INFLUENCE OF BENTHIC SEDIMENTS ON MACROINVERTEBRATE COMMUNITY STRUCTURE IN AGRICULTURAL HEADWATER STREAMS

by

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A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Biology at Purdue Fort Wayne

Fort Wayne, Indiana

August 2020

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To my parents, Doug and Lisa, for always keeping faith in me even in the moments when I did not it have it in myself and to my dogs Toa Brian and Clay Matthews for always being happy to see me after a long day and cheering me up on the days I needed it most.

ACKNOWLEDGMENTS

I am thankful for the love and support that I have received from my family and my friends during this journey. I first thank my primary advisor, Dr. Robert Gillespie for guidance, assistance, and expertise. My collaborators Dr. Peter Smiley Jr. and Dr. Jordan Marshall were each instrumental in my journey of ecological statistics and have provided me with many tools to succeed in my research. I thank the following institutions for their support: ARS-NSERL in West Lafayette IN, for the funding of this research through the USDA's CEAP program; and for contributing pesticide, nutrient, and sediment data; 2) ARS lab at Bowman Farm of Waterloo IN, for their support and processing of sediment and water samples; 3) ARS-SDRU in Columbus OH, for collection of sediment and water samples and work with macroinvertebrate collections; and 4) Purdue University Fort Wayne Biology Department for their support and assistance. I also thank the many graduate and undergraduate students, my lab and field crew members who without this project would not be complete. Special thanks go to Darren Shoemaker for enduring all field sampling days that were physically exhausting 10-hour days; Deanne Jensen for her help in the field and her expertise in the identification of over 250 macroinvertebrate samples collected.

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ABSTRACT

Aquatic macroinvertebrates of channelized headwater streams in agricultural landscapes are exposed to alterations in chemistry and physical characteristics of benthic sediments. These habitat alterations are known to influence communities of aquatic macroinvertebrates. Benthic sediments can have a wide range of impacts and influences on aquatic macroinvertebrates. I hypothesized that sediments would play a significant role in determining macroinvertebrate community structure within agriculturally dominated headwater streams. I evaluated the influences of sediment chemistry characteristics and physical characteristics on aquatic macroinvertebrate communities in Cedar Creek, Indiana and Michigan, and the Upper Big Walnut Creek, Ohio, during 2017 and 2018. Macroinvertebrates were collected twice per year using artificial substrate and leaf pack samplers and identified to the family level. Sediments were sampled two times per year and analyzed for seven physical characteristics and twenty sediment chemistry characteristics. Principle component analyses were used to create axes that are indicators of gradients of sediment chemistry and physical characteristics that occur among the samples. Macroinvertebrate community metrics used in the analyses included abundance, Shannon Diversity Index, Hilsenhoff Biotic Index scores, Invertebrate Community Index scores, percentage of collector-filters, percentage of scrapers, percentage of Chironomidae and a Berger-Parker Reciprocal Index of dominance. Linear Mixed Effect Model analyses revealed that both sediment chemistry and physical characteristics influence macroinvertebrate community metrics. Aquatic macroinvertebrate abundance was negatively correlated with increasing concentrations of simazine and decreasing concentrations of calcium. Percentages of Chironomidae were positively correlated with increasing percentages of sand and decreasing percentages of clay and decreasing diversity of sediment particle sizes. My data supported the hypothesis that benthic sediments play an important role in determining aquatic macroinvertebrate community structure in headwater streams of agriculturally dominated landscapes. Gradients of chemical characteristics containing simazine and calcium were observed to be negatively correlated with macroinvertebrate abundance. Gradients of physical characteristics including percentages of sand and clay along with the diversity of particle sizes were observed to be positively correlated with percentage of chironomids. My research increases the knowledge that benthic sediments, chemically and physically, can lead to alterations in aquatic macroinvertebrate communities within Midwestern headwater streams.

CHAPTER 1. INTRODUCTION

Headwater streams are the uppermost reaches of watersheds (USEPA, 2015) compromising the smallest first to third-order streams in the watershed (Harrel et al., 1967; Strahler, 1957). Headwater streams collect snowmelt, precipitation, and groundwater which all flow downstream to subsequent larger order streams (Moss, 2010). Headwater streams are essential for transport of nutrients, sediment, and biota to river and lakes (Colvin et al., 2019; Meyer et al., 2007). These streams can be perennial, intermittent, or ephemeral. Perennial streams maintain year-round flow, intermittent streams dry up during parts of the year, and ephemeral streams only have flowing water in response to precipitation events (Nadeau et al., 2007). Headwater streams in Indiana and Ohio ocomprise 58.0 to 100.0 % (Nadeau et al., 2007).

Headwater streams are essential as they provide unique habitats for many organisms. This is due to their shallow water depths, low water velocities, natural sinuosity, riffle/run/pool characteristics, and a wide range of benthic sediments (Colvin et al., 2019; Lau et al., 2006; Meyer et al., 2007). Macroinvertebrates are found in large numbers and often great diversity in headwater streams due do their unique habitat qualities (Clarke et al., 2008; Cushing et al., 2001; Meyer et al., 2007; Moss, 2010). Macroinvertebrates in headwaters are often present in large abundances with aquatic insect larvae comprising the majority (Metcalfe-Smith, 2009). Due to macroinvertebrate abundances and their considerable biodiversity, they make excellent bioindicators for stream health and their health of the overall stream communities (macroinvertebrates, fishes, algae, etc.) (Hooda et al., 2000; Li et al., 2010; Smith et al., 1997). Although there are other abundant and diverse organisms within headwaters, macroinvertebrates are used because they are often large enough to be easily observed, collected and identified, they have also been studied extensively as stream health bioindicators (Agency, 2015).

The unique habitats that headwaters can provide means that some macroinvertebrates, such as stoneflies and caddisflies, can only be found within these streams (Meyer et al., 2007), characteristics such fast flowing cold waters. Ephemeroptera, Plecoptera, and Trichoptera are three insect taxa that have genera that only inhabit headwaters (Erman et al., 1995; Stout et al., 2003). Researchers have found as many as 60 species of stoneflies and 78 species of caddisflies that inhabit headwaters that are considered high-quality and free of agricultural influence (Erman et al., 1995; Stout et al., 2003). Non-biting midges (*Chironomidae*) are often found in large abundances and have great diversity in headwaters and they can be found in benthic sediments of a wide variety of sizes and types (Bazzanti, 2000; Butakka et al., 2014; Yamamuro, 2004), traits such as these make chironomids and other macroinvertebrates excellent indicators stream health. Headwater streams offer unique habitats and can harbor organisms that can only be found within them and it's this biodiversity that we can use to determine stream health.

Over the years headwater streams have been degraded by alteration of habitat, introduction of agricultural chemicals (applied pesticides), nutrients, and increases and sediments (Armstrong et al., 2012). Headwater streams in the Midwestern U.S. have been created or modified to serve as drainage ditches for agriculture (Freeman et al., 2007; Mattingly et al., 1993). Headwaters are channelized by removing their natural sinuosity, dredged deeper and steeper, and cleared of large debris (Davis et al., 2003). These habitat modifications are done to remove excess water off of fields, improve the drainage of the fields, and to prevent flooding (D'Ambrosio et al., 2009). Although this benefits agriculture it is detrimental to the natural diversity of the habitat and its biota.

Initial channelization and regular maintenance removes natural habitats that support many aquatic organisms (Jamie et al., 2006). The process of dredging streams is often completed with little regard to the flora and fauna that are unable to avoid or escape this process (Shaw et al., 2015; Stammler et al., 2008; Wenger et al., 2017). Channelization can facilitate the addition of many agricultural contaminants and increased fine grain sediments (Kuenzler et al., 1977) that many of these macroinvertebrates had not been previously exposed to. Channelization also creates a more homogenous instream habitat that lacks a diverse benthic sediment profile and distinct riffle/run/glide characteristics (Watters, 1992); this stress has been observed to alter macroinvertebrate communities by increasing pioneer species, decreasing biodiversity along with altering compositions of existing communities (Płaska et al., 2016; Schoof, 1980).

Headwater streams are also subjected to the input of agricultural chemicals (applied pesticides) and excess nutrients (D'Ambrosio et al., 2009; Megahan, 1999; Roley et al., 2012). Millions of pounds of pesticides are used annually in the United States for agriculture (Gilliom et al., 2006), often large quantities are not retained in the soil and drain off from irrigation and/or rainfall (Gilliom et al., 2006). Drainage of these pesticides is facilitated by both surface and subsurface drainage and leaching into the groundwater which all drains into these channelized headwater streams (Williamson et al., 2014). These agrichemicals and applied nutrients have documented negative impacts on macroinvertebrates that include reduced foraging behavior, decreased reproductive success to lethality of Chironomidae larvae and other taxa; these impacts are documented from single pesticides to mixtures (Belden et al., 2006);. Macroinvertebrates also have sensitivities to nutrients similar to pesticides. Compounds like nitrates can impact varying life stages of macroinvertebrates. Larval instar caddisflies can migrate from their retreats while gammarids show unnatural locomotion at concentrations that show little or no effect on the adult stages. Similar to pesticides nutrients can also be lethal, nitrates have LC50's observed in early instar to adults of many aquatic macroinvertebrates (Camargo et al., 2005).

These agrichemicals not only flow in the streams water column but they can also settle into the benthic sediments (Megahan, 1999). Sediments in many ways from contamination or their characteristics can be stressors that are common in agricultural headwater streams (USEPA, 1997). Contaminants in benthic sediments have been observed to significantly decrease biomass reduce Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa and decrease biodiversity (Moran et al., 2017). Benthic sediments can possess qualities such as, particle size, substrate type, and their adsorption potentials, that make them uninhabitable to macroinvertebrates or hinder certain life processes like reproduction (Suedel et al., 1994). Fine sediments can fill the interstitial spaces between larger substratum needed by many macroinvertebrates and possibly cause physical damage to the macroinvertebrates (Hoy, 2001). The loss of diverse sediments can cause macroinvertebrate taxa biodiversity to decrease by decreasing aquatic plant communities or changing their community structure and the macroinvertebrate communities that depend on these plants (Duan et al., 2009).

Macroinvertebrates in Midwestern headwater streams have been studied extensively but few of the studies look at both benthic sediment chemistry and sediment physical characteristics in the same study. Research on mussels assessed the influences that temporal trends, water chemistry, and instream habitat variables had the communities but did not look into whether the substrates of the streams influenced the populations or the communities (Taylor, 2016). Macroinvertebrate communities have been correlated with land cover and surrounding land use, which influence the abiotic environment but stream benthic sediments were not considered (Cooper et al., 2006). Certain sediment physical characteristics have been included in studies such sedimentation and percentage of silt, sediment chemistry was not included. McKinney et al. observed that instream habitat which included percent silt and substrate richness was more important for macroinvertebrate community composition than water chemistry but sediment chemistry was not included (McKinney, 2012). However, silt is only one sediment variable that may influence macroinvertebrates.

Studies have been done looking at concentrations of sediment pollutants and their impacts on macroinvertebrates others have looked at the physical characteristics of sediments on macroinvertebrates (Dalu et al., 2017; Fanny et al., 2012; Friberg et al., 2003; Moran et al., 2017; Palmer et al., 2000). Few studies, however, have looked at both sediment physical and chemical variables together and observed their influences on macroinvertebrates. Understanding the influences of sediment chemistry and sediment composition is important for understanding how to improve aquatic macroinvertebrate communities in degraded habitats of headwater streams.

Sediment chemical and physical properties by themselves are known to impact macroinvertebrates both negatively and positively, so it is important to determine their influence on aquatic macroinvertebrates in Midwestern agriculturally dominated headwater streams. Understanding the importance of headwaters can beneficial for regulating agricultural chemicals and managing resources including the stream and its biota. Understanding the role of sediments in headwaters of the Midwest can be useful to researchers, resource managers and policy makers. The purpose of this study was to determine if benthic sediment chemistry and particle size characteristics influence aquatic macroinvertebrate community structure. As aquatic macroinvertebrates spend much of their larval forms in or in close to proximity to sediments. The objective of my study is to determine if benthic sediment physical and or chemical properties significantly influence aquatic macroinvertebrates in agricultural streams in Indiana, Michigan and, Ohio. I predict sediment physical characteristics to influence more macroinvertebrate metrics than their chemical characteristics.

CHAPTER 2. METHODS

2.1 Study Sites

A total of eight sites were selected for this study. Sampling locations for this project were part of the Conservation Effects Assessment Project (CEAP). These CEAP sites have been under study in our lab since 2006 looking at agricultural impacts on headwaters of the Midwestern United States. Four sites were located in the Saint Joseph River Watershed (SJR) in Indiana and Michigan (Figure 1). Four sites were chosen in the Upper Big Walnut Creek Watershed (UBWC) of Central Ohio (Figure 2). These sites were chosen based on catchment size and similar land use (Table 1). The catchment sizes of streams in SJR are larger than those in UBWC, however, the mean canopy cover is greater at sites in the UBWC catchment than those in SJR. The percentage of agricultural land use of the catchments ranged between 54% to 83% in SJR and 48% to 95% in UBWC.

These sites were chosen because of the availability of multiple years of water chemistry, physical habitat data and access to multiple sampling locations on private land as they are a part of CEAP and the Agricultural Research Service National Soil Erosion Research Laboratory (ARS/NSERL). Three sites were channelized headwater streams and one site was an unchannelized agricultural headwater stream (Table 1) to ensure that our samples represented a range of sediment physical and chemical characteristics.

2.2 Sediment Sampling

Sampling locations were composed of a 150-meter long reach that were further broken down into 25-meter long segments. Segments were arranged down- to upstream, segment 1 being the most downstream so sampling proceeded upstream to segment 6 (Figure 3). Prior to sampling one segment from each site was randomly selected. No segment was repeatedly sampled and every segment in each site was sampled once during my two-year study. Then locations for sediment sampling within a segment was determined using stratified random selection combinations of 24 possible longitudinal positions (1-meter intervals from1 meter upstream downstream border to 24 meters upstream to downstream border) and three possible latitudinal positions (left, center, right) in each segment. Sediment sampling occurred twice a year and the collection for spring sediments

(May – June) occurred two weeks before spring macroinvertebrates occurred. Sediment collections from fall (September – October) occurred the week after macroinvertebrates were sampled.

Benthic sediments were sampled with a hand corer at the six randomly selected locations within each randomly selected segment during each spring and fall seasons and composited into a single sample from the segment. Cores collected were 7.6 centimeters in depth, if a single core could not be obtained two or three smaller cores were obtained in the immediate vicinity. Cores were placed into a 20 L high-density polyethylene bucket, large visible organic matter was removed, and sediments were homogenized using an electric power drill with a special mixing bit. Sediment samples were kept on ice during transport and stored at 6° C in a cold room, this allowed the sediments in the water to settle out.

Bed sediments were separated evenly between two 500-mL Nalgene or glass bottles. Enough sediment was placed into each bottle to ensure a minimum of 50 g dry weight after water was evaporated. Samples were heated in an oven at 50° C for 12 hours, weighed, and placed back into the oven for 2 hours, and reweighed. This process was continued as needed until the sample reached constant weight (± 1.0%). One half of the bottled sediment sample per segment was used for grain size and organic content analyses. Sediments were sorted through a series of sieves (50.8 mm, 16.0 mm, 2.0 mm, and < 2.0 mm) for 30 minutes using an automated shaker. After 30 minutes large clumps were broken into smaller fragments and the sample was placed back into the automated shaker for another 30 minutes. This process was repeated as necessary. Sediments retained in each sieve and collection pan were weighed. A 5-gram subsample was sent to the National Soil Erosion Research Lab (NSERL), West Lafayette, IN and analyzed for sand, silt and clay fractions with a Malvern Mastersizer 3000 with a hydro EV accessory. A 5-gram subsample of sediment >2.0 mm was ashed in a muffle furnance for 16 hours at 375° C, cooled to room temperature in a desiccator and weighed.

The other half of the bottled sediment sample per segment was analyzed for pesticide, nutrient and physiochemical properties. Nutrients, pesticides and physiochemical analyses were completed at the NSERL, West Lafayette, IN. Conductivity was measured using a Mettler-Toledo SevenCompact conductivity meter with a Mettler #731-ISM electrode. pH was measured using an Orion Star A21 pH meter with a Thermo Orion 9165BNWP CHN-2000 elemental analyzer. Concentrations of ammonia, nitrate, carbon, nitrogen, aluminum, calcium, copper, iron, potassium, magnesium, manganese, phosphorus, sulfur, and zinc were analyzed using colorimetric analyses.

Simazine, S-Metolachlor, Atrazine, and an Atrazine metabolite (2OH-Atrazine) were analyzed using a Waters Acuity Ultra Performance Liquid Chromatography system interfaced with a computer running MassLynx v 4.1 chromatography manager software.

2.3 Macroinvertebrates

Macroinvertebrate sampling occurred twice a year in 2017 and 2018. Sampling periods consisted of June through July (Spring–Summer) collection and August through September (Summer–Fall) collection. Hester-Dendy's (HDs) and leaf packs (LPs) were placed within segments that were sampled for sediments. Three HDs were affixed to a single cement block. The LPs were created using a 50:50 ratio of dried leaves of *Fraxinus americanus* (green ash) and *Cornus florida* (white flowering dogwood) having 30 g of leaves in each pack, enclosed in 1.2 cm^2 mesh netting. Samplers were placed in the corresponding segments for 28 days, one of each sampler types were placed in a down- mid- and upstream positions. A total of 9 HDs and 3 LPs were placed in a segment. One HD and LP sampler type was placed in a downstream, midstream, and upstream position in each segment for 28 days. The HDs were placed strategically on each bank and in the center of the stream channel. All LPs were placed near stream banks. All samplers were positioned to remain submerged, even during base flow. Samplers were repositioned if they became exposed above water levels. Attempts were made to position HDs and LPs so they were not close to each other.

HDs and LPs were collected in either 2-L wide mouth polyethylene terephthalate jars or 2.7 liter Whirl-Paks and returned to the lab. Stream water that was collected with the macroinvertebrate samplers was drained using a 2.0 mm sieve and sample contents were preserved in 70% reagent alcohol. If samples were not preserved on the day of collection, they were stored at 4.0 - 10.0° C, for no longer than one week. Insects were identified to family level non-insects were identified to a minimum taxon of order using identification keys (Brinkhurst et al., 2007; Cummins et al., 1996; Merritt et al., 2008; Thorp et al., 2011). All macroinvertebrate organisms were counted and preserved in 70% reagent alcohol.

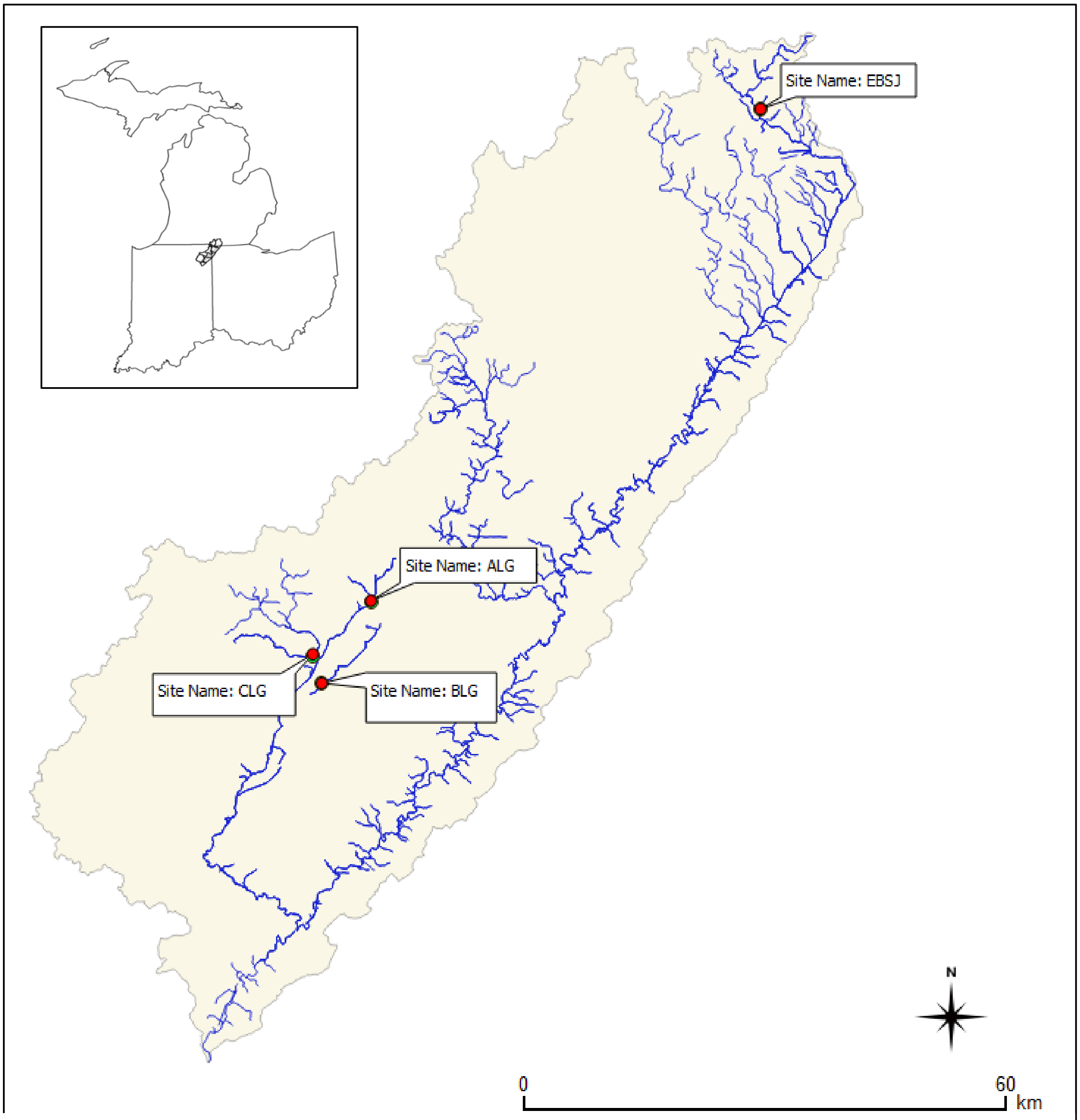


Figure 1 Map of the Saint Joseph River Watershed (SJR) study area that encompasses northeast Indiana and southern Michigan. The three sampling sites in the southern part of the watershed were located in the Cedar Creek subwatershed.

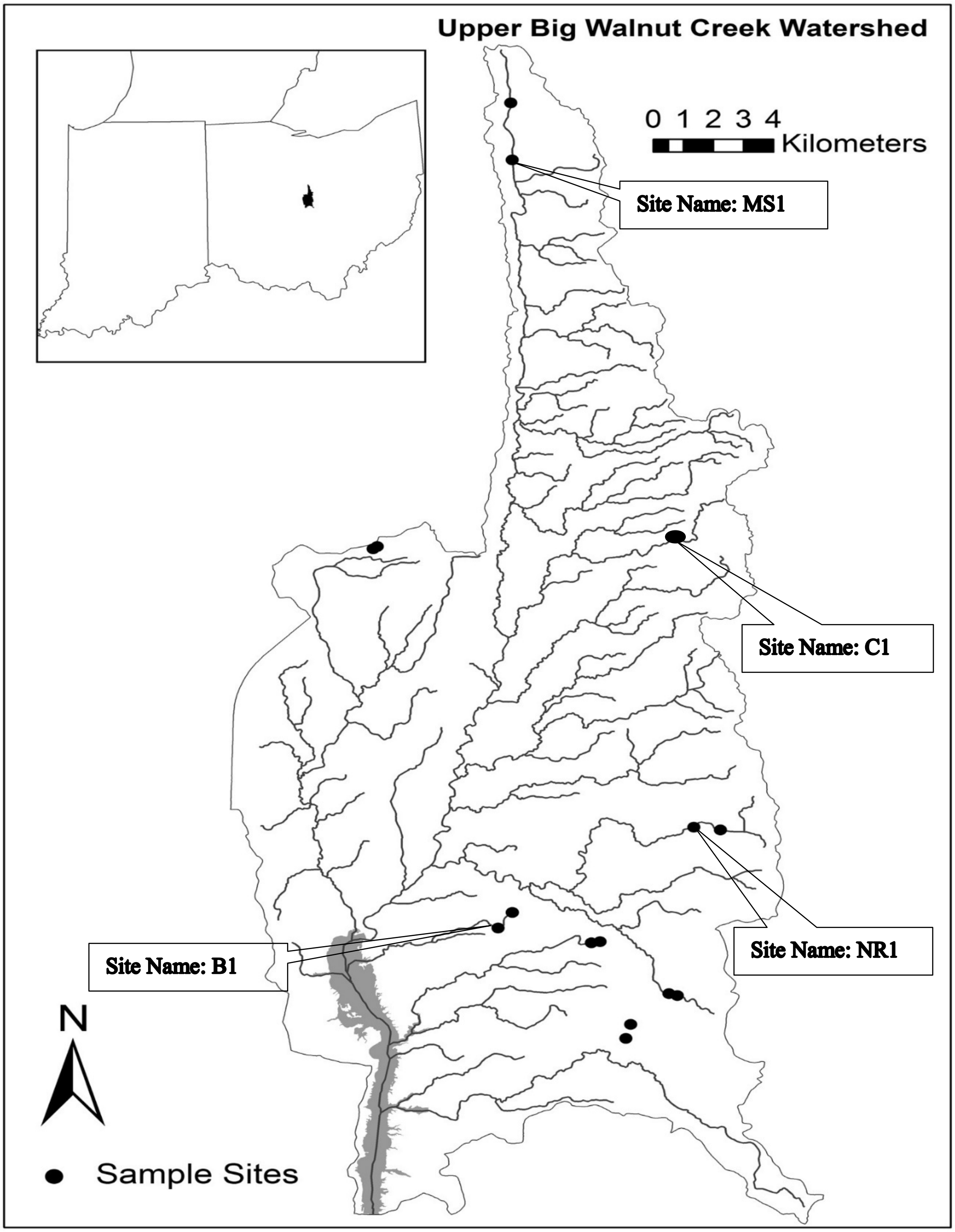


Figure 2 Map of the Upper Big Walnut Creek Watershed (UBWC) study area within Central Ohio. The shaded region represents the Hoover Reservoir. Sampling site locations designated by points with site names.

Table 1 Mean percent canopy cover, watershed size, and percent agricultural land use at sites in the Saint Joseph River and Upper Big Walnut Creek Watersheds. EBSJ and C1 are unchannelized agricultural headwater streams.

Site	Percent Canopy Cover	Watershed Size (km^2)	Percent Agriculture
ALG	0.0	20.4	60.7%
BLG	0.0	13.8	69.0%
CLG	0.0	14.0	71.5%
EBSJ	21.3	21.6	29.3%
B1	0.3	3.8	75.1%
C1	86.5	4.4	55.7%
MS1	1.7	9.7	63.5%
NR1	13.3	7.0	86.9%

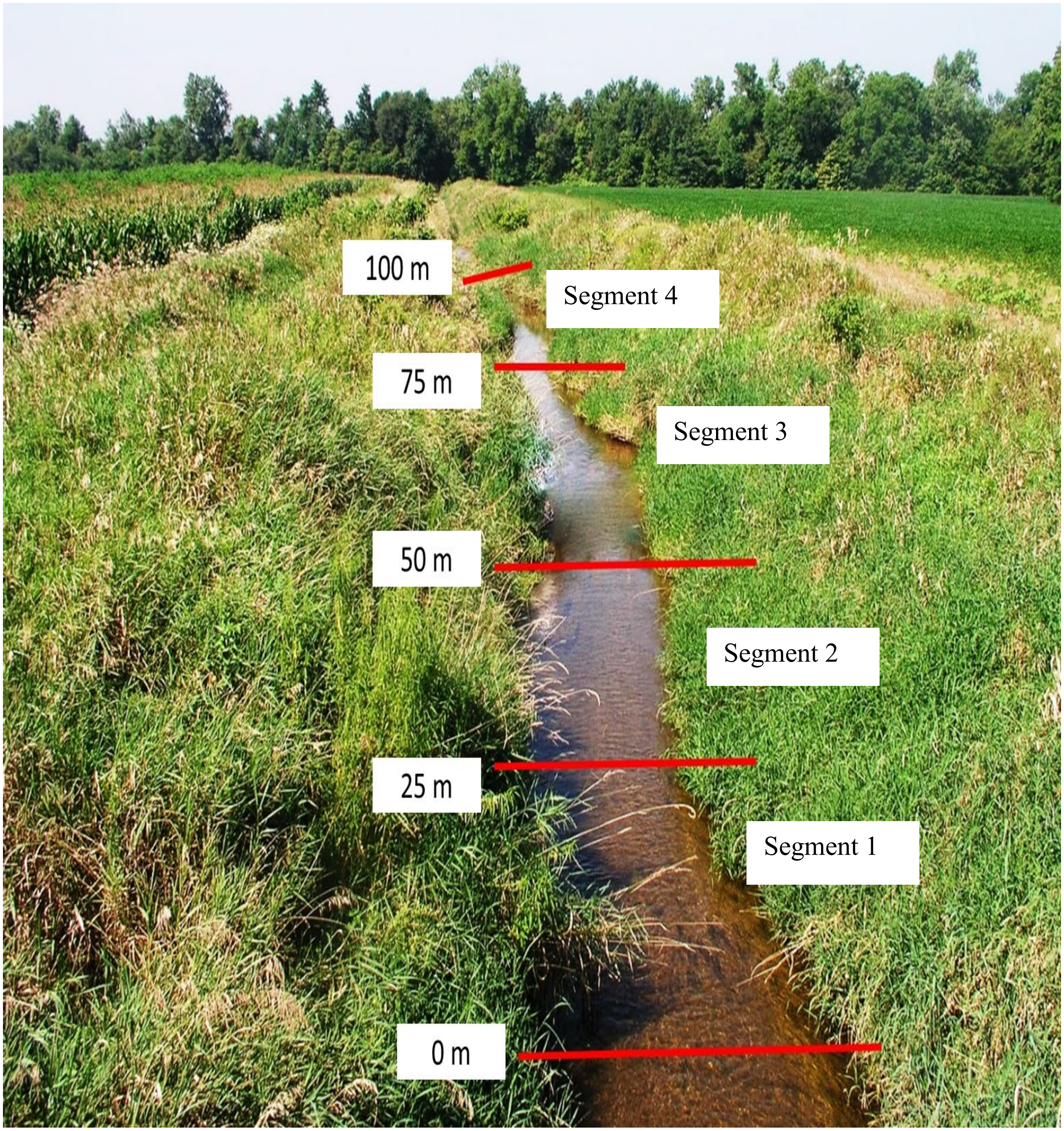


Figure 3 Photo representation of a 150-meter reach and 25-m segments at one site. Segments are not to scale and not all segments are represented in this photo. Site pictured is ALG in the Saint Joseph River Watershed within Indiana.

2.4 Statistical Analyses

2.4.1 Sediment Predictor Variables

Eight physical characteristic variables for sediments were calculated and Twenty sediment chemistry variables were obtained for this study (Table 2). In total 28 total sediment predictors were used in my statistical analyses, I categorized the variables into one of two groups of either physical characteristics or chemical characteristics.

I completed a Principle Components Analysis (PCA) on each of the groupings to create axes that represented the gradients of physical and chemical sediment characteristics among samples. The axes served as my independent variables in statistical analyses. Additionally, the PCA reduced the number of possible predictor variables from 28 to 5 for further analyses (Tables 4 & 5) and reduced the potential for multicollinearity. Three sediment chemistry axes and two sediment physical characteristic axes were retained for further analyses. I ran Pearson Correlation between the pairs of PCA axes to detect strong multicollinearity among independ variables. Variables were determined to be multicollinear if $r \geq 0.60$ or $r \geq -0.60$ and were excluded from further statistical analyses if inflation values were greater than 5.

2.4.2 Macroinvertebrate Response Variables

Fifteen macroinvertebrate response variables were calculated for this study (Table 3). Response variables that were significantly correlated (Pearson Correlation, $r \geq 0.60$) were removed from further analyses. After eliminating strongly correlated variables I retained 8 macroinvertebrate response variables for further analyses (Table 3). Macroinvertebrate response variables were tested for normality using a Shapiro-Wilk test ($w \geq 0.9$), and were transformed if normality was not observed.

2.4.3 Linear Mixed Effects Model Analyses

Linear mixed effects analysis was used to determine the strength of the sediment PCA axes at predicting macroinvertebrate metrics. Multicollinearity was avoided by using PCA axes created from the sediment predictor variables. Analyses were run as multivariate models with all five PCA axes run with each macroinvertebrate response variable. The fixed effects of these linear models were the 5 PCA axes. Random effects included were included in the analyses, these included, site, season, year, and sequences of additive and nested variations of these four random effects were included as needed. The singularity of each of the models was checked using the VarCorr and isSingular packages in RStudio to determine if the model is overfit and if the variances are close to zero. The best random effects models for each macroinvertebrate response variable were chosen based on the Akaike Information Criterion (AIC). Normality of residuals from selected models were assesses using qqPlot and Shapiro-Wilks tests ($w \geq 0.9$). Models with non-normal residuals were re-assessed with the transformed macroinvertebrate response variable using either $\log(x + 1)$ or arcsine squareroot transformations. The homogeneity assumptions of the models were verified by plotting model residuals versus the fitted values to determine if any trends occurred in these plots. Significance levels for all tests performed were $p<0.05$. All statistical analyses performed were done in RStudio R version 3.6.2 (Team, 2020)

Table 2 Calculations and criteria used to calculate sediment predictor variables.

Sediment Predictors	Criteria/Equations
% Organic Content	(Weight of sample before – Weight after ashing)*100
% Large Gravel	% of sediments 16.0 - 50.8 mm in size form a segment
% Small Gravel	% of sediments 2.0 - 15.9 mm in size form a segment
% Sand	% of sediments 53.0 μm
% Silt	% of sediments 2.0 - 52.9 μm
% Clay	% of sediments < 2.0 μm
Shannon Diversity	$-\sum(pi * \ln[pi])$
% Total Carbon	% Total organic carbon in sediments
% Total Nitrogen	% Total organic nitrogen in sediments
N-NH3	Concentration of ammonia in sediments
N-NO3	Concentration of nitrate in sediments
Aluminum	Concentration of extractable Al in sediments
Calcium	Concentration of extractable Ca in sediments
Copper	Concentration of extractable Cu in sediments
Iron	Concentration of extractable Fe in sediments
Potassium	Concentration of extractable K in sediments
Magnesium	Concentration of extractable Mg in sediments
Manganese	Concentration of extractable Mn in sediments
Phosphorus	Concentration of extractable P in sediments

Table 2 Continued

Sediment Predictors	Criteria/Equations
Sulfur	Concentration of extractable S in sediments
Zinc	Concentration of extractable Zn in sediments
Sediment Conductivity	The total capacity of sediments to conduct electrical current
Sediment pH	The acidity or alkalinity of the sediments
2OH-Atrazine	Concentration of Atrazine metabolite in sediments
Simazine	Concentration of Simazine in sediments
Atrazine	Concentration of Atrazine in sediments
S-Metolachlor	Concentration of S-metolachlor in sediments
Total Pesticide Concentrations	Total concentration of all pesticides in sediments

Table 3 Calculations and criteria used to calculate macroinvertebrate response variables.

Macroinvertebrate Responses	Criteria/Calculations
Abundance	Total # of macroinvertebrates in a segment
Shannon Diversity	$-\sum(pi * \ln[pi])$
Hilsenhoff Biotic Index	$(\sum[pi * ai])/N$
% Collector-Filterers (CFs)	([# of CF's in a segment]/N)*100
% Scrapers (Scrs)	([# of Scr's in a segment]/N)*100
% Chironomidae	([# of Chironomidae in a segment]/N)*100
Berger-Parker Reciprocal Index	(1/[total # of most abundant taxon / N])
Invertebrate Community Index	Total # of taxa in segment
	Total # of Mayfly taxa in segment
	Total # of Caddisfly taxa in segment
	Total # of Diptera taxa in segment
	% of Mayflies in segment
	% of Caddisflies in segment
	% of Tanytarsini midges in segment
	% of Other Diptera and Non-insects in segment
	% of Tolerant Organisms in segment
	Total # of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa in segment

Table 4 Loadings of PCA axes of sediment physical characteristics of headwater streams within the Saint Joseph River and Upper Big Walnut Creek Watersheds. Loadings within each axis that are bolded signify those characteristics that form the majority of the weight of my interpretations of each axis.

Sediment Physical Predictors	PCA Axis 1	PCA Axis 2
Percentage Organic Content	**0.35**	-0.10
Percentage Large Gravel	**-0.40**	-0.14
Percentage Small Gravel	**-0.41**	-0.14
Percentage Sand	-0.04	**0.69**
Percentage Silt	**0.43**	-0.24
Percentage Clay	**0.40**	**-0.39**
Shannon Diversity Index of Sediments	-0.25	**-0.47**
Percentage of Variance Explained by Axis	47	22

Table 5 Loadings of PCA axes of sediment chemistry characteristics of headwater streams within the Saint Joseph River and Upper Big Walnut Creek Watersheds. Loadings within each axis that are bolded signify those characteristics that form the majority of the weight of my interpretations of each axis.

Sediment Chemical Predictors	PCA Axis 1	PCA Axis 2	PCA Axis 3
Total Carbon	-0.2263	-0.2794	-0.01
Total Nitrogen	-0.3195	-0.0975	-0.0361
Concentrations of Ammonium (NH4+)	-0.2213	-0.2326	-0.0215
Concentrations of Nitrate (NO3-)	-0.0634	0.1414	**-0.4076**
Aluminum (Al) concentrations	-0.2965	0.2409	0.0397
Calcium (Ca) concentrations	-0.019	**-0.3643**	-0.2477
Copper (Cu) concentrations	0.0339	0.2082	**-0.4084**
Iron (Fe) concentrations	-0.2205	-0.1608	**0.2868**
Potassium (K) concentrations	**-0.3644**	0.0676	-0.1137
Magnesium (Mg) concentrations	-0.3274	0.1589	-0.1836
Manganese (Mn) concentrations	0.0326	-0.1135	-0.3511
Phosphorus (P) concentrations	0.0397	-0.2688	0.2593
Sulfur (S) concentrations	-0.2461	-0.2831	-0.096
Zinc (Zn) concentrations	-0.2558	-0.0546	-0.3606
Sediment Conductivity	-0.2915	-0.2547	0.0568
Sediment pH	**0.1778**	0.0713	-0.2219
2OH-Atrazine concentrations	-0.2325	0.2556	0.2226
Simazine concentrations	-0.0406	**0.3295**	-0.004
Atrazine concentrations	-0.2644	0.2249	0.1647

Table 5 Continued

Sediment Chemical Predictors	PCA Axis 1	PCA Axis 2	PCA Axis 3
Metolachlor concentrations	-0.2117	0.2932	0.1199
Percentage of Variance Explained by Axis	31	21	10

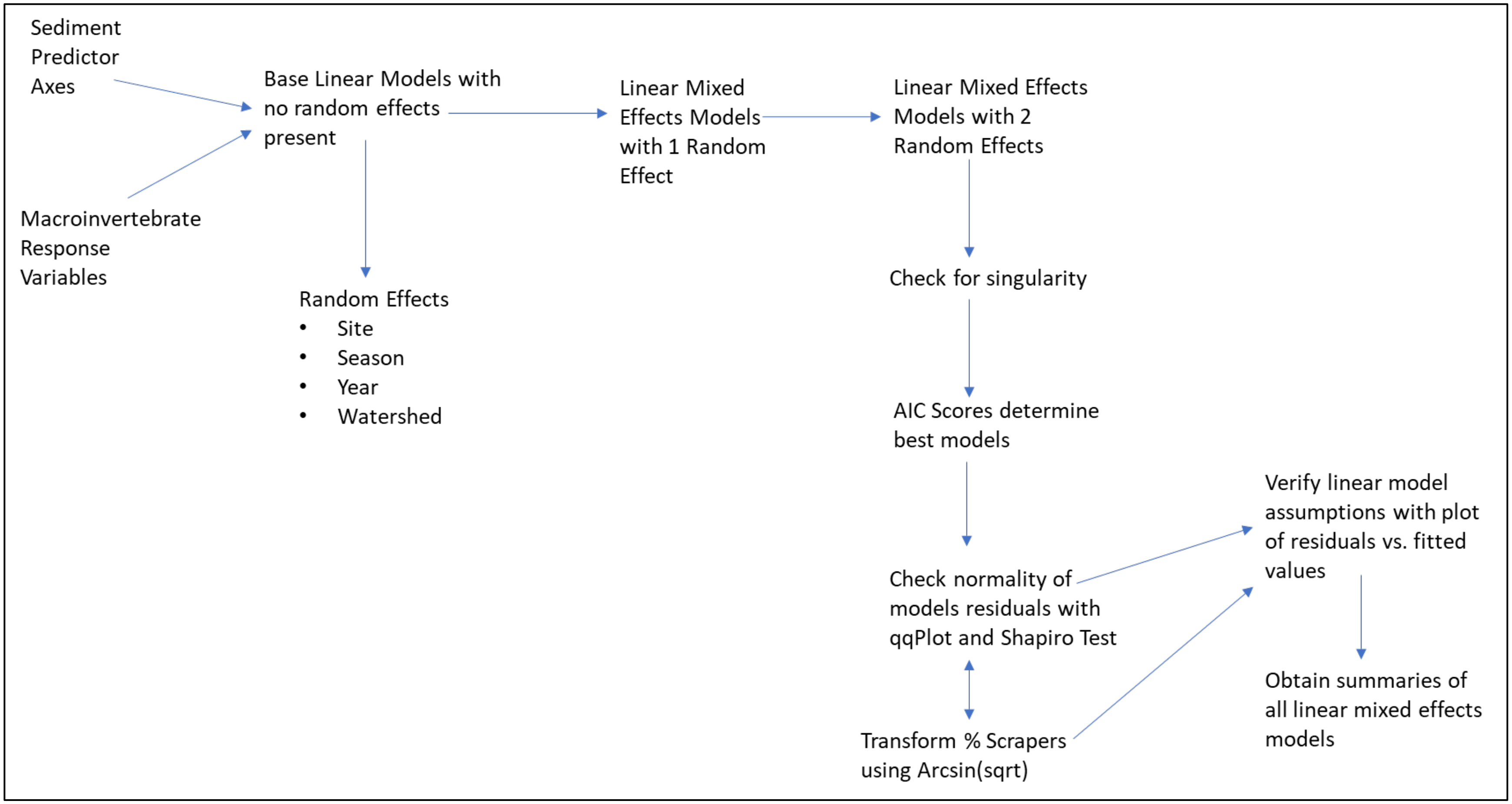


Figure 4 Flow chart of the statistical methods used to analyze the influence of selected sediment predictor axes on aquatic macroinvertebrate communities

CHAPTER 3. RESULTS

3.1 Macroinvertebrates

More than 20,000 organisms were collected among 57 macroinvertebrate taxa from both watersheds (Table 6). Over 40% of macroinvertebrates were from the family Chironomidae. Abundance of macroinvertebrates among SJR sites ranged from 100 to 4,002 (average = 1,485), while those from UBWC ranged from 206 to 1,938 (average = 939; Table 7). Shannon Diversity Index were similar between SJR 1.43 and UBWC 1.49 (Table 7). HBI scores ranged from 2.9 to 6.9 (average = 5.3) at SJR and from 5.7 to 7.4 (average = 6.4) at UBWC. The average HBI score at UBWC suggests a greater proportion of tolerant taxa than that of SJR.

Percentages of collector-filterers at SJR ranged from 0 to 17.9 % (average = 7.36 %) while those from UBWC ranged from 0 to 14.3 % (average = 2.96 %). Percentages of scraping macroinvertebrates at SJR ranged from 1.3 to 35.9 % (average = 6.73 %) while those from UBWC ranged from 0 to 56.5 % (average = 12.0 %). Percentages of Chironomidae at SJR ranged from 1.3 to 79.5 % (average = 43.46 %) while those from UBWC ranged from 4.7 to 78.9 % (average = 39.53 %). ICI scores at SJR ranged from 4 to 38 (average = 22) while those at UBWC ranged from 4 to 28 (average = 15.6). BPRI score ranged from 0 to 3.3 (average = 1.84) for sites in SJR. BPRI score ranged from 1.3 to .8 (average = 2.14) for sites in UBWC.

3.2 Sediments

Calcium concentrations at SJR ranged from 2.99 to 5.38 ppm (average = 3.87 ppm) while those from UBWC ranged from 1.35 to 4.23 ppm (average = 2.66 ppm; Table 8). Concentrations of Simazine at SJR ranged from 0 to 0.72 ppb (average = 0.38 ppb) while those from UBWC ranged from 0.23 to 4.79 ppb (average 1.24 ppb). The Shannon Diversity Index at SJR ranged from 1.08 to 1.45 (average = 1.27) while those from UBWC ranged from 1.04 to 1.55 (average = 1.44; Table 8). Percentage of sand fractions at SJR ranged from 0 to 62.67 % (average = 28.94 %) while those from UBWC ranged from 10.83 to 34.18 % (average = 21.67 %; Table 8). Percentage of clay fractions at SJR ranged from 0.86 to 25.25 % (average = 11.15) while those from UBWC ranged from 3.86 to 36.07 % (average = 17.36 %; Table 8).

Table 6 List of taxa, total number, and relative proportion of macroinvertebrates collected during 2017 – 2018.

Taxa	Taxonomic Level	Total #	%
	59	**20161**	
Aeshnidae	Family	17	0.1
Amphipoda	Order	1156	5.7
Baetidae	Family	3	0.0
Belostomatidae	Family	7	0.0
Bivalvia	Class	335	1.7
Caenidae	Family	148	0.7
Calopterygidae	Family	36	0.2
Capniidae	Family	4	0.0
Ceratopogonidae	Family	42	0.2
Chironomidae	Family	8603	42.7
Coenagrionidae	Family	391	1.9
Collembola	Subclass	57	0.3
Corduliidae	Family	2	0.0
Corixidae	Family	5	0.0
Corydalidae	Family	3	0.0
Culicidae	Family	19	0.1
Decapoda	Order	21	0.1
Diplopoda	Class	13	0.1
Dryopidae	Family	5	0.0
Dytiscidae	Family	7	0.0
Elmidae	Family	1504	7.5
Empididae	Family	125	0.6
Ephydridae	Family	6	0.0
Gastropoda	Class	1825	9.1
Gyrinidae	Family	2	0.0
Haliplidae	Family	11	0.1
Helicopsychidae	Family	11	0.1
Heptageniidae	Family	324	1.6
Hirudinea	Subclass	457	2.3
Hydrachnidae	Family	113	0.6
Hydrophilidae	Family	32	0.2
Hydropsychidae	Family	437	2.2
Hydroptilidae	Family	452	2.2
Isopoda	Order	953	4.7
Lepidostomatidae	Family	1	0.0

Table 6 Continued

Taxa	Taxonomic Level	Total #	%
Leptoceridae	Family	101	0.5
Leptohyphidae	Family	11	0.1
Leptophlebiidae	Family	358	1.8
Lestidae	Family	1	0.0
Leuctridae	Family	1	0.0
Limnephilidae	Family	20	0.1
Nematoda	Phylum	6	0.0
Nematomorpha	Phylum	108	0.5
Nemertea	Phylum	6	0.0
Oligochaeta	Subclass	928	4.6
Perlodidae	Family	1	0.0
Polycentropodidae	Family	3	0.0
Psychomyiidae	Family	2	0.0
Psychodidae	Family	357	1.8
Pyralidae	Family	1	0.0
Scirtidae	Family	2	0.0
Simuliidae	Family	23	0.1
Staphylinidae	Family	1	0.0
Tabanidae	Family	5	0.0
Tipulidae	Family	9	0.0
Turbellaria	Class	1075	5.3
Veliidae	Family	15	0.1
	59	**20161**	

Table 7 Minimum, maximum and average values of aquatic macroinvertebrate community response metrics from the Saint Joseph River Watershed, Indiana and Michigan and the Upper Big Walnut Creek, Ohio.

	Saint Joseph River			Upper Big Walnut Creek		
	Min	Max	Average	Min	Max	Average
Abundance	100	4002	1484.75	206	1938	938.69
Shannon Diversity Index of Macroinvertebrates	0	2.3	1.43	0.9	2.1	1.49
Hilsenhoff Biotic Index	2.9	6.9	5.31	5.7	7.4	6.41
% Collector-Filterers	0	17.9	7.36	0	14.3	2.96
% Scrapers	1.3	35.9	6.74	0	56.5	11.99
% Chironomidae	1.3	79.5	43.46	4.7	78.9	39.53
Berger-Parker Reciprocal Index	0	3.3	1.84	1.3	3.8	2.14
Invertebrate Community Index	4	38	22	4	28	15.63

Table 8 Minimum, maximum and average values of benthic sediment predictor metrics from the Saint Joseph River Watershed, Indiana and Michigan and the Upper Big Walnut Creek, Ohio.

	Saint Joseph River			Upper Big Walnut Creek		
	Min	Max	Average	Min	Max	Average
% Organic Content	1.1	6	2.64	1.8	8.9	4.31
% Large Gravel	0	38	7.24	0	25.2	10.9
% Small Gravel	6.77	38.14	25.45	0	51.65	27.75
% Sand	4.94	62.67	28.94	10.38	34.18	21.67
% Silt	3.64	52.24	27.47	4.94	49.85	22.32
% Clay	0.86	25.25	11.15	3.86	36.07	17.36
Shannon Diversity Index of Sediments	1.08	1.45	1.27	1.04	1.55	1.41
% Total Carbon	1.29	4.57	2.83	1.1	3.95	2.38
% Total Nitrogen	0.02	0.18	0.11	0	0.31	0.13
N-NH3	1.36	60.89	20.33	5.28	62.23	17.31
N-NO3	0.01	0.73	0.16	0.01	2.09	0.44
Aluminum (Al)	0.01	0.16	0.06	0.05	0.46	0.22

Table 8 Continued

	Saint Joseph River Min	Max	Upper Big Walnut Creek Average	Saint Joseph River Min	Max	Upper Big Walnut Creek Average
Calcium (Ca)	3	5.38	3.87	1.36	4.23	2.67
Copper (Cu)	0	0	0	0	0.01	0
Iron (Fe)	0.46	2.29	0.98	0.17	3.43	0.97
Potassium (K)	0.02	0.09	0.05	0.03	0.17	0.08
Magnesium (Mg)	0.1	0.28	0.16	0.13	0.48	0.29
Manganese (Mn)	0.04	0.24	0.14	0.03	0.18	0.11
Phosphorus (P)	0.02	0.05	0.03	0	0.03	0.02
Sulfur (S)	0.04	0.41	0.12	0	0.22	0.08
Zinc (Zn)	0	0.01	0	0	0.01	0
Sediment Conductivity	221.1	721.4	424.68	85.5	736.4	374.98

Table 8 Continued

	Saint Joseph River Min	Max	Upper Big Walnut Creek Average	Saint Joseph River Min	Max	Upper Big Walnut Creek Average
Sediment pH	7.28	9.98	7.76	6.74	8.41	7.64
2OH-Atrazine	2.71	27.54	14.07	14.41	99.95	47.85
Simazine	0	0.72	0.38	0.23	4.79	1.24
Atrazine	0.35	9.97	5.22	2.95	18.61	11.13
S-Metolachlor	0	5.59	1.52	0	24.68	9.36
Total Pesticide Concentrations	3.25	37.91	21.19	30.29	124.64	69.59

3.3 Linear Mixed Effects Model Analyses

The best random effect for macroinvertebrate abundance was season (Table 9), while site was the best random effect for HBI scores and percentages of scraper macroinvertebrates (Table 11, Table 13). Year was the best random effect for percentages of Chironomidae (Table 15). ICI and BPRI scores both shared seasons nested into year as the best random effect (Table 14, Table 16). Site nested within year was the best random effect for the Shannon Diversity (Table 10). Watershed nested within seasons was identified as the best random effect for the percentage of collector-filterers (Table 12).

Two models showed significant effects ($p < 0.05$) with a fixed sediment predictor variable (Table 17). Macroinvertebrate abundance showed a significant negative relationship with sediment chemistry axis 2 ($p < 0.05$). Sediment chemistry axis 2 was a gradient of concentrations of the nutrient calcium and the herbicide Simazine, where increasing site scores contained samples with increasing simazine concentrations and decreasing calcium concentrations. Thus, macroinvertebrate abundance decreased with increasing simazine and decreasing calcium concentrations (Appendix A). The percentages Chironomidae taxa showed a significant positive relationship with sediment physical characteristics axis 2 ($p < 0.05$). Sediment physical characteristics axis 2 was influenced mostly by percentages of both sand and clay and with the Shannon Diversity of grain sizes, where increasing PCA site scores indicated increasing percentage sand and decreasing Shannon Diversity index and percentage clay. Percentage Chironomidae increased with increasing percentage sand and decreasing Shannon Diversity Index and percentage clay (Appendix B). The remaining 6 macroinvertebrate response variable models did not show any significant relationships ($p > 0.05$) with the two PCA axes of sediment physical characteristics or the three PCA axes of sediment chemical characteristics (Table 17).

Table 9 Best random effect for aquatic macroinvertebrate response models from sites in the Saint Joseph River and Upper Big Walnut Creek, Watersheds. Akaike Information Criteria (AIC) are reported for each model. Only AICs of the best random effect models are reported

Macroinvertebrate Response Model	Random Effect(s)	AIC
Abundance	Season	462.52
Shannon Diversity Index	Year/Site	69.31
Hilsenhoff Biotic Index	Site	98.71
Percent Collector-Filters	Season/Watershed	203.06
Percent Scrapers (Transformed)	Site	16.53
Invertebrate Community Index	Year/Season	214.63
Percent Chironomidae	Year	279.02
Berger-Parker Reciprocal Index	Year/Season	101.99

Table 10 Influence of sediment predictor variables on aquatic macroinvertebrate response variables within agricultural headwater streams in the Saint Joseph River and Upper Big Walnut Creek watersheds during 2017 to 2018. Bolded terms indicate the sediment predictor variable axis that had the greatest influence on each of the macroinvertebrate responses. Plus (+) and minus (-) signs indicate the direction of the influence each macroinvertebrate response model.

Aquatic Macroinvertebrate Response Metric	Sediment Predictor Axes	p-Value	Influence
Abundance	Sediment Chemistry axis 1	0.34	
	Sediment Chemistry axis 2	**0.04**	**-**
	Sediment Chemistry axis 3	0.47	
	Sediment Physical Characteristics axis 1	0.20	
	Sediment Physical Characteristics axis 2	0.40	
Shannon Diversity Index of Aquatic Macroinvertebrates	Sediment Chemistry axis 1	0.62	
	Sediment Chemistry axis 2	0.36	
	Sediment Chemistry axis 3	0.95	
	Sediment Physical Characteristics axis 1	0.19	
	Sediment Physical Characteristics axis 2	0.13	
Hilsenhoff Biotic Index	Sediment Chemistry axis 1	0.84	
	Sediment Chemistry axis 2	0.06	
	Sediment Chemistry axis 3	0.83	
	Sediment Physical Characteristics axis 1	0.67	
	Sediment Physical Characteristics axis 2	0.07	
Percentage of Collector-Filterer Aquatic Macroinvertebrates	Sediment Chemistry axis 1	0.97	
	Sediment Chemistry axis 2	0.90	
	Sediment Chemistry axis 3	0.66	
	Sediment Physical Characteristics axis 1	0.92	
	Sediment Physical Characteristics axis 2	0.07	

Table 10 Continued

Aquatic Macroinvertebrate Response Metric	Sediment Predictor Axes	p-Value	Influence
Percentage of Scraper Aquatic Macroinvertebrates	Sediment Chemistry axis 1	0.15	
	Sediment Chemistry axis 2	0.36	
	Sediment Chemistry axis 3	0.15	
	Sediment Physical Characteristics axis 1	0.20	
	Sediment Physical Characteristics axis 2	0.17	
Invertebrate Community Index	Sediment Chemistry axis 1	0.07	
	Sediment Chemistry axis 2	0.07	
	Sediment Chemistry axis 3	0.56	
	Sediment Physical Characteristics axis 1	0.20	
	Sediment Physical Characteristics axis 2	0.51	
Percentage of Chironomidae	Sediment Chemistry axis 1	0.23	
	Sediment Chemistry axis 2	0.97	
	Sediment Chemistry axis 3	0.20	
	Sediment Physical Characteristics axis 1	0.48	
	Sediment Physical Characteristics axis 2	**0.04**	+
Berger-Parker Reciprocal Index of Dominance	Sediment Chemistry axis 1	0.91	
	Sediment Chemistry axis 2	0.10	
	Sediment Chemistry axis 3	0.84	
	Sediment Physical Characteristics axis 1	0.57	
	Sediment Physical Characteristics axis 2	0.91	

CHAPTER 4. DISCUSSION

For conservation and restoration of agriculturally dominated headwater streams to be successful both land and resource managers need to understand what contributes most to degraded aquatic macroinvertebrate communities. Many studies have observed negative impacts and influences that contaminated sediments and sediment physical characteristics have on aquatic macroinvertebrates (Fanny et al., 2012; Friberg et al., 2003; Moran et al., 2017; Pereira et al., 2017). Few studies, however, have evaluated the influence that both sediment chemistry and physical characteristics have on a range of aquatic macroinvertebrate community response variables within midwestern agriculturally headwater streams. The objective of my study was to determine whether benthic sediment characteristics, chemical and or physical, influence aquatic macroinvertebrate community metrics in Midwestern agriculturally dominated headwater streams. I expected that greater fine grain sediments and greater pesticide concentrations would decrease taxa diversity, decrease the presence of pollution-sensitive taxa, and decrease abundance. I expected greater particle size diversity, greater amounts of large grain sediments and, concentrations of pesticides will likely increase the taxa richness, abundance and presence of pollution-sensitive taxa.

I expected the physical characteristics of the benthic sediments would influence more macroinvertebrate community metrics than sediments chemical characteristics. I anticipated that sediment chemistry would influence macroinvertebrates and the physical characteristics of fine grains and less diverse sediment profiles would have negative impacts on aquatic macroinvertebrates. The results of my study indicate that there is no single sediment variable that can explain variations in the aquatic macroinvertebrate communities in these headwater streams. My results also indicate that different macroinvertebrate community metrics are influenced by different sediment predictors variables. I concluded from my study that few chemical and physical characteristics, although not all characteristics, of benthic sediments are influential on the aquatic macroinvertebrate communities in these agriculturally dominated headwater streams. Although the majority of the macroinvertebrate response variables did not show a significant relationship with either sediment chemistry or physical variables, these sediment variables may still have non-statistically significant influences on the macroinvertebrate communities.

Sediment chemical properties were the most influential variables on aquatic macroinvertebrate abundance. I observed that macroinvertebrate abundance decreased with increasing concentrations of Simazine and decreasing concentrations of calcium in benthic sediments. Although published studies have documented the effects of simazine and calcium in sediments on macroinvertebrates, independently, I did not find research articles that reported simultaneous effects of these sediment chemicals on macroinvertebrates. Many studies, however, look at the effects and impacts that these compounds have on aquatic macroinvertebrates singularly.

Although toxicity assays between Simazine and aquatic macroinvertebrates in streams are limited researchers have observed decreases, from water exposure, in species richness and evenness along with alterations in emergence timing (Dewey 1986). Researchers have also observed other s-triazine herbicides have indirect effects on macroinvertebrates as these herbicides cause reductions in the macrophytes needed for food and habitat (Dewey, 1986; Huber, 1993). The highest concentration of Simazine in sampled sediments was 0.72 ppb in SJR. The highest concentration of Simazine in sampled sediments observed was 4.79 ppb in UBWC. The observed concentrations of Simazine in my sites were collected from sediments however they did not reach levels that would have impacts from water exposure. This concentration in UBWC is greater than the LC_{50} for benthic species of Daphnia at 1.1 ppb in 48-hour experiments however, this LC_{50} is recorded from water (Service, 1980). Simazine can alter the aquatic vegetation that these macroinvertebrates need for food and habitat (Walker, 1964), altering the habitat and available food can alter what macroinvertebrates can exist and in what numbers in a given location. The EC_{50} for duckweed has been observed at 166 ppb over 4 days and 450 ppb over 3 days for green algae, these concentrations were obtained from water samples (Service, 1980).

The concentrations of calcium were found to be positively correlated with aquatic macroinvertebrate abundances in these headwater streams. Concentrations of calcium were higher in SJR and (average = 3.9 ppm) than in UBWC (average = 2.7 ppm). Calcium as a nutrient is important for macroinvertebrates in many capacities (Clark, 1958; White, 2003). This nutrient is important for diet, physiological functions and metabolism and many other functions in macroinvertebrates and many living organisms This nutrient is also essential for plant life and growth (White, 2003). Calcium is an essential nutrient however, the threshold for this nutrient in macroinvertebrates was not considered in this study. It is feasible that a plateau can be reached for calcium concentrations and increasing concentrations beyond the threshold will not influence macroinvertebrate abundances. Increases in this nutrient within sediments may also cause increases in aquatic vegetation which as mentioned is necessary for aquatic macroinvertebrates for food and habitat. As calcium is an essential nutrient it can be inferred that sites with lower concentrations of this nutrient in their benthic sediments may be unable to support large populations of aquatic macroinvertebrates.

Although abundance of aquatic macroinvertebrates was negatively correlated with sediment chemical characteristics the influences were most likely indirect. Simazine is a nonselective residual herbicide that can reduce aquatic vegetation which is important for aquatic macroinvertebrates. Simazine may be lethal to pollutant sensitive species such as stoneflies if it is a chronic exposure (Johnson et al., 1980). Calcium, as mentioned, is an important nutrient for many life forms and plays many roles for animals and plants and alterations in concentrations may alter their populations and structuring. Calcium like Simazine will likely cause indirect effects on macroinvertebrates.

The percentage of Chironomidae were found to be significantly influenced by sediment physical characteristics. I observed that the percentage Chironomidae increased with increasing percent sand and decreasing sediment diversity index and percent clay. Research has observed similar findings but observed Chironomids can be selective with the sediments that they inhabit. Although most research looks at singular variables they discern similar findings in headwater streams of the Midwest.

Midges of Chironomidae inhabit a wide range of benthic habitats with a wide range of sediment sizes (Bazzanti, 2000). Chironomidae are tolerant organisms and thought to be indicators of habitat degradation (Carew et al., 2007). As I only identified these organisms to the family level, I was unable to determine if they were of a certain genus that preferred one type of benthic substrate over another. These organisms can inhabit sediments ranging from very fine to large cobble and from homogenous sediments to very diverse sediments provided there is adequate organic content necessary for food (Bazzanti, 2000; Butakka et al., 2014; De Haas et al., 2006; Schmid, 1993).

Sandy fraction of sediments are often positively correlated with the increased percentages in Chironomidae and was observed in the multivariate results of my study. Research has observed that streams with high proportions of sandy sediments are often dominated by chironomid midges and other macroinvertebrates that are able to burrow (Yamamuro, 2004). With a greater proportion of sandy sediments, it would be likely that macroinvertebrates that prefer this substratum would likely increase. The percentages of both chironomid organisms and sandy benthic sediments were greater in SJR than they were in UBWC.

As part of the multivariate modeling percentage Chironomidae was negatively correlated with the diversity index of benthic sediment sizes and the percentage of clay. Although organisms of this family can inhabit a wide range of benthic sediment habitats reducing the diversity of the benthic sediments can lead to decreases in this taxon (Júnior et al., 2016). It is possible that these watersheds are host to genera that have preferential sediment habitats. Increased percentages of the clay fraction of sediments in sites were also associated with a decrease in the Chironomidae taxa. Fine-grain sediments such as clay have been observed to negatively influence aquatic macroinvertebrates and community metrics (Wood et al., 1997). Larval chironomids reside in and or atop of the benthic sediments (Pinder, 1986). Increases in clay sediments can be lethal to midge larval stages as they can become smothered. Increasing percentages of clay reduces the diversity of benthic sediments which can lead to a loss of habitat and food sources.

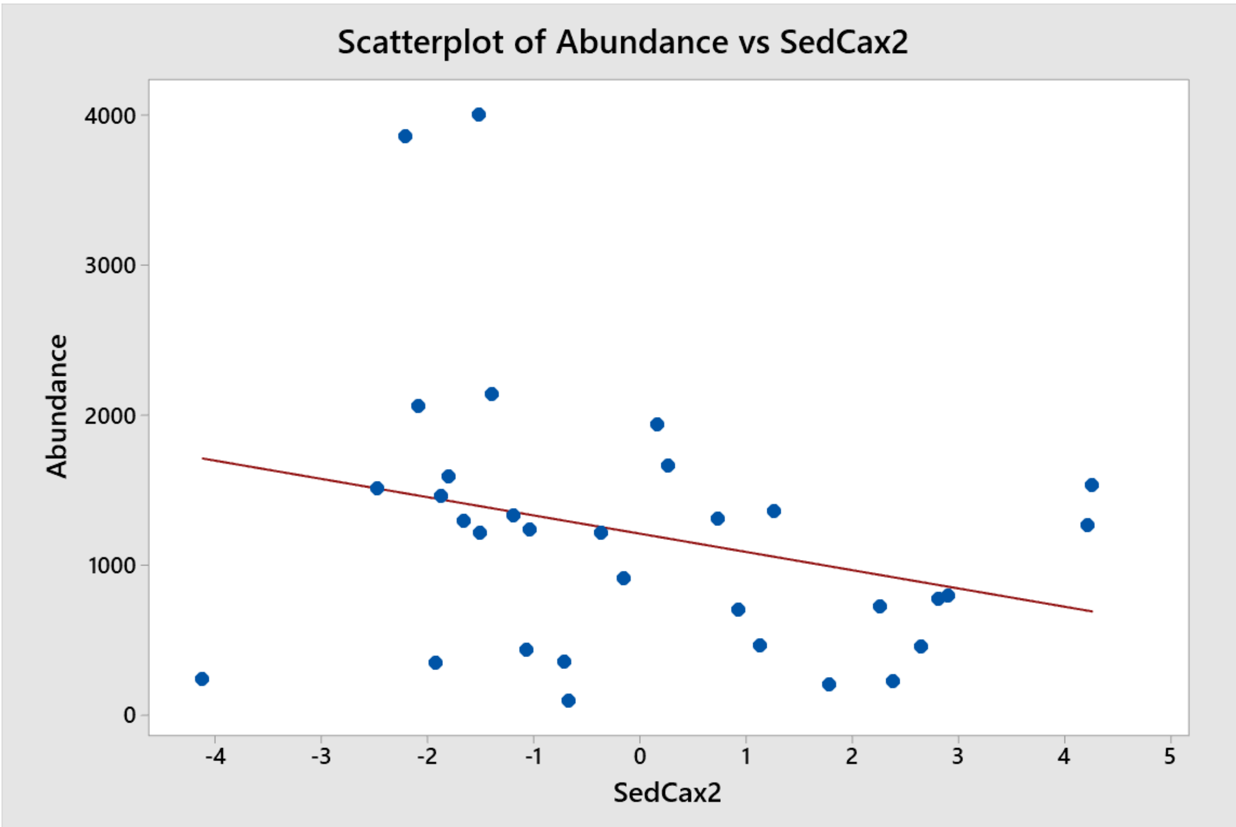
Percentage of Chironomidae in my study were found to be positively correlated with sediment physical characteristics, diversity of sediment and the percentage of sandy fractions. The influences that sediment physical characteristics have on aquatic macroinvertebrates are likely to be direct as chironomids live most/all of their larval stages within sediments. Chironomidae are often the most abundant family of freshwater macroinvertebrates and often the most diverse in stream ecosystems (Dewalt et al., 2010; Pinder, 1986). Lacking further classification of these organisms makes drawing conclusions on the specific effects that physical sediment characteristics have on them difficult.

I was surprised that metrics of aquatic macroinvertebrates were not correlated with more sediment characteristics. As many aquatic macroinvertebrates reside in sediments or use them for foraging it was unexpected that they were influenced by few variables. Very few macroinvertebrate metrics were significantly correlated with sediment physical characteristics but that does not necessarily mean they do not play a role. It may be that within my sampled watersheds the concentrations of agricultural chemicals found within the sediments were not great enough to cause negative influences. Further studies and bioassays will need to be conducted to determine what concentrations of these chemicals in sediments impact aquatic macroinvertebrates.

Further studies should include other habitat variables such as water chemistry, discharge, and riparian habitat. Other research has observed that contaminated benthic sediments may alter pollutant sensitive aquatic macroinvertebrates when the sediments are exposed to bioturbation brought on by macroinvertebrates (van der Meer et al., 2017). Macroinvertebrate sensitivities, such as physiological and chemical impairments or lethality are subject not only to classes of pesticides but concentrations for acute and chronic toxicities (Dinh Van et al., 2014). This study provides information to determine in these headwater streams what is significantly influencing these macroinvertebrate communities in a short timeframe however, it is likely that there are more sediment predictors including sedimentation and adsorption/desorption rates influencing these communities over long periods of time.

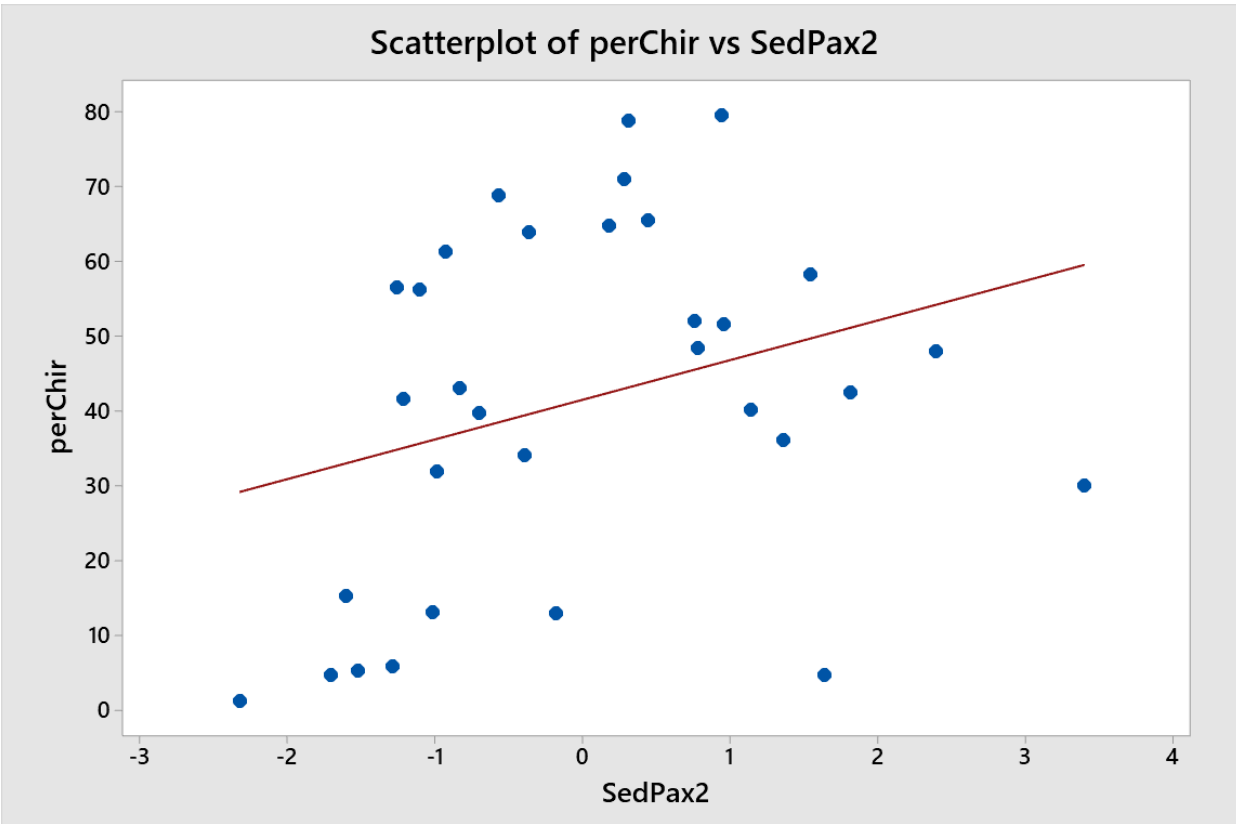
In conclusion, benthic sediment gradients of physical and chemical characteristics are significant environmental variables that can influence communities of aquatic macroinvertebrate communities. Although only two macroinvertebrate metrics were significantly influenced in my study both metrics are important indicators of stream health. Macroinvertebrate abundance and percentage of Chironomidae within agriculturally dominated headwater streams of the Saint Joseph River and Upper Big Walnut Creek watersheds are influenced by benthic sediment gradients. Fluctuations in macroinvertebrate abundances could indicate fluctuations of the chemical concentrations within the sediments. Fluctuations in the percent chironomids could indicate fluctuations in benthic substratum and or disturbances in the benthic habitats. Both macroinvertebrate abundance and percent chironomids are important metrics of stream health, however, they are only two of many that need to be considered. Macroinvertebrate abundance and percent chironomids are important to the overall stream health as they are often a key food source for many organisms within the stream (Armitage, 1995). Alterations of these two metrics can indicate potential disturbances in the macroinvertebrate and overall stream community. The use of multivariate gradients shows that multiple sediment variables can be influential however, they do not show the individual influences that they may have on aquatic macroinvertebrates. Further analyses on the observed influential variables within the PCA axes will elucidate their exact influence and how strong they are. It is likely that aquatic macroinvertebrates in other agriculturally dominated watersheds will be influenced by contrasting sediment variables depending upon the substratum, pesticides, nutrients, concentrations, and a wide variety of variables. My results support that macroinvertebrate abundance is significantly negatively correlated with increased Simazine and decreased calcium concentrations. My results also support the percent of chironomids is significantly positively correlated with increasing percentage of sandy sediments and decreasing percentages of clay sediments and decreasing sediment particle diversity. The results of my study increase our knowledge that benthic sediments can be significantly influential variables on aquatic macroinvertebrate communities within agriculturally dominated headwater streams in the Midwestern United States.

APPENDIX A. ABUNDANCE VS. SEDIMENT CHEMISTRY AXIS 2 PLOT

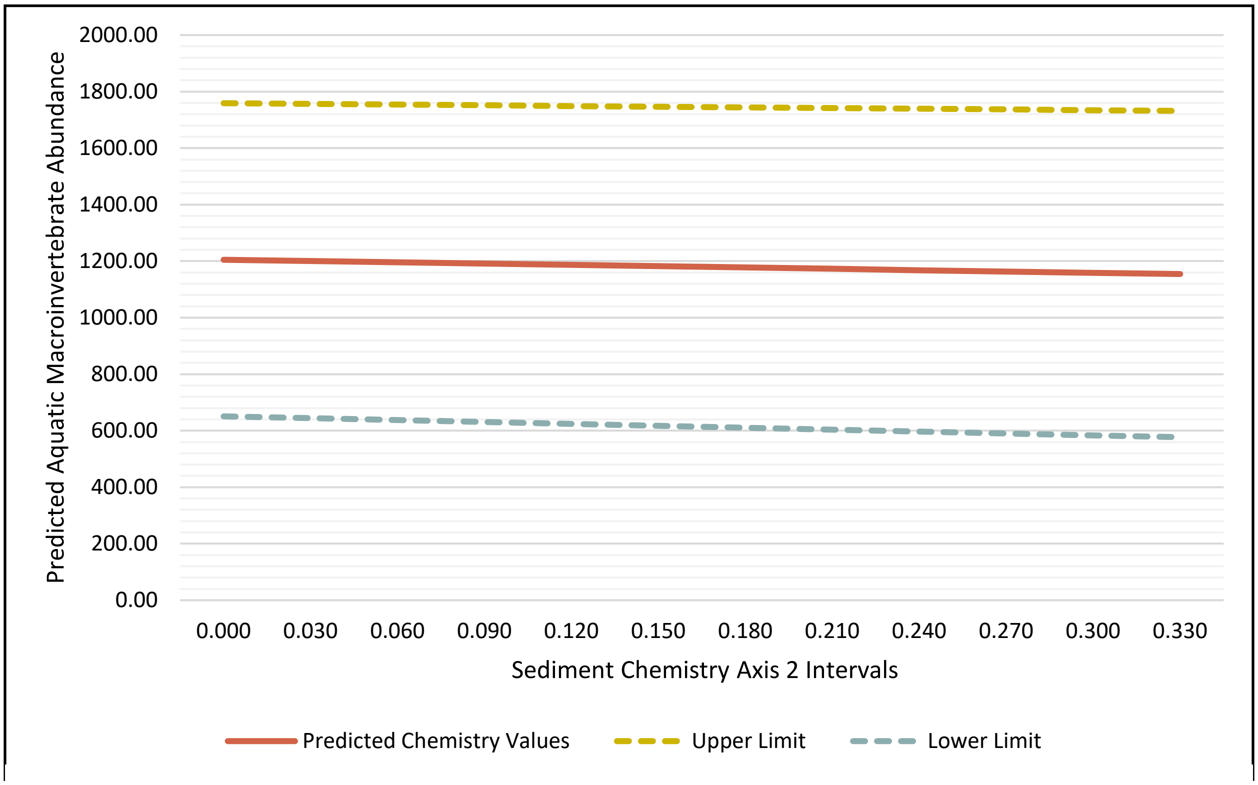


Aquatic macroinvertebrate abundances plotted against sediment chemistry axis 2 in the Saint Joseph River Watershed, Indiana and Michigan and the Upper Big Walnut Creek, Ohio

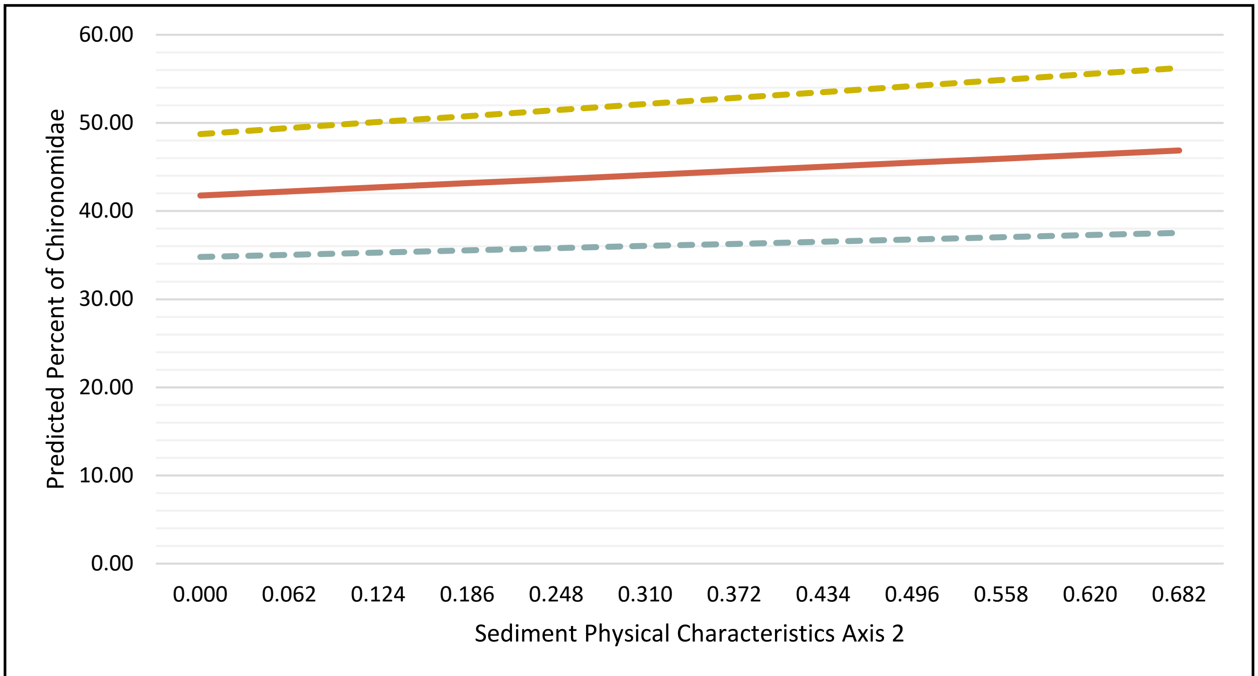
APPENDIX B. PERCENT CHIRONOMIDAE VS. SEDIMENT PHYSICAL CHARACTERISTICS AXIS 2 PLOT



Percentage of Chironomidae organisms plotted against sediment physical characteristics axis 2 in the Saint Joseph River Watershed, Indiana and Michigan and the Upper Big Walnut Creek, Ohio.



Predicted aquatic macroinvertebrate abundance against sediment chemistry axis 2 in both the Saint Joseph River Watershed, Indiana and Michigan and the Upper Big Walnut Creek, Ohio. Solid line is the predicted abundance value, dashed lines are the upper and lower limits



Predicted percentage of Chironomidae against sediment physical characteristic axis 2 in both the Saint Joseph River Watershed, Indiana and Michigan and the Upper Big Walnut Creek, Ohio. Solid line is the predicted abundance value, dashed lines are the upper and lower limits.

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