. However, areas with low flow (more stable) had macroinvertebrates with high reproduction rates and high mobility were able to colonize these areas (Lenat et. al., 1981).

Wang et. al. (2013) looked at post construction sites and observed the sediment load. They determined that heavy rainfall would input large amounts of sand into the system. When construction sites are moving large amounts of substrate without the proper protocols leads to severe effects to the ecosystem. Wang et. al. (2013) found that there are lasting effects of post construction sediment including 7 months of time to return to a reasonable amount of sediment in a stream.

Sedimentation Effects and Management

Sedimentation is present, first as suspended solids with no lasting affects in low amounts of sediment. In high amounts sedimentation fills suitable habitat, such as coarse stone which is habitat for macroinvertebrates (Chutter, 1969). Jowett (2003) determined that increased instability of substrates have lower abundances of macroinvertebrates. However, in low velocity streams sand has higher recruitment than it would in faster velocity streams. (Waters, 1984). It is well document in literature that sedimentation causes issues to macroinvertebrate abundance and diversity (Luedtke & Brusven, 1976; Zweig & Rabeni, 2001; Townsend & Scarsbrook, 1997). Managing for sedimentation can lead to better water quality and overall a more functional river.

In a 15-year study done on Michigan's Hunt Creek, Alexander and Hansen (1986) found that sedimentation can have adverse effects on trout. They found increased sedimentation can lead to reduced habitat that is readily available to trout. Sedimentation fills in good substrate for spawning fish, and deep pools, where trout reside in the warmer summer months (Alexander and Hansen, 1986). Another study by Alexander and Hansen (1983) showed sedimentation can fill in deep pools and other substrate. Sedimentation decreases diversity and abundance by creating a uniform with a highly erodible surface. The increased sedimentation, lack of habitat, predation, and loss of habitat are all important factors in which we need to observe.

This review looks at the current and past methodology of sedimentation and the effects on benthic ecology and trout populations. Sedimentation has lasting impacts on the ecology of rivers. It can alter the benthic community decreasing biodiversity and abundance. As a food source for trout, it is important that they are in high abundance. Trout habitat is altered which increases water temperature and decreases available habitat. Future management for sedimentation should reduce the sources such as construction (urbanization) logging, and unkept roads. Lee et al. (2000) shows the importance of buffer zones in controlling sediment runoff. Jones et al. (2012) shows the importance of macrophyte beds that trap sediment from complex rooting systems. Macrophytes create an area of lower velocity which can help deposit sediments around the beds (Jones et al., 2012). Lastly, the most important factor to consider is continued monitoring. Monitoring allows us to track important changes within the system acting as an early detection system.

Extended Methodology

Field Sampling

We quantified macroinvertebrate abundance on five substrates across five sites on the North Branch. A Hess sampler was used with approximately equal effort to quantify abundances on sand and gravel substrates. Working from downstream to upstream we placed the sampler in the substrate and disturbed that substrate with a shovel. Working from the outside of the sampler to the inside, substrate was disturbed and collected in the sampler. The sample extracted and transferred to 250 ml bottle with 70% ethanol to preserve the sample for later identification in the lab. This was carried out for both sand and gravel substrates across our five sites.

Coarse woody debris structures were located at each sample site. Three individual structures were sampled at each site representing our replicates. Loppers were used rather than a saw to reduce the loss of macroinvertebrates. We used branches with similar diameters ranging from 0.5 to 2 centimeters. Macroinvertebrates were hand-picked in the field and preserved in 70% ethanol for later identification.

Macrophytes beds were carefully selected and individually picked out for quantitative sampling. A Hess sampler was placed over the macrophyte bed, and a D-net was placed into the sampler. The macrophyte was plucked from the substrate and placed in the D-net (Alonso and Camargo (2010). The number of leaves were noted for surface area and the entire plant with macroinvertebrates were placed in a 250 ml bottle and preserved in 70% ethanol for later identification in the lab.

Soft sediment was sampled using an Eckman Dredge with a pole attachment. The Dredge was place on the top few centimeters of the sediment and collected. The sample was immediately sieved, and the remaining sample was transferred to a 250 ml bottle and preserved in 70% ethanol for later identification.

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Appendix A

Table 1. Individuals/m^2 on five substrates at Ford Road sample site.

	Ford Road				
Taxa	Gravel	Sand	CWD	Macrophytes	Soft Sediment
Ephemeroptera Heptageniidae epeorus	15.504				
Ephemeroptera Heptageniidae stenonema	232.558		761.145	33.508	
Ephemeroptera Ephemerellidae drunella	534.884		24.597		
Ephemeroptera Ephemerellidae ephemerella (A)	50.388		687.974		
Ephemeroptera Ephemerellidae Ephemerella (adult A)					
Ephemeroptera Ephemerllidae Ephemerella (B)	27.132		2521.193		
Ephemeroptera Ephemerellidae Teloganopsis deficiens	135.659		1077.593		13.889
Ephemeroptera Ephemerellidae Eurylophella		3.876			
Ephemeroptera Ephemeridae Ephemera	7.752	15.504	24.597		
Ephemeroptera Baetidae Baetis	89.147	23.256	538.923		
Ephemeroptera Caenidae Caenis					13.889
Ephemeroptera Leptohyphidae Tricorythodes					
Ephemeroptera (adult Poor)					
Ephemeroptera Baetiscidae Baetisca		7.752			13.889
Ephemeroptera Leptophlebiidae Leptophlebia	7.752				
Plecoptera Perlidae Paragnetina	7.752		43.562		
Plecoptera nemouridae Podmosta			45.033		
Plecoptera Perlodidae Isoperla	3.876		856.589		
Plecoptera Chloroperlidae Early instar					
Perlodidae unable to ID	3.876				
Trichoptera Helicopsychidae Helicopsyche (larva)	135.632		43.562		
Trichoptera Helicopsychidae Helicopsyche (pupa)					
Trichoptera Hydropsychidae Hydropsyche (larva)	81.395		9812.321	33.508	

Trichoptera Hydropsychidae (pupa)	27.132		90.066		
Trichoptera Glassosomatidae Protoptila (larva)	3.876				
Trichoptera Glassosomatidae (pupa)					
Trichoptera Glossosomatidae Glossosoma	15.504				
Trichoptera Brachycentridae Brachycentrus	3.876		224.911	101.556	
Trichoptera Brachycentridae pupa	3.876				
Trichoptera Brachycentridae Micrasema	19.380	7.752	90.066	47.382	
Trichoptera Leptoceridae Oecetis	23.256		2789.096		13.889
Trichoptera Leptoceridae Setodes					
Trichoptera Leptoceidae Mystacides		11.628			305.556
Trichoptera Lepidostomatidae Lepidostoma	3.876	3.876			
Trichoptera Lepidostomatidae Pupa	3.876				
Trichoptera Limnephilidae Anabolia					
Trichoptera Limnephilidae Pycnopsyche		3.876		47.382	
Trichoptera Rhyacophilidae Rhyacophila					
Trichoptera Hydroptilidae Hydroptila	120.155		175.718		
Trichoptera Hydroptila pupa	34.884				
Trichoptera Hyroptila Oxyethira			24.597		
Trichoptera Thremmatidae Neophylax	15.504		1339.967		
Trichoptera Polycentripodidae Nyctiophylax			180.131		
Trichoptera Polycentripodidae Cynellus fraternus					
Trichoptera Philopotamidae Chimarra	77.519		69.629		
Trichoptera Philopotamidae Dolophilodes					
Trichoptera Psychomiidae Tinodes					
Trichoptera Sericostomatidae Agarodes		3.876			
Trichoptera (unable to ID)					
Hemiptera Cicadellidae					
Hymenoptera Formicidae					

Diptera Chironomidae (large)	46.512	93.023			666.667
Diptera Chironomidae (small)	585.271	364.341	10628.197	33.508	4347.222
Diptera Chironomidae (pupa)	62.016	38.760	203.257		194.444
Diptera Chironomidae (adult)		3.876			
Diptera Empididae hemerodromia	62.016	3.876	135.099		
Diptera Empididae metachela					
Diptera Empididae pupa	3.876		45.033		
Diptera Athericidae atherix					
Diptera Simuliidae (Adult)					
Diptera Simuliidae simulium (pupa)			114.662		
Diptera Simuliidae simulium (larve)	19.380		1761.499	128.272	
Diptera Ceratopogonidae bezzia		7.752			333.333
Diptera Ceratopogonidae Ceratopogon					
Diptera Tabanidae (poor condition)					
Diptera Tabanidae chrysops	3.876				27.778
Diptera Tabanidae Tabanus		7.752			
Diptera Limoniidae Pilaria					
Diptera Limoniidae Antocha					
Diptera pupa (unknown)					
Coleoptera Elmidae Dubiraphia (larvae)		19.380			152.778
Coleoptera Elmidae Macronychus (larvae)	162.697		878.750		
Coleoptera Elmidae Macronychus (adult)	11.628				
Coleoptera Elmidae Optioservus	58.140				
Coleoptera Elmidae Ordobrevia	135.659		45.033		
Coleoptera Curculionidae					
Odonata Gomphidae (early instar)					
Odonata Gomphidae Ophiogomphus	23.256				
Odonata Gomphidae Dromogomphus (larvae)	3.876				27.778

Odonata Calopterygidae Calopteryx			45.033		
Megaloptera Corydalidae Nigronia					
Annelida Hirudinea (3-6 cm)					
Annelida Hirudinea (over an inch)					
Annelidae Oligochaeta (large)	69.767				13.889
Annelidae Oligochaeta (Small)	174.419	65.891			125.000
Gastropoda Physidae					
Gastropoda Viviparidae					
Gastropoda Hydrobiidae	89.147	65.891			55.556
Gastropoda Planoridae (large)					27.778
Gastropoda Tateidae	42.636	50.388			
Mollusca Sphaeriidae (large)	19.380	31.008			291.667
Mollusca Sphaeriidae (medium)	69.767	54.264	45.033		305.556
Mollusca Sphaeriidae (small)	19.380	100.775			263.889
Trombidiformes Hydracarina	11.628	3.876			
Isopoda Asellidae (large)					
Isopoda Asellidae (small)					
Amphipoda Gammaridae (Large)					
Amphipoda Gammaridae (small)		7.752	45.033		97.222
Cyclopoida					13.889
Cypriniformes cyprinidae	410.853				
Araneae Pisauridae Dolomedes					

Table 2. Individuals/m^2 on five substrates at Twin Bridges sample site.

	Twin Bridges				
Taxa	Gravel	Sand	CWD	Macrophytes	Soft Sediment
Ephemeroptera Heptageniidae epeorus	38.76		333.57		
Ephemeroptera Heptageniidae stenonema	62.02		295.36		
Ephemeroptera Ephemerellidae drunella	1065.89	3.88	933.16	311.52	
Ephemeroptera Ephemerellidae ephemerella (A)	1224.81		4123.25	89.77	
Ephemeroptera Ephemerellidae Ephemerella (adult A)					
Ephemeroptera Ephemerllidae Ephemerella (B)			94.36		
Ephemeroptera Ephemerellidae Teloganopsis deficiens	116.28		823.86		13.89
Ephemeroptera Ephemerellidae Eurylophella					
Ephemeroptera Ephemeridae Ephemera	3.88				
Ephemeroptera Baetidae Baetis	96.90		569.57	153.14	
Ephemeroptera Caenidae Caenis					
Ephemeroptera Leptohyphidae Tricorythodes					
Ephemeroptera (adult Poor)					
Ephemeroptera Baetiscidae Baetisca					
Ephemeroptera Leptophlebiidae Leptophlebia					
Plecoptera Perlidae Paragnetina			39.81		
Plecoptera nemouridae Podmosta	3.88				
Plecoptera Perlodidae Isoperla	7.75	3.88	57.40		
Plecoptera Chloroperlidae Early instar					
Perlodidae unable to ID					
Trichoptera Helicopsychidae Helicopsyche (larva)	3449.61	271.32		62.90	
Trichoptera Helicopsychidae Helicopsyche (pupa)	65.89	174.42			
Trichoptera Hydropsychidae Hydropsyche (larva)	236.43	3.88	2510.15	158.84	
Trichoptera Hydropsychidae (pupa)	42.64				
Trichoptera Glassosomatidae Protoptila (larva)	147.29	3.88			

Trichoptera Glassosomatidae (pupa)	15.50				
Trichoptera Glossosomatidae Glossosoma	38.76				
Trichoptera Brachycentridae Brachycentrus	139.53		1651.10	5787.49	
Trichoptera Brachycentridae pupa					
Trichoptera Brachycentridae Micrasema	286.82		2302.08	134.66	
Trichoptera Leptoceridae Oecetis	7.75		148.57		
Trichoptera Leptoceridae Setodes	131.78	3.88			
Trichoptera Leptoceidae Mystacides	3.88	3.88	17.59		
Trichoptera Lepidostomatidae Lepidostoma	54.26	7.75			
Trichoptera Lepidostomatidae Pupa					
Trichoptera Limnephilidae Anabolia					
Trichoptera Limnephilidae Pycnopsyche					
Trichoptera Rhyacophilidae Rhyacophila					
Trichoptera Hydroptilidae Hydroptila					
Trichoptera Hydroptila pupa					
Trichoptera Hyroptila Oxyethira					
Trichoptera Thremmatidae Neophylax	3.88				
Trichoptera Polycentripodidae Nyctiophylax					
Trichoptera Polycentripodidae Cynellus fraternus			55.09		
Trichoptera Philopotamidae Chimarra	11.63				
Trichoptera Philopotamidae Dolophilodes					
Trichoptera Psychomiidae Tinodes					
Trichoptera Sericostomatidae Agarodes					
Trichoptera (unable to ID)					
Hemiptera Cicadellidae					
Hymenoptera Formicidae					
Diptera Chironomidae (large)	3089.15				597.22
Diptera Chironomidae (small)	674.42	31.01	8411.33	1342.93	6125.00

Diptera Chironomidae (pupa)	50.39		73.57		125.00
Diptera Chironomidae (adult)					
Diptera Empididae hemerodromia	100.78		62.38		
Diptera Empididae metachela	7.75				
Diptera Empididae pupa	65.89				
Diptera Athericidae atherix	232.56		40.70		
Diptera Simuliidae (Adult)					
Diptera Simuliidae simulium (pupa)	7.75		478.21	8145.80	
Diptera Simuliidae simulium (larve)			3258.98	6348.57	
Diptera Ceratopogonidae bezzia	11.63				27.78
Diptera Ceratopogonidae Ceratopogon					
Diptera Tabanidae (poor condition)					
Diptera Tabanidae chrysops					
Diptera Tabanidae Tabanus	34.88				
Diptera Limoniidae Pilaria					
Diptera Limoniidae Antocha	11.63		79.08		
Diptera pupa (unknown)					
Coleoptera Elmidae Dubiraphia (larvae)					
Coleoptera Elmidae Macronychus (larvae)	620.16	7.75	826.54		
Coleoptera Elmidae Macronychus (adult)	116.28		97.21		
Coleoptera Elmidae Optioservus	27.13		17.59		
Coleoptera Elmidae Ordobrevia	42.64				
Coleoptera Curculionidae					
Odonata Gomphidae (early instar)	3.88				
Odonata Gomphidae Ophiogomphus	7.75				
Odonata Gomphidae Dromogomphus (larvae)					
Odonata Calopterygidae Calopteryx					
Megaloptera Corydalidae Nigronia	3.88				

Annelida Hirudinea (3-6 cm)					27.78
Annelida Hirudinea (over an inch)					
Annelidae Oligochaeta (large)	174.42	7.75			
Annelidae Oligochaeta (Small)	46.51	7.75			555.56
Gastropoda Physidae					
Gastropoda Viviparidae					
Gastropoda Hydrobiidae					
Gastropoda Planoridae (large)					
Gastropoda Tateidae					
Mollusca Sphaeriidae (large)		7.75			138.89
Mollusca Sphaeriidae (medium)	3.88	34.88			402.78
Mollusca Sphaeriidae (small)		31.01	35.19		180.56
Trombidiformes Hydracarina	31.01		20.79		
Isopoda Asellidae (large)	7.75				27.78
Isopoda Asellidae (small)	3.88				652.78
Amphipoda Gammaridae (Large)	3.88				27.78
Amphipoda Gammaridae (small)	3.88				55.56
Cyclopoida					55.56
Cypriniformes cyprinidae					
Araneae Pisauridae Dolomedes					

Table 3. Individuals/m^2 on five substrates at Copper Fisherman sample site.

	Copper Fisherman				
Taxa	Gravel	Sand	CWD	Macrophytes	Soft Sediment
Ephemeroptera Heptageniidae epeorus	7.75				
Ephemeroptera Heptageniidae stenonema			31.86		
Ephemeroptera Ephemerellidae drunella	639.53		377.33	53.56	
Ephemeroptera Ephemerellidae ephemerella (A)	1779.07		3556.66	538.29	
Ephemeroptera Ephemerellidae Ephemerella (adult A)					
Ephemeroptera Ephemerllidae Ephemerella (B)	3.88		54.24	159.38	
Ephemeroptera Ephemerellidae Teloganopsis deficiens	46.51		216.94		
Ephemeroptera Ephemerellidae Eurylophella					
Ephemeroptera Ephemeridae Ephemera	54.26	3.88			
Ephemeroptera Baetidae Baetis	34.88	3.88	965.92		
Ephemeroptera Caenidae Caenis					
Ephemeroptera Leptohyphidae Tricorythodes					
Ephemeroptera (adult Poor)			54.24		
Ephemeroptera Baetiscidae Baetisca					
Ephemeroptera Leptophlebiidae Leptophlebia	3.88				
Plecoptera Perlidae Paragnetina	3.88		30.66		
Plecoptera nemouridae Podmosta					
Plecoptera Perlodidae Isoperla	3.88		655.71		
Plecoptera Chloroperlidae Early instar					
Perlodidae unable to ID					
Trichoptera Helicopsychidae Helicopsyche (larva)	465.12	228.68	61.32		
Trichoptera Helicopsychidae Helicopsyche (pupa)	7.75	19.38			
Trichoptera Hydropsychidae Hydropsyche (larva)	15.50		482.32	451.99	

Trichoptera Hydropsychidae (pupa)	3.88				
Trichoptera Glassosomatidae Protoptila (larva)	31.01				
Trichoptera Glassosomatidae (pupa)	23.26				
Trichoptera Glossosomatidae Glossosoma	7.75			53.56	
Trichoptera Brachycentridae Brachycentrus	31.01		2525.51	2961.24	
Trichoptera Brachycentridae pupa					
Trichoptera Brachycentridae Micrasema	27.13	3.88	1499.86	159.38	
Trichoptera Leptoceridae Oecetis					
Trichoptera Leptoceridae Setodes	3.88	7.75			
Trichoptera Leptoceidae Mystacides					
Trichoptera Lepidostomatidae Lepidostoma	7.75				
Trichoptera Lepidostomatidae Pupa					
Trichoptera Limnephilidae Anabolia					
Trichoptera Limnephilidae Pycnopsyche					
Trichoptera Rhyacophilidae Rhyacophila	15.50				
Trichoptera Hydroptilidae Hydroptila					
Trichoptera Hydroptila pupa					
Trichoptera Hyroptila Oxyethira					
Trichoptera Thremmatidae Neophylax					
Trichoptera Polycentripodidae Nyctiophylax					
Trichoptera Polycentripodidae Cynellus fraternus			63.72		
Trichoptera Philopotamidae Chimarra					
Trichoptera Philopotamidae Dolophilodes					
Trichoptera Psychomiidae Tinodes			126.24		
Trichoptera Sericostomatidae Agarodes		3.88			
Trichoptera (unable to ID)					
Hemiptera Cicadellidae					
Hymenoptera Formicidae					

Diptera Chironomidae (large)	34.88	11.63			569.44
Diptera Chironomidae (small)	186.05	93.02	5232.70	961.00	10041.67
Diptera Chironomidae (pupa)	42.64	46.51	62.52		319.44
Diptera Chironomidae (adult)					
Diptera Empididae hemerodromia					
Diptera Empididae metachela					
Diptera Empididae pupa	7.75		63.72		
Diptera Athericidae atherix	3.88		179.28		
Diptera Simuliidae (Adult)			31.86		
Diptera Simuliidae simulium (pupa)			867.77	2188.34	
Diptera Simuliidae simulium (larve)			9510.24	9845.87	
Diptera Ceratopogonidae bezzia	3.88	3.88			
Diptera Ceratopogonidae Ceratopogon					13.89
Diptera Tabanidae (poor condition)					
Diptera Tabanidae chrysops					41.67
Diptera Tabanidae Tabanus		19.38			
Diptera Limoniidae Pilaria					27.78
Diptera Limoniidae Antocha					
Diptera pupa (unknown)					
Coleoptera Elmidae Dubiraphia (larvae)					
Coleoptera Elmidae Macronychus (larvae)	34.88		155.70		
Coleoptera Elmidae Macronychus (adult)	19.38		31.86		
Coleoptera Elmidae Optioservus	3.88				
Coleoptera Elmidae Ordobrevia					
Coleoptera Curculionidae					
Odonata Gomphidae (early instar)					
Odonata Gomphidae Ophiogomphus	3.88			69.93	
Odonata Gomphidae Dromogomphus (larvae)					

Odonata Calopterygidae Calopteryx					
Megaloptera Corydalidae Nigronia					
Annelida Hirudinea (3-6 cm)					
Annelida Hirudinea (over an inch)					
Annelidae Oligochaeta (large)	73.64	7.75			138.89
Annelidae Oligochaeta (Small)	27.13	42.64		69.93	361.11
Gastropoda Physidae	3.88				
Gastropoda Viviparidae					
Gastropoda Hydrobiidae					
Gastropoda Planoridae (large)					
Gastropoda Tateidae					
Mollusca Sphaeriidae (large)		15.50			125.00
Mollusca Sphaeriidae (medium)		62.02			166.67
Mollusca Sphaeriidae (small)		96.90			208.33
Trombidiformes Hydracarina					
Isopoda Asellidae (large)					152.78
Isopoda Asellidae (small)					1763.89
Amphipoda Gammaridae (Large)					
Amphipoda Gammaridae (small)	3.88				13.89
Cyclopoida					
Cypriniformes cyprinidae					27.78
Araneae Pisauridae Dolomedes	3.88				

Table 4. Individuals/m^2 on five substrates at Dam 4 Road sample site.

	Dam 4 Road				
Taxa	Gravel	Sand	CWD	Macrophytes	Soft Sediment
Ephemeroptera Heptageniidae epeorus			199.52		
Ephemeroptera Heptageniidae stenonema			228.49		
Ephemeroptera Ephemerellidae drunella	1065.89	50.39	1245.56	110.19	
Ephemeroptera Ephemerellidae ephemerella (A)	391.47		2656.59		
Ephemeroptera Ephemerellidae Ephemerella (adult A)	112.40				
Ephemeroptera Ephemerllidae Ephemerella (B)	23.26		788.59		
Ephemeroptera Ephemerellidae Teloganopsis deficiens	19.38		1401.80		
Ephemeroptera Ephemerellidae Eurylophella					
Ephemeroptera Ephemeridae Ephemera	3.88				
Ephemeroptera Baetidae Baetis	15.50	3.88	637.52		
Ephemeroptera Caenidae Caenis					
Ephemeroptera Leptohyphidae Tricorythodes		7.75			
Ephemeroptera (adult Poor)					
Ephemeroptera Baetiscidae Baetisca					
Ephemeroptera Leptophlebiidae Leptophlebia	3.88		35.80		
Plecoptera Perlidae Paragnetina					
Plecoptera nemouridae Podmosta			35.80		
Plecoptera Perlodidae Isoperla	3.88		466.98		
Plecoptera Chloroperlidae Early instar					
Perlodidae unable to ID					
Trichoptera Helicopsychidae Helicopsyche (larva)	189.92	31.01		110.19	
Trichoptera Helicopsychidae Helicopsyche (pupa)	3.88				
Trichoptera Hydropsychidae Hydropsyche (larva)	27.13		2037.43	66.81	

Trichoptera Hydropsychidae (pupa)					
Trichoptera Glassosomatidae Protoptila (larva)	7.75				
Trichoptera Glassosomatidae (pupa)					
Trichoptera Glossosomatidae Glossosoma					
Trichoptera Brachycentridae Brachycentrus			3092.46	286.17	
Trichoptera Brachycentridae pupa					
Trichoptera Brachycentridae Micrasema	139.53	7.75	1737.59	396.37	
Trichoptera Leptoceridae Oecetis	3.88		114.24		
Trichoptera Leptoceridae Setodes					
Trichoptera Leptoceidae Mystacides					13.89
Trichoptera Lepidostomatidae Lepidostoma		3.88			
Trichoptera Lepidostomatidae Pupa					
Trichoptera Limnephilidae Anabolia					
Trichoptera Limnephilidae Pycnopsyche					
Trichoptera Rhyacophilidae Rhyacophila					
Trichoptera Hydroptilidae Hydroptila					
Trichoptera Hydroptila pupa					
Trichoptera Hyroptila Oxyethira					
Trichoptera Thremmatidae Neophylax					
Trichoptera Polycentripodidae Nyctiophylax					
Trichoptera Polycentripodidae Cynellus fraternus			1348.15		
Trichoptera Philopotamidae Chimarra					
Trichoptera Philopotamidae Dolophilodes	3.88				
Trichoptera Psychomiidae Tinodes					
Trichoptera Sericostomatidae Agarodes					
Trichoptera (unable to ID)					
Hemiptera Cicadellidae		3.88			
Hymenoptera Formicidae		3.88			

Diptera Chironomidae (large)	7.75	73.64	938.01		263.89
Diptera Chironomidae (small)	492.25	399.22	18260.35	1117.26	11319.44
Diptera Chironomidae (pupa)	34.88	104.65	337.17		
Diptera Chironomidae (adult)			42.64		
Diptera Empididae hemerodromia	27.13				
Diptera Empididae metachela					
Diptera Empididae pupa	11.63				
Diptera Athericidae atherix	34.88		341.09		
Diptera Simuliidae (Adult)			35.80		
Diptera Simuliidae simulium (pupa)			2579.20	2437.94	
Diptera Simuliidae simulium (larve)	3.88		74146.53	12681.17	
Diptera Ceratopogonidae bezzia	3.88				55.56
Diptera Ceratopogonidae Ceratopogon					13.89
Diptera Tabanidae (poor condition)					
Diptera Tabanidae chrysops					27.78
Diptera Tabanidae Tabanus	69.77	7.75			
Diptera Limoniidae Pilaria					
Diptera Limoniidae Antocha					
Diptera pupa (unknown)					
Coleoptera Elmidae Dubiraphia (larvae)					
Coleoptera Elmidae Macronychus (larvae)	275.19	23.26	173.46		13.89
Coleoptera Elmidae Macronychus (adult)	23.26		35.80		
Coleoptera Elmidae Optioservus					
Coleoptera Elmidae Ordobrevia	46.51				
Coleoptera Curculionidae					13.89
Odonata Gomphidae (early instar)					
Odonata Gomphidae Ophiogomphus	15.50				
Odonata Gomphidae Dromogomphus (larvae)					

Odonata Calopterygidae Calopteryx					
Megaloptera Corydalidae Nigronia					
Annelida Hirudinea (3-6 cm)					27.78
Annelida Hirudinea (over an inch)					13.89
Annelidae Oligochaeta (large)	143.41	379.84			1194.44
Annelidae Oligochaeta (Small)	89.15	279.07	290.61		944.44
Gastropoda Physidae					
Gastropoda Viviparidae					
Gastropoda Hydrobiidae					
Gastropoda Planoridae (large)					
Gastropoda Tateidae					
Mollusca Sphaeriidae (large)	3.88	23.26			208.33
Mollusca Sphaeriidae (medium)		31.01			652.78
Mollusca Sphaeriidae (small)		15.50			611.11
Trombidiformes Hydracarina					
Isopoda Asellidae (large)		7.75			277.78
Isopoda Asellidae (small)	3.88	11.63			7555.56
Amphipoda Gammaridae (Large)					41.67
Amphipoda Gammaridae (small)		42.64			888.89
Cyclopoida					
Cypriniformes cyprinidae					
Araneae Pisauridae Dolomedes					

Table 5. Individuals/m^2 on five substrates at Kellogg's Bridge sample site.

	Kellogg's Bridge				
Taxa	Gravel	Sand	CWD	Macrophytes	Soft Sediment
Ephemeroptera Heptageniidae epeorus	3.88		205.72		
Ephemeroptera Heptageniidae stenonema	15.50				
Ephemeroptera Ephemerellidae drunella	837.21	11.63	2020.17		
Ephemeroptera Ephemerellidae ephemerella (A)	864.34	3.88	8659.07	213.24	
Ephemeroptera Ephemerellidae Ephemerella (adult A)			27.02		
Ephemeroptera Ephemerllidae Ephemerella (B)	3.88		76.21		
Ephemeroptera Ephemerellidae Teloganopsis deficiens	11.63		516.12		
Ephemeroptera Ephemerellidae Eurylophella					
Ephemeroptera Ephemeridae Ephemera		3.88			
Ephemeroptera Baetidae Baetis	34.88	7.75	297.20		
Ephemeroptera Caenidae Caenis					
Ephemeroptera Leptohyphidae Tricorythodes		46.51			
Ephemeroptera (adult Poor)					
Ephemeroptera Baetiscidae Baetisca					
Ephemeroptera Leptophlebiidae Leptophlebia					
Plecoptera Perlidae Paragnetina	3.88				
Plecoptera nemouridae Podmosta					
Plecoptera Perlodidae Isoperla			152.42		
Plecoptera Chloroperlidae Early instar	11.63				
Perlodidae unable to ID					
Trichoptera Helicopsychidae Helicopsyche (larva)	1023.26	27.13	34.29		
Trichoptera Helicopsychidae Helicopsyche (pupa)	69.77				
Trichoptera Hydropsychidae Hydropsyche (larva)	15.50		3296.32		

Trichoptera Hydropsychidae (pupa)	11.63				
Trichoptera Glassosomatidae Protoptila (larva)	170.54				
Trichoptera Glassosomatidae (pupa)					
Trichoptera Glossosomatidae Glossosoma					
Trichoptera Brachycentridae Brachycentrus	46.51		995.17	2759.57	
Trichoptera Brachycentridae pupa					
Trichoptera Brachycentridae Micrasema	100.78	3.88	3128.64	494.06	
Trichoptera Leptoceridae Oecetis					
Trichoptera Leptoceridae Setodes	27.13				
Trichoptera Leptoceidae Mystacides					
Trichoptera Lepidostomatidae Lepidostoma			34.29	67.02	
Trichoptera Lepidostomatidae Pupa					
Trichoptera Limnephilidae Anabolia					13.89
Trichoptera Limnephilidae Pycnopsyche					
Trichoptera Rhyacophilidae Rhyacophila					
Trichoptera Hydroptilidae Hydroptila	3.88				
Trichoptera Hydroptila pupa					
Trichoptera Hyroptila Oxyethira					
Trichoptera Thremmatidae Neophylax	3.88				
Trichoptera Polycentripodidae Nyctiophylax	3.88				
Trichoptera Polycentripodidae Cynellus fraternus			76.21		
Trichoptera Philopotamidae Chimarra					
Trichoptera Philopotamidae Dolophilodes					
Trichoptera Psychomiidae Tinodes					
Trichoptera Sericostomatidae Agarodes					
Trichoptera (unable to ID)					13.89
Hemiptera Cicadellidae					
Hymenoptera Formicidae					

Diptera Chironomidae (large)	27.13	19.38			444.44
Diptera Chironomidae (small)	468.99	131.78	7387.03	628.09	1736.11
Diptera Chironomidae (pupa)	34.88	7.75	34.29		41.67
Diptera Chironomidae (adult)					
Diptera Empididae hemerodromia	38.76	3.88	381.04		
Diptera Empididae metachela					
Diptera Empididae pupa	81.40	7.75			
Diptera Athericidae atherix	7.75		228.62		
Diptera Simuliidae (Adult)		3.88			
Diptera Simuliidae simulium (pupa)				50.68	
Diptera Simuliidae simulium (larve)	7.75	7.75	1215.79	1401.85	83.33
Diptera Ceratopogonidae bezzia	3.88				
Diptera Ceratopogonidae Ceratopogon					
Diptera Tabanidae (poor condition)					13.89
Diptera Tabanidae chrysops					
Diptera Tabanidae Tabanus	38.76				
Diptera Limoniidae Pilaria					
Diptera Limoniidae Antocha					
Diptera pupa (unknown)					97.22
Coleoptera Elmidae Dubiraphia (larvae)					
Coleoptera Elmidae Macronychus (larvae)	569.77	3.88	2179.49		13.89
Coleoptera Elmidae Macronychus (adult)	23.26	3.88	76.21		
Coleoptera Elmidae Optioservus					
Coleoptera Elmidae Ordobrevia	15.50				
Coleoptera Curculionidae					
Odonata Gomphidae (early instar)					
Odonata Gomphidae Ophiogomphus	23.26				
Odonata Gomphidae Dromogomphus (larvae)					

Odonata Calopterygidae Calopteryx					
Megaloptera Corydalidae Nigronia					
Annelida Hirudinea (3-6 cm)					111.11
Annelida Hirudinea (over an inch)					
Annelidae Oligochaeta (large)	96.90	19.38			527.78
Annelidae Oligochaeta (Small)	34.88	7.75			1347.22
Gastropoda Physidae	19.38				13.89
Gastropoda Viviparidae					55.56
Gastropoda Hydrobiidae	3.88				
Gastropoda Planoridae (large)					
Gastropoda Tateidae					
Mollusca Sphaeriidae (large)					55.56
Mollusca Sphaeriidae (medium)	3.88				222.22
Mollusca Sphaeriidae (small)					69.44
Trombidiformes Hydracarina	11.63		685.87		
Isopoda Asellidae (large)					69.44
Isopoda Asellidae (small)	7.75				583.33
Amphipoda Gammaridae (Large)					
Amphipoda Gammaridae (small)	7.75				
Cyclopoida					
Cypriniformes cyprinidae					
Araneae Pisauridae Dolomedes					

Appendix B

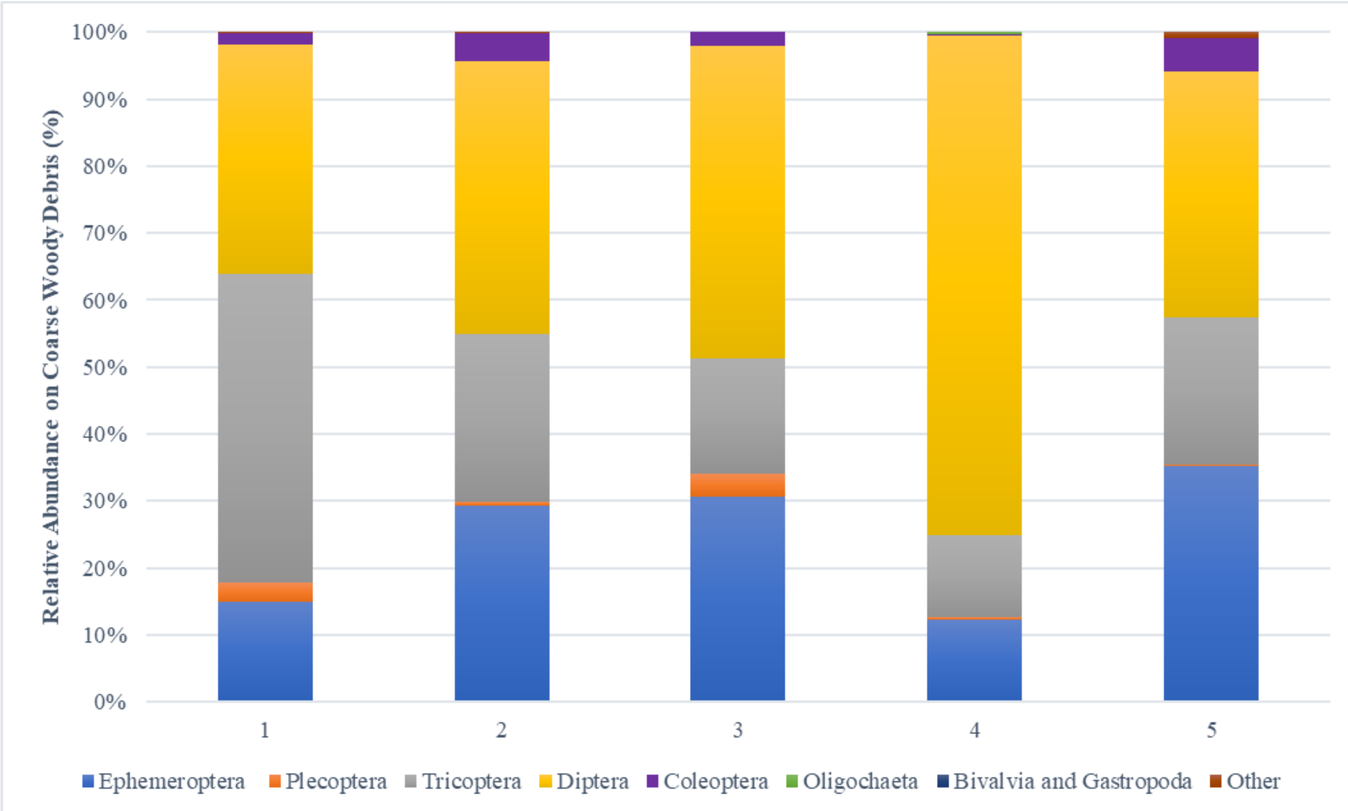

Figure 1. Relative abundance of major macroinvertebrates groups on coarse woody debris at 5 sample sites on the North Branch. Site 1 = Ford Road, site 2 = Twin Bridges, site 3 = Copper Fisherman, site 4 = Dam 4 Road, and site 5 = Kellogg's Bridge. 'Other' is a combination of Odonata, terrestrial invertebrates, Hydracarina, Isopoda, and Amhipoda.

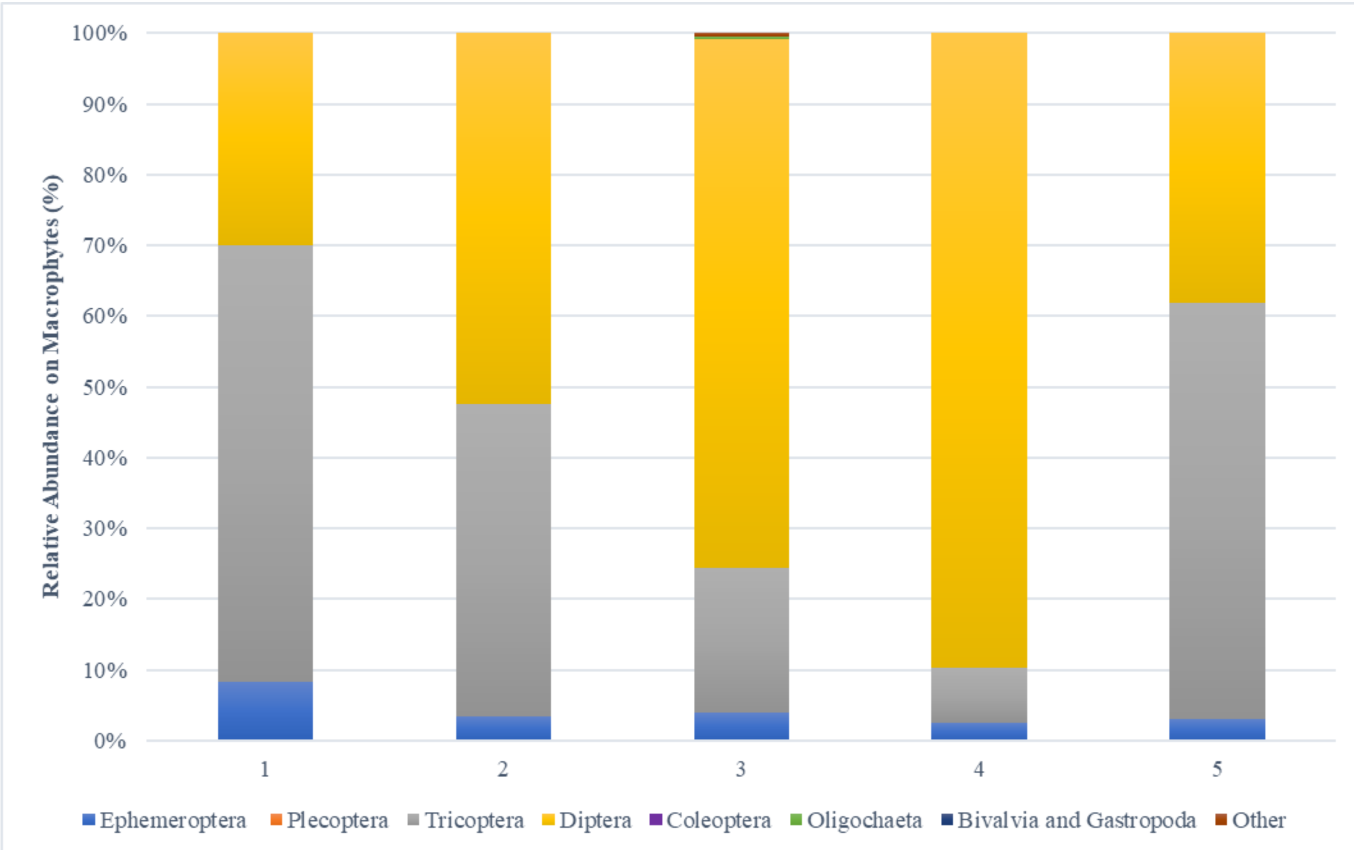

Figure 2. Relative abundance of major macroinvertebrates groups on macrophytes at 5 sample sites. Site 1 = Ford Road, site 2 = Twin Bridges, site 3 = Copper Fisherman, site 4 = Dam 4 Road, and site 5 = Kellogg's Bridge. 'Other' is a combination of Odonata, terrestrial invertebrates, Hydracarina, Isopoda, and Amhipoda.

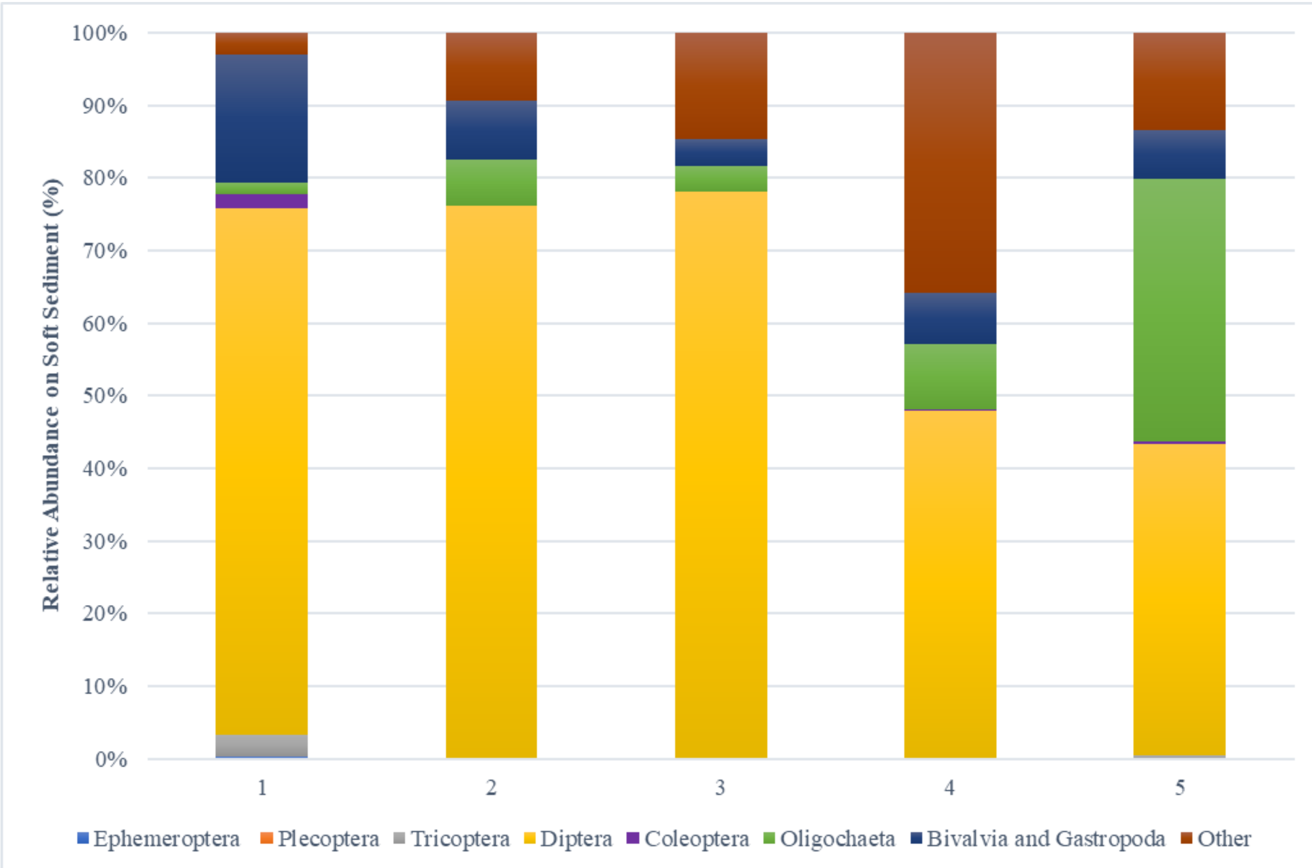

Figure 3. Relative abundance of major macroinvertebrates groups on soft sediment at 5 sample sites. Site 1 = Ford Road, site 2 = Twin Bridges, site 3 = Copper Fisherman, site 4 = Dam 4 Road, and site 5 = Kellogg's Bridge. 'Other' is a combination of Odonata, terrestrial invertebrates, Hydracarina, Isopoda, and Amhipoda.

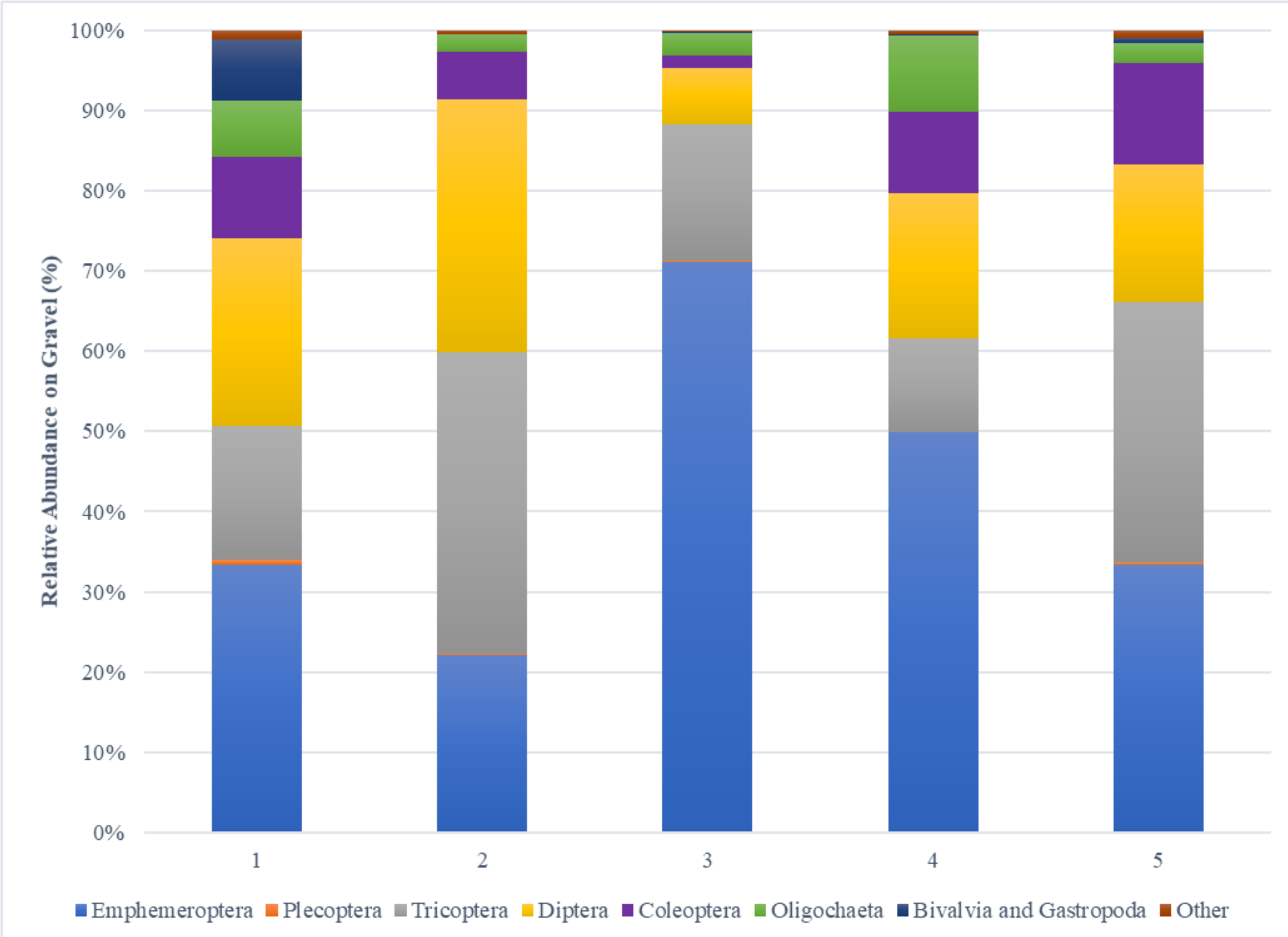

Figure 4. Relative abundance of major macroinvertebrates groups on gravel at 5 sample sites. Site 1 = Ford Road, site 2 = Twin Bridges, site 3 = Copper Fisherman, site 4 = Dam 4 Road, and site 5 = Kellogg's Bridge. 'Other' is a combination of Odonata, terrestrial invertebrates, Hydracarina, Isopoda, and Amhipoda.

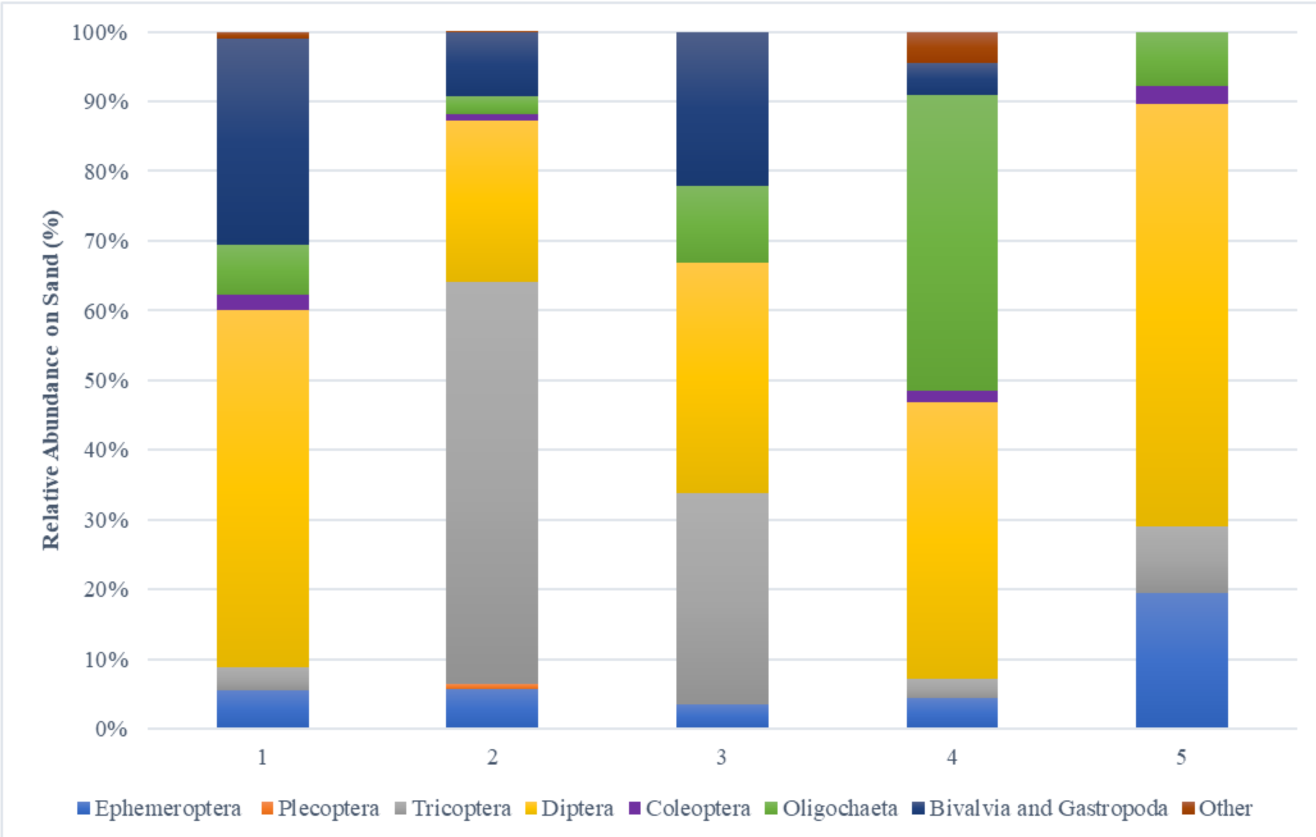

Figure 5. Relative abundance of major macroinvertebrates groups on sand at 5 sample sites. Site 1 = Ford Road, site 2 = Twin Bridges, site 3 = Copper Fisherman, site 4 = Dam 4 Road, and site 5 = Kellogg's Bridge. 'Other' is a combination of Odonata, terrestrial invertebrates, Hydracarina, Isopoda, and Amhipoda.