# EFFECT OF ABIOTIC FACTORS ON THE DIVERSITY AND ABUNDANCE OF AQUATIC MACROINVERTEBRATES IN THE EAST AND WEST BRANCHES OF THE MAPLE RIVER, MICHIGAN, USA

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**Abstract**

Streams are home to a wide variety of aquatic organisms including fish and benthic macroinvertebrates. Variance in diversity and abundance of macroinvertebrates in stream ecosystems is influenced by water chemistry and substrate availability. Different substrates provide macroinvertebrates with varying habitats, protection, and resources. Four distinct sites on the East and West branches of the Maple River around Pellston, MI were sampled and studied to examine the combined effects of abiotic factors on macroinvertebrate diversity and abundance. At each site, three substrates (cobble, gravel, and sand) were studied. Rocky riverbed substrates (i.e. gravel and cobble) contained greater numbers and more diverse macroinvertebrate families than sandy substrates. Stable isotope analysis of the water from each site showed a correlation between the abundance of individuals and groundwater concentration but did not show a correlation between diversity of families and groundwater concentration.

## 1 Introduction

Macroinvertebrates are abundant eukaryotic organisms in aquatic ecosystems and perform important functions including decomposition of organic matter and formation of the base of many aquatic food chains (Nelson, 2007). The presence and diversity of macroinvertebrates in streams is an indicator of water quality and overall stream health. Three orders of aquatic insects, Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) are particularly sensitive to anthropogenic pollution and unfavorable water conditions. As a result of this sensitivity, they are often the principal taxa studied in the investigation of stream water quality (Jerves-Cobo *et al.,* 2017). These taxa thrive in favorable conditions which include high dissolved oxygen (> 5 ppm), neutral pH, and cold water temperatures (Chadde, 2017). A majority of macroinvertebrates live in streams during their larval stage for up to one year, prior to eventual metamorphosis into terrestrial adult insects. However, some macroinvertebrates are aquatic throughout the entire life cycle (Berg *et al*., 2008).

Variable habitats within stream ecosystems create variation and complexity in macroinvertebrate communities to accommodate adaptations to survive and flourish (Bond *et al*., 2000). Abiotic factors such as stream flow rate, substrate type, water temperature, dissolved oxygen levels, stable isotope levels, and nutrient availability influence the distribution and richness of macroinvertebrate populations (Brooks *et al*., 2005). Abiotic factors that alter water quality are affected by the ambient environment through which a stream flows.

The Maple River is located in Emmet County in Michigan's lower peninsula and is part of the Cheboygan River watershed (Godby, 2014). The substrates of this region are influenced by glacial geology (Nadelhoffer *et al.,* 2010). The Maple River lies atop unsorted till deposited by glaciers, which allows for groundwater movement (Godby, 2014). The Maple River is primarily groundwater fed, with hydrologic sources including groundwater springs, marshes, and precipitation. The quality of water is influenced by anthropogenic sources including exogenous drainage and runoff (Zhang *et al*., 2015). The Maple River has two branches, each accumulating different nutrients and pollutants. The West Branch of the Maple River originates from the Pleasantview Swamp. It travels through woodlands, past the Pellston airport, and through the village of Pellston. The East Branch of the Maple River begins at the Douglas Lake outlet and meanders through woodlands until it connects with the West Branch at the Maple River Dam in Lake Kathleen (Godby, 2014). The Maple River Dam influences the water quality of the river by increasing the water temperature by as much as 3°C during summer (Godby, 2014).

The Conservation Resource Alliance (CRA) is pursuing the removal of the Maple River Dam, scheduled to begin in 2018 (CRA, 2017). Removal of the dam is predicted to result in a return of the river to pre-dam temperatures (Godby, 2014). An opportunity exists to gather data assessing the abiotic and biotic factors of the Maple River before and after the removal of the dam, including the effect it will have on the abundance and diversity of macroinvertebrates. The CRA plans to restore in-stream habitat at sites sampled in this study (CRA, 2017). Our findings regarding the preferred habitats of macroinvertebrates could be useful to the CRA in their efforts to improve the stream ecosystem.

In this study, we measured and analyzed abiotic factors of stream ecosystems at four different sites of the Maple River to examine patterns of macroinvertebrate diversity and abundance. Macroinvertebrates prefer gravel and cobble habitats located in riffles (fast, shallow water), over sand and silt habitats in pools of slow, deep water (Meng-zhen *et al.,* 2012; Beauger *et al.,* 2006; Duan *et al.,* 2008). Variations in substrate and hydrologic source produce differences in macroinvertebrate communities. Gravel has been shown to support greater macroinvertebrate populations (Meng-zhen *et al.,* 2012). Our first hypothesis is that gravel will have the greatest abundance and diversity of macroinvertebrate taxa of the three observed substrates.

Groundwater concentration can be determined using stable isotope analysis. Stable isotopes are atoms which are the same elements, but have a different number of neutrons and therefore mass. Stable water isotopes include $\sigma^{18}O$ for oxygen, which corresponds to $\sigma^{16}O$, and $\sigma^{2}H$ (or Deuterium, D), which corresponds to $\sigma^{1}H$ for hydrogen (Gat, 1996; Mook, 2001). Groundwater has a lower concentration of heavy isotopes ($\sigma^{2}H$ and $\sigma^{18}O$) than surface water (Nyende *et al.,* 2013).

Groundwater-fed streams tend to demonstrate more stable and less polluted environments. Temperature and water levels do not fluctuate as extensively in contrast to streams fed by runoff and precipitation. Interactions between groundwater and surface water influence dissolved oxygen concentration, nutrient levels, water temperature, and light intensity (Zimmer *et al*., 2015). However, little research has been conducted on the specific effects of groundwater concentration on macroinvertebrate communities. We hypothesize that higher concentrations of groundwater will be correlated with more diverse and abundant macroinvertebrate communities due to greater stability of water conditions.

## 2 Methods

### *2.1 Site Selection*

Twelve samples of macroinvertebrates were collected from four sites on the Maple River. Two sample sites were on the East Branch and two sample sites were on the West Branch (Figure 1). Site 1 is located on East Branch of the Maple River on Douglas Lake Road; Site 2 is located on the East Branch of the river at Robinson Road; Site 3 is located on the West Branch of the river at US 31; and Site 4 is located on the West Branch of the river by the Philip J. Braun Nature Reserve (Figure 1). A sample was taken from each of the three substrate types; sand, gravel, and cobble. Cobble is defined as rock larger than six centimeters diameter, gravel is defined as rock greater than one millimeter (0.01 cm) but less than six centimeters diameter, and sand is defined as any granules less than one millimeter (0.01 cm) diameter. Samples were taken of water with a fast flow rate ( > 45 cm/s) and a slow flow rate (< 45 cm/s) in each substrate on each branch of the Maple River.

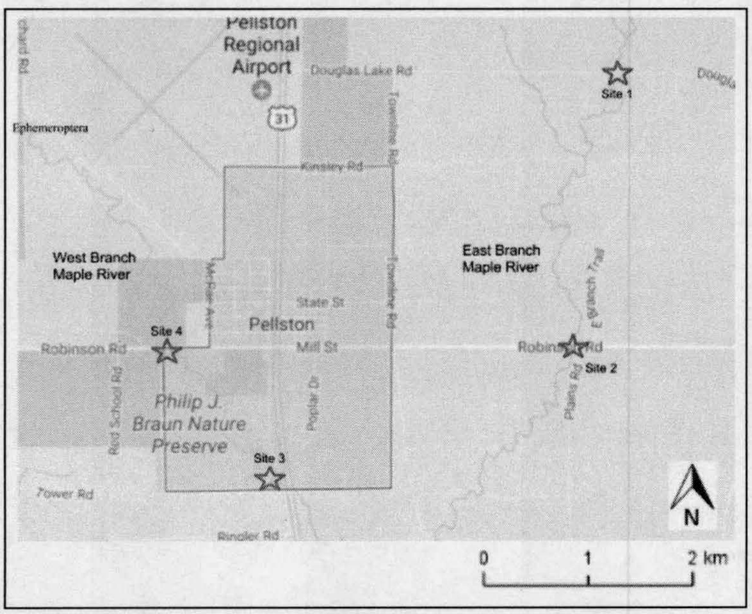


**Figure 1.** Site locations on the the East and West Branches of the Maple River in the Pellston, Michigan area. Specific sampling site locations are indicated by stars on the map. (Source: Google Maps, 2017).

*2.2 Sampling Technique - Water Chemistry*

Surface and groundwater samples were collected at all four sampling sites. Water samples were taken at upriver sites first to avoid contaminating downriver sites by agitating upriver substrates. To collect surface water, a Piezometer constructed by the University of Michigan Biological Station Chemistry Laboratory was used to pump the water from the river into rinsed acid-washed 500-milliliter plastic jars. The jars were filled to the brim and sealed to prevent evaporation. Groundwater was obtained by inserting a 50-centimeter section of Polyvinyl Chloride (PVC) pipe into the sandbank until water permeated the sand. The pipe was removed and emptied of sand, then re-inserted into the hole. Groundwater was collected from the pipe with a piezometer, using the same method as described above.

Dissolved oxygen (mg/L) measurements for each site were taken by slowly swirling a YSI Model 55 Dissolved Oxygen Probe in each jar of surface water. Water samples were then analyzed at the University of Michigan Biological Station Chemistry Lab for stable isotopic composition, nitrogen, and total hardness. Percent groundwater at each site was estimated using isotopic composition. The stable isotopes studied were hydrogen ($\sigma^{1}H$ and $\sigma^{2}H$, or Deuterium) and oxygen ($\sigma^{16}O$ and $\sigma^{18}O$).

*2.3 Sampling Technique - Macroinvertebrates*

For each of the twelve samples, a thin-mesh dip-net was used to collect macroinvertebrates. Macroinvertebrates were collected from two separate four-centimeter rocks in the gravel substrate, and from a single six-centimeter rock in the cobble substrate that represented the most typical rock size and morphology of the collection site. Macroinvertebrates were placed in a labeled plastic Nalgene jar containing a 70% Ethanol solution for preservation. At each sampling site, we measured pH using a pH multimeter and measured conductivity (μS) and water temperature (°C) using a conductivity probe. Depth (cm) and flow rate (cm/s) measurements were taken at each section of substrate sampled.

*2.4 Macroinvertebrate Classification*

The twelve macroinvertebrates samples were identified under a light microscope using a dichotomous key (Birmingham, 2005). Upon completion of macroinvertebrate identification, each family was separated into individual vials filled with 70% Ethanol solution for preservation. All were labeled with order and family name, collection location, substrate, and stream flow rate. The total number of macroinvertebrates and their respective families in each sample were determined and recorded.

*2.5 Calculations*

A statistical software was used to perform a Chi Square Test of Independence to compare the abundance of macroinvertebrates found in different substrates and hydrologic sources. The same method was utilized to compare the total number of families and indicator families at each site. A Biotic Index (BI) calculation was performed to determine the levels of stream pollution based on macroinvertebrate diversity and abundance. The BI categorizes macroinvertebrate orders based on sensitivity to organic pollution such as sewage and other oxygen-consuming contaminants (Sharpe *et al*., 2015). Macroinvertebrates collected were identified and assigned to either Class I (Pollution Sensitive Taxa), Class II (Moderately Tolerant Taxa) or Class III

(Pollution Tolerant Taxa). Pollution Sensitive Taxa include Ephemeroptera, Plecoptera, and Trichoptera; Moderately Tolerant Taxa include Amphipoda, Coleoptera, Megaloptera, and Odonata; and Pollution Tolerant Taxa include Diptera. The following BI computation was performed:

$$\textit{Biotic Index} = 2(n\ \textit{Class I}) + (n\ \textit{Class II})$$

Where *n* is equal to the number of taxa. If the result is greater than or equal to 10, the stream is considered "clean" with little to no pollution. A result between 3-9 indicates "moderate pollution," while a result between 0-2 indicates "gross pollution" (Sharpe *et al.*, 2015).

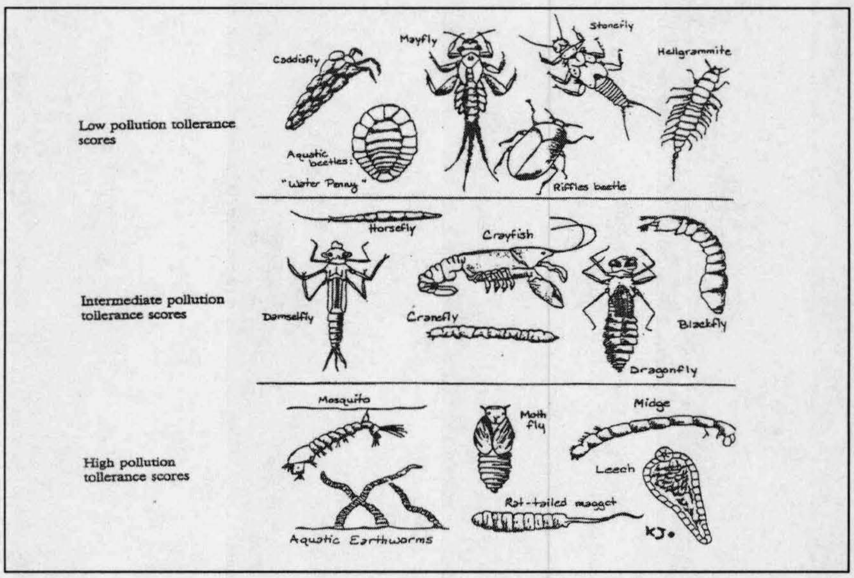


**Figure 2**. General pollution tolerance categories for common aquatic macroinvertebrates in the Maple River watershed (Source: Zimmerman, 1993).

Diversity indices were calculated to compare the diversity of macroinvertebrate communities in the three substrates. The Shannon Diversity Index (H) was calculated to compare the diversity of communities in the three substrates. Shannon's Equitability ($E_H$) was calculated to compare the evenness of the communities on the different substrates (Gross, 2000). The

Sørensen Index (S) was calculated to compare the similarity between substrates in families present. The Sørensen Index is a value between 0 and 1, with higher values indicating that the two communities are more similar and have a larger proportion of families in common (Krebs, 2014). These calculations were also used to compare the diversity of communities in high and low groundwater concentration samples.

An ANOVA test was performed to analyze the variance of abiotic factors between substrates, including temperature, conductivity, pH, depth, flow rate, dissolved oxygen, total hardness, and total nitrogen to determine if these factors could have affected our results. An Independent Samples t-Test was conducted to compare these same abiotic factors between sites with high and low groundwater concentration.

## 3 Results

### *3.1 Results by Site*

There were a total of 84 macroinvertebrates from 11 families collected at Site 1, while 179 macroinvertebrates from 15 families were collected at Site 2 (Table 1); 273 macroinvertebrates from 18 families were collected from Site 3; and 250 macroinvertebrates from 17 families were collected at Site 4 (Table 2). Site 3 had the greatest abundance of aquatic macroinvertebrates and highest richness of the four sites. There was an overall total of 786 macroinvertebrates collected from 25 different families, 19 of which were indicator families (Tables 1 and 2).

**Table 1.** Total collection of macroinvertebrates from Sites 1 and 2 on the East Branch of the Maple River, as indicated on the map (Figure 1). Indicator families are shown with an asterisk.

| SITE 1 | Sand (slow: 33.87 cm/s) | Gravel (slow: 22.04 cm/s) | Cobble (fast: 48.2 cm/s) |
|---|---|---|---|
| | 1 Amphipoda<br>2 Diptera<br>2 Trichoptera, Limnephilidae* | 6 Amphipoda<br>1 Diptera<br>1 Ephemeroptera, Heptageniidae*<br>2 Megaloptera, Corydalidae<br>1 Odonata, Aeshnidae<br>2 Odonata, Gomphidae<br>1 Plecoptera, Perlidae*<br>1 Trichoptera, Helicopsychidae*<br>1 Trichoptera, Hydroptilidae* | 53 Amphipoda<br>2 Ephemeroptera, Caenidae*<br>3 Ephemeroptera, Heptageniidae*<br>2 Megaloptera, Corydalidae<br>1 Odonata, Aeshnidae<br>1 Odonata, Gomphidae |
| **SITE 2** | **Sand (fast: 62.8 cm/s)** | **Gravel (fast: 68.8 cm/s)** | **Cobble (slow: 30.4 cm/s)** |
| | | 6 Diptera<br>1 Ephemeroptera, Baetidae*<br>12 Ephemeroptera, Caenidae*<br>3 Ephemeroptera, Ephemerellidae*<br>4 Ephemeroptera, Heptageniidae*<br>1 Ephemeroptera, Metretopodidae*<br>11 Megaloptera, Corydalidae<br>2 Odonata, Aeshnidae<br>1 Plecoptera, Perlodidae*<br>25 Trichoptera, Glossosomatidae*<br>1 Trichoptera, Helicopsychidae*<br>28 Trichoptera, Hydropsychidae*<br>11 Trichoptera, Hydroptilidae*<br>4 Trichoptera, Philopotamidae* | 1 Diptera<br>1 Ephemeroptera, Baetidae*<br>4 Ephemeroptera, Caenidae*<br>1 Ephemeroptera, Heptageniidae*<br>3 Megaloptera, Corydalidae<br>1 Plecoptera, Perlidae*<br>45 Trichoptera, Glossosomatidae*<br>1 Trichoptera, Helicopsychidae*<br>10 Trichoptera, Hydropsychidae*<br>1 Trichoptera, Hydroptilidae*<br>1 Trichoptera, Philopotamidae* |

There were a total of 84 macroinvertebrates from 11 different families collected at Site 1, 6 of which were indicator families (Table 1). Five macroinvertebrates from 3 families were found in sand; 17 macroinvertebrates from 9 families were found in gravel; and 62 macroinvertebrates from 6 families were found in cobble. There were a total of 179 macroinvertebrates from 15 different families collected at Site 2, 12 of which were indicator families. Zero macroinvertebrates were found in sand; 110 macroinvertebrates from 14 families were found in gravel; and 69 macroinvertebrates from 11 families were found in cobble.

**Table 2.** Total macroinvertebrates collected from Sites 3 and 4 on the West Branch of the Maple River, as indicated on the map (Figure 1). Indicator families are shown with an asterisk.

| **SITE 3** | **Sand (slow: 38.66 cm/s)** | **Gravel (fast: 70.90 cm/s)** | **Cobble (slow: 43.68 cm/s)** |
|---|---|---|---|
| | 1 Ephemeroptera, Caenidae*<br>1 Ephemeroptera, Metretopodidae*<br>1 Trichoptera, Brachycentridae* | 3 Coleoptera<br>8 Diptera<br>21 Ephemeroptera, Caenidae*<br>5 Ephemeroptera, Heptageniidae*<br>1 Plecoptera, Perlodidae*<br>58 Trichoptera, Brachycentridae*<br>28 Trichoptera, Glossosomatidae*<br>1 Trichoptera, Limnephilidae*<br>18 Trichoptera, Lepidostomatidae*<br>1 Trichoptera, Philopotamidae* | 8 Diptera<br>16 Ephemeroptera, Caenidae*<br>2 Ephemeroptera, Ephemerellidae*<br>1 Ephemeroptera, Heptageniidae*<br>1 Ephemeroptera, Leptophlebiidae*<br>7 Ephemeroptera, Leptohyphidae*<br>4 Plecoptera, Nemouridae*<br>1 Plecoptera, Taeniopterygidae*<br>56 Trichoptera, Brachycentridae*<br>22 Trichoptera, Glossosomatidae*<br>3 Trichoptera, Hydropsychidae*<br>2 Trichoptera, Hydroptilidae*<br>1 Trichoptera, Limnephilidae*<br>2 Trichoptera, Lepidostomatidae* |
| **SITE 4** | **Sand (fast: 56.44 cm/s)** | **Gravel (slow: 39.54 cm/s)** | **Cobble (fast: 85.66 cm/s)** |
| | 5 Diptera<br>1 Odonata, Gomphidae | 7 Coleoptera<br>12 Diptera<br>33 Ephemeroptera, Caenidae*<br>1 Ephemeroptera, Heptageniidae*<br>8 Ephemeroptera, Leptohyphidae<br>2 Plecoptera, Perlidae*<br>68 Trichoptera, Brachycentridae*<br>1 Trichoptera, Glossosomatidae*<br>2 Trichoptera, Hydropsychidae*<br>10 Trichoptera, Philopotamidae* | 26 Diptera<br>15 Ephemeroptera, Caenidae*<br>1 Ephemeroptera, Ephemerellidae*<br>1 Ephemeroptera Leptophlebiidae*<br>8 Ephemeroptera, Leptohyphidae*<br>1 Plecoptera, Nemouridae*<br>1 Plecoptera, Perlidae*<br>2 Trichoptera, Brachycentridae*<br>1 Trichoptera, Hydroptilidae*<br>41 Trichoptera, Lepidostomatidae* |

There were a total of 286 macroinvertebrates from 18 different families collected at Site 3, 16 of which were indicator families (Table 2). Three macroinvertebrates from 3 families were found in sand; 144 macroinvertebrates from 10 families were found in gravel; and 126 macroinvertebrates from 14 families were found in cobble. There were a total of 250 macroinvertebrates from 17 different families were collected at Site 4, 14 of which were indicator families. Six macroinvertebrates from 2 families were found in sand; 144

macroinvertebrates from 10 families were found in gravel; and 100 macroinvertebrates from 11 families were found in cobble.

**Table 3.** Averages for the water chemistry measurements taken at each site.

| **Location** | **Conductivity (µS)** | **Temperature (°C)** | **pH** | **Total Nitrogen (ug/L)** | **Dissolved Oxygen (mg/L)** | **Total hardness (mg/L)** |
|---|---|---|---|---|---|---|
| Site 1 | 236.1 | 18.2 | 7.60 | 19.80 | 7.90 | 40.66 |
| Site 2 | 242.2 | 18.2 | 7.45 | 23.80 | 8.52 | 45.01 |
| Site 3 | 264.7 | 14.8 | 7.51 | 4.00 | 8.68 | 53.99 |
| Site 4 | 253.7 | 16.2 | 7.56 | 11.70 | 8.50 | 52.63 |

Averages for water chemistry measurements at each site indicate that dissolved oxygen, total hardness, conductivity, and pH remain fairly constant, while total nitrogen differs greatly between sites (Table 3). Biotic Index calculations for Site 1, Site 2, Site 3, and Site 4 are all greater than ten (12, 26, 31, and 30 respectively). Therefore, the Maple River can be classified as "clean" with little to no organic pollution.

### *3.2 Results by Substrate*

**Table 4.** Community diversity indices for each substrate.

| | Sand | Gravel | Cobble |
|---|---|---|---|
| Number of Individuals | 14 | 415 | 357 |
| Richness (no. of families) | 7 | 22 | 22 |
| No. of indicator families | 4 | 15 | 17 |
| Diversity (H) | 1.57 | 2.32 | 2.34 |
| Evenness ($E_H$) | 0.805 | 0.749 | 0.758 |
| Similarity between substrates in families present; Sørensen Index (S) | Between sand and gravel: 0.483 | | |
| | Between gravel and cobble: 0.864 | | |
| | Between sand and cobble: 0.414 | | |

There was a total of 768 macroinvertebrates from 25 different families collected, 19 of which were indicator families (Table 4). Fourteen macroinvertebrates from 7 families were found in sand; 415 macroinvertebrates from 22 families were found in gravel; and 357 macroinvertebrates from 22 families were found in cobble (Table 4). The diversity of macroinvertebrate communities found in the gravel and cobble substrates are similar, though slightly higher in cobble, with H = 2.32 for gravel and H = 2.34 for cobble (Table 4). Both the gravel and cobble substrates have higher diversity than the sand substrate (H = 1.57). The communities found in the sand substrates exhibited more evenness ($E_H$ = 0.805) than either the gravel ($E_H$ = 0.749) or cobble ($E_H$ = 0.758) substrates. The Sørensen Index indicates that the gravel and cobble substrates are more similar in regards to families present (S = 0.846) than either are to sand (S = 0.483, S = 0.414; Table 4).

The ratios of the number of individuals of each family within each substrate were compared across all substrates and against each other using a Chi-Square Test of Independence. The results indicate that there is a significant difference between the number of individuals within each family across the three substrates ($p < 0.001$). A Chi-Square Test for Goodness of Fit was conducted to determine if there was a significant difference in the abundance and diversity of macroinvertebrates in each substrate. There was a statistically significance difference between the total number of individuals across the three substrates ($p < 0.001$). In addition, there was a statistically significant difference between gravel and cobble substrates ($p < 0.001$). The total number of families were compared across each substrate. There was a statistically significant difference between all three substrates ($p = 0.012$). However, there was not a significant difference between the gravel and cobble substrates at the 5% significance level. Similarly, there was a significant difference between indicator families throughout all substrates ($p = 0.017$), but not between the gravel and cobble substrates.

**Table 5.** Averages of abiotic factor measurements for each substrate. P-values calculated with ANOVA test comparing variances. If $p < 0.05$, there is a statistically significant difference between the three substrates in the factor tested.

| **Variable** | **Sand** | **Gravel** | **Cobble** | **P-value** |
|---|---|---|---|---|
| Temperature (°C) | 16.85 | 16.85 | 16.85 | 1 |
| Conductivity (µS) | 249.175 | 249.175 | 249.175 | 1 |
| pH | 7.53 | 7.53 | 7.53 | 1 |
| Depth (cm) | 44 | 32.5 | 44 | 0.542 |
| Flow Rate (cm/s) | 47.95 | 50.32 | 51.99 | 0.963 |
| Dissolved Oxygen (mg/L) | 8.4 | 8.4 | 8.4 | 1 |
| Total Hardness (mg/L) | 48.07 | 48.07 | 48.07 | 1 |
| Total Nitrogen (mg/L) | 14.83 | 14.83 | 14.83 | 1 |

The ANOVA test was used to analyze the variance of abiotic factors tested at the 5% significant level. The test results show that there is no significant difference between the three substrates for any of the abiotic factors tested (Table 5).

### *3.3 Results by Groundwater Concentration*

**Table 6.** Results of the stable isotope analysis. Stable isotope concentrations reveal relative groundwater concentrations at the different sites, with lower numbers indicating higher groundwater concentration. VSMOW stands for versus standard mean ocean water.

| **Sites** | **Water Source** | **dD VSMOW** | **$d^{18}O$ VSMOW** |
|---|---|---|---|
| Site 1 | ground | -82.51 | -12.12 |
| | surface | -56.45 | -4.24 |
| Site 2 | ground | -72.36 | -11.38 |
| | surface | -59.76 | -6.12 |
| Site 3 | ground | -81.88 | -11.89 |
| | surface | -73.93 | -10.35 |
| Site 4 | ground | -79.76 | -10.98 |
| | surface | -71.60 | -8.92 |
| **Standard Deviation** | | 0.47 | 0.43 |

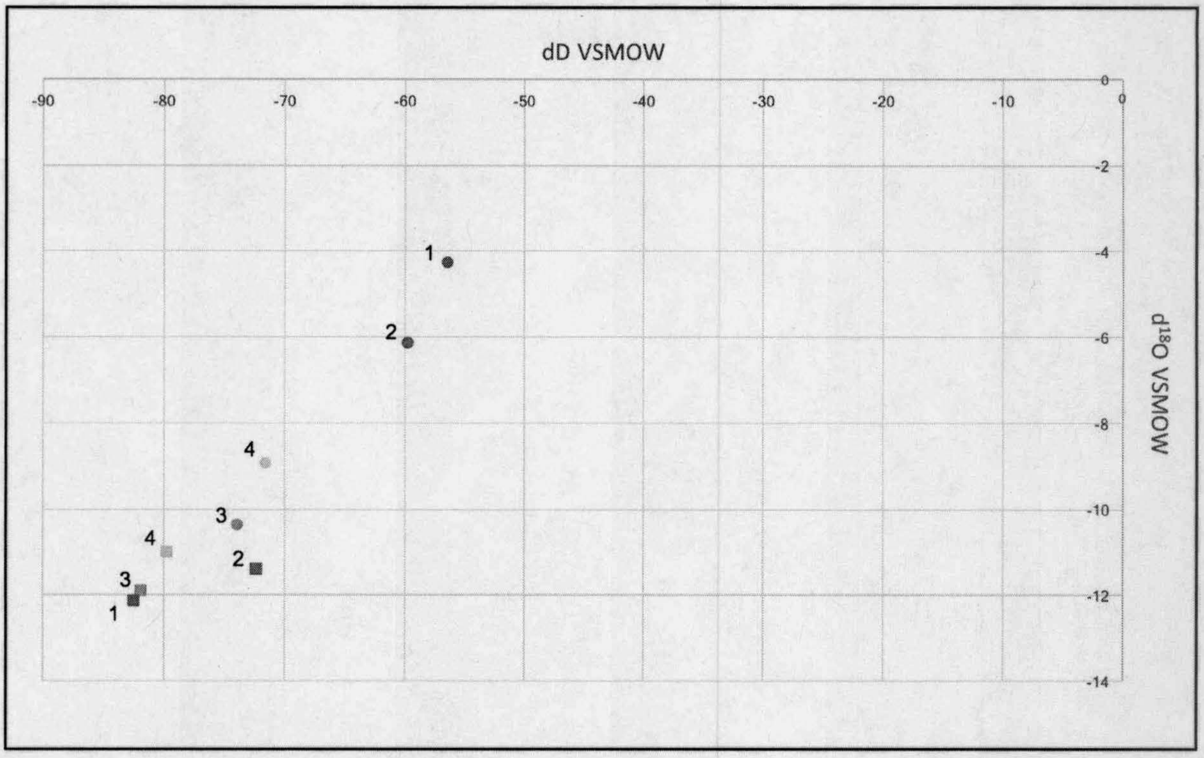


**Figure 3.** Scatter plot of the results of stable isotope analysis. Groundwater is represented by squares, surface water is represented by circles. Site 1 is blue, Site 2 is red, Site 3 is green, and Site 4 is yellow. Additionally, points are labeled with their corresponding sites. Sites with river water points further from the corresponding groundwater point have lower groundwater concentration.

The results of the isotopic analysis (Table 6) indicate that each site has both groundwater and runoff as hydrologic sources. As expected, at each site the groundwater has a lower concentration of both deuterium and $\sigma^{18}O$ than the river water. We calculated 95% confidence intervals for each value to confirm that the sites were significantly different from one another. Graphically, the East Branch sites are well removed from the West Branch sites (Figure 3). These results demonstrate that the West Branch of the Maple River has a significantly lower concentration of deuterium and $\sigma^{18}O$ than the East Branch (Table 6 and Figure 3). Therefore, the West Branch has a higher concentration of groundwater than the East Branch.

**Table 7.** Community diversity indices for the East and West Branches.

| | **East Branch** | **West Branch** |
|---|---|---|
| Number of Individuals | 236 | 523 |
| Richness (no. of families) | 18 | 20 |
| No. of indicator families | 13 | 17 |
| Diversity (H) | 2.183 | 2.032 |
| Evenness ($E_H$) | 0.756 | 0.678 |
| Similarity between East and West Branches in families present; Sørensen Index (S) | 0.684 | |

In the East Branch, 263 macroinvertebrates from 18 families were collected, 13 of which are indicator families. In the West Branch, 523 macroinvertebrates from 19 families were collected, 17 of which are indicator families (Table 7). Based on the diversity indices (Table 7) the diversity of macroinvertebrate communities found in the East and West Branches are similar. However, the diversity is higher in the East Branch ($H = 2.183$) compared to the West Branch ($H = 2.032$). The communities found in the East Branch show more evenness ($E_H = 0.756$) than those found in the West Branch ($E_H = 0.678$). The Sørensen Index indicates that the two branches are similar in regards to families present ($S = 0.684$; Table 7).

A Chi-Square Test of Independence was conducted to analyze the difference in macroinvertebrate community composition between the East Branch (lower groundwater concentration) and the West Branch (higher groundwater concentration). We found a significant difference in macroinvertebrate communities between the East and West Branches ($p < 0.001$). A Chi-Square Test of Goodness of Fit was conducted to analyze differences in abundance and diversity. We found a significant difference in the total number of macroinvertebrate individuals

($p < 0.001$). The West Branch had a higher abundance of macroinvertebrates than the East Branch. We did not find a significant difference between the total number of families nor the number of indicator families at the 5% significance level.

**Table 8.** Averages of abiotic factor measurements for the East and West Branches. P-values calculated with an Independent Samples t-Test. If $p < 0.05$, there is a statistically significant difference between the two branches in the factor tested.

| Variable | East Branch | West Branch | P-value |
|---|---|---|---|
| Temperature (°C) | 18.2 | 15.5 | < 0.001 |
| Conductivity (μS) | 239.15 | 259.2 | < 0.001 |
| pH | 7.53 | 7.54 | 0.787 |
| Depth (cm) | 45 | 35.33 | 0.313 |
| Flow Rate (cm/s) | 44.35 | 55.81 | 0.319 |
| Dissolved Oxygen (mg/L) | 8.21 | 8.59 | 0.04 |
| Total Hardness (mg/L) | 42.84 | 53.31 | < 0.001 |
| Total Nitrogen (mg/L) | 21.8 | 7.85 | < 0.001 |

An Independent Samples t-Test was used to analyze the differences in means of the abiotic factors tested. There is a significant difference in water temperature ($p < 0.001$), conductivity ($p < 0.001$), dissolved oxygen ($p = 0.04$), total hardness ($p < 0.001$), and total nitrogen ($p < 0.001$) between the two branches (Table 8). The Independent Samples t-Test shows there is no difference in the pH, depth, or flow rate between the two branches at the 5% significance level.

## 4 Discussion

The data supported our first hypothesis that there would be a greater abundance of macroinvertebrates in gravel compared to the cobble and sand. However, gravel and cobble do not have a significant difference in diversity. In addition, the data supported our second hypothesis that a correlation exists between high relative concentration of groundwater and greater abundance of macroinvertebrate communities. Groundwater concentration did not have an apparent correlation with the diversity of macroinvertebrate communities.

The three macroinvertebrate indicator taxa (Ephemeroptera, Plecoptera, and Trichoptera) were found at all sites, which indicates that the Maple River has relatively high water quality. Additionally, the Biotic Index scores were found to be greater than 10. This factor combined with the presence of these indicator taxa suggests that the Maple River does not have significant levels of pollution. Moreover, the data demonstrate that the Maple River has high dissolved oxygen content, neutral pH, and relatively cold water temperatures (Table 3).

The ratios of individuals within each order from each substrate were significantly different, which suggests that the community composition of macroinvertebrates differs greatly between substrates. The total number of macroinvertebrate individuals in the Maple River differs significantly between each substrate. In addition, the results illustrate that there was a higher number of individuals found in gravel substrates compared to the sand or cobble substrates (Table 4). There is a significant difference in the richness of total families and the richness of indicator families between sand and the other two substrates, but not between the gravel and cobble substrates. This indicates that there is higher diversity in gravel and cobble substrates compared to sand substrates, but not between the gravel and cobble substrates.

The diversity indices used to compare the three substrates further support the hypothesis that gravel and cobble substrates have higher macroinvertebrate diversity than sand (Table 4). Gravel and cobble substrates were found to have similar family evenness as well as higher diversity in macroinvertebrates when compared to sand (Table 4). The rocky substrates (i.e cobble and gravel) provide macroinvertebrates a habitat with protection from predators. These two substrates are also similar in habitat and resource availability, which causes the community composition to be more similar (Brown & Brussock, 1991).

Possible confounding variables include stream flow rate, dissolved oxygen, water temperature, conductivity, total hardness and nitrogen, depth, and pH. The ANOVA test results indicate that there was not significant variance of these abiotic factors between substrates (Table 9). This allowed for the elimination of these factors as confounding variables in this study. However, seasonality may affect the composition of macroinvertebrates communities observed due to the differences in phenology (Alba-Tercedor, 2017; Brand & Miserendino, 2012). The families present during our sampling timeframe may not be representative of the year-round macroinvertebrate communities that exist in the Maple River. In addition, heavy rainfall occurred for several days before data collection. The resulting increase in water flow may have impacted the composition of macroinvertebrate communities.

Macroinvertebrates' preference for rocky substrates (i.e. gravel and cobble) compared to sandy substrates could be tested further in the future. An evolutionary advantage or selective pressure that was not examined in this study may influence macroinvertebrate habitat preference. Time and funding constraints did not allow investigation of this phenomena. Riffles and pools could also be examined within each substrate to provide more precise information about macroinvertebrate habitat preference and further improve the results of this study.

Community composition differed significantly between sites with high and low groundwater concentration. There was a significant difference in the total number of macroinvertebrates. However, there was not a significant difference in the total number of families nor the total number of indicator families between high and low groundwater concentrations. Therefore, there is no difference in diversity of macroinvertebrate communities. Similarly, the diversity indices used to compare the East and West Branches do not support our hypothesis that higher relative groundwater concentration is correlated with greater diversity of macroinvertebrate communities. The branches have similar values for macroinvertebrate family diversity and evenness. Furthermore, the Sørensen Index value suggests that the two branches have similar community composition (Table 7).

The same confounding variables tested for substrate were also tested with regard to groundwater concentration. However, the Independent Samples t-Test indicate that only pH, depth, and stream flow rate were not different between high and low groundwater concentrations. Thus, these factors can be ruled out as confounding variables. Water temperature, conductivity, dissolved oxygen, and total hardness and nitrogen were all significantly different between the sites with high concentrations of groundwater and sites with low concentrations of groundwater (Table 8). Therefore, these factors could be confounding variables for our results regarding groundwater concentration. Other confounding variables would be the same as those discussed above. Additionally, the excessive rainfall preceding sampling could have increased the relative amount of surface water in the Maple River to levels above its yearly averages.

The results of our study suggest that restoration efforts should be concentrated on increasing the gravel and cobble substrates present in the river. Both substrates demonstrate high macroinvertebrate abundance and diversity in the Maple River. Additionally, the East Branch is more susceptible to anthropogenic pollution due to its higher sourcing from surface runoff. Therefore, stream restoration and conservation efforts should be more concentrated on this branch of the Maple River.

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**Literature Cited**

Alba-Tercedor, J., Sainz-Bariáin, M., Poquet, J. M. & Rodŕıguez-Lopez, R. (2017). Predicting River Macroinvertebrate Communities Distributional Shifts under Future Global Change Scenarios in the Spanish Mediterranean Area. *PLOS ONE*, *12*(1). doi:10.1371/journal.pone.0167904

Angelier, E. (2003). Ecology of Streams and Rivers. Enfield, NH: *Science.*

Armitage, P. D., Moss, D., Wright, J. F. & Furse, M. T. (1983). The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water sites. *Water research*, *17*(3), 333-347. https://doi.org/10.1016/0043-1354(83)90188-4

Ayres, M. (2015). Water conductivity in stream ecosystems. *Dartmouth University.* Retrieved from http://www.dartmouth.edu/~bio31/conductivity.htm

Beauger, A., Lair, N., Reyes-Marchant, P. & Peiry, J. L. (2006). The distribution of macroinvertebrate assemblages in a reach of the river Allier (France), in relation to riverbed characteristics. *Hydrobiologia, 571*(1), 63-76. doi:10.1007/s10750-006-0217-x

Berg, M. B., Cummins K. W. & Merritt, R. W. (2008). *An Introduction to the Aquatic Insects of North America.* Dubuque, Iowa: Kendall Hunt Publishing Company.

Birmingham, M, (2005). Benthic Macroinvertebrate Key. *Iowater.*

Bond, N. R., Downes, B. J. & Hindell, J. S. (2000). What's in a site? Variation in lotic macroinvertebrate density and diversity in a spatially replicated experiment. *Austral Ecology*, *25*, 128-139. doi:10.1046/j.1442-9993.2000.01019.x

Brand, C. & Miserendino, M. L. (2012). Life cycle phenology, secondary production, and trophic guilds of caddisfly species in a lake-outlet stream of Patagonia. *Limnologica*, *42*(2), 108-117. doi:10.1016/j.limno.2011.09.004

Brooks, A. J., Haeusler, T., Reinfelds, I. & Williams, S. (2005). Hydraulic microhabitats and the distribution of macroinvertebrate assemblages in riffles, *Freshwater Biology*, *50,* 331–344. doi:10.1111/j.1365-2427.2004.01322.x

Brown, A. & Brussock, P. (1991). Comparisons of benthic invertebrates between riffles and pools. *Hydrobiologia, 220*(2), 99-108. doi:10.1007/BF00006542

Chadde Schumaker, J. (2017). Macroinvertebrates and Fishes as Bioindicators of Stream Water Pollution. *Western U.P. Center for Science, Mathematics & Environmental Education, Michigan Technological University.* Retrieved from wupcenter.mtu.edu/education/stream/Macroinvertebrate.pdf

Conservation Resource Alliance (2017). *Lake Kathleen Dam Removal Kick-Off*. Rivercare. Retrieved from http://www.rivercare.org/news/lake-kathleen-dam-removal-kick-off

Conservation Resource Alliance (2017). *Spring Update on the Carp and Maple Rivers.* Rivercare. Retrieved from https://www.rivercare.org/news/spring-update-on-the-carp-maple-rivers

Duan, X., Wang, Z. & Tian, S. (2008). Effect of streambed substrate on macroinvertebrate biodiversity. *Environmental Science & Engineering in China, 2*(1), 122-128. doi:10.1007/s11783-008-0023-y

Environmental Protection Agency (2012). *5.9 Conductivity.* Retrieved from http://water.epa.gov/typeirsl/monitoring/vms59.cfm

Gat, J. R. (1996). Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review Earth Planet. Sci.* 24, 225–262.

Godby, N. (2014). Maple River: Status of Fishery Resource Report. *Michigan Department of Natural Resources. Michigan.gov,* State of Michigan.

Google Maps. (n.d). [Pellston Michigan]. Retrieved June 2, 2017.

Gross, L. J. (2000). Diversity Indices: Shannon's H and E. *Alternative Routes to Quantitative Literacy for the Life Sciences.* Retrieved from http://www.tiem.utk.edu/~gross/bioed/bealsmodules/shannonDI.html

Heinen, J. T. & Vande Koople, R. (2003). Profile of a Biosphere Reserve: The University of Michigan Biological Station, USA, and Its Conformity to the Man and Biosphere Program. *Natural Areas Journal, 23*(2), 165-173.

Hem, J. D. (1992). Study and Interpretation of the Chemical Characteristics of Natural Water. *U.S. Geological Survey Water Supply*. Retrieved from https://pubs.usgs.gov/wsp/wsp2254/pdf/wsp2254a.pdf

Jerves-Cobo, R., Everaert, G., Iñiguez-Vela, X., Córdova-Vela, G., Díaz-Granda, C., Cisneros, F., Nopens, I. & Goethals, P. L. M. (2017). A Methodology to Model Environmental Preferences of EPT Taxa in the Machangara River Basin (Ecuador). *Water*, *9*, 195. doi:10.3390/w9030195

Krebs, C. J. (2014). Estimating Community Parameters. *Ecological Methodology* (Chapter 12). Retrieved from http://www.zoology.ubc.ca/~krebs/downloads/krebs_chapter_12_2014.pdf

Macan, T. T. (1970). A guide to freshwater invertebrate animals. London: *Longman.*

Mook, W. M. E. (2001). *Environmental Isotopes in the Hydrological Cycle. Principles and Applications*. UNESCO/IAEA Series.

Nadelhoffer, K. J., Hogg, A. J., & Hazlett, B. A. (2010). *The changing environment of northern Michigan: a century of science and nature at the University of Michigan Biological Station*. Ann Arbor, MI: University of Michigan Press.

Nelson, S. M. & Andersen, D. C. (2007). Variable Role of Aquatic Macroinvertebrates in Initial Breakdown of Seasonal Leaf Litter Inputs to a Cold-Desert River. *The Southwestern Naturalist, 52*(2), 219-228. Retrieved from http://www.jstor.org/stable/20424817

Nyende, J., van Tonder, G. & Vermeulen, D. (2013). Application of Isotopes and Recharge Analysis in Investigating Surface Water and Groundwater in Fractured Aquifer under Influence of Climate Variability. *Journal of Earth Science and Climatic Change, 4*(4). doi:10.4172/2157-7617.1000148

Sharpe, W. E, Kimmel, W. G. & Buda, A. R. (2002). Biotic Index Guide. *Pennsylvania State University.* Retrieved from extension.psu.edu/natural-resources/water/watershed-education/watershed-publications/BICcard.pdf

Utah State University Extension. (2016). *Water Quality - Aquatic Macroinvertebrates* [Data File]. Retrieved from extension.usu.edu/waterquality/whats-in-your-water/aquatic_macroinvertebrates

Xu, M., Wang, Z., Pan, B. & Zhao, N. (2012). Distribution and species composition of macroinvertebrates in the hyporheic zone of bed sediment. *International Journal of Sediment Research*, *27*(2), 129-140. https://doi.org/10.1016/S1001-6279(12)60022-5

Zhang, W. S., Swaney, D. P., Li, X. Y., Hong, B., Howarth, R. W., and Ding, S. H. (2015). Anthropogenic point-source and non-point source nitrogen inputs into Huai River basin and their impacts on riverine ammonia–nitrogen flux. *Biogeosciences,* 12, 4275-4289, doi:10.5194/bg-12-4275-2015, 2015.

Zimmer, M. & Lautz, L. (2015). Pre- and post-restoration assessment of stream water–groundwater interactions: Effects on hydrological and chemical heterogeneity in the hyporheic zone. *Freshwater Science, 34*(1), 287-300. doi:10.1086/679514