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Dispersal Capabilities of Two Plecopteran Species and Macroinvertebrate Community from Four Watersheds in Northeast Ohio

Alison L. Yasick
Cleveland State University

DISPERSAL CAPABILITIES OF TWO PLECOPTERAN SPECIES AND MACROINVERTEBRATE COMMUNITY FROM FOUR WATERSHEDS IN NORTHEAST OHIO.

ALISON L. YASICK

Bachelor of Science in Biology

Indiana University of Pennsylvania

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Masters of Science in Biology

Indiana University of Pennsylvania

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We hereby approve this dissertation
For
Alison L. Yasick
Candidate for the Doctoral Degree in Regulatory Biology for the
Department of Biological, Geological, and Environmental Sciences
and
Cleveland State University
College of Graduate studies by:

____Date:____________________
Dr. Julie Wolin, BGES/CSU
Chairperson

____Date:____________________
Dr. Robert Krebs, BGES/CSU
Committee Member

____Date:____________________
Dr. B. Mike Walton, BGES/CSU
Committee Member

____Date:____________________
Dr. Joe Kieper, Director of Virginia Museum of Natural History
Committee Member

____Date:____________________
Dr. Wentworth Clapham, BGES/CSU
Internal Examiner

____Date:____________________
Dr. Karen Munroe, Department of Biology and Geology, Baldwin Wallace University
External Examiner

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Two roads diverged in a yellow wood
And sorry I could not travel both
And be one traveler, long I stood
And looked down one as far as I could
To where it bent in the undergrowth;

Then took the other, as just as fair
And having perhaps the better claim,
Because it was grassy and wanted wear;
Though as for that, the passing there
Had worn them really about the same,

And both that morning equally lay
In leaves no step had trodden black.
Oh, I kept the first for another day!
Yet knowing how way leads on to way,
I doubted if I should ever come back.

I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood and I—
I took the one less traveled by,
And that has made all the difference.

Robert Frost *The Road Not Taken,* 1920

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ABSTRACT

This dissertation focused on the insect order Plecoptera, and hypothesized that *Allocapnia recta* populations would have lower genetic diversity than *Leuctra tenuis* between adjacent Chagrin and Grand Rivers due to wing structure and season of terrestrial adult emergence. Genetic variations within the 16s rRNA region of mtDNA in *A. recta*, a winter emerging adult with rudimentary wing structure, and *L. tenuis*, a summer emerging adult with fully developed wings, were compared and revealed significant genetic variability between *A. recta* samples from the two rivers (F_{ST} = 0.20) but not between *L. tenuis* samples (F_{ST} = 0.07). Further genetic variation investigation used *A. recta*, populations, within and between the Chagrin River and Grand River, hypothesized that differences in populations is a function of distance, and that greater distance leads to greater genetic variability. To strengthen the robustness of this work, samples were collected from two additional watersheds, the Rocky and Cuyahoga Rivers. Genetic variation of *A. recta* populations differed significantly across all four watersheds, especially between the Cuyahoga and Grand Rivers (G'_{ST} = 1), Rarity of movement regardless of distance suggests that other factors have a more profound effect than previously thought – factors that include human influences.

The unresolved genetic variation of *A. recta* and potential human influence resulted in a holistic examination of macroinvertebrate community structure and ecology within the four watersheds. Both legacy land use and anthropogenic disturbance effects on seasonal variation

were examined and it was hypothesized that: (1) greatest species diversity and richness among stoneflies and other macroinvertebrates will occur during the summer months, when weather conditions in Ohio are more conducive. (2) The greatest species diversity and richness among stoneflies and other macroinvertebrates will occur where the landscape has been historically less disturbed. The results revealed inconsistencies in seasonal diversity between sites; regardless of legacy land-use and anthropogenic influence. Results of this research show the significance of examining both aquatic and terrestrial stages in order to collect accurate and robust data on macroinvertebrate community structure. Furthermore, year-long macroinvertebrate sampling must be conducted even during extreme events in order to construct a better understanding of macroinvertebrate communities.

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CHAPTER I

INTRODUCTION

Stream biodiversity is constantly threatened by human encroachment through many complex pathways. Loss of diversity may occur as a result of land use alterations including changes in water chemistry, riparian vegetation removal, changes in light penetration, water temperature, and organic inputs. Such a loss of biodiversity can alter stream community structure. This research investigates landscape characteristics and land use effects on different scales of biodiversity from species level alpha diversity, to ecosystem level beta diversity, and finally effects on gamma diversity from a regional perspective.

In order to address questions related to alpha diversity, dispersal in two species of stoneflies were studied. Stoneflies are weak fliers as adults and have a terrestrial range limited, in general, to fifteen meters from the stream embankment (Schultheis et al., 2002). The nature of their wing structure and flight mechanics limits their aquatic-terrestrial dispersal capability and should affect their genetic diversity. By studying the genetic variability of stonefly subpopulations between watersheds, a genetic relationship can be established and utilized as evidence of intra- and/or interconnectivity between adjacent systems (Schultheis et al., 2002; Kauwe et al., 2004). This research studies multiple watersheds that have been separated from each other by a great enough distance and for a sufficient amount of time to have genetic variation within the stonefly populations.

Previous works have recognized that *Allocapnia recta* (Claassen, 1924) emerge during winter months with a rudimentary wing structure, a flight deficiency that is not known to have a direct correlation to emergence period (Ross and Ricker, 1971). However, the time of emergence and corresponding wing structure may have an effect on genetic heterogeneity of the stonefly population. This research explores population genetics of two species of stoneflies, *Allocapnia recta* and *Leuctra tenuis* (Picket, 1841), to determine if 1) time of year of adult terrestrial emergence and wing morphology have an effect on dispersal capability in stoneflies, and 2) if spatial distance is a factor contributing to the genetic variation within stonefly populations. It is hypothesized that between the two, *Allocapnia recta* will have the greatest genetic diversity among all sites due to its winter emergence and rudimentary wing structure and that *Leuctra tenius* populations, a summer emergent, will be low. Alternatively, there will be no significant difference in the amount of genetic variation between either of the two species. Secondly, it is hypothesized that the greater the distance *Allocapnia recta* populations are from each other, the greater the genetic diversity between their populations. Alternatively, there will be no significant difference in the amount of genetic variation between *Allocapnia recta* populations regardless of distance. At the ecosystem and regional level, how does the aquatic macroinvertebrate community assemblage contribute to the overall health and biodiversity of a stream ecosystem? Is species diversity and richness in macroinvertebrate populations affected by seasonal variation? Is there a difference in macroinvertebrate community assemblage and bioiversity in watersheds adjacent to managed land versus land currently or historically disturbed? Such understanding enables development of meaningful, empirical relationships and their use in developing more effective land management policies. In addition, a thorough understanding of stream health as a consequence of surrounding land use enables more direct actions for successful remediation, restoration, and future projects that insure the continued health and biodiversity of a stream ecosystem.

Determining whether the species diversity of stoneflies and macroinvertebrate communities is correlated to the health of stream systems and their surrounding habitats is not a trivial undertaking. While some macroinvertebrate orders, and other aquatic organisms, may remain active and even thrive in channels with poor water quality, stoneflies and similar pollution-intolerant macroinvertebrates require relatively high water quality for survival.

Less than ideal water quality, accompanied by a lack of suitable habitat, reduces species diversity and species abundance at a site. Such reductions have a direct impact on the genetic variability of the population by lowering the number of potential mates for reproduction.

Thus the third hypothesis is there will be both greater taxa diversity and richness among macroinvertebrate populations in watersheds circumvented by managed and/or designated protected lands when compared to watersheds surrounded by land use that have been demonstrated through previous research to reduce macroinvertebrate community diversity (i.e. urban, agricultural, residential, etc.). This hypothesis is not relegated to modern land use alone. The use of land both within the watersheds and adjacent to the stream channels included in this research have a dynamic and well-documented history. The direct effect of these historic land uses on macroinvertebrate communities was not researched until the latter decades of the twentieth century, often only focused on individual species and their populations. This work expands on previous studies by exploring the current structure of macroinvertebrate communities as a direct link to historic land use.

Including the preceding hypotheses, the purpose of this research is to:

1. Conceive, develop, and execute a multidisciplinary approach to studying stoneflies and other macroinvertebrate communities through the combination of entomology, population genetics, and landscape ecology.
2. Study the significance of dispersal capacity by examining two species of stoneflies (*A. recta* and *L. tenuis*) with differing temporal emergence periods and distinctive wing structures, characteristics that have the ability to isolate unique populations despite the lack of physical boundaries.
3. Measure the habitat quality required for maintaining species richness and diversity of plecopterans in a lotic community.
4. Measure and compare the species richness and diversity of *A. recta*, *L. tenuis,* and other macroinvertebrate communities within four Northeast Ohio watersheds, each surrounded by a unique land use, to determine the overall impacts reflecting legacy land use effects and current land use practices.

1.1 Biomonitoring as an Index of Stream Health

1.1.1 *Brief History of Aquatic Entomology*

Many of the preliminary advances in the scientific community's knowledge of aquatic insects correspond with the global explorations during the sixteenth and seventeenth centuries. In 1675, Dutch anatomist Jan Swammerdam was the first to study the natural history of the burrowing Ephemeroptera (McCafferty, 1998). Swammerdam's contributions, including detailed information on the transformation of aquatic insects from naiad to adult, the identification of external gills as an important respiratory structure, and the identification of dimorphic sexual characteristics between males and females was a cornerstone for the evolution of a new discipline (McCafferty, 1998).

Building on the work of Carolus Linnaeus, John Christian Fabricius created the first insect taxonomy as an apprentice of Linnaeus during the eighteenth century (Merritt and Cummings, 1996; McCafferty, 1998). Thomas Say and Benjamin Walsh were the two most prominent American figures to emerge, both understanding and advancing the importance of aquatic entomology (Merritt and Cummings, 1996). By the late nineteenth century, aquatic entomology emerged as a formal discipline of study and had developed a firm place in American scientific research - particularly as a result of extensive Ephemeroptera research by James G. Needham of Cornell University (McCafferty, 1998).

The first use of aquatic macroinvertebrates to assess the quality of water, particularly in regards to its general health and portability, was developed in Germany during the early twentieth century (Merritt and Cummings, 1996). Newly developed methodology employing biotic factors not only enabled researchers to decree a body of water as polluted, but also the degree to which it was polluted. Among the earliest studies conducted was an assessment of sewage outputs entering natural stream systems, a public health issue necessity heavily addressed in Europe at the time. It was recognized that an increase in sewage led to a decrease in dissolved oxygen and negative effects on aquatic life (Merritt and Cummins, 1996). These pioneering studies empirically led to the concept of indicator species as observations correlated a decrease in macroinvertebrate abundance and diversity with certain types of environmental alterations (Cairns and Pratt, 1993; Clements et al., 2013).

During the twentieth century, macroinvertebrates received a lot of attention due to their relative successes and failures in aquatic habitats related to environmental dynamics (Merritt and Cummings, 1996). Aquatic macroinvertebrates are an essential part of the aquatic food web for other organisms, including fish, amphibians, shorebirds, waterfowl, and other animals that forage on aquatic or terrestrial stage insects (McCafferty, 1998). By 1972, entomologists understood that altered conditions in a natural area, such as a stream, can lead to dire short-term and irreversible long-term effects that impact the quality and the community structure of organisms that inhabit streams.

1.1.2 *Modern Perspectives*

During the 1970s, North American aquatic ecologists shifted to quantitative methods outlining consistency in sampling techniques, replication of sample units, and the use of detailed statistical analyses (Resh and Jackson, 1993; Hauer and Lamberti, 1996; Merritt and Cummings, 1996). As biomonitoring and the use of indicator communities continue to evolve, two distinctive methodological paradigms have emerged among aquatic ecologists. In the face of both increasing financial and time constraints, one faction has reverted to traditional qualitative approaches to water quality monitoring practices. The second, an efficient yet approachable bioassessment procedure, has introduced a more salient means of quantitative and qualitative practices (Resh and Jackson, 1993). Aquatic insects are often preferred over other aquatic organisms such as fish, algae, and protozoans (Hellawell, 1986); the importance of aquatic insects and other benthic macroinvertebrates is difficult to overestimate.

Life cycle characteristics of aquatic macroinvertebrate can be monitored to gauge both subtle and prolific changes to water quality in the systems they inhabit. Any change to the structure of macroinvertebrate communities can be measured, both quantitatively (i.e., statistical measures of taxa diversity) and qualitatively (i.e., habitat analyses), and used to determine the various degrees of suboptimal conditions; providing a clear benefit over the use of chemical and other water quality analyses alone. Whereas water chemistry analyses through traditional methods can provide a snap-shot reflecting the upstream health of a sampling site on a particular day at a particular time, macroinvertebrate biomonitoring is able to ascertain varying temporally defined pollutants –continuous, intermittent, or accidental - at any number of spatial levels ranging from a single point source to degradation across an entire region.

Just as important as the value of macroinvertebrate biomonitoring over traditional methods (water chemistry analysis) is the recognition that macroinvertebrates do not uniformly respond to all types of impacts. For some macroinvertebrate species, their distribution and abundance is a function of the physiochemical aspects of the habitat as opposed to the quality of the water alone. When using macroinvertebrates in biomonitoring, it is considered good practice to consider both the biological and physical features of a stream to fully analyze the water quality at a site.

1.1.3 *Macroinvertebrates in Context*

With the development and evolution of different biomonitoring indices, such as the *Benthic Index of Biotic Integrity* (Kerans and Karr, 1994) and the *Rapid Bioassessment Protocol* (Barbour et al., 1997), larger categories of macroinvertebrates as bioindicators for quickly identifying stream quality have been developed. Aquatic macroinvertebrates are routinely used to determine the extent of certain pollutants such as organic and inorganic compounds from urban, agricultural, and industrial wastes in lotic system. Aquatic macroinvertebrate life cycles are impacted by changes in water chemistry, benthic habitat availability, and surrounding land use patterns making them excellent biological indicators (Koop et al. 2008). Several advantages of using macroinvertebrates include: (a) they are widespread and affected by a wide range of environmental stressors, (b) communities typically contain a diverse group of species which offers a wide range of stress responses, (c) in the aquatic life stage macroinvertebrates are not very mobile - allowing for spatial examination of disturbance effects; and (d) they have a relatively long life cycle that allows for temporal examination of disturbance effects (Gaufin, 1973; Hellawell, 1986; Rosenberg and Resh, 1993; McCord et al, 2007). Researchers can predict responses to remediation efforts by identifying changes in the biomass of macroinvertebrate populations, especially benthic forms, due to their sensitivity to pollutants (Letterman and Mitsch, 1978; Johnson et al., 1993; Death and Winterbourne, 1995). These advantages, coupled with regard for the scientific integrity and cost-effectiveness of evaluating the quality of stream habitats, enable and justify qualitative sampling and analysis of aquatic macroinvertebrates (Ohio Environmental Protection Agency, 2000).

1.2 Paradigmatic Evolution in Systems Studies

Throughout the past two decades, there has been an evolution in how scientists and natural resource managers examine entire lotic ecosystems. The traditional paradigm was that community-level organisms were influenced by rapidly changing events and that only physical characteristics directly adjacent to the stream affected the biota. However, more recent methodological constructs in macroecology incorporate a more balanced view of biodiversity and community structure; linking them to a combination of ecological and historical processes (Williams et al., 2003). Evolving ecological perspectives acknowledge the importance of physical and biological relationships in aquatic ecosystems, relationships that are both dependent on spatial and temporal factors (Gorman and Karr, 1978; Williams et al., 2003). How these evolving ecological perspectives neglect the importance of both spatial and temporal attributes in favor of one or the other is not clearly understood. Ecologists that study the complexity of factors impacting stream systems continue to largely neglect historical factors that act as filters for fauna on a regional scale, and are capable of predetermining the species diversity within a watershed (Tonn, 1990; Hugueny, 1997; Ricklefs et al., 1999; Williams et al., 2003; Allan, 2004) – factors that have the most influence on the distribution of aquatic organisms. It is clear that in order to understand a stream’s ecosystem; systemic studies cannot be isolated to assessments of diversity at a community level alone (Baattrup-Pederson, et al. 2008). Researchers must necessarily include the study of stream morphology and population structure as it relates to the surrounding landscape, both historically and in the present, on a spatial and temporal level (Yasick et al., 2007; Houghton and Wasson, 2013). This link has been traditionally underrepresented and needs to become a significant part of lotic system assessments.

1.2.1 *Land Use Dynamics: An Introduction to Historical Processes*

Multiple human activities have and continue to bring about changes in the geomorphology of the landscape due to complex and lasting alteration in the physical structure and hydrology of river systems that may never be completely restored (Allan, 2004). Applied historical studies of land use continue to evolve, the consensus of ecologists today is that, at a minimum, it is important to know and understand the historical land use to properly monitor ecosystems in the future (Swetnam et al., 1999). Distinguishing between past and present land use and its impact on ecosystems is cloaked under the de-notation of *legacy effects* (Allan, 2004). For example, agriculture has taken on a smaller role in the local economy in the southern region of the Appalachian Mountains (Allan, 2004). As the value of land for agrarian purpose has waned, the abandonment has resulted in natural dynamics of land reverting back to forests. Even with this natural change in land, studies continue to show that the flora and fauna within and surrounding such a region is more similar to streams in agricultural areas than present-day primary forested areas (Maloney and Weller, 2011). Land evaluation becomes more complicated when the land use surrounding riverine systems becomes cyclic, such as when primary forested landscapes are converted to agricultural lands and then later converted to urban landscapes or back to forests (Harding et al., 1998; Allan 2004; Maloney et al., 2008).

Legacy effects rarely result from natural processes (deforestation due to natural fires, riparian destruction or stream modification from extreme flood events, etc.). Harding (2003) implicated humans as the primary cause of the irreversible loss of taxonomic diversity due to historical manipulation in most of the endemic vertebrates in terrestrial, marine, and lake systems of New Zealand (Harding et al. 1998 ;Harding et al., 1998) revealed that the practice of repetitive burning destroyed the landscape vegetation, to clear large tracts of land that included increased erosion in riparian zones, and eliminated soil seed banks when humans colonized present-day New Zealand more than 1000 years ago. The physical effects that riparian zone removal has on stream ecosystems - alteration of bank stability, alteration of substrate characterization, and increased temperature regime – is well understood. But the lasting effects on the regional flora and fauna as a consequence of riparian removal throughout an entire watershed is yet to be completely comprehended (Roth et al., 1996; Wang et al., 1997; and Harding et al., 1998); particularly given the innumerable combinations of land use change that has occurred over space and time (Harding, 2003; Bojkova et al., 2012).

Many legacy effects are remnants of forest clear-cutting, a land management practice nearly as old as civilization itself. Clear cutting, was often performed near riverine systems where the channel served as a means of transporting fallen timber. This practice led to the removal of thousands of square kilometers of riparian vegetation and has had a lasting effect on the diversity of present-day aquatic biota. The more ubiquitous effects include bank instability and increased sedimentation, the introduction of more competitive invasive species, and water contamination (Harding, 2003; Allan, 2004; Burcher and Banfield, 2006).

Macroinvertebrate species diversity and populations that depend on relatively stable conditions, low sedimentation, and forested habitats become extinct or migrate from riverine systems subjected to clear-cutting. Stone and Wallace (1998) discovered that the aquatic biota was low in species abundance, taxonomic richness, and biomass. The most pollution intolerant taxa, Ephemoroptera, Plecoptera, and Tricoptera (EPT) abundance remained much lower when compared to pre-logging levels sixteen years after logging was halted and reforestation started to return around a North Carolina stream. These legacy effects were predicted to last several more decades.

Despite the fact that an area of land may return to its natural condition through cycles of deforestation/riparian vegetation removal and reforestation, it is not certain that the biota will recover at the same rate or at all. In addition, such cycles do not guarantee that a reforested tract of land will function in the same manner that it did prior to reforestation. The age and size of forests plays an important role in organismal community structure (Foster et al., 2003). Once displaced from their original habitat, populations re-establish very slowly. If there are large physical structures – dams, bridges, artificial waterfalls, etc. – as a consequence of land management, recolonization as a consequence of land management practices, may never occur (Foster et al., 2003).

1.2.2 *The Changing Landscape: Land Use and Macroinvertebrate Communities*

One way of defining macroinvertebrate communities in stream systems is by organizing macroinvertebrates into functional feeding groups – those with the greatest potential of relaying important information about the process-level aquatic ecosystem attributes (Rawer-Jost et al., 2000). Vannote et al. (1980) developed the *River Continuum Concept* (RCC) in an undisturbed stream, a theory that predicts key ecosystem properties along a continuum of the stream system. Although the RCC was developed for forested riverine systems, the concept can be modified to fit other forms of lotic ecosystems because it illustrates the response of macroinvertebrate communities to changes in their food resources. Changes in the functional feeding groups can be used to monitor shifts in the relative abundance of defined macroinvertebrate functional feeding groups - particularly in response to land use change.

In the insatiable pursuit of land acquisition, humans are rapidly converting once undisturbed landscapes into urbanized and agricultural regions. This practice of land conversion contributes to a variety of dynamics affecting nutrient loading, erosion, animal grazing, chemical contamination, and building human infrastructures within cities and suburban regions (Burcher and Benfield, 2006). A significant problem with streams in urbanized and agricultural regions is the creation of impervious surface leading to an increase in overland flow. This increases the frequency and intensity of run off and leads to increased water level fluctuations and flash flooding (Moglen, 2000; Moore and Palmer, 2005). Furthermore, in agricultural streams, erosional dynamics and a decrease in riparian vegetation are two of the most significant concerns affecting streams. The increase in fine sedimentation in the substrate can lead to elevated in-stream embeddedness and shallower streams (Wang et al., 2002; Vondracek et al., 2005). Shallower streams, in conjunction with the loss of riparian vegetation, experience an increase in water temperature; a changing dynamic inversely proportional to dissolved oxygen concentrations. Whereas these negative effects due to changing land use are most traditionally related to urbanization, agricultural areas are fully capable of acting like urbanized areas when impervious surfaces result from large areas of compacted soils.

While traditional studies of negatively impacted riverine systems and land use change have focused on the industrialization and urbanization impacts of the past century, preliminary studies have demonstrated measurable human impairment – often with more abstract impacts resulting from historical and modern agricultural land use (Vitousek, 1994; Bruns, 2005). Studies that include biological examination of agricultural conditions are done so within the context of gradients of agricultural land use and intensity, as opposed to unmodified, virgin land (Reynoldson et al. 1997; Genito et al., 2002; Louhi et al. 2011). Biological integrity and habitat quality are negatively correlated to the intensity of agricultural land use upstream from study sites (Roth et al., 1996; Herbst, et al. 2012). A study conducted by Harding et al. (1999), in a New Zealand river, showed replacement of pollution sensitive (EPT) orders with those that are pollution tolerant is a common community response in riverine systems subjected to agriculturally derived pollutants.

Currently, the greatest concerns in water quality are those due to non-point source pollutants (Chambers et al., 2006). Throughout the past few decades, much work has been done to eliminate point-source pollution by upgrading old industrial or sewage treatment operations and incorporating design improvements. The greatest concentrations of non-point pollutants commonly detected in and around agricultural areas are those from nutrient and organic matter loading, sedimentation, and contaminates (i.e. herbicides, pesticides, fertilizers) (Lenat, 1994; Vondracek et al., 2005; Palmer et al. 2010). Increasing nitrogen and phosphorus can cause excessive aquatic plant growth, loss of plant species, depletion of oxygen, and deleterious changes in the abundance and diversity of macroinvertebrates, fish and other organisms that depend on, or part of, a stream ecosystem (Smith et al., 1999; Chambers et al., 2006 Robinson, 2012; Zhang et al. 2012). In order to mitigate human impact on lotic ecosystems, it is essential to understand and relate the patterns of land cover changes to the process of changes in land use and relate those changes to within the freshwater ecosystem (Bruns, 2005).

1.3 Empirical Focus

Stoneflies are weak fliers as terrestrial adults (Schultheis et al., 2002) and the nature of their wing structure and flight mechanics limits their aquatic-terrestrial dispersal capability. Species with high dispersal (i.e. gene flow) have little genetic differentiation among their populations. Thus, the measure of genetic differentiation among populations is a good indicator of dispersal among populations. Genetic diversity within and among populations is affected by the degree of population isolation, population size, length of isolation, and environmental differences between sites (Hughes et al., 1999). Where dispersal is confined, the amount of genetic homogeneity among different populations begins to decrease and genetic drift, selection, and mutation within the separate groups can lead to greater genetic variability (Hedrick, 2000).

1.4 Plecopteran Community

Stoneflies are a small order of exopterygote insects including about 2000 species worldwide (500 species in North America). They have a relatively long, but fragmented, fossil record dating back to the Permian Period (Cushing & Allan, 2001). Members of the Plecoptera spend the majority of their life as aquatic naiads. The naiads emerge as terrestrial adult insects throughout the year and may live anywhere from several hours to several weeks. The length of time an individual remains in either stage is species specific (Schmidt et al., 1995). Furthermore, time of year for the adult phase is also species specific. As terrestrial adults, stoneflies are sexually mature and seek out mates. Once a male impregnates a female, the female will return to the water to lay her eggs. Due to their dependence on the aquatic environment, stoneflies do not fly far from a water source (Cushing and Allan, 2001).

1.4.1 *Dispersal*

Stoneflies live most of their life in their immature naiad stage, inhabiting streams and swimming for dispersal. Their adult, terrestrial lives are short-lived and primarily isolated to their natal riparian zone. Plecopteran species show significant variability in wing morphology and musculature, and variability in flight as adults (Malmqvist, 2000; Winterbourne, 2005). This variability in flight, and hence dispersal range, can be categorized among plecopterans through extremes from full wing, full flight capabilities to apterous and flightless. Dispersal is measured as the distance from an organism's natal habitat to the breeding habitat among individuals of a species (MacNeale et al., 2005). Dispersal among plecopterans may be passive (e.g. migrating as a result of wind directionality, hydrologic gradient in open channel stream systems, etc.), but it is more likely the result of intentional movement leading to more desirable attributes conducive to survival. The outcome of dispersal causes the population, as a whole, to spread out spatially and is critical – in fact, a necessity – for the long-term survival of the species.

The role of dispersal is critical. Highly specialized behaviors have evolved among insects for dispersal, and those behaviors have become part of the physiology and ecology of a species. Among the most important of these physiological behaviors is the development of flight; enabling greater access to resources, mate acquisition, and predator avoidance. As a result of dispersal, many species variables are affected –

including natal population demographics, the populations of adjacent habitats, colonization of new or previously uninhabited regions, and the rates at which populations become genetically distinct from each other (Briers et al., 2003). Malmqvist (2000) suggested that in addition to species population variables, such as population size, length of isolation, etc., colonization and range size may lead to the commonality or rarity of a species. Insects with long wings are good dispersers and potentially good colonizers, whereas some species become rare as a result of short wings and poor colonization ability. However, even with all of its advantages, flight has been lost repeatedly among insects (McCulloch et al., 2009).

Plecopterans have two dispersal mechanisms: adult flight and larval drift. Huntsman et al. (1999) showed that flying insects, in general, could disperse long distances either by muscular powered flight or by wind action. Even in species like stoneflies that are not strong fliers, flying still gives the insect a greater dispersal advantage than insects that do not fly. Dispersal is also achieved through larval drift where stoneflies move downstream with the accompanying current. Stoneflies also have the ability to actively swim upstream either in search of food or for predator avoidance. Unfortunately, the significance of adult flight and larval drift as mechanisms of dispersal is difficult to quantify with concrete data (Brederveld et al., 2011).

While not all macroinvertebrates have the affinity to drift, extreme biological and physiological disturbances are a major seasonal variant affecting their assemblages (Muller, 1974). Most drift studies have only addressed the daily movement of macroinvertebrates and few studies have documented their seasonal movement or investigated the levels at which a disturbance can disrupt the normal pattern of drift

(Brittan and Eikeland, 1988; Tockner and Waringer, 1997; Robinson et al., 2002). A greater intensity of studies related to drift and seasonal disturbance can lead to a better understanding of the alternative aspects of lotic ecosystem function (Robinson et al., 2002). Seasonal changes in macroinvertebrate drift have important implications for both organic matter exchanges with the floodplain channels and organism dispersal/migration (Romito et al. 2010)

Plecoptera mobility in the naiad stage has always been relegated to swimming. The most rudimentary form of stonefly flight began with surface skimming before evolving into more complex flight patterns; increasing flight velocity at each stage. In *surface skimming,* thrust is provided by wing flapping and maintaining continuous contact with the water surface, removing the need for total aerodynamic weight support. Several variants of flight connected to surface skimming led Marden et al. (2000) to index the evolution of surface skimming into five distinct stages, swimming/swimming-skimmer, six-and four-leg skimmers, hind-leg skimming and jumping , with each stage of evolution leading to full flight capability. Wing variability results from a variety of factors ranging from habitat stability to elevation and ambient air temperature. While the general environmental factors affecting flight are understood, there still remains uncertainty regarding the interrelationship of environmental factors and flight capability. The main argument for reduced wing structure and flight capabilities, as presented by Malmqvist, 2000, is the relationship of wing development and fecundity. Because egg production and wing development depend on the same metabolic energy resources, population members may choose to disproportionately allocate metabolic energy towards one or the other; respectively leading to greater reproduction capability or dispersal capability.

Consistent observation of plecopteran flight – whether active (full flight mechanisms) or passive (variations of surface skimming) – reveals a consistent directional pathway of movement upstream from the site of emergence. McNeale et al. (2005) studied the direction and distance that *Lectura ferruginea* traveled through analytical assessment using the stable isotope ^{15}N. By incorporating the isotope in four stream systems over a period of four years, enriching the ^{15}N nutrient concentration in *L. ferruginea* naiads, captured emerging adults were assessed for N-enrichment. The results supported qualitative observations that *L. ferruginea*, which are strong fliers, had flight vectors oriented upstream; in some cases with head winds nearing 5km/hr.

Whereas the directionality of emerging plecopterans continues to be studied and better understood, questions continue to surround the reason(s) for these flight patterns. One current theory is that upstream movement and female ovipositon of eggs is an adaptive trait that has evolved in plecopterans (Winterbourne and Crowe, 2001). Other research suggests that upper reaches of a stream have greater productivity and biomass availability (Hall et al., 2001). Among the favorable conditions that may facilitate upstream directionality is a decrease in predation and interspecies competition, as well as favorable physical factors like lower pollutant levels, sedimentation, and other anthropogenic factors.

1.4.2 Study Organisms

This research is focused on two species of Plecoptera: *Allocapnia recta* (Claassen 1924) from the family Capnidae and *Leuctra tenuis* (Pictet 1841) from the family Leuctridae, and general assemblages of aquatic macroinvertebrates. *Allocapnia recta* and *L. tenuis* were chosen due to the difference in time of the calendar year that they emerge as terrestrial adults. *Allocapnia recta* and *Leuctra tenuis* emerge at nearly opposite times of the year and are faced with contrasting environmental factors related to the time of year. Specific habitat requirements for plecopterans, both as naiads and adults, include pristine water conditions with a high oxygen concentration and little to no anthropogenic impact.

The dispersal potential of *Allocapnia recta* and *Leuctra tenuis* will be measured indirectly using the genetic markers, mitochondrial deoxyribose nucleic acid (mtDNA). Insect mtDNA contains thirteen protein-coding regions, twenty-two transfer ribose nucleic acid (tRNA) genes, two ribosomal RNA (rRNA) genes and one non-coding region (the origin of mtDNA replication) (Simon, 1991; Schultheis, 2002). Genetic variation within and between species populations arises more quickly in mtDNA than in the nuclear genome due to its faster rate of nucleotide substitution, maternal mode of inheritance and lack of recombination. Moreover, patterns of evolutionary relationships are easier to trace in uniparental systems than in nuclear DNA. Moritz et al. (1987), along with Murdoch and Herbert (1997), validate mtDNA analysis as one of the most powerful means of genetic analysis available to examine patterns of phylogeographic relationships.

1.5 General Aquatic Quality

Analysis of landscape patterns show that all ecosystems elements, whether terrestrial or aquatic, respond to disturbances differently depending on how the alterations occur, intensity and duration of the disturbance, and patterns or conditions under which the ecosystem is to recover (Burcher et al., 2007; Louhi et al. 2011). As aquatic ecologists learn more about the co-variable interactions between the aquatic and terrestrial environment, it becomes too complex to detangle the relationships that exist between each of the variables (Richards et al., 1996; Wang et al., 1997; Vondracek et al., 2005). Johnson et al. (2007) stated that watershed land cover contributes materials and energy to the stream, which together determine the cumulative stressor load to which a stream ecosystem is subjected at any given time. Van Sickle (2003) and King et al (2005) also commented on the relationship between land and water variables by illustrating two seemingly independent factors, such as benthic substrate and allochthonous coarse particulate matter, function dependently to affect nutrient availability and habitat parameters. In addition, these two factors function together to determine the diversity and abundance of the macroinvertebrate community found within the ecosystem. Ruhl (1995) determined that land use practices, vegetation, geology, and soil structure all attribute to the biological response and chemical and physiological factors of a stream system. These biological responses include degree of susceptibility to chemical and organic pollutants entering waterways, habitat loss and degradation due to changing land-use activities, local extinctions triggered by the loss of key predators, the spread of predatory or competitive invasive species, and response to climate change (Allan and Flecker, 1993; Harding, 2003).

Historically, streams have been assessed through spatially or temporally constrained water quality inferences, a practice that, in large part, continues today. Understanding the impact of historical and land use legacy effects and the consequence of modern land use alterations on riverine systems is a task of monumental proportion. The current rate at which land reclassification occurs has escalated well beyond the current systemic understanding of the stream systems and watersheds they directly impact (Pond, 2012).

1.6 Specific Aquatic Quality

Riverine system investigators realize that the health and maintenance of stream biodiversity is constantly threatened by human encroachment through many complex pathways; complexities affecting the ecological integrity of the ecosystem, habitat health, water quality, and the local biota (Sponseller et al., 2001; Megan et al., 2007). Land use alterations are known to be a dominant stressor, particularly, but not exclusively on freshwater ecosystems, with the greatest impacts associated with watershed modifications (i.e. substrate alterations and increase water temperature) and human contamination of aquatic resources (i.e. organic and inorganic input) (Carpenter et al., 1992; Bruns, 2005; Kruse et al. 2013). Such encroachment within a watershed also presents implications for the downstream ecological integrity and may compromise the viability of the ecosystem (Norris et al., 2007). Water chemistry, light penetration, organic inputs, and water temperature (Megan et al., 2007) among others, become increasingly vulnerable as the ecological integrity of the ecosystem is affected; with temperature change as a primary consequence of riparian vegetation removal (Scrimgeour et al. 2013).

The loss of streamside vegetation, such as the conversion of a forested landscape into agriculture land, increases the amount of solar radiation reaching the stream channel, subsequently leading to increased water temperatures. Alteration of the thermal regimes in a stream habitat is critical to the natural history and ecology of macroinvertebrates (Vannote & Sweeney, 1980 and Quinn et al., 1994). The premature development of macroinvertebrates, brought on by a rise in water temperature has many negative effects including compromising mate acquisition for both male and females, female fecundity, and egg development.

In addition to changes in thermal regime, loss of riparian vegetation often leads to an increased percentage of impervious surfaces. Whether riparian alterations lead to agricultural land use or more conventional urban constructs, increased impervious surface reduces levels of evapotranspiration and infiltration, altering natural flow regimes and catalyzing bank erosion (Maloney et al. 2009). As a consequence of escalated bank erosion, sedimentation rates increase and the substrate embeddedness is negatively impacted - often reducing macroinvertebrate species diversity and densities (Lenat and Crawford, 1994; Quinn et al., 1997; Sponseller et al., 2001; Maloney et al., 2009).

Allan (1997) demonstrated that while water chemistry and sediment yield are primarily governed by geology, hydrology, soils, and vegetation at the watershed level, it is riparian vegetation that mediates water quality and quantity, sedimentation, and nutrient sinks and/or sources. The riparian vegetation affects the timing and amount of discharge, in-stream temperature, influences habitat structure, hydraulic complexity, channel morphology, and nutrient input. However, the ability of vegetation to act as a sink in agricultural areas is limited (Lowrance et al., 2001).

Land cover/land use level investigations have repeatedly shown that species numbers and composition are relatively dependent on the environmental factors to which the communities are exposed. Very few species are collected in areas where environmental factors beyond optimum requirements, resulting not only in loss of taxonomic richness, but also genetic diversity (Ruse, 2000; Probst et al., 2005). Through their research in an Australian stream, Townshed et al. (1997) showed that population density of burrowing macroinvertebrates was greater in reaches below pasteurized land than in reaches downstream of forested areas. In a similar study conducted by Delong and Brusven

(1998), scrapers were the dominant feeding group downstream of agricultural land, where there was less canopy cover and more light penetration in the stream allowing for increased algae growth, the main food resource for scrapers. The authors found shredders, however, dominated in reaches downstream of forests. Shredders thrive on the increase allochthanous material (leaf litter). Presence of these functional feeding types illustrates the greater reliance on autotrophic food sources in altered landscapes due to the effect of riparian vegetation modification in agricultural/pasteurized land in contrast to original forested habitats (Vannote et al., 1980; Genito et al., 2002; Utz, 2009).

1.6.1 Biodiversity of Macroinvertebrate Communities

Assessing biodiversity of macroinvertebrates in lotic systems is an essential component of basic ecological inquiry and applied ecological assessments (Ward and Tockner, 2001). Aspects of taxonomic diversity and composition in aquatic ecosystems are used to quantify water quality and measure the efficacy of remediation and restoration efforts. Aquatic ecologists realize that the health and maintenance of stream biodiversity is constantly threatened by human encroachment through complex pathways; complexities affecting the ecological integrity of the ecosystem, habitat health, water quality, and local biota. Land use alterations are known to be a dominant stressor on the reduction of stream biodiversity, with the greatest impacts associated with watershed modifications and human contamination of aquatic resources (Ward and Tockner 2001; Evan-White et al., 2009).

Agricultural practices illustrate the loss of biodiversity through human impact better than most other examples. Reduced biodiversity in streams with high nutrient levels is thought to be caused by direct nutrient toxicity from non-point source pollution, which can lead to indirect alteration of physical and biological factors such as increase in primary production and reduced dissolved oxygen concentrations. Furthermore, the increase in suspended sediment by increased livestock grazing in and around the river will also have a negative impact on the biodiversity of stream macroinvertebrates (Evans-White et al., 2009). Suspended sediments can cause significant respiratory problems among macroinvertebrates. The sediments can settle on the bottom of the stream and coat the external gills of the more sensitive taxa (i.e. EPT). Increases in stream temperature associated with removal of riparian trees can also cause respiratory stress in macroinvertebrates and reduce their success at survival, or alter their growth and development. Each of these mechanisms plays a role in reducing macroinvertebrate diversity in nutrient-enriched streams (Evans-White et al., 2008; Pfrender et al., 2010). Faced with loss or displacement of biodiversity, populations of macroinvertebrates exhibit either resilience or resistance (Southwood 1977). Resilient species have the capacity of returning to their prior taxon richness and density after disturbance. Their resilience resides in their ability to reproduce at an early age, their short reproductive cycles, regeneration potential, and their ability to recolonize from refugia (Southwood, 1977). Resistant species have the ability to withstand the disturbance without significant loss of taxon richness or density. Their resistance is facilitated by their ability to create a firm attachment to the substrate, a streamlined body form, and invulnerable life stages (i.e. diapause or hibernation during peak weather extremes) (Townshed et al., 1997; Statzner and Beche, 2010; Demars et al., 2012).

Aquatic macroinvertebrates are best known for their use as indicator organisms in aquatic ecosystems. They are an important food resource for fish, amphibians, and waterfowl, and their involvement in the breakdown and recycling of organic matter and nutrients make them critical components of stream ecosystems. Their distinction as indicators also includes their invaluable usage in assessing the health of riverine systems and they are used more often than any other assemblage of aquatic organisms.

1.6.2 Spatial Perspective

Riverine ecologists recognize that rivers and streams are complex patches of habitat and environmental gradients that characterize aquatic and terrestrial connectivity and spatial complexity (Schlosser, 1991; Fausch et al., 2002; Allan, 2004; Norris et al., 2007). The systemic interdependence linking macroinvertebrates to their aquatic and terrestrial surroundings enables scientists to use changes in stream environments that lead to shifts in the macroinvertebrate community, such as changes in substrate, dissolved oxygen concentrations, water temperature and allochthonous input as a measure of disturbance levels.

Currently, the practice of embedding forested land parcels within agricultural landscapes is a short-term remedy. The forest fragments increase allochthonous input, stable stream morphology, reduce flow variation, and buffer water chemistry factors known to increase benthic macroinvertebrate community diversity and stability (Nakamura and Yamada, 2005; Harding et al., 2006). However, Harding et al. (2006) illustrated that fragmented forests represent an intermediate habitat. The researchers found that the size of the fragment and vegetation type were the most significant factors in the success of maintaining a refuge for macroinvertebrates between the forested and agricultural landscapes (Harding et al., 2006). However, in a study of community structure in the family Formicidae, Ivanov and Keiper (2010) observed that even though species richness increased at the interface between forested and urban land use, the increase was due to increases in opportunistic and generalist species. Similar patterns are found in fragmented landscapes surrounding stream ecosystems. Although total numbers and diversity of macroinvertebrates may increase at the edge of forested and agriculture/urban land cover (i.e. dipterans), there are losses in species diversity, especially among the more sensitive orders (i.e. ephemeropterans, plecopterans, and trichopterans). As demand for landscapes as areas of agriculture, urban, and industrial land increases on a global scale, land fragments may be the most reasonable solution, though not the best ecological alternative.

1.6.3 Reach and Organism Perspective

Historically, riverine system processes have been studied from a reach perspective (Sponseller et al., 2001; Allan, 2004; Johnson et al., 2007; Norris et al., 2007). Reach-scale perspective analyses, while efficient due to their small scale, are spatially constrained, raising concerns that lotic communities and populations are studied at a scale far too small to develop an adequate understanding of organisms and the processes in which they are an integral part. The aquatic organism perspective, also known as the organism-centered view, examines a riverine system from the perspective of individual organisms and recognizes aquatic landscapes as a variety of microhabitats – leaf litter, stands of macrophytes, and streambed substrate – essential to macroinvertebrate species diversity (Lancaster and Belyea, 1997).

Plecopterans and other aquatic macroinvertebrates exhibit a vast array of morphology, physiology, and behavior adaptations that enable them to exist in many aquatic ecosystems; including temporal and aestival pools, cold and hot springs, running and standing waters, intermittent streams, and saline lakes. Rarely are the aquatic conditions and habitat so extreme that macroinvertebrates are absent (Ward, 1992; Utz et al., 2009). Seventy to ninety percent of all macroinvertebrates collected at a stream are of the class Insecta (Voshell, 2005). Within the class Insecta, thirteen orders contain species with aquatic or semi-aquatic life stages; five orders (Ephemeroptera, Plecoptera, Trichoptera, Odonata, and Megaloptera) have aquatic stages possessed by all species in the order (Hauer and Lamberti, 1996). The remaining eight orders contain both aquatic and terrestrial representatives. With rare exceptions, species in the Coleoptera and Hemiptera (suborder Heteroptera) contain immature and adult aquatic stages while all other orders are amphibiotic - characterized by terrestrial adults (Ward, 1992).

1.6.4 Temporal Perspective

The spatiality of macroinvertebrate settings - including the location of the watershed, stream orders within its basin, the relative proportions of various land uses combined with topography, and physical features of the system - can relay important systems information to aquatic ecologists (Megan et al., 2007). However, stream dynamics are not limited to spatial variations; many streams experience networks of annual expansion and contraction (Stanley et al., 1997; Robinson et al., 2002; Zhang et al., 2012) leading to temporal variations directly related to disturbance patterns of a riverine system. As the streams experience networks of annual physical change, benthic communities experience change on a more temporal, seasonal level (Hynes, 1970; Death, 1995; Reece et al., 2001; Romito et al., 2010) - particularly those changes affecting the availability of food, stability of habitat, and drift. These within-stream seasonal variations, among others, are known to contribute to the variability of macroinvertebrate assemblages within a stream system.

1.6.4.1Seasonal Weather/Storms

Research by Townshed and Hildrew (1994) demonstrated the potential impact of a second important temporal variant: seasonal weather patterns and accompanying storm events. The frequency of intense storm events has the potential of changing stream patterns from minor to major degrees. In their 1994 publication, Townshed and Hildrew's study of weather patterns and storm events supported the long held perception that the disturbance of a stream is a constituent of the temporal regime as opposed to the spatial regime. Disturbance, or stress, was defined as an event that caused removal of residential organisms with time.

1.6.4.2 Food availability

Aquatic macroinvertebrate diversity and composition in a stream ecosystem is largely dependent on surrounding environmental factors to which the community is exposed and their function as nutrient recyclers; representing their linkage between lower and higher tropic levels, and as a food resource for fish and amphibians (Megan et al., 2007; Ferreira et al., 2013). As nutrient recyclers, macroinvertebrates are essential to riverine systems. Of prime importance is the effect shredders have on recycling carbon back into the system through the breakdown of large particulate organic carbon in the form of autochthonous and/or allochthonous materials. In addition, grazers, deposit, and suspension feeders use nutrients in the form of dissolved organic material and biofilms composed of algae, protozoans, bacteria, and/or fungus build up. While in lower order streams or headwater streams, macroinvertebrate predators may be at the top of the food chain, preying on such organisms as other macroinvertebrates, fish fry, and salamander eggs (Wallace and Webster, 1996; Huryn and Wallace, 2000; Malmqvist, 2002).

The constituents of a macroinvertebrate community in any system are directly related to the availability of food. In regions dominated by deciduous foliage, there is an increase in both the quantity and quality of course particulate organic matter (CPOM) during the autumn season. During this season, CPOM levels enable shredders to dominate (Murphy and Giller, 2000). As autumn transitions to winter and CPOM is broken down into fine particulates, filter feeders dominate until the season changes once again and increased solar intensity - facilitated by a sparse canopy – leads to algal blooms and a riverine system dominated by grazers and collector-gathers.

In order to determine the relationship between diversity in macroinvertebrate community structure and surrounding environment towards achieving the best overall predictive models for biomonitoring, a combination of both spatial knowledge and temporal knowledge of the entire watershed is required. A study conducted by Murphy and Giller (2000) illustrates this by demonstrating the direct dependence of quantity and quality of detritus for macroinvertebrate consumption (temporal perspective) on the type of land use (spatial perspective), and more importantly, riparian vegetation bordering the streams. This research, an extension of the dietary continuum (Petersen and Cummins, 1974), designed predictive models of macroinvertebrate assemblages based on the decay rate of specific types of detritus. The model revealed that detritus from each classification reaches palatability after a sequentially longer period in the stream; classifying decay rates under the general constructs slow, medium, and fast. The conclusions showed that the more diverse the riparian vegetation, and consequently the range of detritus decay, the longer the sustainability of food availability for detritivours well beyond coarse particulate matter (CPOM) and the autumn leaf fall.

In order to better understand the effects of temporal variation, legacy land use, and current land use practices on macroinvertebrate community and functional feeding group diversity at the at the local and regional level, this study investigated the macroinvertebrate communities of four Northeast Ohio watersheds on a seasonal basis between January 2004 and December 2005, while comparing past and present land use conditions to the current macroinvertebrate community..

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CHAPTER II

THE EFFECTS OF DISPERSAL ABILITY IN WINTER AND SUMMER STONEFLIES ON THEIR GENETIC DIFFERENTIATION

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2.1 ABSTRACT

1. Plecopteran species disperse less than most other aquatic insects. Within stoneflies, members of different families vary in the degree of wing morphology and season of adult emergence.

2. The dispersal limitations were tested to determine if there were increased differences among nearby populations by comparing genetic variation within the 16s rRNA region of mitochondrial DNA in two stoneflies: *Allocapnia recta*, which emerges in winter and often has rudimentary wings, and *Leuctra tenuis*, which emerges in summer with fully developed wings.

3. There was significant genetic variability between the samples of *A. recta* from two adjacent rivers (F_{st} = 0.20), but not between samples of *L. tenuis* (F_{st} = 0.07).

4. Distinct clades in *A. recta* were found to occur within the minimum spanning tree specific to the Chagrin River, contributing to a significant difference in gene diversity between the two rivers. Haplotypes in *L. tenuis* appeared randomly distributed between the two rivers; however, nucleotide diversity was significantly less in samples from the Grand River.

5. Further investigation is required to determine if these species migrated into both watersheds and populations have since diverged by genetic drift, or whether their poor dispersal potential led to different genetic lineages entering each stream.

Key Words: dispersal, genetic drift, 16s rRNA region, *Allocapnia recta, Leuctra tenuis* haplotype diversity, stoneflies

2.2 Introduction

Dispersal ability of organisms is a key ecological factor that influences the structure of a population (Miller *et al*. 2002). In freshwater communities, genetic divergence may arise because a stream system flows through several habitats, each habitat acting effectively as a biogeographic barrier from either a location within the stream or other near-by watersheds (Monaghan *et al*., 2002; Monaghan *et al.*, 2005). Isolation by physical barriers in combination with genetic drift, or differing pressures of natural selection within each habitat has the potential of increasing genetic divergence between streams (Monaghan *et al.* 2002).

Most flying aquatic insects can navigate between adjacent rivers (Petersen *et al.*, 2004). However there are some species that possess wings, but have limited flight. Sanderson *et al*. (2005) noted that the composition of the community from neighboring streams were generally similar, with some differences observed in weak dispersers such as Ephemeroptera. Smith *et al*. (2006) similarly reported population divergence in mayflies across catchments, although differences between adjacent streams were less.

However, stoneflies (Order: Plecoptera) are weak fliers (Nebeker & Gaufin, 1967) even compared to mayflies and therefore their movement among river systems is more likely to be inhibited by habitat fragmentation than that of stronger flying insects. Schultheis *et al.* (2002) identified greater movement of *Peltoperla tarteri* (Stark & Kondratieff 1987) within streams as opposed to among streams in the Southern Appalachians. In western Montana, Hughes *et al.* (1999) similarly identified population variation in *Yoraperla brevis* (Ricker). High gene flow within streams is possible because larvae can disperse downstream, but long distance dispersal between streams requires adult flight (DePietro *et al.*, 1997; Hughes *et al.*, 1999). Therefore, understanding a stream's ecosystem requires not only the assessment of diversity on a community level, but also knowledge of population structure and morphology as they relate to the landscape (Bohonak & Jenkins, 2003).

To test the structure, genetic divergence was examined in *Allocapnia recta* (Claassen) and *Leuctra tenuis* (Pictet), two Plecopteran species that vary in wing morphology and emergence period (Gaston 1994 and Malmqvist 2000). *Allocapnia recta* emerge during the, coldest time of year between November and March. Although some males and females of *A. recta* have wings the full length of their body, they are commonly collected as apterous or with rudimentary wing structure (Frison, 1942; Nebeker & Gaufin, 1967; Stark *et al.*, 1998). *Leuctra tenuis* emerge between July and September, when the weather is warmer and adults in the family Leuctridae are relatively strong fliers at these temperatures than other stonefly species. When ambient air temperatures are cooler than 13°C, *Leuctra* species have been observed to move upstream skimming across the water surface with their hind-legs (Marden *et al.*, 2000). Although *L. tenuis* is a poor flying insect in comparison to other insects, it is predicted to have a greater dispersal potential than *A. recta* for movement among watersheds (Briers *et al.*, 2004).

2.3 <u>Materials and Methods</u>

Adult specimens of *Allocapnia recta* (Family Capniidae) and *Leuctra tenuis* (Family Leuctridae) were collected along the Chagrin and Grand Rivers, two adjacent tributaries of Lake Erie on Ohio's north coast (Figure II.1). Collections were made between 2003 and 2005. *Allocapnia recta* was obtained from November to February and *L. tenuis* from

June to August. Specimens were collected within 15 m of the stream. Specimens of *Allocapnia recta* were collected as the insects were observed crawling on the snow. A beating sheet was used to collect *L. tenuis* from low hanging tree limbs or from ground vegetation.

Each individual specimen was placed in a 1.5 ml microcentrifuge tube containing 95% ethanol. If a male and female were captured in copula, the mating pair was placed in the same tube. In the laboratory, the old ethanol was replaced with fresh 95% ethanol for optimal preservation of the insect.

Collected specimens were identified to species based on the structure of the male genitalia (Ross & Rickter, 1974). The lower abdomen was removed from the male specimens and stored for species documentation. The only females used in this study were those found associated with a male in the field.

Each stonefly, less the lower abdomen, was soaked in distilled water for ten minutes to remove ethanol. DNA was isolated using the QIAGEN DNeasy ® Tissue kit and applying the *rodent tail tissue protocol* (following methods from Schultheis *et al.*, 2002). An elution of 100µl was used to increase DNA concentration.

The 16s rRNA gene, which codes for the large mitochondrial ribosomal subunit, was used to assess levels of genetic differentiation at the population level. Universal animal primers of the 16s rRNA gene amplified an approximate 500 base pair-long region of the mitochondrial DNA (mtDNA). The forward primer (16sB) was 5'- CCG GTT TGA ACT CAG ATC ATG T -3' and the reverse (16sA) was 5'- CGC CTG TTT AAC AAA AAC AT -3' (Palumbi, 1997; optimized for insect use). In the stonefly specimens, the universal primers produced a faint 100-200bp secondary product that interfered with sequencing quality. Therefore, internal primers were developed specifically for each species to improve sequencing. For *A. recta* the forward primer was (SF_arF) 5'- TCG AAC AGA CCT AAA CTT TG -3' and the reverse was (SF_arR) 5'- AAT AAT TTA AAG TCT GAC CTG C -3'. For *L. tenuis* the forward primer read as (SF_ltF) 5'- GAA CAT CTA CAC CCA AAA TYA C -3' and the reverse as (SF_ltR) 5'- TCT GAC CTG CCC GCT GAT TA -3'.

Each polymerase chain reaction (PCR) for both stonefly species was set up in 50µl as follows: 16µl of deionized water; 5µl of each primer (2.5µM); 5µl of dNTP's; 10µl of $MgCl_2$ (2.5µM); 0.2µl of FisherBiotech *Taq* DNA polymerase (concentration of 5U/µl with 5µl of 10X Assay Buffer A), and 2µl of template DNA. PCR reactions were cycled 40 times in a Perkins Elmer GeneAmp PCR system 2400. The PCR conditions were set with an initial denaturation phase of 5 minutes at 94ºC and all subsequent denaturation for 30 seconds. The annealing phase was 30 seconds at 49ºC, and extension was at 72ºC for 30 seconds. After all cycles were completed a final extension for 7 minutes at 72ºC was performed.

The amplified DNA region was sequenced at Cleveland State University's DNA sequencing facility on a Beckman CEQ-8000 capillary autosequencer. All sequences were run in both the forward and reverse directions. Mitochondrial DNA sequences were aligned and read using the Sequencher software package (Sequencher v. 4.0, Gene Codes Corp.) and conservatively screened by eye to eliminate any ambiguous scoring. Therefore, it was not likely to miss one variation present or to score a new haplotype. Analysis of variation among haplotypes was performed with Arlequin v.3.01 (Excoffier *et al.*, 2005), and the minimum spanning trees were produced by Network v. 4.1.1.2 (Fluxus Technology Limited).

2.4 <u>Results</u>

Tables I and II illustrate the diversity of haplotypes in both species for which gene and nucleotide diversity levels were similar. Distinct polymorphisms were common within the 16s rRNA region in both *Allocapnia recta* and *Leuctra tenuis* (DQ915179-DQ915181). Between the Chagrin and Grand Rivers, the internal primers enabled accurate sequencing of a 492 base pair region of the mtDNA in 36 *A. recta* specimens and of 459 bases from 30 individuals of *L. tenuis*. A Blast search (Altschul et al., 1997) in GenBank using the most frequent haplotypes of both *A. recta* and *L. tenuis* best matched *Pteronarcys princeps*, the ebony salmonfly (accession number AY687866), which is a stonefly of the western US followed by insects from other related orders.

2.4.1 *Allocapnia recta*

Wright's F_{ST} scores for *A. recta* indicated that separation of samples between the two watersheds can explain 20% of the variation in haplotype diversity (Table I, F_{ST} = 0.20; p-value >0.0). This difference between samples from the Chagrin and Grand Rivers was significant. Over half of the specimens possessed one of two haplotypes (Table I). The most common haplotype (H01) occurred frequently in both watersheds, but haplotype H02 was collected only once in the Chagrin River. Conversely haplotype H03 (*n* = 5) was only observed in the Chagrin River. All other haplotypes were found once in one of the rivers. Overall gene and nucleotide diversities across the two watersheds were 0.83 and 0.67 respectively. Both gene and nucleotide diversities were consistently greater in the Chagrin River than in the Grand River (Table I). This pattern is apparent in the minimum spanning tree where Chagrin River samples derived from one large and divided clade (Figure II.2).

2.4.2 *Leuctra tenuis*

In contrast to the results observed in *A. recta*, the FST score for samples of *L. tenuis* was just 0.065 (p-value = 0.14), a result not significantly different from zero (Table II). The two most prevalent haplotypes were identified in samples from both watersheds, indicated that haplotypes in *L. tenuis* were randomly distributed between the rivers. Therefore, gene diversity varied little and no distinct clades occurred within the minimum spanning tree specific to either watershed (Figure II.3).

The measure of nucleotide diversity in *L. tenuis* from each river, however, gave an unexpected result. Individuals of *L. tenuis* from the Chagrin River showed a significantly higher level of nucleotide diversity (0.95) than did specimens from the Grand River (0.38) (Table II).

2.5 Discussion

The winter stonefly varied genetically between the neighboring Chagrin and Grand Rivers in north-east Ohio, whereas the summer stonefly did not. Therefore the time of emergence or the reduced wing structure of *A. recta* a much weaker flier (Marden *et al.*, 2000), likely contributes to the limited ability of this species to disperse between the watersheds. The ability of some stoneflies to disperse long distances is likely a function of wind speed. *Allocapnia* species have been observed to sail on the surface of the water using wind power to propel themselves from one location to another. As the adults emerge on mid-stream rocks or ice, they stand on top of the water surface tension, and raise their wings in response to gusts of wind, thus sailing to the shore (Marden &

Kramer 1995). Some members of the genus *Allocapnia* may also glide down from trees and other riparian vegetation during strong winds. Marden and Kramer (1995) determined that an insect with rudimentary wing structures such as *A. recta,* sailing across the water surface is more effective than gliding. Furthermore, temperature has an effect on the dispersal of winter stoneflies. Adult *Allocapnia* species were in higher abundance on sunny days when temperatures exceeded 5 °C, with limited wind; during the harsher and colder days of winter, fewer adults were observed crawling along the snow (pers. obs.). Most sought cover under piles of dead vegetation, woody debris, or snow packs on days of extreme cold temperatures.

Leuctra tenuis can disperse farther than *A. recta*. When summer air temperatures are less than 13 °C, *Leuctra* stoneflies can use a hind-leg skimming mechanism to raise the body, and reduce drag on the water surface (Kramer & Marden, 1997). On warmer days, *L. tenuis* flew over the stream searching for mates (pers. obs.). In addition to the mechanism of flight and temperature, the sample sites within the Chagrin and Grand Rivers are deeply incised channels, making transportation between streams difficult for even the stronger flying stoneflies.

Regardless of flight proficiency, few individuals will migrate across watersheds because adult gravid females remain near their natal streams to deposit their eggs after mating, while males will either search for other females for mating or die. This tendency not to disperse may restrict gene flow. While neonates, after hatching, may immediately start to swim downstream in search of food and to avoid predators (Kuusela & Huusko, 1996), their movement is limited due to their size and they need not cross between rivers (Hughes *et al.* , 1999; Schultheis *et al.* , 2002).

One caveat of the results is the possibility that cryptic species were encountered that vary in mtDNA sequences rather than variation within each species. In *A. recta,* three haplotype clades occurred in the samples from the Chagrin River, but only two in the Grand River, and in *L. tenuis* , Grand River samples predominantly possessed haplotypes basal within the observed clade. If a cryptic sibling species was present in the Chagrin River that was responsible for the apparent population structure, conclusions about dispersal would be unchanged; variation in *Allocapnia* instead would suggest structure at a community rather than a population level.

As a final note, in salamanders, fish and arthropods, populations in previously glaciated regions tend to have less genetic variation than their populations of origin (Tilley, 1997; Bernatchez & Wilson, 1998; Reiss *et al.* 1999), a pattern consistent with the lower genetic variation found in the Grand River than in the Chagrin River samples.

Table I. Haplotype frequencies for *Allocapnia recta* as they relate to location. Single haplotypes were pooled. Gene diversity was estimated using Nei (1987), and nucleotide diversity was calculated using Arlequin v.3.01 (Excoffier *et al.*, 2005). ((H01, 02, etc. refers to haplotype number, *h* (gene diversity), and π (haplotype diversity) SE (standard error))

Site	H01	H02	HO3	H04	H05	Pooled Single Haplotypes (H06-H12)	Totals	*h*	SE	π x100	SE x 100
Chagrin River	7	1	5	2	0	5	20	0.85	0.01	0.68	0.09
Grand River	5	7	0	0	2	2	16	0.73	0.02	0.52	0.08
Totals	12	8	5	2	2	6	36	0.83	0.01	0.67	0.07
Haplotype Frequencies	0.33	0.22	0.14	0.06	0.06	0.03/each					

Table II Haplotype frequencies for *Leuctra tenuis* as they relate to location. Single haplotypes were pooled. Gene diversity was estimated using Nei (1987), and nucleotide diversity was calculated using Arlequin v.3.01 (Excoffier *et al.*, 2005). ((H01, 02, etc. refers to haplotype number, *h* (gene diversity), and π (haplotype diversity), SE (standard error))

Site	**H01**	**H02**	**H03**	**Pooled Single Haplotypes (H04-H10)**	**Totals**	***h***	**SE**	**π X 100**	**SE X 100**
Chagrin River	6	5	0	4	15	0.76	0.02	0.95	0.14
Grand River	7	1	4	3	15	0.74	0.02	0.38	0.07
Totals	13	6	4	7	30	0.77	0.01	0.64	0.07
Haplotype Frequencies	0.43	0.20	0.13	0.03/each					

Figure II.1. Map of study area in Cuyahoga, Lake, and Geauga counties, Ohio, USA. Circles represent sampling locations.

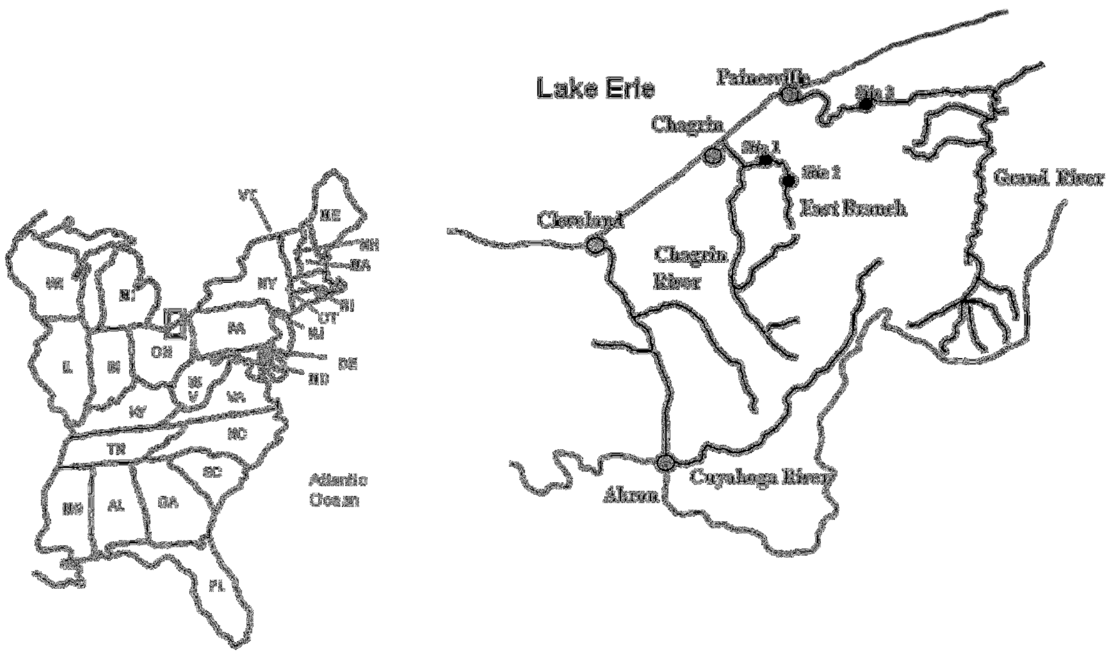


Figure II.2 Haplotypes of *Allocapnia recta* in the Chagrin and Grand Rivers. Circle diameter represents the sample size of each haplotype and levels of shading denote the frequency either in the Chagrin River (black) or Grand River (gray). Numbers indicate the base position changed in the sequence.

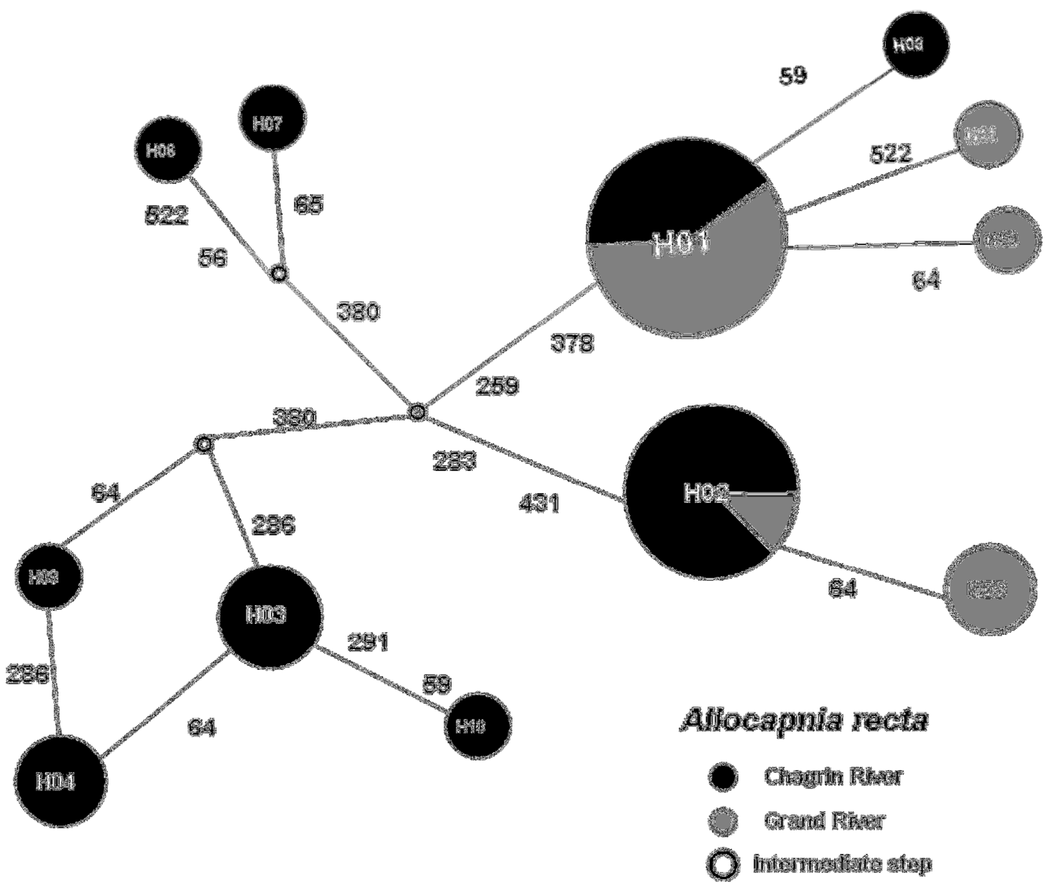
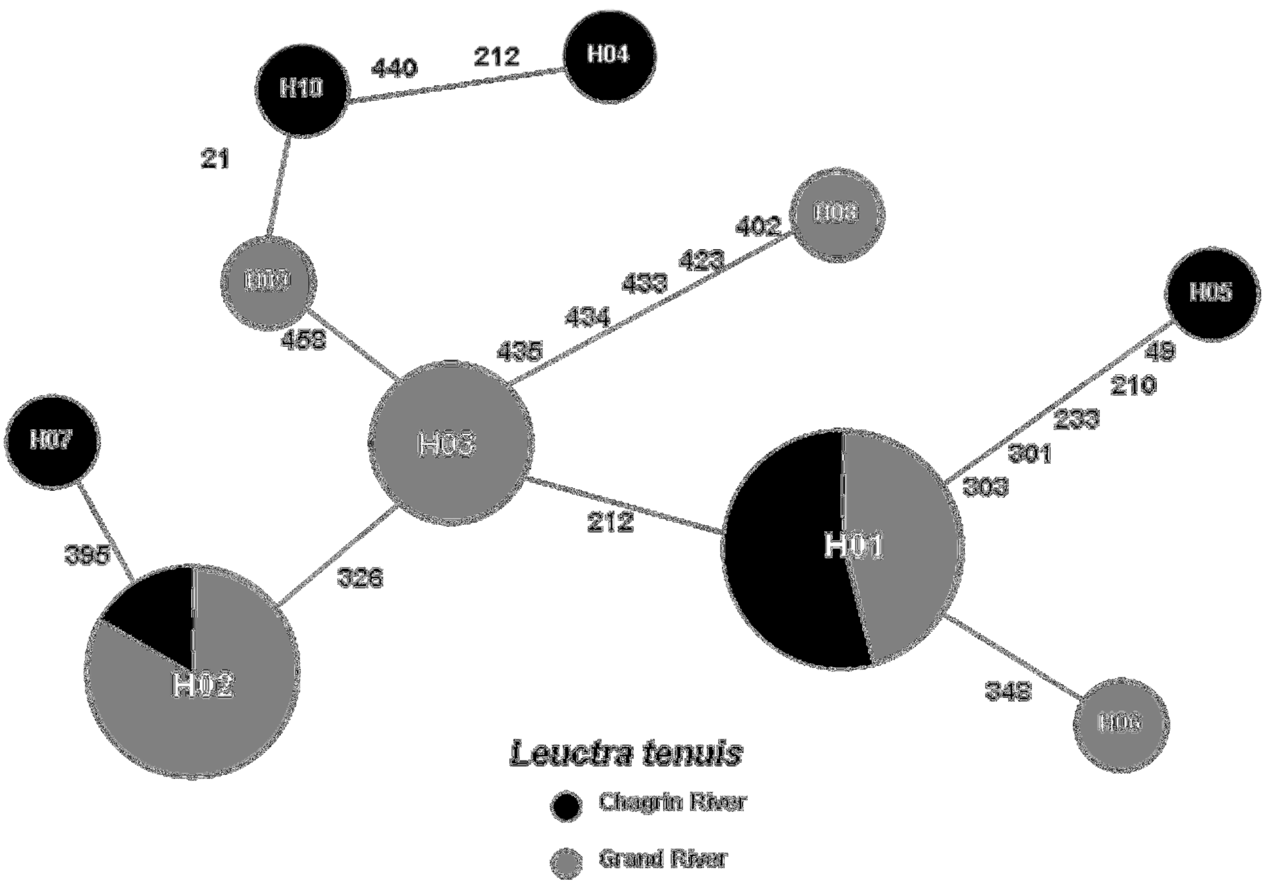


Figure II.3 Haplotypes of *Leuctra tenuis* in the Chagrin and Grand Rivers. Circle diameter represents the sample size of each haplotype and levels of shading denote the frequency either in the Chagrin River (black) or Grand River (gray). Numbers indicate the base position changed in the sequence.



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CHAPTER III

DISPERSAL ANALYSIS WITHIN THE SPECIES *ALLOCAPNIA RECTA* (ORDER PLECOPTERA) BETWEEN FOUR WATERSHEDS IN NORTHEAST OHIO

3.1 Introduction

Flight is of prime importance in most insect species and affects their dispersal behavior. Adult stoneflies exhibit significant variability in wing morphology and musculature among species, and thus a high degree of flight variability (Malmqvist, 2000; Winterbourne, 2005). Dispersal in stoneflies may be passive (e.g. migrating as a result of wind directionality, hydraulic gradient of open channel stream systems, etc.), but dispersal, as it relates to flight capabilities, can generally be categorized in adult stoneflies through the delineation of a continuum of extremes ranging from full wing, full flight capabilities to apterous, flightless members. In the most rudimentary forms of adult flight starting with surface skimming most likely evolved into more complex forms of flight that required well developed wings with increasing flight velocity at each stage of evolution (Marden et al., 2000; Marden, 2008). Although their adult terrestrial stage is short-lived, it is important for mating and reproductive processes. Furthermore, as dispersal behaviors are altered, intraspecies relationships are affected – including natal population demographics, the population of adjacent habitats, colonization of new or previously uninhabited regions, and the rates at which populations become genetically distinct from each other (Briers et al., 2003).

In a previous study on two stonefly species varying in dispersal potential, Yasick et al. (2007) compared genetic variability between *Allocapnia recta*, a short-winged, winter emerging stonefly, and *Leuctra tenuis,* a long-winged summer emerging stonefly. Populations of *A. recta* were significantly more diverse between the adjacent watersheds, the Chagrin and Grand Rivers, in Ohio. The results suggest that rudimentary wing structure and time of year of the adult terrestrial stage limit flight capability (Marden et al., 2000) and isolated even the nearby Chagrin and Grand Rivers populations. With limited population studies of stoneflies in the literature, this research expands on previous analysis by determining how extensive population isolation in Northeast Ohio watersheds may be, despite the close proximity of the watersheds to one another. Furthermore, this research also addresses several questions relevant to understanding how the distance of between populations contributes to levels of divergence in post-glacial systems.

Wing morphology cannot be an exclusive reason for population isolation. Maintaining the correlation asserting that rudimentary wing morphology leads to limited flight distance in stoneflies, the genetic variability of *A. recta* populations between and among the research collection sites will be directly proportional to the distance between sampling sites. Here we test whether genetic differences between *A. recta* populations is a function of distance – either linear distance along waterways or direct distance overland between watersheds. The collecting sites farthest from each other should be the most different. Thus we assess genetic variation in four Lake Erie tributaries in Northeast Ohio,, the Rocky, Cuyahoga , Chagrin, and Grand Rivers. .

3.2 Materials and Methods

Adult specimens of *Allocapnia recta* (Family Capniidae) were collected between 2004 to 2007 at sites within four adjacent tributaries– the Cuyahoga, (N41.2314; W –81.5086 and N41.2335; W –81.5021) Chagrin (N41.5960: W81.2512 and N61.6071; W81.2875) , Rocky (N41.2115; W –81.6831), and Grand Rivers (N41.7217; W81.0830) (Figure III.1) –. Samples were obtained during the peak of annual winter adult emergence from November to February. Specimens were collected within the stream channel and within 15m of the stream embankments in the riparian zone. Collection within the channel was performed manually using forceps to procure samples from tree trunks, or on snow and ice between the embankments. A beating sheet was used to collect *A. recta* from low-lying tree limbs or upon remnants of ground vegetation along the stream. Preferred collecting days were when temperatures exceeded 0ºC with few to no clouds (based on personal observations). Individual *A. recta* were placed in a 1.5 ml microcentrifuge tube containing 95% ethanol. If a male and female were captured in copula, the mating pair was placed in the same tube.

Collected *A. recta* specimens were identified to species based on the structure of the male genitalia (Ross & Rickter, 1971). Following identification, the lower abdomen was removed from male specimens and stored for species documentation. The only female samples used in this study were those captured in copula. To further verify species identification, a cladogram was constructed using available sequence data from species within the same family as *A. recta*, Capniidae [Used by permission MD Terry (Figure

III.2)]. Prior to DNA extraction, specimens were soaked in distilled water for fifteen minutes to remove the ethanol. Samples were then blot dried on Kimwipe tissue and placed into a microcentrifuge tube for DNA (mtDNA) extraction. DNA was amplified according to the methods and primers outlined in Yasick et al. (2007). The forward primer was (SF_arF) 5'- TCG AAC AGA CCT AAA CTT TG -3' (20 nucleotides in length) and the reverse primer was (SF_arR) 5'- AAT AAT TTA AAG TCT GAC CTG C -3' (22 nucleotides in length).

Early sequencing of *A. recta* was conducted at Cleveland State University's DNA sequencing facility on a Beckman CEQ-8000 capillary autosequencer. Those samples were run in both the forward and reverse directions. Later samples were sequenced at the Cleveland Clinic Lerner Research Institute's Genomic Core Facility using a Biosystems model 37 30xl DNA analyzer using the forward primer only. Using the forward primer only, sequences were reduced from 492 base pairs (results published in Yasick et al. 2007) to 467 base pairs (see Table III for a complete list of newly sequenced specimens and previously sequenced specimens used in this section). Mitochondrial DNA sequences were aligned and read using the Sequencher® software package (Sequencher v. 4.10.1, Gene Codes Corp.) and conservatively screened to eliminate any ambiguous scoring.

Analysis of variance among haplotypes was performed with *DnaSP* v. 5.10.01 (Rozas et al. March 2010) and the minimum spanning trees were produced with Network v. 4.6.0.0 (Fluxus Technology Limited 2005). Pairwise comparison was used to determine where the greatest genetic differences, or similarities, exist when comparing samples across the four watersheds. Hedrick (2005) and Merimans and Hedrick (2011), propose G'_{ST} as a standardized method of measuring genetic variation between populations and results in a more meaningful score – resultant values range from 0 to 1 – and increased validity; particularly with smaller sample sizes when compared to F_{ST}. A G'_{ST} score of 1 indicates haplotypes are completely different, while a score of 0 is indicative of identical haplotypes.

Table III. Haplotypes scaled to shortened sequences used from those originally identified in Yasick et al. 2007, a publication that that only included the Grand and Chagrin River sites, and the more recently identified haplotypes collected from all four sampling locations. Letter codes indicate collection site: Rocky River (RR), Cuyahoga site A,(CU1); Cuyahoga site D (CU2); East Branch Chagrin (CH1); Stebbins Gulch (CH2); and Talcott Creek (GR)

Insect	Published Haplotypes (492 bp range)	Revised Haplotypes (467 bp range)	Insect	Published Haplotypes (492 bp range)	Revised Haplotypes (467 bp range)	Insect	Published Haplotypes (492 bp range)	Revised Haplotypes (467 bp range)
05CU22		1	05CH1_59	5	3	05CU288		6
05CU23		1	05CH1_81	5	3	44CH2_6		6
7CU4		1	05CH1_82	6.6	3	07RR136		7
9CU2		1	05CH1_96	6	3	07RR150		7
21CU8		1	05CH1_97	6	3	07RR152		8
20CU27		1	31CH2_7		4	07RR155		8
29CU9		1	33CH2_9		4	07RR149		9
05CU87		2	43CH2_5		4	07RR153		9
05CU95		2	07RR125		4	07RR157		10
8CU3		2	07RR126		4	07RR159		10
07RR134		2	07RR127		4	07RR160		10
15RR1		2	07RR135		4	07RR167		11
05CH2_25	1	2	07RR173		4	07RR128		12
05CH2_21	1	2	17RR3		4	07RR130		12
05CU215		3	38RR4		4	07RR158		13
05CU80		3	39RR5		4	07RR156		14
05CU85		3	40RR6		4	05CH1_98	6.5	15
05CU86		3	41RR7		4	4GR8		16
05CU89		3	42RR8		4	13GR7		16
05CU91		3	04CH2_4	3	4	14GR10		16
05CU292		3	04CH2_12	3	4	1GR1		17
05CU93		3	05CH2_26	3	4	2GR2		17
05CU94		3	05CH2_28	3	4	3GR3		17
05CU100		3	05CH2_30	3	4	5GR9		17
07CU141		3	05CH2_31	3	4	11GR5		17
07CU143		3	05GR44	3	4	12GR6		18
19CU26		3	05GR45	3	4	05GR37	7	19
26CH2_2		3	05GR47	3	4	05GR38	7	19
32CH2_8		3	05GR48	3	4	05 GR39	8	19
07RR124		3	05CH2_54	4	4	05GR42	7	19
07RR151		3	05GR109	9	4	05GR43	8	19
07RR165		3	05GR110	10	4	05GR46	7	19
04CH2_1	5	3	23CH1_1		5	05GR55	7	19
04CH2_2	5	3	23CH2_1		5	05GR60	7	19
05CH1_36	5	3	04CH2_5	3	5	05GR83	7	19
			05CU79		6	05GR84	7	19

Figure III.1: Collecting sites within the Rocky (RR), Cuyahoga (CU1 and CU2), Chagrin (CH1 and CH2), and Grand (GR) Rivers, Northeast Ohio USA. Large circles indicate sampling sites and smaller circles represent major metropolitan areas. Two sites were used within the Chagrin and Cuyahoga Rivers for more meaningful data collection.

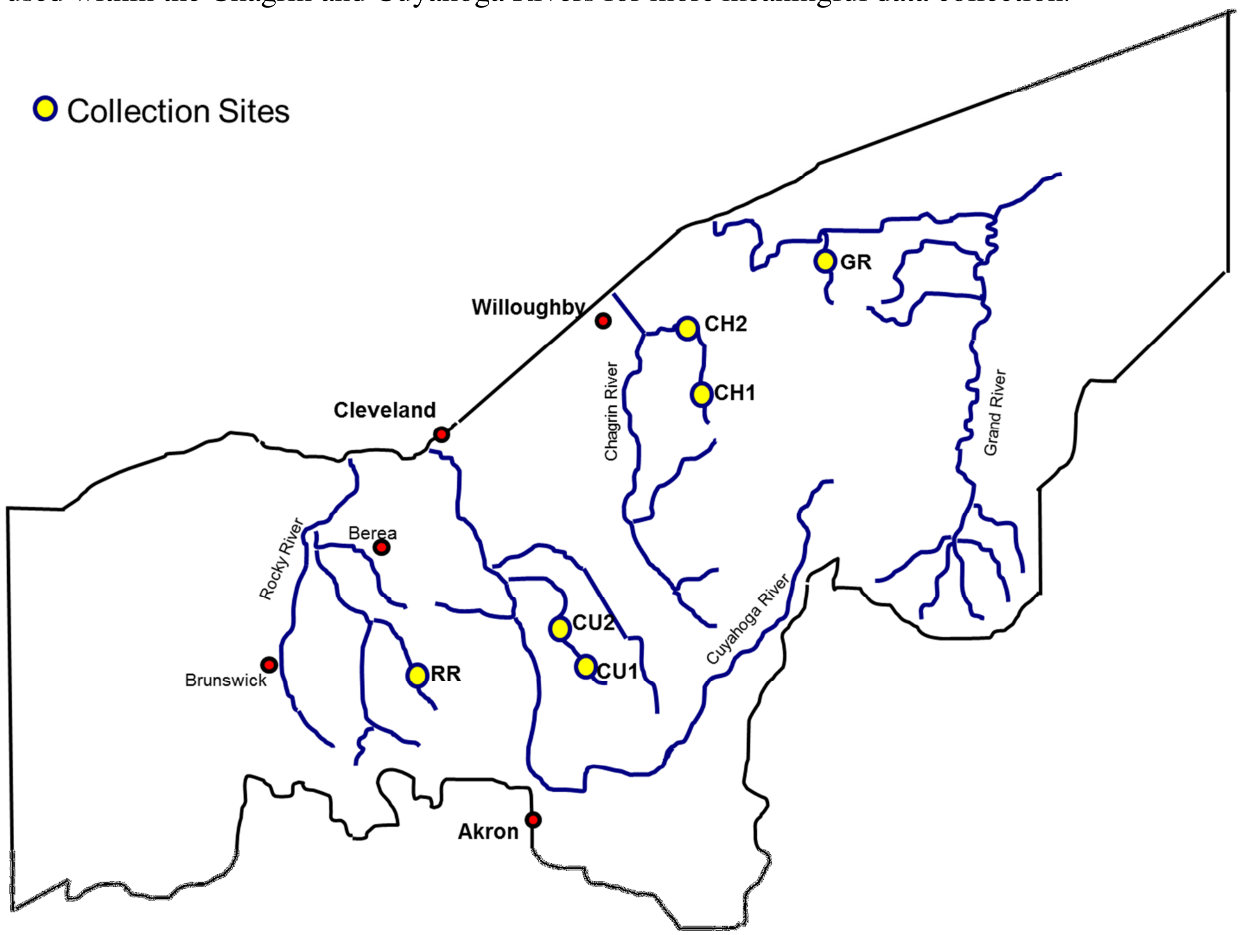
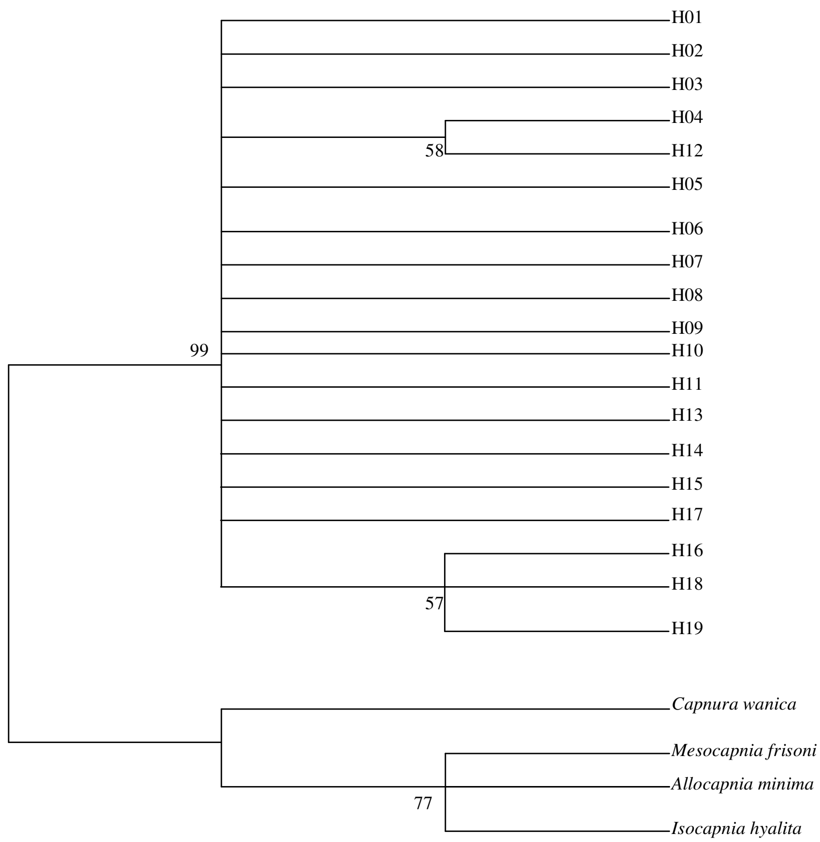


Figure III.2 MEGA Phylogeny Tree. Using MEGA (Molecular Evolutionary Genetic Analysis) version 5.05 (Tamura et al. 2011) a phylogeny tree was constructed with haplotypes identified in Table III. The cladogram includes outgroups used by permission from MD. Terry, PhD (Associate Professor at University of Texas-Pan America). Outgroups are identified by genius and species, along with *A. recta* are members of the family Capnidae. The outgroups were used to validate the relatedness of the *A. recta* haplotypes



3.3 Results

Haplotypes were obtained from 107 specimens of *Allocapnia recta* from four watersheds in Northeast Ohio; the Grand (n=25), Cuyahoga (n=25; pooled from CU1 and CU2), Chagrin (n=27;pooled from Ch1 and Ch2), and the Rocky River (n=30). Nineteen unique haplotypes were identified and distinct polymorphisms were found using the 16sRNA region of the mitochondria DNA (KC881036-KC881054). The F_{st} score for *A. recta* indicated that separation of samples between the four watersheds explained 37% (P <0.05) of the variation in haplotype diversity, while 63% of the variation is represented within-group variation (Table IV).

Table IV Molecular Analysis of Variance (MANOVA) of 16s RNA variation among and within groups of *A. recta* samples collected in the four sample sites were analyzed in Arlequin.

Source of Variation	d.f.	Sum of Squares	Variance Components	% of Variation
Among	3	58.86	0.69	36.66
Within	103	122.85	1.19	63.34
Total	106	181.70	1.88	
Fixation Index	Fst=0.367			

From Table IV, not one haplotype was present across all four watersheds, not even the two most common haplotypes, H3 and H4, which were represented by 26 and 27 individuals, respectively, and collected at from three of the four sites. H3 was absent in *A. recta* samples collected in the Grand River and H4 was absent in *A. recta* samples collected in the Cuyahoga River. The third most common haplotype, H19 (n=10) was collected in the Grand River only. H1 and H2 haplotypes were the fourth most common haplotypes identified (both n=7). All seven specimen samples with H1 haplotypes were collected in the Cuyahoga River, while the H2 haplotype was unusual for its presences in multiple watersheds was collected in the Cuyahoga, Rocky, and Chagrin Rivers. Nine haplotypes (H5-H10, H12, H16, and H17 were present in two to five copies in only one watershed. Haplotypes H11, H13-15, and H18 were only observed once and were pooled together (see Table V).

Samples from Rocky River had the greatest haplotype diversity with eleven total haplotypes found; including eight unique haplotypes. Six haplotypes were collected from samples in the Chagrin River with two unique haplotypes; five haplotypes were collected in the Grand River with three unique haplotypes; and, four haplotypes were collected in the Cuyahoga River with one unique haplotype. This pattern is graphically apparent when employing the minimum spanning tree where samples were derived from one large and divided clade (Figure III.2). Haplotype and nucleotide diversities are estimated in Table V and indicate that the overall haplotype and nucleotide diversity was 0.86 and 0.47, respectively. Table VII is representative of the calculated population pairwise estimate using both F_{ST} and G'_{ST} scores. Greatest pairwise difference exist between sample populations collected in the Cuyahoga and Grand Rivers ($G'_{ST} = 1.0$). Least pairwise difference is between the Chagrin and Rocky River ($G_{ST} = 0.31$). All other sample specimens and locations are statistically significantly different from each other using a p-value < 0.05. By comparing sites based on distance from each other, Table VIII illustrates that overland distance is not the likely driving force between distance population genetic structures. Sites like the Rocky River and the Grand River should be completely different from each, while sites such as the Cuyahoga and the Chagrin, Rocky and the Cuyahoga, and the Chagrin and the Grand should not be significantly different from each other.

Table V Haplotype frequencies for *Allocapnia recta* as they relate to sampling location. Single haplotypes were pooled and haplotypes collected in more than one location are highlighted in grey.

Site	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H12	H16	H17	H19	Pooled*	Total
Cuyahoga	7	3	13	0	0	2	0	0	0	0	0	0	0	0	0	25
Rocky	0	2	3	11	0	0	2	2	2	3	2	0	0	0	3	30
Chagrin	0	2	10	10	3	1	0	0	0	0	0	0	0	0	1	27
Grand	0	0	0	6	0	0	0	0	0	0	0	3	5	10	1	25
Total	7	7	26	27	3	3	2	2	2	3	2	3	5	10	5	107
Frequency	0.07	0.07	0.24	0.25	0.03	0.03	0.02	0.02	0.02	0.03	0.02	0.03	0.05	0.09	0.05	1

Table VI Gene diversity (*h)* was estimated in alignment with the work of Nei (1972); and nucleotide diversity (π) was calculated using Arlequin v. 3.5 (Excoffier et al. 2011). SE is standard error.

Sites	**Totals**	**h**	**hSE**	**π x 100**	**π SE x 100**
Cuyahoga	25	0.66	0.071	0.19	0.12
Rocky	30	0.85	0.055	0.37	0.18
Chagrin	27	0.73	0.054	0.25	0.14
Grand	25	0.76	0.051	0.32	0.17
Totals	107	0.86	0.020	0.47	0.18

Table VII Pairwise Population differences based on location using both Fst (the upper number) and G'st (the lower number) scores.

	Rocky	**Cuyahoga**	**Chagrin**	**Grand**
Rocky	--------------			
Cuyahoga	0.47 0.86	__________		
Chagrin	0.07 0.31	0.35 0.45	___________	
Grand	0.34 0.68	0.62 1.0	o.29 0.72	___________

Figure III.3 Haplotypes of *Allocapnia recta* in the four watersheds. The diameter of each circle represents the sample size of each haplotype and levels of shading denote the location: Rocky River (white), the Cuyahoga River (light grey), Chagrin River (dark grey) or the Grand River (black). Numbers indicate the haplotype number and the dashes represent the number of base changes from each haplotype.

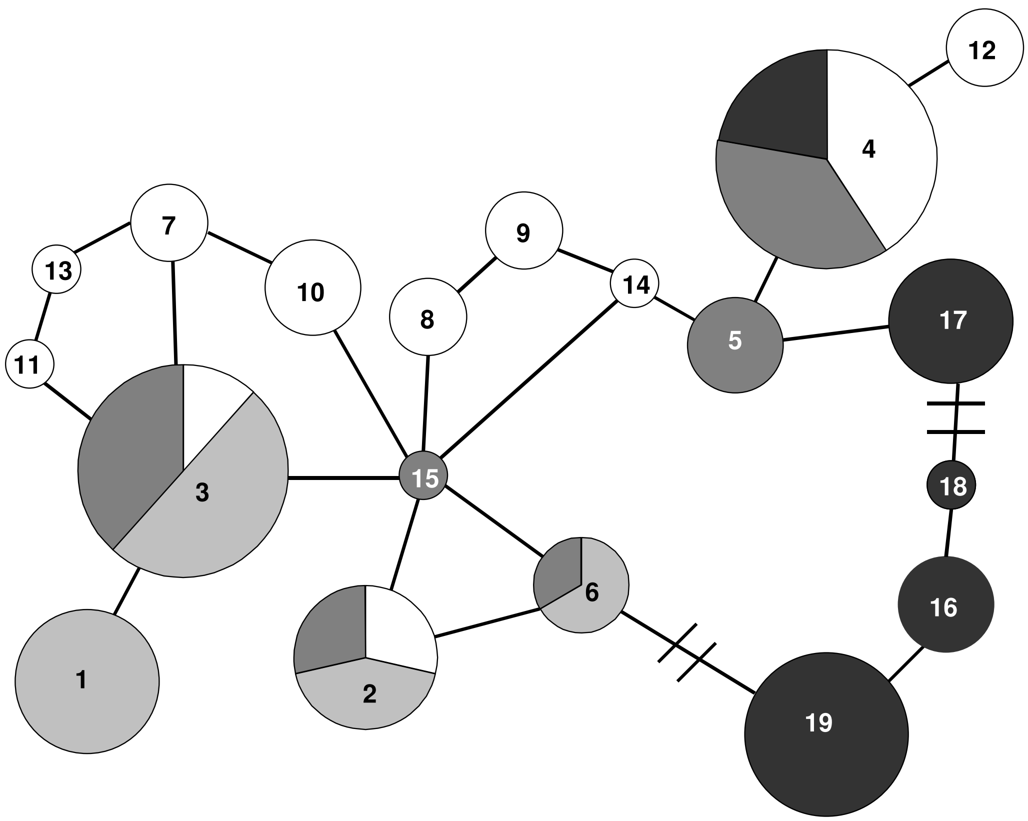


Table VIII Pairwise comparisons based on distance by flight. The first group is one step away from each other, while the second and third groups are two and three steps away from each other respectively. More steps refer to greater distance between the watersheds. Overall, the table illustrates that distance is not a major factor on genetic variability.

Flight Distance	**Collecting Site**		**Fst**	**G'st**	**Significance**
	Rocky/Cuyahoga		0.47	0.86	***
One Step	Cuyahoga/Chagrin		0.36	0.45	**
	Chagrin/Grand		0.29	0.72	***
		Average	0.37	0.68	
Two Steps	Rocky/Chagrin		0.01	0.31	*
	Cuyahoga/Grand		0.58	1.00	***
		Average	0.36	0.65	
Three Steps	Rocky/Grand		0.34	0.68	***

3.4 Discussion

The goal was to assess patterns of dispersal among *Allocapnia recta* and to further explain the dispersal processes observed in Yasick et al. (2007). The initial predication was that distance would be the driving factor towards explaining intraspecific dispersion and why collected specimens of *Allocapnia recta* varied genetically between all four watersheds. The four watersheds sampled illustrated different haplotypes from each other, suggesting that dispersal of *Allocapnia recta* between neighboring watersheds is minimal. This inference is drawn from the results that no single haplotype was found among all four watersheds, even among the five most common haplotypes. The samples collected from the Cuyahoga River and Grand River were completely different from each other ($G'_{ST} = 1$) while the samples collected from the Rocky River and Chagrin River, the two non-adjacent watersheds were the most similar ($G'_{ST} = 0.31$).

Thus linear overland distance between the four watersheds cannot explain the observed variation. If distance was a major contributor, then the Rocky River and the Grand River should have had the most differences among populations, while comparisons between the Rocky River and Cuyahoga Rivers; Cuyahoga and the Chagrin Rivers; or the Chagrin and the Grand Rivers should not be the most similar. Instead no discriminating pattern occurred and therefore, other factors, such as post-glacial migration, land use (both historic and modern), and resource competition dynamics are more likely explanations (Alp et al., 2012; Shulthesis et al., 2012).

3.4.1 *Post-Glacial Migration*

The lack of dispersion, among *A. recta* is caused by a number of variables. Rudimentary wing structure (especially apterous males w), winter-time adult terrestrial emergence period, and the behavior in females to return to the natal stream to oviposit are three of the most common and well understood. In addition, the current distribution of *Allocapnia recta* populations in northeast Ohio may have also been affected by post-glacial changes in topography that disconnected streams that were once interconnected to each other in the past (Hynes, 1988). Regional glacial periods and the consequential changes to the watershed landscape in northeast Ohio may be responsible for the limited interactions of stonefly species following glaciation (White and Totten, 1982 and Szabo et al., 1988).

Records of known glacial events coupled with the presence of *A. recta* in and around streams once covered by continental ice sheets establish a relationship between biogeography and the history of the landscape (Ross & Ricker 1971, Ford 1987, and Hynes 1988). Prior to glaciation, the headwaters of the Cuyahoga River, Chagrin River, and Grand River were in close proximity to each other; creating a natural passageway connecting the streams and the amphibious organisms (i.e. *A. recta*) between them (Austin et al., 2002). The Laurentide Ice Sheet had a profound effect on the region's geomorphology. The transgressing ice sheet originated in Labrador and advanced in a southeasterly direction, first into the Great Lakes basin and then into present day Northeast Ohio. The entire landscape in and around Lake Erie was isostatically compressed by the weight of the 3km thick ice sheet during the Wisconsin Glacial cycle, a glacial period that ended only 15,000 years ago (Lo and Soster, 1981; White and Totten, 1982; DP Cronin; personal communication May 2013). Once the ice retreated, the rigid crust experienced glacial isostatic adjustment – a slow uplifting due to the removal of the glacier's weight.

As a result of glaciation, the hydrological and geomorphic systems in the region dramatically changed (White and Totten, 1982). In riverine systems like the Grand River and Cuyahoga River, glacial dynamics and ice movement disrupted flow patterns and changed the direction of flow. The Grand River turned westward and the Cuyahoga flowed north (White and Totten 1982). Thus the Grand, Cuyahoga, and Chagrin Rivers lost their interconnectivity and separated populations of aquatic insects. As the glacial ice melted, re-colonization of *A. recta* may have occurred first in the Rocky River, and expanded eastward. Thus the post-glacial population dynamic helps explain why specimens of *A. recta* collected from the Rocky River are the most diverse, although they share some haplotypes with *A. recta* from the other three watersheds but also have many unique haplotypes when the same comparison is made (as supported by findings in Yasick et al., 2007). The region continues to experience glacial isostatic adjustment over long periods of time, and may be continually placing organisms like *A. recta* into closer proximity to each other and possibly allow organisms to migrate to streams that are currently out of reach for poor dispersers (Coffey, 1958 and Habel et al. 2005).

3.4.2 *Land Use*

Aquatic insects employ aerial dispersion for a variety of reasons. While predator avoidance and mate competition are the primary interactions that drive aerial dispersion among aquatic insects, it can also be used for site selection if the aquatic conditions of the habitat become suboptimal (Lehrian et al., 2010; Bogan and Boersma, 2012; Krosch et al., 2012). Aquatic species are integrated with the movement of a stream and dispersal can be passive or active. As a result of unilateral water flow, dispersion among many aquatic macroinvertebrates tends typically to follow a downstream bias (Alp et al., 2012).

Given the wide array of geological, climatological, and natural phenomenon that affect the Earth's surface, land fragmentation is not an unusual phenomenon when assessing the paleoecology of specific sites over geologic time. However, on shorter time scales – ranging years to centuries of human habitation – land fragmentation often results in anthropogenic effects on land use, reducing stream habitat quality. Although drift dispersal is considered the primary mechanism for colonization of a new or disturbed habitat (Williams and Hynes 1976, Gore 1982, and Bogan and Boersma 2012), limits to aerial dispersal need to be considered, even for minimal dispersal distance over fragmented terrestrial habitats.

Allocapnia recta dispersal is further limited by a complicated mix of historical and anthropogenic factors leading to land fragmentation that can be used to explain the low dispersal and distribution of *A. recta* in Northeast Ohio as with other aquatic insects with similar flight restraints (Alp et al., 2012). As such, dispersal and re-population into a new aquatic habitat or one that is recovering from land fragmentation due to natural or anthropogenic disturbances is not likely among *A. recta* population in this region. Lyle et al. (2007) states not all disturbances are bad. Species can adapt to a wide range of natural disturbance regimes, suggesting that species populations may be able to evolve in response to disturbance if given enough time. Unfortunately, anthropogenic disturbances tend to be more traumatic and unpredictability in regards to dispersal.

Each of four watersheds historically have been dominated by agricultural land use and anthropogenic disturbances. The Cuyahoga River (Burkes and McClaugherty, 2008), and Grand River (Grand River Partners 2003; Natural Conservatory 2009) watersheds have a history of intensive row crop farming, while the Rocky River, (Lo and Soster, 1981) and

Chagrin River watershed was primarily pastoral (Chagrin River Watershed Partners 2013; Case Western 1997). Although row crop farming and other forms of intensive cultivation strongly impact stream conditions, the influence of pastoral agriculture is less pronounced (Meador and Goldstein, 2003; Allan, 2004).

Streams draining in agricultural lands support fewer pollution sensitive aquatic insect species than streams draining in forested landscapes (Meador and Goldstein 2003; Allan 2004). Currently, three of the four sampling sites – Rocky River, Cuyahoga River, and Chagrin River – are under the auspices of conservation land management systems – the Cleveland Metroparks, Cuyahoga Valley National Park, and Holden Arboretum, respectively (the Grand River sampling site location is within privately owned land). However, the Rocky River watershed remains primarily enveloped by pastoral farming and cultivated crops with isolated areas of mixed forest along some reaches. With the Cuyahoga River collection site located within the boundaries of the Cuyahoga Valley National Park, the primary land use is currently mixed forest. Regardless, several areas adjacent to the park system and the Cuyahoga River watershed as a whole are dominated by cultivation and pastoral farming; with land use and cash crops similar to those in the Rocky River watershed. Located east of the Cuyahoga River, the Chagrin River collection site is within the boundaries of the Holden Arboretum, which is a protected mixed forest habitat of both deciduous and evergreen trees. The low-density, developed region surrounding the Holden Arboretum remains, or is marked by the remnants of, agricultural use. The Grand River is circumscribed primarily by mixed forests followed by low-to medium density developed property and mixed forest habitat [land use conclusions based on Multi-Resolution Land Characteristics Consortium (MRLC), 2013 and personal observation of the terrestrial habitat].

Both current and long-term land disturbances on macroinvertebrate populations continue to occur (Harding et al., 1998; Allan, 2004). While many changes in the past century have looked to protect the habitat, legacy land use continues to play a role in macroinvertebrate distribution and population structure. Conservation by regional, state, and federal agencies may protect the immediate regions adjacent to the stream embankments (i.e. Cuyahoga Valley National Park, Hinckley Reservation, and Holden Arboretum), but little can be done to avert the consequences of surrounding agricultural land use and the drainage that makes its way into the streams.

3.4.3 *Resource Competition*

Analyses relevant to fragmentation, land use, and post-glacial migration each present reasonable explanations for the current haplotype distribution within poor dispersing species. Another hypothesis presented by McCauley et al., 2009 likewise provides an explanation for the *A. recta* haplotype distribution, by using resource competition as a way of explaining differences in haplotype diversity even between neighboring streams. According to McCauley et al., aquatic insects, including poor dispersers, when they emerge as terrestrial adults, are likely to avoid adjacent riverine habitats even if they are of good quality. Using species abundance and habitat quality as methodological variables, McCauley et al., (2009) concluded that aquatic insects will disperse greater distances to avoid genetically similar members of the species for mate, food, and other resource competition. Despite *A. recta* being a poor disperser, they can still disperse longitudinally and respond to poor habitat quality and limited food resources by moving out of a particular stream reach through downstream drift. Thus avoidance for resource completion can be a potential explanation that leads to genetic differentiation and haplotype differences between the subpopulations of the four watersheds.

3.5 Conclusion

Although this study has limited ability to infer the specific processes that have contributed to current genetic structure of *Allocapnia recta*, distance between watersheds was not the primary factor. Other factors such as a combination of post-glacial migration, land fragmentation and land use, and resource competition are all possibilities for population separation. In a dispersal study conducted by Finn et al., 2006, using a species of blackfly, a much stronger flier than stoneflies, they determined that distance was a factor of dispersal. However, their population pairwise comparison illustrated that landscape features were more influential than overland distance. Landscape features such as high ridgelines, and areas lacking stream and riparian zone corridors lead to greater intra-population genetic diversity. Both the Chagrin and Grand River collecting sites were greatly incised and would be difficult for such weak fliers as *A. recta* from moving from one stream site to the next with ease. Furthermore, since all streams within this study were surrounded by current and historic agricultural land use would also influence the size of the riparian zone, and hinder the ability for passive fliers to migrate from one stream site to another. Peterson et al. (2006) suggested that female stoneflies are more likely to remain near their natal streams for ovipositing her eggs than fly another stream, especially if the migration were made difficult by hindering landscape uses.

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CHAPTER IV

SEASONAL AND LEGACY LAND USE EVALUATION OF MACROINVERTEBRATE COMMUNITIES IN FOUR WATERSHEDS IN NORTHEAST OHIO

4.1 Introduction

The role of spatial heterogeneity and temporal variation in determining biological communities has long been a central topic of stream ecology (Hynes, 1970; Winemiller et al., 2010). For nearly 60 years aquatic organisms have been used to evaluate lotic ecosystems, with benthic macroinvertebrates among the most commonly studied. Benthic macroinvertebrates are often favored over fish, algae, and macrophytes for several reasons, among the most important are the cost efficiencies in collection, identification, and analysis. Macroinvertebrates are long-lived, exhibit fidelity to a stream ecosystem, and are found in abundances that enable the use of meaningful statistical analyses. Benthic macroinvertebrates are also particularly sensitive to sedimentation, habitat loss, and chemical pollution and therefore capable of indicating long-term local habitat quality and legacy land use impacts (Usseglio-Polatera et al., 2000). Legacy land is used to describe anthropogenic disturbance that continues to influence ecological systems long after the initial disturbance is over (Harding et al., 1998). Due to the long life cycle and long-lived aquatic stages of stoneflies and other macroinvertebrates, comparison of their relative abundance and taxonomic diversity across regional stream habitats may provide insight as to how historic changes in land use may influence present day communities.

The conversion of forested land to agriculture and/or urban land use has long been considered a major stressor to aquatic ecosystems. Agricultural lands increase the input of herbicides/pesticides and fine sediments, catalyze the loss of riparian complexity and in-stream habitat, and change the stream hydrology (Allan, 2004; Harding et al., 1998; Zhang et al., 2012). Urban land uses also bring about changes that greatly affect stream systems. Runoff from increased impervious surfaces modify channel morphology, increase sediment loads, and change the overall hydrology of a stream system (Zhang et al, 2012). In addition, each transition in land use affects organic matter exchanges with the floodplain and surrounding lands, and can negatively impact the dispersal ability of stoneflies and other macroinvertebrates requiring macroinvertebrates to travel farther to reach more suitable stream habitats (Vibrickas et al., 2011). Increased awareness of the effects of land use on streams has spearheaded conservation and protection efforts of stream ecosystems from a watershed perspective including embankments and riparian zones by regional, state, and federal agencies. To further complicate anthropogenic effects, changes in land use and impact on stream ecosystems, which include species richness and community diversity, may last for decades even after the land has been altered to another land use type (Harding et al., 1998). Legacy land use effects are particularly important factors to consider when studying stream ecosystem recovery. Streams that are impacted by impairments such as urbanization or agriculture lead to changes in macroinvertebrate community structure. These impairment induced changes typically lead to communities where most taxa exhibit non-seasonal life cycles and are present throughout the year (Soulsby et al., 2001; Johnson et al. 2012). Thus, it can be inferred that macroinvertebrate communities in impaired environmental conditions will exhibit less seasonal variation than more taxonomically diverse streams not compromised by anthropogenic effects, and will contain taxa exhibit seasonal growth and diversification patterns.

Seasonal dynamics play an important role in macroinvertebrate assemblage composition within a stream. Taxonomic abundance and richness in aquatic macroinvertebrates change seasonally, as do hydrology and thermal regimes (Spoka et al., 2006). Flooding occurs more frequently during the spring and fall, freezing during the winter, and drought in the summer, and result in within-year changes; especially in low-order streams; the majority of streams investigated for this research (Beche et al., 2006; Zhang et al., 2012). These changes in thermal regime and hydrology greatly influence emergence time, reproduction, growth and development of stoneflies and other macroinvertebrates.

While seasonal patterns in macroinvertebrate communities and life history strategies are known, there have been few studies that examine seasonality of functional feeding groups at the community level. In general, seasonality and temporal variability in benthic macroinvertebrate communities have only been examined in terms of macroinvertebrate taxonomic identification.

In order to better understand the seasonality of macroinvertebrate functional feeding groups at the community level, a two year study of stoneflies and macroinvertebrate communities was conducted seasonally, in four watersheds of Northeast Ohio. Macroinvertebrate communities were compared spatially (based on land use surrounding each sample site) and temporally (by season). This work hypothesizes:

1. The greatest species diversity and richness among stoneflies and other macroinvertebrates will occur during the summer months, when weather conditions in Northeast Ohio are more conducive, while the lowest diversity will occur during the winter months, when weather conditions in Northeast Ohio are the most inhospitable.
2. The greatest species diversity and richness among stoneflies and other macroinvertebrates will occur in regions where the landscape has been historically less disturbed, and the lowest diversity will occur at sites that have been historically impacted by humans even if the stream is currently surrounded by protected and managed lands.
3. Current land use, in addition to flight ability and emergence success, has the potential to affect the overall community structure of macroinvertebrates at the collection sites.

Although the primary objective of the this study was to determine the relationship that spatial and temporal changes have on the macroinvertebrate community located within the stream, it was also important to consider the results of chapter 3, and the influence of spatial and temporal factors on *Allocapnia recta* population structure.

4.2 <u>Materials and Methods</u>

This two year study ran from January 2004 to December 2004 (YEAR 1), and January 2005 to December 2005 (YEAR 2). The four seasons were defined as mid-March to early June (spring), late June to mid-September (summer), late September to early December (fall), and late December to early March (winter). In addition to macroinvertebrate data, physical/chemical data were collected from the four watersheds and six collecting sites designated in Chapter 3: one collecting site within the Rocky River (N41.2115: W –81.6831), two sites within the Cuyahoga River (N41.2314; W –81.5086 and N41.2335; W –81.5021), , two collecting sites within the Chagrin River (N41.5961; W –81.2521 and N41.6071; W –81.2875), , and one collecting site in the Grand River (N41.7258; W –81.0774), for all seasons between 2004 and 2005.

The Rocky River watershed consists of west, east, and main branches, with the collecting site in the East Branch. The land surrounding the collecting site is dominated by agriculture and paralleled by bridle paths. The East Branch of the Rocky River flows south into Hinckley Lake where it is impounded by the Hinckley Dam. The collection site was located in the channel downstream from the Hinckley Dam within the Hinckley Reservation of the Cleveland Metroparks. Using a nearby access road, the collection site is 2.410km along an earthen trail that follows the bridle path; both eventually crossing the river. The river is very dynamic in this area due, in part, to the dam and its sinuous path experiences a wide range of water depths from very shallow in some areas to more than 1.0m deep in others.

The two Cuyahoga River collecting sites were located within the Cuyahoga Valley National Park (CVNP), within the headwaters of the Boston Run tributary. Boston Run flows parallel to state Route 303, approximately 420m west of Happy Days Nature Center. The headwaters of Boston Run originate in a forested area within the CVNP. The upstream collection site was designated site CU1. The second site, approximately 200 meters downstream of the first site, was designated CU2.

Two collecting sties were located in the East Branch of the Chagrin River. The first was in the East Branch of the Chagrin River itself; the collection site was located within the Holden Arboretum on Wisner Road. The riparian zone on the left bank was approximately 60 meters deep while the riparian zone on the right bank was a steep embankment. The second collection site was located within Stebbins Gulch, a first order tributary to the East Branch of the Chagrin River, also located within Holden Arboretum, along an extension of Wilder Road south of Mitchells Mill Road; near row crop and livestock farming to the north. Most of the land outside of Holden Arboretum, as well as downstream, is privately owned and characterized as rural residential. The land upstream from Holden Arboretum is dominated by a large horse ranch

The Grand River collection site was located within the tributary Talcott Creek, a second order stream. The Grand River has been protected within the Lake County Metroparks since 1974, but remains adjacent to various land uses around the stream without protective designations. As a remnant of the Wisconsin glaciation and other glacial events, the river is deeply entrenched with steep embankments and slopping hills; hindering urbanization in comparison to other areas in Northeast Ohio. As a result, the land around the stream and, in particular, the sampling site, is a low-intensity residential area. Despite the presence of some residents, the predominant land use is crop and pastoral agriculture.

To determine stream conditions at each collection site, water samples were collected and analyzed using HACH chemical testing. Benthic macroinvertebrates were collected, identified to genus in most cases, and community structure was analyzed for each collecting site. Additional data, including stream habitat assessment and physical characterization, were collected in the field using Ohio Environmental Protection Agency's *Qualitative Habitat Evaluation Index*.

4.2.1 ***Water Chemistry***

Dissolved oxygen, temperature, and pH were measured *in situ* at each site using YSI Environmental 550A Dissolved Oxygen (DO) instrument (YSI Environmental Incorporated Yellow Springs, OH). The YSI Environmental 550A was calibrated prior to each use and DO readings were set to mg/L. Recordings for dissolved oxygen, temperature, and pH were acquired in the thalweg, upstream from the researcher.

Water chemistry samples for ammonia, nitrate, and orthophosphate were collected in a 1L polyurethane bottle by submerging the bottle beneath the stream's surface. Once collected, the sample was placed in a cooler, on ice, and transported to the lab for analysis. Samples were analyzed for ammonia, nitrate, and orthophosphate concentrations using an AQUAMATE ThermoSpectronic Spectrophotometer (St. Louis, Missouri) using HACH methods, reagents, and standards. To test for orthophosphate, the HACH PhosVer3 (Ascorbic Acid) Method was used. A 10mL subsample was placed into a clean, acid washed Erlenmeyer flask using a plastic pipette. The reagent PhosVer3 phosphate powder pillow was added to the flask and the solution mixed. After a two minute reaction time period a 2mL cuvette was filled with the solution and placed in the spectrophotometer along with distilled water blank. The spectrophotometer was set at wavelength 890nm (per procedural instructions) and output values were recorded in mg/L.

The HACH Cadmium Reduction Method was used to test for nitrate. A 10mL subsample was placed into a clean, acid washed Erlenmeyer flask using a plastic pipette. NitraVer 5 Nitrate Reagent Powder Pillow was added to the flask and shaken vigorously for one minute followed by a five minute reaction time. After the reaction period, a 2mL cuvette was filled with the solution and placed in the spectrophotometer along with distilled water blank. The spectrophotometer was set at the wavelength 400nm (per procedural instructions) and results were measured in mg/L.

The HACH Nessler Method was used to test for ammonia. A 50mL graduated cylinder was filled with the stream water sample to the 25mL mark. A second 50mL graduated cylinder was filled with deionized water and used as the blank. Three drops of Mineral Stabilizer were added to each cylinder, stoppered, and inverted three times to mix. Three drops of Polyvinyl Alcohol Dispersing Agent were added next to each cylinder, stoppered, and inverted three times. Finally, 1.0mL of Nessler Reagent was added to each cylinder, stoppered, and inverted three times. Following a one-minute reaction time, a subsample was placed in 2mL cuvette. The spectrophotometer was set at the wavelength 425nm (per procedural instructions) and output values were measured in mg/L. All reagents; PhoVer 3 phosphate pillow, NitraVer 5 nitrate reagent powder pillow, Mineral Stabilizer, Polyvinyl Alcohol Dispersing Agent, and Nessler Reagent were supplied by the HACH company.

4.2.2 *Habitat/Physical Characterization Assessment*

Habitat evaluation was done using the Ohio Environmental Protection Agency's Qualitative Habitat Evaluation Index (2006), modified to best fit the habitat and needs for aquatic macroinvertebrates. Four metrics were evaluated: substrate, in-stream cover, riparian zone and bank erosion, and riffle-run habitat quality. Substrate is a two-fold metric that measures type and quality of substrate. Larger substrates, like boulders, cobble, and gravel are preferred for most aquatic macroinvertebrates, while substrate such as artificial substrates, silts, or muck are scored lower as they can interfere with insect respiration, especially those with external gills such as Ephemeroptera, Plecoptera, and Tricoptera (EPT). In-stream cover represents areas of shelter that provide macroinvertebrates protection from predators, competitors, or provide a resting place to conserve energy away from current forces. The in-stream cover metric is measured under four conditions: extensive (> 75%), moderate (25-75%), sparse (5-25%), and minimal (< 5%). Riparian zone and bank erosion (RZ/BE) is the third metric. Riparian zone measures the quantity of the vegetative area around the stream and the quality of floodplain vegetation. This metric includes the zone width, floodplain quality, and extent of erosion. The maximum score of 100% includes: little to no erosion, riparian width of 750m or more, and forested or swamp floodplain vegetation. The lowest score includes conditions that show signs of severe erosion, absences of riparian zone, and urban, construction, or pastoral/row crop activity within the floodplains. The final metric is the riffle-run habitat quality. A mixture of flow and depth in a stream provide a variety of habitats to support diverse communities of macroinvertebrates. Riffles are shallow regions of the stream where water runs fast and is agitated by rocks. Dissolved oxygen concentrations in these areas are extremely high and may be near 100% saturation. Habitat specialists and macroinvertebrates that require high levels of oxygen due to external gills are the most diverse in these regions. Runs are deeper regions of a stream, but not as deep as pools. Although oxygen concentrations are lower in runs in comparison to riffles, runs provide additional habitat proximal to riffles where macroinvertebrates may be outcompeted in riffle or pool habitats. Riffle depth, run depth, riffle/run substrate, and riffle run embeddedness were also measured. The highest quality riffle depth is greater than 10cm deep, run depth greater than 50cm deep, and substrate is either boulder or cobble, with no embededdness. Poor quality areas are riffles less than 5cm deep, run depths less than 50cm deep, and substrate of more than 75% fine gravel or sand.

4.2.3 ***Benthic Macroinvertebrates***

Benthic macroinvertebrate samples were collected using a 500μm mesh kick-net with a collection surface area of 84.60cm^2. Two kick-net collections were performed at each site, one from the riffle and one from the pool, for a period of two minutes. Kick-nets were then placed on a tarp and macroinvertebrates were collected from the kick-net using forceps. The kick-nets and the tarp were then rinsed into a tub to ensure all captured samples were collected.

Macroinvertebrates were collected during all four seasons, identified to genus (oligocheates and chironomids were identified to family) and recorded in the field. Samples that required further identification, and all samples collected during the winter due to less than favorable weather conditions were collectively placed in a 1L sample jar containing 75% ethanol and returned to the laboratory. Upon arrival at the laboratory, the collected sample was emptied into a small basin and individual specimens removed and placed into a new 20mL plastic specimen jar with 75% ethanol alcohol. The specimen bottle was labeled with the date, location, and weather conditions from the sampling. Specimens were identified under magnification, using reference materials (McCafferty (1998), Peckarsky (1990), Merrit and Cummings (1996), Thorp and Covich (2001), and Voshell (2002), and additional resources.

All samples collected on-site from the kick-net and tarp were rinsed into a collecting tub and immediately transferred to 1L sample jars containing 75% ethanol alcohol before being transported to the lab.

4.2.4 *Statistical Analyses and Metrics*

Several metrics and statistical programs were utilized to evaluate the relationship of macroinvertebrate communities including stream characteristics and water quality, habitat characteristics and quality, riparian zone quality, and seasonal distribution among and between the Rocky, Cuyahoga, Chagrin, and Grand Rivers.

4.2.4.1 *Shannon Diversity and Evenness Indices*

Shannon Diversity Index measures macroinvertebrate taxonomic richness and diversity at the sample sites, while the Evenness Index determines how similar in number each macroinvertebrate taxa is at the collecting sites, together the indices were used to quantify taxa distribution. The underlying measure of this particular statistical method is that the more diverse the macroinvertebrate sample populations are, and the more similar their proportional abundance in a stream ecosystem, the more difficult it becomes to predict which species will be the next one collected from the sampling site. If diversity is very low – predominantly represented by a single, common species with all other specimens being rare – and a large number of members of the species are collected, the Shannon Diversity index will approach zero, therefore no uncertainly in predicting the taxonomic species of the next randomly collected specimen. Thus in the case of Shannon Diversity and Evenness Indices, macroinvertebrate community diversity was compared between and among sites, per season, per years.

4.2.4.2 *Cluster Analysis*

Cluster analysis was used to explore and analyze the data. The objective of cluster analysis is to sort samples into groups (clusters) so that the degree of association is strong between members of the same cluster and weak between members of different clusters. Since cluster analysis is a descriptive tool, it was used to reveal associations and structure in data, which though not immediately evident become clear once associations were determined. An agglomerative cluster analysis, using Euclidean Distance was performed using SPSS 19.0 for Windows (© 2010) to comparing sites, seasons, and percent abundance of macroinvertebrate taxa.

4.2.4.3 *Canonical Correspondence Analysis*

To analyze data relevant to the distribution of macroinvertebrate taxa and specific physical factors measured within the four watersheds in this study, Canonical Correspondence Analysis (CCA) was conducted using the software program CANOCO (ter Braak and Smilauer 2002). Canonical Correspondence Analysis is a direct gradient analysis that compares response variables (species) against environmental variables in order to determine which factors are most important in determining the presence and abundance of species in each sample.

Canonical correspondence analysis was used to compare seasonal macroinvertebrate distribution between collecting sites and years with seasonal environmental variables. The relative abundance of each macroinvertebrate taxa (genus) and eleven physical characteristics, including orthophosphate, ammonia, nitrate, dissolved oxygen, pH, water temperature, percent canopy cover , substrate quality, in-stream cover, riparian zone/bank erosion, and riffle/run habitats, were used in the analyses. Data for each season and from each of the four sampling locations were imported into CANOCO to complete the data set. Manual forward selection in the CANOCO software was used to determine significant environmental variables.

4.2.4.4. *Analysis of Variance and Functional Feeding Groups*

A statistical model, analysis of variance (ANOVA), using SPSS 19.0 for Windows (© 2010) was used in two ways. ANOVA was used to first analyze the physical variations between and among seasons and sites, and second to relate the physical variables in relation to the functional feeding groups (FFG) collected for each of the eight seasons.

Functional feeding groups were determined using Merritt and Cummings (1996) and McCafferty (1998). This is a classification method based on morpho-behavioral mechanisms for food acquisition and enables study of a much smaller group of macroinvertebrates based on how they obtain food and how they function in processing energy in the stream ecosystem. Additionally, FFG establish a link between aquatic food resource categories and the adaptations required for their exploitation. Food resource categories include coarse particulate organic matter (CPOM) – food particles greater than 1.0mm, fine particulate organic matter, (FPOM) – food particles with a size ranging from 0.45µm to 1.0mm, periphyton – sessile organisms such as heterotrophic microbes and algae, and prey – a general category including other macroinvertebrates, small amphibians, fish and fish eggs. The five defined categories of macroinvertebrates based on aquatic food resources in FFG analysis include: a.) *scrapers* – consuming mainly algae; b.) *shredders* – consuming mainly leaf litter but also decomposing wood debris; c.) *collector-gather* – consuming collected FPOM from the stream substrate; d.) *collector-filters* – consuming collected FPOM suspended in the water column; and, e.) *predators* – consuming other consumers.

4.3 Results and Discussion

Diversity indices, multivariate analysis, and functional feeding group evaluations were utilized in evaluating macroinvertebrate communities. Although some of these metrics may seem redundant, they measure different aspects of macroinvertebrate assemblage structure, function, and processes; lending a greater depth of understanding. A total of 62 species, representing 49 families and 13 orders (Table IX) were identified among the 6,243 macroinvertebrate specimens collected seasonally during 2004 and 2005. The number of individuals identified collectively at each site collectively over the two year period was lowest at Cuyahoga Site A in spring with 87 specimens collected and highest in the summer at Rocky River with 498 specimens (Table X). Six taxa were commonly collected throughout the study period at all six sites; *Hydropsyche* (order Tricoptera), *Stenelmis* and *Psephenus* (order Coleoptera), *Baetis* (order Ephemeroptera), *Simulium* (order Diptera), and the family Chironomidae (order Diptera). Although, the presence of the same taxa may indicate similarity among sites, it may also indicate that these taxa are generalists and have certain biological traits such as desiccation resistance, respiration mechanisms, body armor, and food preferences that allow them to survive in many different ecological habitats.

4.3.1 *Analysis of Variance and Physical Data*

4.3.1.1 *Seasonal Variation within Sites.*

To test for change in environmental conditions that affect macroinvertebrate communities, eleven variables; dissolved oxygen, temperature, pH, ammonia, nitrate, orthophosphate, substrate, in-stream cover, riparian zone, bank erosion, and riffle/run quality (Table XI) were tested and compared seasonally using one-way analysis of variance (ANOVA) using SPSS. Several seasonally based environmental factors were statistically significant different from each other; water temperature (C°) (p=0.000), dissolved oxygen (mg/L) (p=0.011), canopy cover (percent coverage) (p=0.011) orthophosphate (mg/L) (p=0.026) and nitrate (mg/L) (p=0.031) (Table XII). Not surprisingly, water temperature was highest in the summer (average = 15.5°C), lowest in the spring (average = 6.3°C), and intermediate during both the fall (average = 14.0 °C) and winter seasons (average = 7.0°C). Dissolved oxygen (DO), like water temperature, also changed seasonally. DO was significantly different among the all seasons (p-value = 0.011) and, in general, DO was highest in the winter (average = 11.66mg/L) and lowest in the spring (average = 7.25mg/L) in Northeast Ohio. Percent canopy cover measured using a densitometer and is the measure leaf density stretching over or adjacent to the stream channel, also changed seasonally at each collection site. Summer had the highest percent canopy cover (average = 78.67%) and the winter season had the lowest (average = 15.83%) (see Table XII).

Orthophosphate and nitrate were also statistically significant with p-values of 0.026 and 0.031, respectively. The highest mean concentrations of orthophosphate was recorded during the spring (average = 0.14mg/L) and a no orthophosphate was detected in the fall (0.00mg/L). Similar to orthophosphate, nitrate was recorded at highest concentrations during spring (average = 0.51mg/L). However, high concentrations were also recorded during the winter season (average = 0.21mg/L), while no nitrate was detected in samples tested during the summer and fall collecting periods (Table XII).

Table IX Macroinvertebrate abundance collected seasonally within the six sample sites for years one and two. The data has been combined yearly for this table

	RRSp	RRSu	RRFa	RRWt	CuyASp	CuyASu	CuyAFa	CuyAW	CuyDSp	CuyDSu	CuyDFa	CuyDW	EBCHSp	EBCHS	EBCHFa	EBCHW	StGSp	StGSu	StGFa	StGWt	GrSp	GrSu	GrFa	GrWt
Ephemeroptera																								
Acentrella sp.	0	0	0	0	0	3	3	2	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1
Arthroplea sp.	2	0	0	0	0	0	0	1	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Baetis sp.	4	16	6	8	2	15	19	23	23	22	23	30	0	15	16	19	29	3	5	5	9	20	18	15
Brachycercus sp.	0	0	2	0	0	7	3	0	3	1	1	0	0	0	6	0	0	0	0	0	0	0	0	1
Ephemerella sp.	8	4	6	0	0	12	13	10	5	6	8	10	0	4	3	8	0	0	0	0	3	0	0	5
Heptagenia sp.	1	0	0	0	0	1	1	7	7	2	0	6	0	7	5	0	2	11	0	0	2	4	0	0
Maccaffertium sp.	7	0	8	3	0	0	0	8	0	5	1	1	8	1	2	1	4	0	0	9	17	0	14	0
Paraleptophlebia sp.	1	2	0	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Procloeon sp.	0	0	0	1	0	0	0	1	1	1	0	0	9	0	1	3	33	0	0	2	25	0	0	0
Siphlonurus sp.	0	0	0	0	0	2	4	0	3	0	0	0	0	2	0	3	0	34	0	0	0	19	0	1
Plecoptera																								
Allocapnia sp.	4	0	1	2	2	3	4	3	8	3	2	2	7	7	3	10	116	0	3	28	55	0	0	2
Acroneuria sp.	13	7	5	1	0	14	23	16	15	25	17	14	0	7	11	7	10	0	10	1	15	6	4	12
Capnia sp.	1	1	6	0	0	9	8	7	5	6	4	4	0	1	1	1	0	0	0	0	2	0	0	2
Haploperla sp.	0	0	0	3	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1
Isoperla sp.	4	0	1	20	6	0	0	1	3	2	0	2	3	0	0	0	13	0	0	16	3	0	0	1
Leuctra sp.	1	0	1	0	0	1	2	1	1	1	1	1	6	0	0	0	5	0	0	0	0	11	0	1
Nemocapnia sp.	0	0	0	0	0	0	0	2	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Nemoura sp.	0	0	0	0	1	0	3	2	1	1	0	0	0	1	1	7	0	0	0	26	4	3	0	0
Paraleuctra sp.	0	2	0	0	0	1	2	2	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1
Tricoptera																								
Dolophilodes sp.	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	4	0	0	5	0	0	0	1
Hydropsyche sp.	59	231	37	11	5	29	49	53	28	25	40	40	22	32	42	4	69	15	26	42	110	85	11	40
Lepidostoma sp.	0	0	0	0	0	1	4	2	1	1	1	3	0	0	1	1	5	0	6	0	0	0	0	0
Limnephilus sp.	2	0	5	0	0	0	1	6	4	0	6	6	0	2	2	0	4	1	1	0	0	0	1	1
Neureclipsis sp.	0	0	0	0	0	1	0	2	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Phryganea sp.	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0
Polycentropus sp.	0	1	1	0	0	1	0	3	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0
Pseudostenophylax sp.	0	2	0	2	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	1	2	3	0	1
Rhacophila sp.	2	0	1	2	0	0	2	0	1	1	3	2	0	0	0	1	0	5	1	18	31	8	1	2
Coleoptera																								
Dineutus sp.	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Gerris sp.	0	0	1	0	0	1	2	1	5	2	1	2	0	0	2	1	0	1	0	0	0	0	0	1
Gyrinus sp.	0	0	0	0	0	0	7	3	2	3	1	0	0	2	6	2	0	1	0	0	2	0	0	1
Helichus sp.	28	0	0	2	0	6	3	1	0	0	0	0	1	0	0	0	25	6	2	0	0	0	0	0
Psephenus sp.	4	2	16	5	0	31	25	26	25	26	21	25	1	12	25	5	38	17	9	10	13	39	5	19
Stenelmis sp.	52	151	10	16	0	35	36	40	21	24	25	40	11	8	32	11	17	18	0	1	13	0	0	20
Odonata																								
Aeshna sp.	2	0	0	0	0	3	1	1	3	3	1	0	0	2	1	1	3	1	0	0	0	0	0	1
Gomphus sp.	0	0	3	0	0	0	2	1	3	0	1	2	0	2	2	0	0	1	0	0	1	1	0	0
Hetaerina sp.	0	1	0	0	0	2	2	0	0	2	1	0	0	0	0	0	0	2	0	0	0	0	3	0
Progomphus sp.	0	0	0	0	0	2	3	1	1	1	1	0	0	2	5	1	0	1	0	0	0	0	0	1
Megaloptera																								
Chauliodes sp.	1	4	2	0	0	2	2	6	2	2	1	1	0	2	6	2	0	1	0	0	0	0	0	0
Corydalus sp.	0	3	1	0	0	3	6	1	0	1	0	3	0	3	0	0	0	0	0	0	0	0	0	0
Nigronia sp.	0	5	2	1	0	3	0	0	0	1	3	3	0	0	2	1	2	1	1	0	1	1	2	7
Sialis sp.	2	2	4	0	0	2	4	1	3	1	3	1	0	1	1	1	0	1	0	1	0	1	0	1
Diptera																								
Atherix sp.	0	0	4	0	0	5	8	7	4	5	2	7	1	8	1	3	0	1	0	0	0	0	0	2
Antocha sp.	0	0	5	1	0	5	4	1	1	3	0	4	0	1	2	1	0	0	0	0	0	0	0	1
Chironomidae	19	20	37	15	41	44	64	135	27	26	35	30	24	21	24	41	24	21	8	27	49	47	32	32
Chelifera sp.	1	0	0	0	0	0	0	0	3	0	0	1	2	0	0	0	41	0	0	0	0	0	0	0
Chrysops sp.	0	0	0	0	0	0	1	1	1	2	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Culex sp.	1	1	1	0	0	3	2	20	2	2	3	3	3	2	2	2	1	0	5	0	11	0	0	2
Dasyhelea sp.	0	0	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Dolichopus sp.	0	0	0	0	10	0	0	4	2	0	0	0	0	1	0	0	1	0	0	0	3	0	0	0
Hexatoma sp.	5	0	2	12	1	3	6	3	6	5	7	6	0	11	9	1	3	0	0	0	6	0	0	5
Leptoconops sp.	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1
Pedicia sp.	0	0	0	0	0	0	0	0	0	5	0	1	0	0	1	0	0	0	0	0	0	0	0	1
Simulus sp.	3	14	24	4	1	16	25	19	21	35	27	30	0	20	21	48	10	0	49	231	10	0	5	14
Stratiomys sp.	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Tipula sp.	7	23	3	1	4	9	10	12	7	9	6	14	0	7	5	4	5	1	1	10	0	0	4	7
Hemiptera																								
Rhagovelia sp.	1	4	1	0	0	2	1	1	2	1	0	1	0	0	0	0	1	0	0	0	1	0	0	0
Others																								
Gammarus sp.	0	0	0	1	3	9	12	12	4	6	9	8	0	6	3	6	0	1	0	0	4	1	0	6
Haplotaxida sp.	0	0	0	1	0	0	0	0	0	0	0	2	3	0	0	0	4	0	0	3	0	0	0	1
Hyalella sp.	0	0	0	0	0	0	0	2	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Lumbricula sp.	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	0	0	0	0	0	0
Unionidae, Family	0	0	0	1	0	0	0	0	0	2	2	6	0	0	0	0	0	0	0	0	0	0	0	0

Table X Total number of individuals collected at each site. Each subsequent column represents the number of individuals collected during each season combined from January 2004-December 2005

Site	Total	Spring	Summer	Fall	Winter
Rocky	1046	235	498	197	116
CuyahCU1	1197	87	287	369	454
CuyahCU2	1115	255	275	265	320
EBCH	748	104	192	253	199
StGulch	1181	471	145	127	438
Grand	946	393	249	101	213

Table XI Seasonal Chemistry and Physical Assessment The mean and range of water chemistry and physical features of the stream collected seasonally within each site from January 2004 until December 2005.

Rocky River

SampleID	PO4(mg/L	NH4mg/L)	NO3(mg/L)	DO(mg/L)	pH	Temp°C	%CC
RR_SP04	0.03	0.44	0.59	9.00	8.00	7.50	75
RR_SP05	0.00	0.00	0.00	0.00	8.05	6.75	74
RR_SU04	0.00	0.13	0.00	8.90	7.50	16.00	75
RR_SU05	0.00	0.11	0.00	8.05	7.50	15.80	80
RR_FAL04	0.00	0.00	0.00	10.21	7.30	13.80	40
RR_FAL05	0.00	0.00	0.00	15.89	7.30	11.50	60
RR_WT04	0.17	0.00	0.00	10.50	8.15	6.20	15
RR_WT05	0.08	0.00	0.00	10.50	7.90	6.90	15
Mean	**0.04**	**0.08**	**0.07**	**9.13**	**7.71**	**10.56**	**54.25**
Range	**0-0.17**	**0-.44**	**0-0.59**	**0-15.9**	**7.3-8.2**	**6.2-15.8**	**15-80**

East Branch Chagrin

SampleID	PO4(mg/L	NH4mg/L)	NO3(mg/L)	DO(mg/L)	pH	Temp°C	%CC
EBCHSp04	0.27	0.62	0.95	9.70	7.50	5.00	40.00
EBCHSp05	0.00	0.00	0.00	10.20	7.15	8.00	41.00
EBCHSu04	0.00	0.00	0.00	10.72	7.50	16.05	40.00
EBCHSu05	0.00	0.00	0.00	11.01	7.60	15.80	45.00
EBCHFa04	0.00	0.00	0.00	11.36	7.55	14.90	25.00
EBCHFa05	0.00	0.00	0.00	9.80	8.14	12.80	20.00
EBCHWt04	0.00	0.00	0.00	12.70	7.45	6.10	0.00
EBCHWt05	0.00	0.00	0.00	13.50	7.60	8.00	4.00
Mean	**0.03**	**0.08**	**0.12**	**11.12**	**7.56**	**10.83**	**26.88**
Range	**0-0.273**	**0-0.6211**	**0-0.955**	**9.7-13.5**	**7.15-8.14**	**5.00-16.05**	**0-45.0**

Cuyahoga Site A

SampleID	PO4(mg/L	NH4mg/L)	NO3(mg/L)	DO(mg/L)	pH	Temp°C	%CC
CuyASp04	0.39	0.56	1.33	5.10	7.95	4.85	85.0
CuyASp05	0.00	0.00	0.00	7.45	8.30	6.00	84.0
CuyASu04	0.00	0.12	0.00	3.85	8.30	15.20	90.0
CuyASu05	0.03	0.08	0.00	4.87	8.30	16.01	97.0
CuyAFa04	0.00	0.00	0.00	4.31	8.25	13.00	60.0
CuyAFa05	0.00	0.00	0.00	3.89	7.84	15.23	50.0
CuyAWt04	0.01	0.00	0.00	11.75	8.35	6.35	10.0
CuyAWt05	0.00	0.60	1.26	10.40	7.75	8.50	10.0
Mean	**0.05**	**0.17**	**0.32**	**6.45**	**8.13**	**10.64**	**60.8**
Range	**0-0.390**	**0-0.603**	**0-1.33**	**3.84-11.75**	**7.75-8.35**	**4.85-16.01**	**10.0-97.0**

Stebbin's Gulch

SampleID	PO4(mg/L	NH4mg/L)	NO3(mg/L)	DO(mg/L)	pH	Temp°C	%CC
StGSp04	0.25	0.53	0.73	10.20	7.20	4.80	91.00
StGSp05	0.00	0.00	0.00	11.50	7.00	7.90	93.00
StGSu04	0.02	0.00	0.00	10.91	7.30	12.00	96.00
StGSu05	0.04	0.00	0.00	11.09	7.40	15.70	96.00
StGFa04	0.00	0.00	0.00	6.45	7.20	14.50	45.00
StGFa05	0.00	0.00	0.00	11.45	8.20	12.90	70.00
StGWt04	0.00	0.00	0.00	12.15	7.00	5.90	30.00
StGWt05	0.00	1.59	0.01	13.80	7.50	8.10	28.00
Mean	**0.04**	**0.27**	**0.09**	**10.94**	**7.35**	**10.23**	**68.63**
Range	**0-0.25**	**0-1.59**	**0-0.73**	**10.2-13.80**	**7.00-8.20**	**4.80-15.7**	**28.00-96.0**

Cuyahoga Site D

SampleID	PO4(mg/L	NH4mg/L)	NO3(mg/L)	DO(mg/L)	pH	Temp°C	%CC
BRD_SP04	0.74	1.00	2.51	6.45	8.20	5.00	90.00
BRD_SP05	0.00	0.00	0.00	8.05	8.00	7.00	87.00
BRD_SU04	0.00	0.05	0.00	7.67	8.25	16.00	93.00
BRD_SU05	0.02	0.11	0.00	8.01	8.40	17.19	99.00
BRD_FAL04	0.00	0.00	0.00	8.40	7.70	14.00	70.00
BRD_FAL05	0.00	0.00	0.00	6.59	8.50	16.05	65.00
BRD_WT04	0.01	0.00	0.00	12.40	7.60	6.05	15.00
BRD_WT05	0.00	0.61	1.30	10.40	8.00	8.00	10.00
Average	**0.10**	**0.22**	**0.48**	**8.50**	**8.08**	**11.16**	**66.13**
Range	**0-0.734**	**0-1.00**	**0-2.51**	**6.45-10.40**	**7.50-8.50**	**5.00-17.19**	**10.00-99.00**

Grand River

SampleID	PO4(mg/L	NH4mg/L)	NO3(mg/L)	DO(mg/L)	pH	Temp°C	%CC
TC_SP04	0.00	0.00	0.00	9.30	7.55	5.20	63.00
TC_SP05	0.00	0.00	0.00	0.00	7.25	7.00	65.00
TC_SU04	0.09	0.00	0.00	12.90	7.60	13.40	65.00
TC_SU05	0.00	0.00	0.00	10.50	8.10	16.80	68.00
TC_FAL04	0.00	0.00	0.00	15.25	8.15	14.15	35.00
TC_FAL05	0.00	0.00	0.00	10.56	7.50	14.80	50.00
TC_WT04	0.00	0.00	0.00	11.65	8.20	7.20	25.00
TC_WT05	0.00	0.00	0.00	10.20	8.30	6.10	28.00
Mean	**0.01**	**0.00**	**0.00**	**10.05**	**7.83**	**10.58**	**49.88**
Range	**0-0.09**	**0.00**	**0.00**	**0-15.3**	**7.3-8.2**	**5.2-16.8**	**25-68**

Table XII Summary results of a one-way analysis of variance of the physical characteristics between seasons. Orthophosphate (PO4), nitrate, dissolved oxygen (DO), temperature, and canopy cover were significantly different between seasons. DF=47.

Physical by Season	**F score**	**P<0.05**
PO4(mg/L)	3.39	0.03
Ammonia(mg/L)	2.27	0.94
Nitrate(mg/L)	3.24	0.03
DO(mg/L)	4.17	0.01
pH	0.28	0.84
Temp(°C)	176.66	0.00
Canopy Cover (%)	34.92	0.00
Substrate Type	1.60	0.20
In-stream Cover (%)	2.37	0.08
RipZon/BE(%)	0.57	0.64
Riffle/Run(%)	0.30	0.82

4.3.1.2 *Seasonal variations Between Sites*

ANOVA was also used to analyze seasonal variables between sites (Table XIII). ANOVA results revealed pH (p=0.0), dissolved oxygen (mg/L) (p=0.046), riparian zone/bank erosion (a metric with a possible score from 0 (no riparian zone and the presence of bank erosion) to 10 (well developed riparian zone and an absences of bank erosion) (RZ/BE) (p=0.0), riffle/run habitat quality (a metric with a possible score of 0 (absences of riffle/run) and 10 (a stream with an extensive level of the combination of riffles and runs)) (p=0.0), and substrate quality (%) (p=0.000) were statistically significant (Table XIII).

ANOVA indicated that pH was statistically significant between Cuyahoga site CU1, compared to East Branch of the Chagrin (p-value = 0.017) and Stebbins Gulch (p-value = 0.0). Significant differences was also observed for the pH variable when between Cuyahoga site CU2, pH value was compared between East Branch of the Chagrin (p-value = 0.035) and Stebbins Gulch (p-value = 0.001). Dissolved oxygen statistically significant between East Branch of the Chagrin and Cuyahoga site CU1 (p-value =

0.050). Riparian zone/bank erosion (RZ/BE) when compared among sites, were determined to be significantly different between Rocky River and Stebbins Gulch (p-value = 0.001), between Rocky and Grand River (p-value = 0.00); between Cuyahoga site CU2 and the East branch of the Chagrin (p-value= 0.00), Cuyahoga site CU1and between the Grand River, EB Chagrin, and Stebbins Gulch (all comparisons had a p-value equal to 0.00)

Riffle/run habitat quality was statistically significant between most of the sites, including between Rocky River and Cuyahoga CU1, East Branch of the Chagrin (both with a p-value = 0.00) and Stebbins Gulch (p-value = 0.002)). Cuyahoga site CU2 was significantly different from the East Branch of the Chagrin and Stebbins Gulch (both p-values = 0.00), and Cuyahoga site CU1 (p-value = 0.002).

Comparison of riffle/run habitat quality between the East Branch of the Chagrin and the other sites, determined it was significantly different from all sites except Stebbins Gulch (p-value = 0.993). Additionally, the Grand River was significantly different from the Cuyahoga site CU1, (p-value = 0.0), East Branch of the Chagrin (p-value = 0.008), and Stebbins Gulch (p-value = 0.035).

Percent substrate quality was significantly different between Cuyahoga site CU1, when compared between East Branch of the Chagrin (p-value = 0.020), Stebbins Gulch (p-value= 0.00), and Grand Rivers (p-value = 0.002) sites. Additional comparisons showed that there was also significant results observed when comparing percent substrate between the Rocky River and Stebbins Gulch (p-value = 0.032) and between Cuyahoga site CU2 and Stebbins Gulch (p-value = 0.005).

Table XIII Summary results of a one-way analysis of variance of the physical characteristics between collecting sites. Significantly different variables were dissolved oxygen (DO), pH, substrate type, percent riparian zone/bank erosion (RipZon/BE), and percent riffle run. Df=47.

Physical by Site	F score	P<0.05
PO4(mg/L)	0.36	0.88
Ammonia(mg/L)	0.80	0.56
Nitrate(mg/L)	1.07	0.39
DO(mg/L)	2.49	0.05
pH	6.04	0.00
Temp(°C)	0.24	1.00
Canopy Cover (%)	1.74	0.15
Substrate Type	7.36	0.00
In-stream Cover (%)	0.93	0.47
RipZon/BE(%)	15.18	0.00
Riffle/Run(%)	25.68	0.00

4.3.2 *Macroinvertebrate Evaluation*

Overall, the most dominant taxa at each collecting site, season, and year were *Baetis, Hydropsyche*, *Simulium, Stenelmis*, and members of the family Chironomidae. These taxa are generalist, and tolerate a variety of anthropogenic impacts. The genus *Baetis* (order Ephemeroptera) is more tolerant of organic wastes and nutrient increases than most members of the order. *Baetis* larvae can develop successfully in water as warm as 32°C and as cold as 4°C (Voshell, 2002) and eggs when laid can hatch immediately or may remain dormant for months under extreme conditions (Merritt and Cummings, 1995). *Hydropsyche* (order Trichoptera), are collector-gathers using nets to collect anything from fine organic matter to coarse particulate matter, while some members are filter feeders. They can survive in moderate levels of pollution but are the densest in streams high in organic matter and nutrients (McCafferty, 1983).

Members of the genus *Stenelmis* (order Coleoptera) can live in a variety of habitats and commonly feed on periphyton. They exchange oxygen by means of a highly developed plastron and are not dependent on dissolved oxygen levels within the stream. Most *Stenelmis* do not reach sexual maturity until their second year in the aquatic larval stage, and have the ability to forgo adulthood and mating during times of extreme stress brought about by anthropogenic or natural events (Merritt and Cummings, 1995).

Like *Hydropsyche*, *Simulium* (order Diptera) are generalist and filter –feeders, feeding on fine organic particulate matter (FPOM), algae, bacteria, and microfilms. Though most dipterans are tolerant of high levels of stream pollution, *Simulium* are sensitive to inorganic pollution, but more tolerant of organic pollution (Voshell, 2002).

Members of the family Chironomidae (order Diptera) were among the most abundant taxa collected in this research. The Chironomidae are a large and diverse family found in almost every aquatic or semiaquatic ecosystem (Merritt and Cummings, 1995). Most are generalist and some members of the family have hemoglobin that allows them to exist in near anoxic environments (Voshell, 2002).

4.3.2.1 Shannon Diversity Index and Evenness Analysis

While most collecting sites showed seasonal or year to year variation in Shannon Diversity and Evenness Index values (Figure IV.1), there was no consistent pattern of change within or across sites. Overall, the second Cuyahoga site, CU2, had the highest diversity for all seasons and years, and was the most consistently diverse (H'= lowest 2.77 to highest 2.97). All other sites varied across seasons and years. In addition, diversity declined significantly at two sites during the two year sampling period; however, both sites were able to recover. The decline occurred in Cuyahoga Site CU1 and Grand River, and reflects effects of a 100-year storm event in August 2003 at Cuyahoga site CU1 and a 50-year storm event in August 2005 in the Grand River.

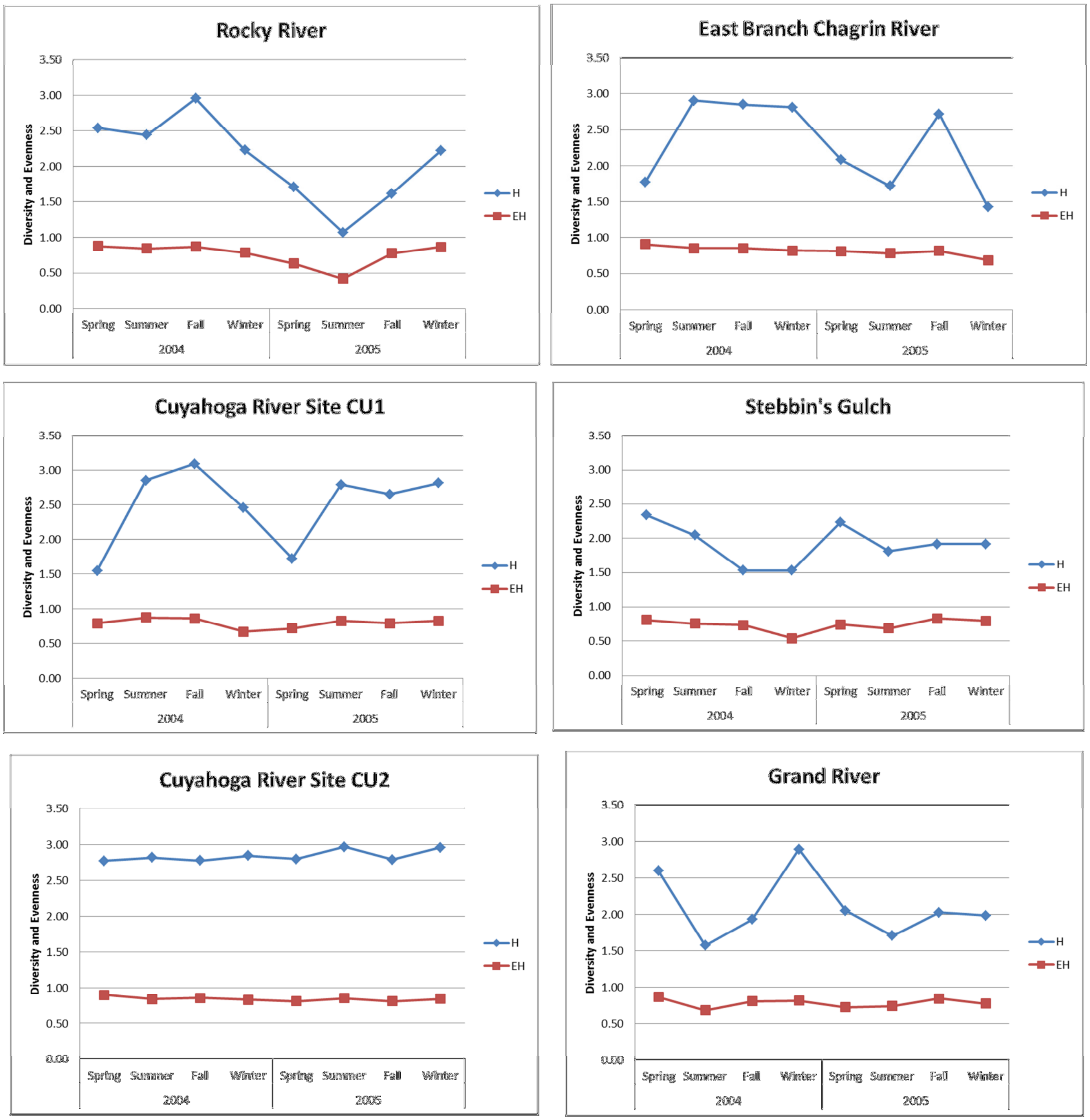
Preliminary macroinvertebrate sampling occurred at Cuyahoga River site CU1 in winter 2002 and summer 2003 to assess adult stonefly populations. This was followed by a 100-year storm event in late summer 2003. The lowest macroinvertebrate diversity at Cuyahoga site CU1 occurred in spring 2004. Previous to the 100-year storm event, this headwater stream had cobble and gravel substrate, dense canopy cover, fast moving cold water, and high dissolved oxygen concentrations. After the storm event in August of 2003, a dense clay layer several centimeters thick collapsed into the river, altering substrate and water chemistry. Despite these changes, macroinvertebrates were still present during spring 2004, though in much lower numbers. By summer 2004, the macroinvertebrate community assembled in Cuyahoga site CU1 showed signs of recovery with the highest site diversity values occurring in summer and fall 2004. The low diversity seen in spring 2005 may be due to the persistence of road runoff related to nearby State Route 303 following spring snowmelt and rainfall. The most diverse period in the Grand River was during winter 2004 sampling ($H = 2.895$/ $E_H = 0.814$), followed by spring 2004 samples. During the summer, the highest number of individuals were collected (n=158) represented by 35 taxa. Similar to Cuyahoga site CU1, a reduction in diversity occurred at the Grand River site during the summer 2005 season/year following a 50-year storm event. One hundred and sixteen specimens were collected post-storm and only ten taxa were represented. As with Cuyahoga CU1, the decrease in the number of specimens and taxa within the Grand River post flood event was most likely the result of the storm and altered substrate.

In the Rocky River, the most diverse sampling period was fall 2004 ($H=2.953/E_H = 0.868$), while the lowest diversity was summer 2005 ($H = 1.073$/ $E_H = 0.418$). While 388 individuals were collected, they were only comprised of 13 taxa. The following seasons the number of individuals remained high, but the total number of taxa was low. Within the East Branch of the Chagrin, the most diverse sample period was during summer 2004 (H = 2.906/ E_H =0.854) and the sampling period with the least diverse macroinvertebrate distribution was collected in winter 2005 (H = 2.895)/ E_H =0.814). During summer 2004, 165 individual macroinvertebrates were collected representing 30 unique taxa. Dominant taxa at the collection site were consistent with the aforementioned taxa above (i.e. *Baetis, Hydropsyche*, *Simulium, Stenelmis*, and the family Chironomidae). Furthermore, taxa that were rare in many of the other collection sites (e.g. *Heptagena* and *Ephemerella* (ephemeropterans), *Allocapnia* and *Acroneuria* (plecopterans), and *Hexatoma* and *Tipula* (dipterans) were present in larger numbers in East Branch of the Chagrin, and may be due to the fact that the East Branch collecting site was a much higher order stream than other sample sites, and included taxa that favor larger order streams. Winter 2005 was the season with the lowest Shannon Diversity and evenness, with 8 taxa representing 59 specimens. Chironomids and *Simulium* accounted for 75% of the taxa collected. Even though other taxa were collected at this time, no more than 10 individuals of any one taxon were collected from the site. The low diversity found in samples from the East Branch is difficult to explain within the scope of this research. At other sampling locations where diversity was low, such as the Cuyahoga River and Grand River, catastrophic storm events and subsequent flooding provided a plausible explanation for the lack of diversity. These low levels of diversity at East Branch of the Chagrin may actually be the result, in part, of a mild summer and fall. Aquatic insects that normally remain in their aquatic stage during poor stream conditions will emerge as terrestrial adults during favorable terrestrial conditions, leaving fewer juveniles in the stream.

In Stebbins Gulch, the most diverse sampling period was during spring 2004 (H = 2.336 / E_H =0.808) and the least diverse community structure was found during fall 2004 (H = 1.528/ E_H =0.735). In spring 2004, 154 specimens representing 18 genera were collected. Although *Hydropsyche* was among the dominant genera with 31 specimens, it only accounted for 20% of the total organisms collected. Abundances of a taxon relatively unique from the other sites, *Chelifera* (n=40) a dipteran, exceeded those of *Hydropsyche*, (n=31) during this sampling period.

Sample collection at Stebbins Gulch during the fall of 2004 had the lowest diversity for all sampling periods during the two years of collecting. Only 58 macroinvertebrate specimens were collected, and the specimens were represented by only 8 taxa. Together, *Simulium* and *Hydropsyche* accounted for 70% of the macroinvertebrate community sampled at the site. Although both genera were dominant during all eight seasons of sampling, it was noteworthy that the community structure lacked representatives from other taxa when compared to the other sampling locations, years, and seasons. Chironomids, *Baetis*, and *Stenelmis* were typically collected at Stebbins Gulch but there were periods, i.e. fall 2004, when the number of specimens in each taxon was marginal to absent. The level of diversity observed at the location improved throughout the course of this work, but the lack of sampling data prior to 2004 prevents development of a meaningful explanation for the lack of community structure in 2004.

Figure IV.1 Shannon diversity (H) and Evenness (E_H) for all six collecting locations by season and year.



4.3.2.2 Cluster Analysis

In previous research, cluster analyses have been used to classify data into discrete groups. While cluster analysis classification is a useful tool, it does not take into account the degree of variability along natural or anthropogenic environmental gradients (Gerth et al., 2013). In this particular study, seasonal variation in temperature, rainfall, and stream flow velocity are among the major factors influencing macroinvertebrate community structure at a stream site. However, the more refined the data, the more likely cluster analysis reflects this gradient (Leslie et al., 2012). In this research, it was important to identify macroinvertebrates to the lowest taxonomic level possible. Macroinvertebrates were identified to genus (except chironomids, which were identified to family), by collecting site, season, and year. The more refined the data, the more useful cluster analysis becomes. An agglomeration cluster analysis using Euclidian distance was conducted in SPSS using the percent abundance macroinvertebrate data for each of the six collecting sites, and eight sample seasons for the 2004 and 2005 sampling period. Data were analyzed based on the resulting dendrogram (Figure IV.2). Clusters were defined based on hierarchical designation. Five major groups were identified and labeled Roman numerals I through V. Delineating the groups further Arabic numbering 1-11 were used to designate the next tier of clusters, letters were used to identify specific relationships within clusters. While similar communities grouped together, outlier sites were also identified. From the results, many similarities existed among the communities. Most clusters occurred based on collecting site and season; year had the least influence on the results. With few exceptions, most sites/season/year within Cluster I which include the more distinct Clusters 1 through 4; Figure IV.2), had high percent abundance of the following taxa: *Hydropshye, Stenelmis, Simulium, Beatis,* and chironomids. Other clusters either shared similar, but unique macroinvertebrate taxa composition or had lower percentages of the aforementioned abundant taxa.

Overall, cluster analysis of the macroinvertebrate community data revealed that fall and winter samples for both years (2004 and 2005), regardless of site, were similar in composition at some level, especially in Cluster I; 1-4. In some cases, spring and summer samples also had similar composition either among or between sites, while most other spring and summer samples were unique, and not clustered together.

Cluster analysis indicated that macroinvertebrate communities in Cuyahoga CU2 had similar composition for all years and seasons (Cluster I; 1a and 2), which was supported by the Shannon Diversity and Evenness indices results. Hypothetically, although several unique taxa were found in all clustered sites and samples, rare taxa (i.e. *Tipula* and *Antocha*, *Nigronia*, and *Acroneuria*) and common species (i.e. *Stenelmis, Simulium, Beatis,* and *Psephenus*) were present in nearly the same abundance across all samples at Cuyahoga site CU2 and could explain the pattern of clustering.

The macroinvertebrate community for Cuyahoga site CU1 samples was similar in composition to Cuyahoga CU2 in fall 2004 and summer and winter 2005, with slightly different community composition in summer 2004 and fall 2005. Samples from winter 2004, and spring 2004 and 2005 clustered together to form Cluster IV, cluster 10. During these three particular seasons and years, macroinvertebrate community diversity was low, a result from the potential influence of roadway runoff due to its close proximity to State Route 303.

Within the larger Cluster V; cluster 11a-b not only did the two Chagrin River samples cluster together, East branch and Stebbins Gulch, but they also cluster by fall and winter seasons, similar to those observed in Cluster I. Fall and winter macroinvertebrate communities collected within Stebbins Gulch (SG) clustered together with fall (2004 and

2005) and winter 2005 samples clustering first, before joining winter 2004 and the Chagrin winter 2005 samples (clusters 11a and b). In Cluster II; cluster 7a-b, three out of the five samples clustering are from the Grand River. Grand River spring 2005, clustered with the East Branch of the Chagrin spring 2005 for the formation of 7a cluster, while Grand River summer 2004 and winter 2005 clustered with the Rocky River fall 2005.

Finally spring and summer samples paired with the East Branch of the Chagrin and the Rocky River collecting sites in the larger Cluster III, specifically cluster 9. The most probably reason for this is that both the East Branch of the Chagrin and the Rocky River collecting sites are much more open systems and support different members of a the macroinvertebrate communities (i.e. less shredders and more grazers and filter-feeders). Except for clustering with the East Branch of the Chagrin and the Grand Rivers, Rocky River (RR) macroinvertebrate communities showed no distinct affinity by site or season.

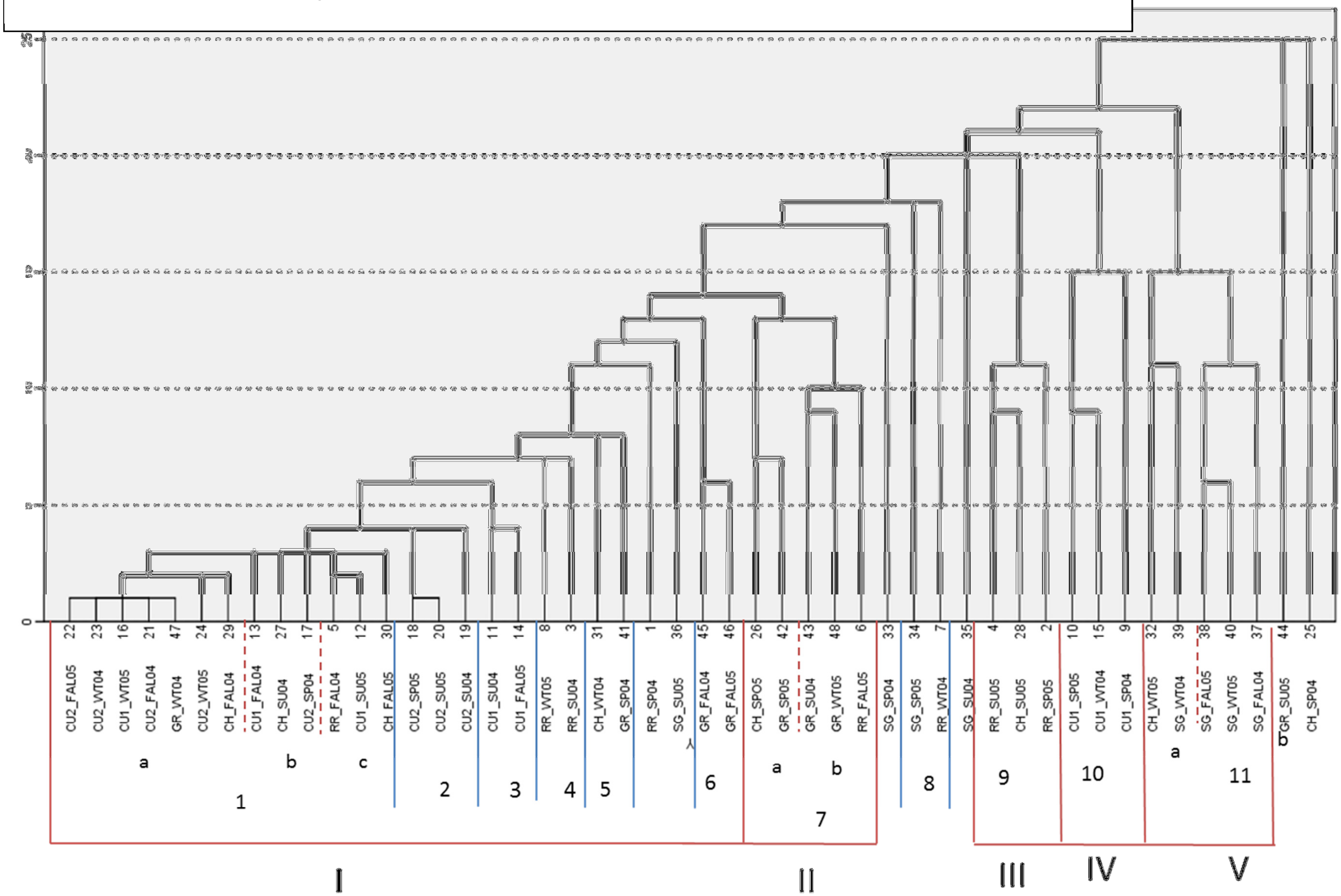
The two identified outliers identified were the East Branch of the Chagrin, spring 2004 and Grand River summer 2005. In August of 2005 Grand River a fifty-year storm event occurred that altered the stream habitat similar to that in Cuyahoga Site CU1 in summer 2003. Northeast Ohio counties of Lake, Geauga and Ashtabula had flood events and several tributaries to the Grand River were either flooded or altered (personal observations). This storm event caused extensive flood damage; especially at the Grand River sample site. Unfortunately, the summer collection occurred after the flood event. The site had been washed out and a stream-side residence abandoned by the owners was collapsing due to water damage. A closer examination of the site and surrounding area revealed that gravel entrained upstream of the collection site, had moved downstream with smaller clastic particles and altered the aquatic habitat, noticeably changing the in-stream dynamics. The effects of increase sedimentation, stream embeddedness, and change in stream pattern from the fifty year storm event on macroinvertebrate community composition, is the most likely explanation for the Grand River summer 2005 sample as an outlier . A total of 115 macroinvertebrate specimens were collected during this sample. Of the 115 specimens collected, thirty-four and thirty-five individuals were represented by *Hydropshye* and *Psephenus* (order Coleoptera), respectively. Collectively, these two genera accounted for 63% of the macroinvertebrates at that time. Both genera are relatively hardy macroinvertebrates and are able to survive extreme conditions caused by storm events, whereas other macroinvertebrates could not survive, or at least stay within that region of the stream. Macroinvertebrates may simply move downstream along with the stronger current during the storm, while others may have moved down into the hyporehic zone for shelter.

In the second outlier, spring 2004 at the East Branch of the Chagrin River, only twenty macroinvertebrate specimens were collected, one of the lowest numbers of macroinvertebrates collected per season at any site. Spring 2004 was the first collection period from this site, and reasons for the low numbers of both individuals and taxa are unknown; subsequent macroinvertebrate collection numbers were much higher. The most common taxa were collected here, as were a few rare taxa including the plecopterans *Allocapnia* and *Leuctra*.

In summary, Cluster I contained taxa that were in low numbers and were common among all members of the cluster (i.e. plecopterans *Acrenuria, Allocapnia,* and *Capnia*, the dipteran *Atherix*, and the ephemeropteran *Ephemeralla*). Other taxa which are normally rare were also high in number within Clusters I and II, *Nigronia* (order

Megaloptera), and dipterans *Tipula* and *Hexatoma* were in relatively high abundance for all sites in the second cluster. Dominant genera such as *Hydropshye, Stenelmis, Simulium, Beatis,* and chironomids are less influential because they make up close to 99% of all macroinvertebrates among collecting sites, seasons, and years combined. Percent abundance of rare and moderate taxa such as *Allocapnia, Isoperla, Tipula,* and *Heptagenia* are more likely to link sites and seasons together. Despite the fact that rare species are smaller in quantity, they are more influential on overall macroinvertebrate community structure than previously realized, and exert more influence on cluster analysis results than the dominant species (Chao et al., 2012).

Figure IV.2 Cluster Analysis: The clusters were defined based on hierarchy of the data. The major clusters were designated I through V. The second tier of grouping macroinvertebrate data into smaller clusters designated 1-11. Some clusters were further specified into a-c. Rocky River (RR), Cuyahoga site A (CU1) Cuyahoga site D (CU2), East branch of the Chagrin (CH), Stebbins Gulch (SG), and Grand River (GR).



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In the cluster analysis, five major clusters were based on temporal factors and that appear to have the most influence on which sites grouped together. Overall, year had little influence on clustering whereas season followed closely by location were the most influential factors in the analysis. Winter samples clustered more often with other winter data , than with spring, summer, or fall. However, winter and fall samples clustered together more often than spring and summer. Location was also a factor that contributed to site clustering. Sampling locations within the same watershed were more often clustered together than with any other sample sites (i.e. the East Branch of the Chagrin and Stebbins Gulch, and the two Cuyahoga River sites). Sample sites located farther east were clustered together and those sites that were farther west were clustered together, i.e., sample sites from the East Branch of the Chagrin River and the Grand Rivers paired together more often as did sites from the Rocky and Cuyahoga Rivers.

Research conducted by Kim et al., 2013 used cluster analysis to determine temporal and seasonal variation in the Nakdong and Suyong Rivers in South Korea. They defined seasonal variation as “winter” (low temperatures and drought) and “summer” (high temperatures and rainfall). Temporal conditions were based on pollution level in the streams. The Nakdong River was less polluted than Suyong River. The results of their cluster analysis indicated that in the less polluted river, the Nakdong, macroinvertebrate community structure clustered according to season, while in the polluted rivers of the Suyong River macroinvertebrate communities did not cluster according to season, but were influenced with metropolitan factors such as increase in sedimentation, bank erosion, road waste, and sewage, along with other point source pollution (Kim et al., 2013). Although most sites in my research were not directly affected by urbanization, they were affected by agriculture, and/or low-residential areas and Allan (2004) showed that agricultural areas may have similar effects on macroinvertebrate community assemblages.

4.3.2.3 Canonical Correspondence Analysis

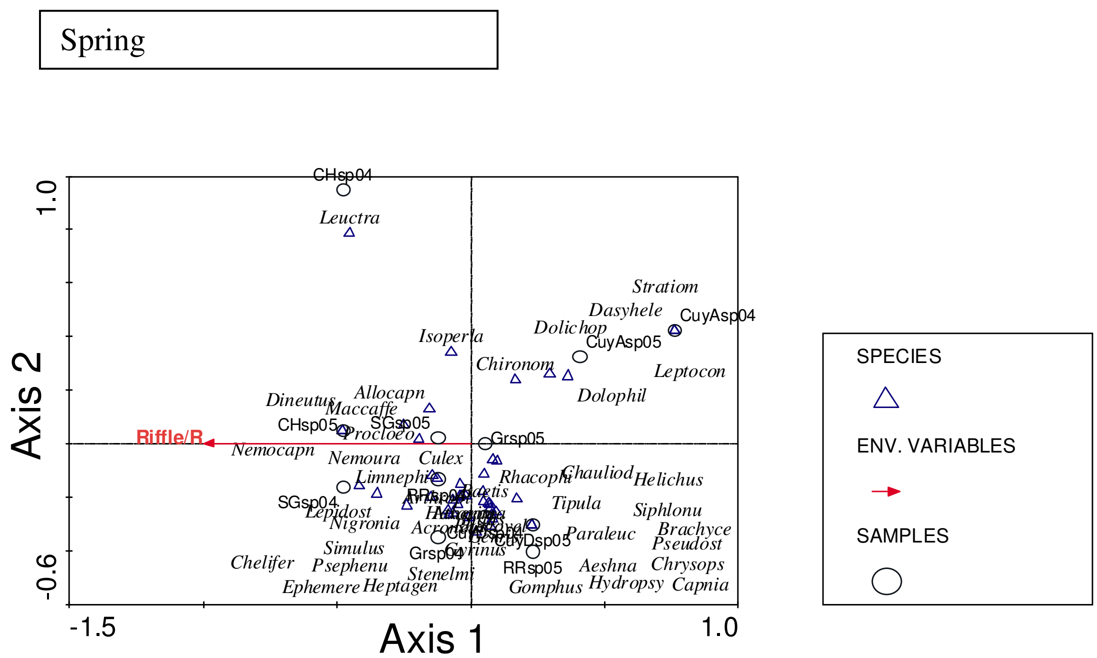
Results from CCA using the manual forward selection identified riffle/run habitat quality as a statistically significant variable for spring ($p = 0.036$; F-ration = 1.65) and accounted for 14.2% of the variance in the species data. Orthophosphate ($p = 0.008$; F-ratio = 3.41) and pH ($p = 0.044$; F-ratio = 2.20) were statistically significant for winter and together accounted for 40% of the variance in the data. Temperature was the dominant environmental factor in summer but was not significant ($p = 0.09$; F-ratio = 1.58), as was dissolved oxygen in the fall ($p = 0.128$; F-ration = 1.60). Riffle/run habitat quality influenced the macroinvertebrate community during the spring of 2004 and 2005. This metric quantifies stream habitat diversity and is directly proportional to the biodiversity of macroinvertebrate community (Voshell, 2002). Taxa and samples (sites and years) located near the center of the CCA triplot are neutral and variance in these data are not explained by the particularly significant environmental variables used, while the data points located near the vectors or opposite them are either positively or negatively influenced by that particular variable (see Figures IV.3 and IV.4 *Spring*). Both sampling sites of the East Branch of the Chagrin River and Cuyahoga site CU1 were strongly affected by percent riffle/run quality during the spring season, Cuyahoga CU1 was negatively correlated with the variable, while East Branch of the Chagrin was positively correlated with it. Same was true for both orthophosphate and pH during the winter analysis.

Macroinvertebrate data from Cuyahoga site CU1 was negatively correlated with riffle/run habitat quality in particularly in the spring of 2004. In August 2003, the 100-year storm event resulted in a replacement of cobble/gravel stream bed material with clay, and riffle/run habitat quality QHEI score was 0.0% in spring 2004. Three major genera, *Dasyhelea*, *Stratiomys,* and *Leptoconops*, were dominant at this site, and normally negatively correlated with riffle/run habitat quality. All three genera are midge taxa common to slower bodies of water with low dissolved oxygen, conditions associated with poorer riffle/run habitat. The East Branch of the Chagrin site was positively correlated to riffle/run habitat quality measured as 87.5%. The stream substrate had a good mix of riffle/run and pool habitats and macroinvertebrates associated with high oxygen levels were collected at this site (i.e. *Allocapnia* and *Nemocapnia* (Plecoptera), *Mccaffertium* (Ephemeroptera), and *Dineutus* (Coleoptera).

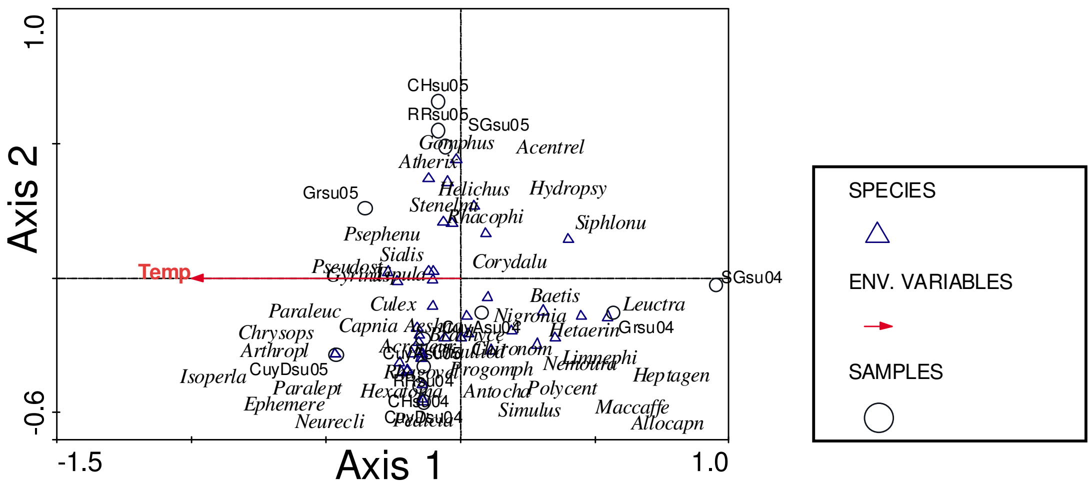
Although no sample was negatively correlated with orthophosphate, Rocky River was positively correlated for 2004 and 2005, and had the highest concentrations of PO_4 (0.175mg/L) and (0.08mg/L) respectively during the winter when compared to any other site or year. All sites within the study area were either currently or historically affected by agriculture (row-crops or pastoral) and low-residential land use. Water contaminants such as fertilizers, herbicides, pesticides, and/or sewage could increase the amount of orthophosphate, especially in the winter. Higher concentrations of orthophosphate are released during snow melt then during other times of the year. The Rocky River 2004 sample had the highest concentration of orthophosphate, 0.175mg/L. Additionally; a bridle path ran perpendicular to the Rocky River site and crossed the river approximately 4m from the collecting site. Horse feces were observed where the bridle path crossed the stream during most seasons when samples were collected. Two plecopteran genera *Isoperla* and *Haploperla*, and three dipteran genera of Diptera: *Helichus*, *Dasyhelea*, and *Hexatoma* were positively correlated with these higher levels of orthophosphate. The dipteran species are more tolerant of pollutants, but the two plecopteran taxa are not. Both stonefly taxa are known to be predaceous in their aquatic stages, and may be present due to food availability (Voshell 2002; McLeod 2006).

The other significant environmental variable, pH ranged from 7 to 8.5. Sites positively correlated with pH were the Grand River in 2004 and 2005, and Cuyahoga site A in 2004. Winter values were measured at 8.2, 8.3, and 8.3 respectively. Most macroinvertebrate genera respond better to pH levels that are slightly basic as opposed to acidic or neutral conditions. The East Branch of the Chagrin winter 2005, however, was negatively correlated with pH levels, with a value of 7. (Figure IV.3 and IV.4 *Winter*). Typically macroinvertebrates prefer basic pH (Voshell 2002). No statistically significant environmental variables were identified for summer and fall.

Figure IV.3 CANOCO Analysis: Canonical Correspondence Analysis of seasonal variation according to 1% or greater macroinvertebrate percent abundance according to season. This figure reflects macroinvertebrates community assemblage. The seasons are identified at the top of the figure and the legend represents the species, environmental variable, and sample. Sites were represented by the following abbreviations: Rocky River (RR), Cuyahoga River site A (CuyA), Cuyahoga site D (CuyD), East Branch of the Chagrin River (CH), Stebbins Gulch (SG), and Grand River (GR).



Summer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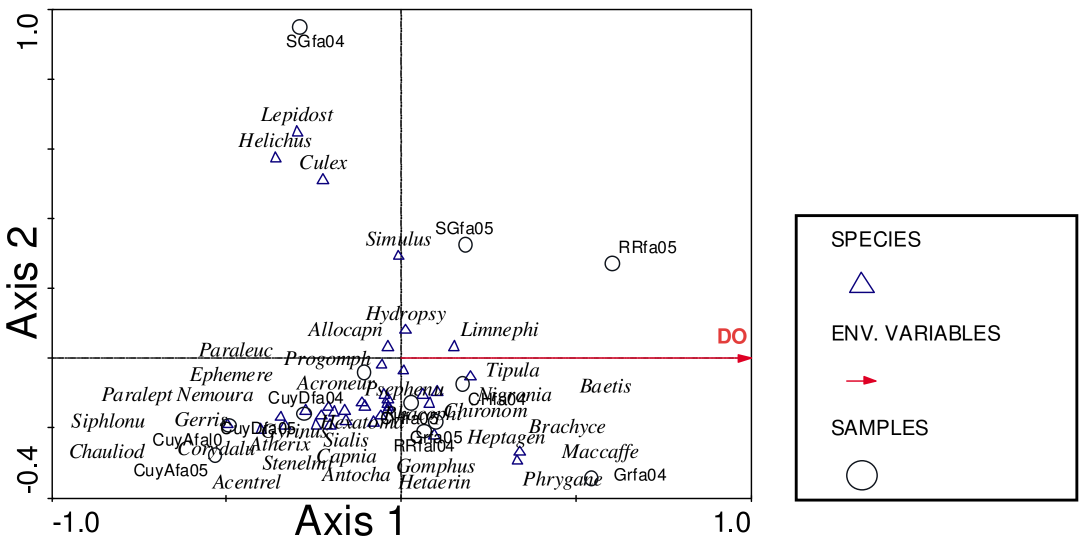
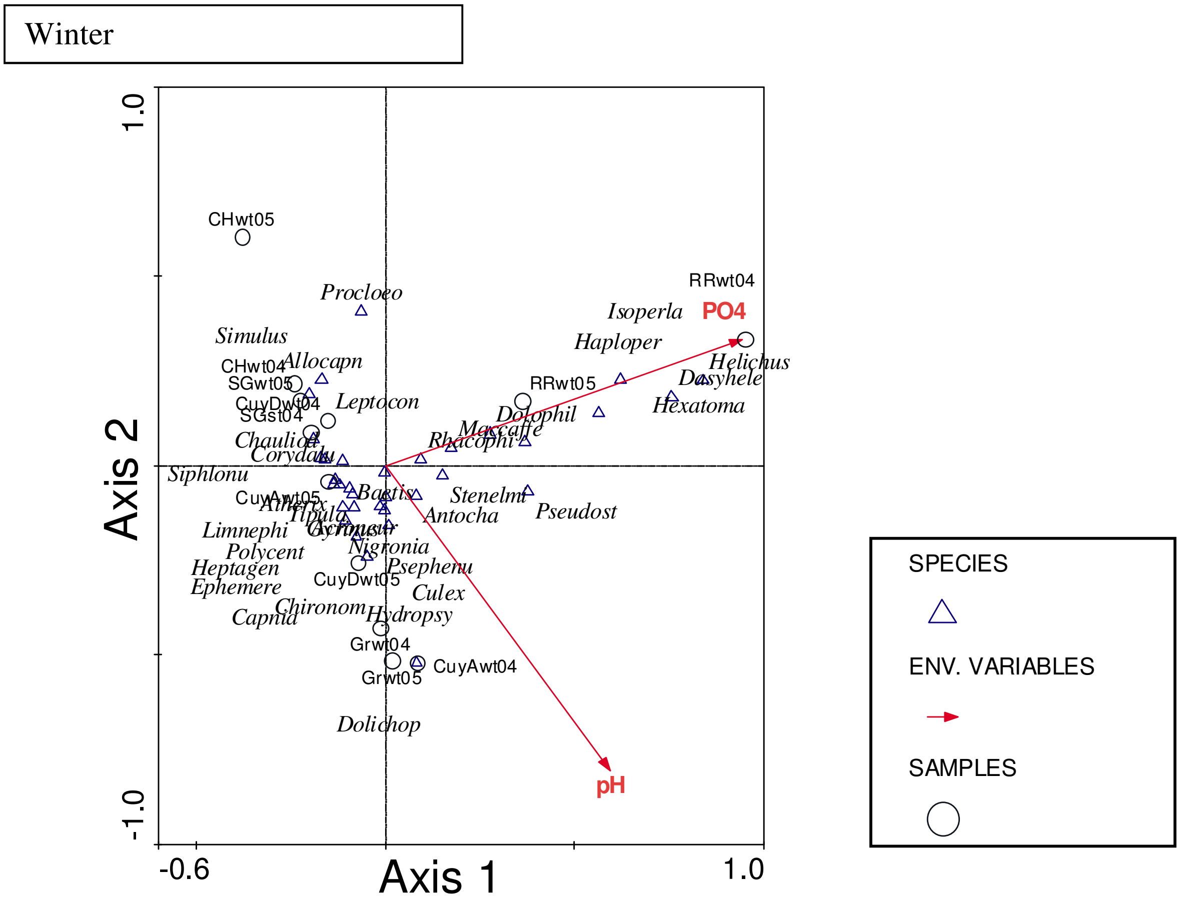
-1.5
1.0
Axis 1
Axis 2
Temp
CHsu05
RRsu05
SGsu05
Grsu05
SGsu04
Grsu04
CuyDsu05
CHsu04
Gomphus
Acentrel
Atherix
Helichus
Hydropsy
Stenelmi
Rhacophi
Siphlonu
Psephenu
Sialis
Corydalu
Pseudost
Culex
Baetis
Leuctra
Paraleuc
Nigronia
Hetaerin
Chrysops
Capnia
Arthropl
Chironom
Limnephi
Nemoura
Isoperla
Progomph
Heptagen
Paralept
Hexatoma
Antocha
Polycent
Ephemere
Simulus
Maccaffe
Neurecli
Allocapn
SPECIES
ENV. VARIABLES
SAMPLES

95

Fall



Axis 2
Axis 1
1.0
-0.4
-1.0
1.0
SGfa04
Lepidost
Helichus
Culex
Simulus
SGfa05
RRfa05
Hydropsy
Allocapn
Limnephi
DO
Paraleuc
Progomph
Ephemere
Tipula
Acroneur
Psephenu
Baetis
Paralept
Nemoura
CuyDfa04
Nigronia
CHfa04
Chironom
Siphlonu
Gerris
Brachyce
Sialis
Heptagen
Chauliod
Atherix
RRfal04
Maccaffe
Capnia
Stenelmi
Gomphus
CuyAfa05
Antocha
Phrygane
Grfa04
Acentrel
Hetaerin
SPECIES
ENV. VARIABLES
SAMPLES


Winter
Axis 2
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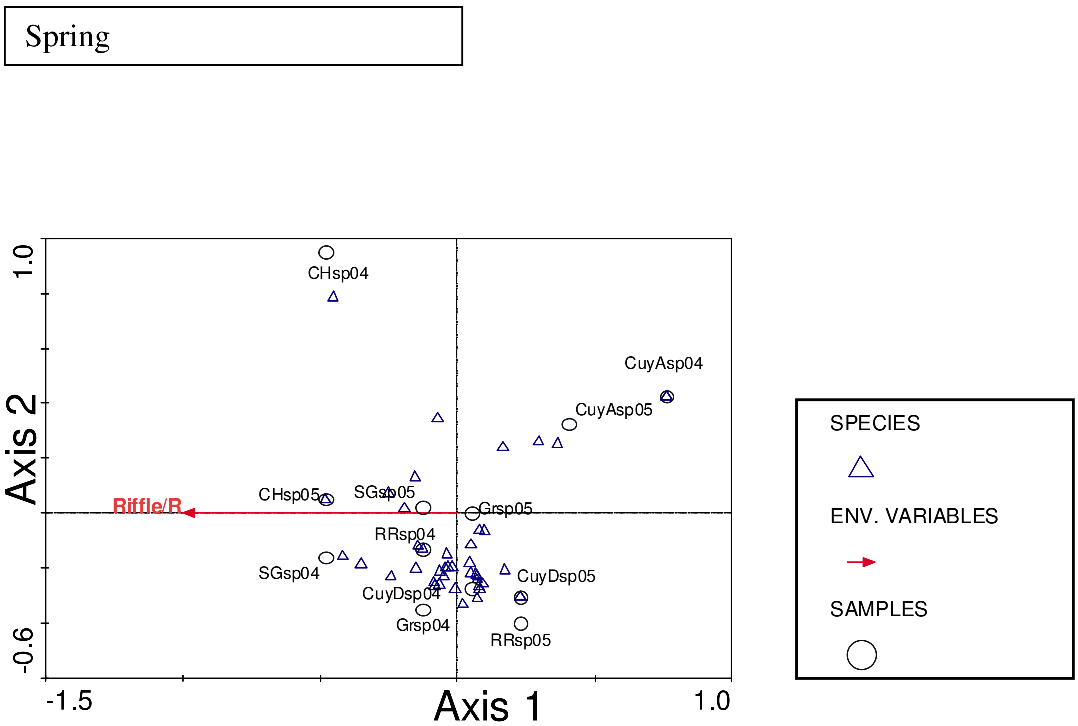
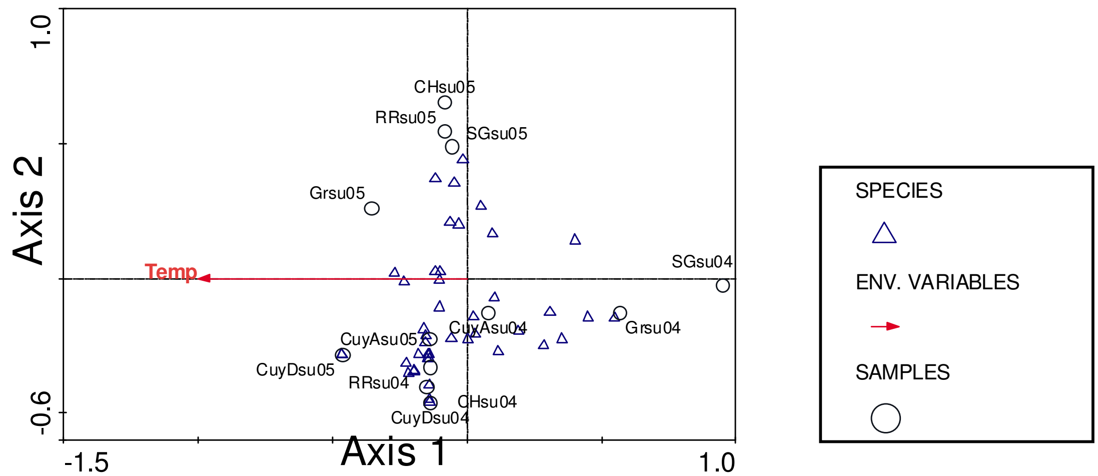
-1.0
Axis 1
-0.6
1.0
CHwt05
Procloeo
RRwt04
Isoperla
PO4
Simulus
Haploper
Helichus
CHwt04
Allocapn
SGwt05
RRwt05
Dasyhele
CuyDwt04
Leptocon
Hexatoma
SGst04
Dolophil
Mescaffe
Chauliod
Rhacophi
Corydalu
Siphlonu
CuyAwt05
Baetis
Stenelmi
Atherix
Antocha
Pseudost
Tipula
Limnephi
Nigronia
Polycent
Psephenu
Heptagen
CuyDwt05
Ephemere
Culex
Chironom
Hydropsy
Capnia
Grwt04
CuyAwt04
Grwt05
Dolichop
pH
SPECIES
ENV. VARIABLES
SAMPLES

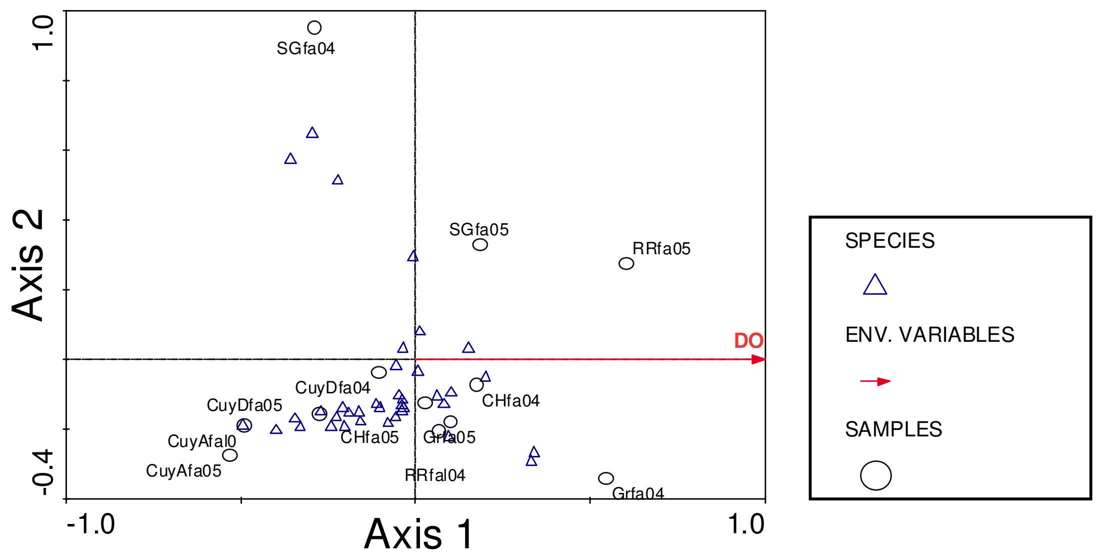
Figure IV.4 CANOCO Analysis: Canonical Correspondence Analysis of seasonal variation according to 1% or greater macroinvertebrate percent abundance according to season Figure IV.4 is similar to figure IV.3, however, macroinvertebrate taxa have been removed and replaced by triangles, so that better observation of how physical factors affect macroinvertebrate community distribution.



Summer


CHsu05
RRsu05
SGsu05
Grsu05
Temp
SGsu04
CuyAsu05
CuyAsu04
Grsu04
CuyDsu05
RRsu04
CHsu04
CuyDsu04
Axis 2
Axis 1
1.0
-0.6
-1.5
1.0
SPECIES
ENV. VARIABLES
SAMPLES

Fall


SGfa04
SGfa05
RRfa05
DO
CuyDfa04
CuyDfa05
CHfa04
CuyAfal0
CHfa05
Grfa05
CuyAfa05
RRfal04
Grfa04
1.0
-0.4
Axis 2
-1.0
1.0
Axis 1
SPECIES
ENV. VARIABLES
SAMPLES

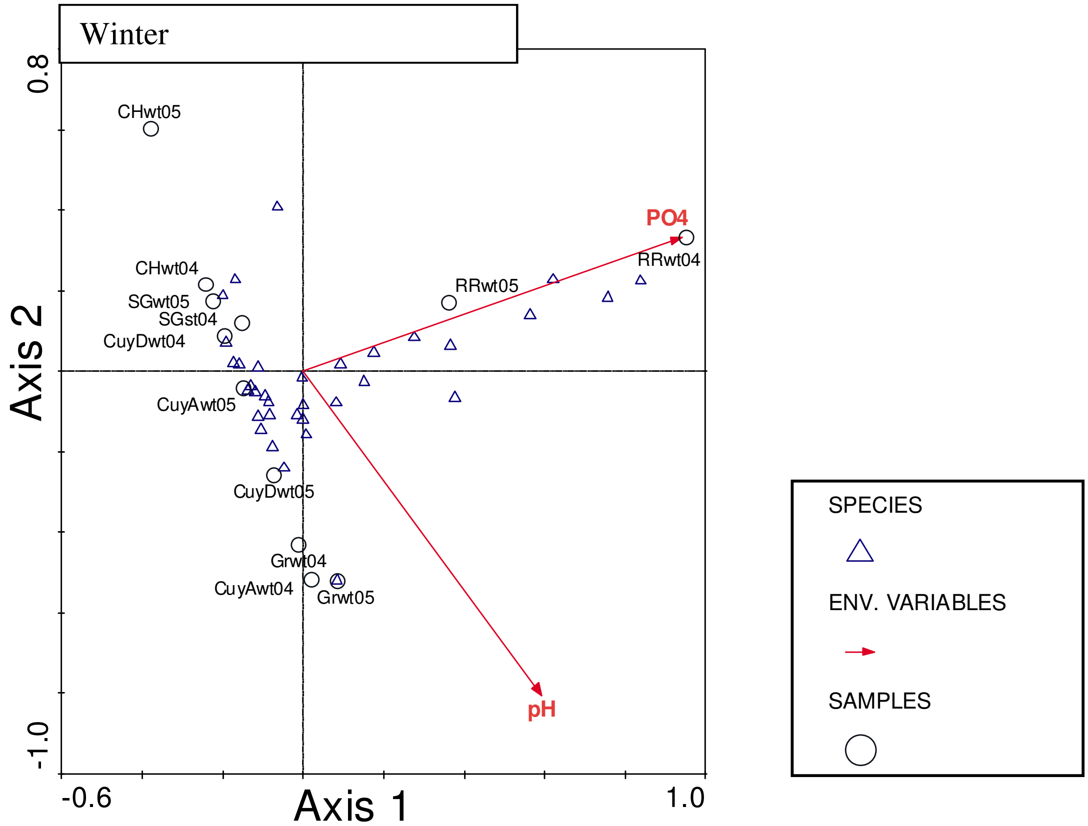

Winter
0.8
-1.0
Axis 2
-0.6
1.0
Axis 1
CHwt05
CHwt04
SGwt05
SGst04
CuyDwt04
CuyAwt05
CuyDwt05
Grwt04
CuyAwt04
Grwt05
RRwt05
RRwt04
PO4
pH
SPECIES
ENV. VARIABLES
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Table XIV Environmental data used for CCA analysis per season.

Spring

Sample	PO4	NH4	NO3	DO	pH	Temp	% Canopy	Substrate	InstreamC	RipZone/E	Riffle/Run
RRsp04	0.0258	0.436	0.5864	9	8	7.5	75	65	30	80	62.5
RRsp05	0	0	0	0	8.05	6.75	74	90	60	80	37.5
CuyAsp04	0.390184	0.555267	1.3256	5.1	7.95	4.85	85.0	55.0	20.0	75.0	0.0
CuyAsp05	0	0	0	7.45	8.3	6	84.0	85.0	30.0	75.0	25.0
CuyDsp04	0.73855	1.002633	2.512567	6.45	8.2	5	90.0	80.0	30.0	85.0	50.0
CuyDsp05	0.001235	0	0	8.05	8	7	87.0	50.0	55.0	95.0	37.5
CHsp04	0.273017	0.621133	0.954867	9.7	7.5	5	40.0	95.0	55.0	80.0	87.5
CHsp05	0	0	0	10.2	7.15	8	41.0	80.0	35.0	65.0	87.5
SGsp04	0.250383	0.533133	0.726	10.2	7.2	4.8	91.0	100.0	60.0	100.0	87.5
SGsp05	0	0	0	11.5	7	7.9	93.0	95.0	35.0	100.0	62.5
Grsp04	0	0	0	9.3	7.55	5.2	63	85	60	60	62.5
Grsp05	0	0	0	0	7.25	7	65	100	45	60	50

Summer

Sample	PO4	NH4	NO3	DO	pH	Temp	% Canopy	Substrate	InstreamC	RipZone/E	Riffle/Run
RRsu04	0	0.1271	0	8.9	7.5	16	75	70	45	80	37.5
RRsu05	0.00175	0.11025	0	8.05	7.5	15.8	80	90	40	80	25
CuyAsu04	0.0024	0.122583	0	3.845	8.3	15.2	90.0	36.0	45.0	75.0	0.0
CuyAsu05	0.02608	0.08205	0	4.87	8.3	16.01	97.0	40.0	40.0	70.0	0.0
CuyDsu04	0	0.052167	0	7.665	8.25	16	93.0	50.0	65.0	85.0	37.5
CuyDsu05	0.0174	0.10635	0	8.01	8.4	17.19	99.0	60.0	55.0	95.0	28.0
CHsu04	0	0	0	10.72	7.5	16.05	40.0	90.0	80.0	75.0	94.0
CHsu05	0	0	0	11.014	7.6	15.8	45.0	50.0	40.0	60.0	62.5
SGsu04	0.017717	0	0	10.905	7.3	12	96.0	100.0	70.0	100.0	75.0
SGsu05	0.0425	0	0	11.09	7.4	15.7	96.0	93.0	70.0	90.0	75.0
Grsu04	0.092684	0	0	12.9	7.6	13.4	65	60	60	60	75
Grsu05	0	0	0	10.5	8.1	16.8	68	95	55	65	37.5

Fall

Sample	PO4	NH4	NO3	DO	pH	Temp	% Canopy	Substrate	InstreamC	RipZone/E	Riffle/Run
RRfal04	0	0	0	10.21	7.3	13.8	40	45	40	80	62.5
RRfa05	0	0	0	15.89	7.3	11.5	60	40	60	80	62.5
CuyAfal04	0	0	0	4.305	8.25	13	60.0	35.0	55.0	50.0	0.0
CuyAfa05	0	0	0	3.89	7.84	15.23	50.0	34.0	35.0	46.0	35.0
CuyDfa04	0	0	0	8.395	7.7	14	70.0	65.0	35.0	95.0	37.5
CuyDfa05	0	0	0	6.59	8.5	16.05	65.0	80.0	60.0	100.0	50.0
CHfa04	0	0	0	11.355	7.55	14.9	25.0	85.0	55.0	65.0	50.0
CHfa05	0	0	0	9.8	8.14	12.8	20.0	90.0	35.0	60.0	100.0
SGfa04	0	0	0	6.45	7.2	14.5	45.0	100.0	65.0	100.0	75.0
SGfa05	0	0	0	11.45	8.2	12.9	70.0	95.0	65.0	100.0	87.5
Grfa04	0	0	0	15.25	8.15	14.15	35	95	65	60	62.5
Grfa05	0	0	0	10.56	7.5	14.8	50	65	45	20	25

Winter

Sample	PO4	NH4	NO3	DO	pH	Temp	% Canopy	Substrate	InstreamC	RipZone/E	Riffle/Run
RRwt04	0.174635	0	0	10.5	8.15	6.2	15	95	40	80	50
RRwt05	0.083	0	0	10.5	7.9	6.9	15	80	60	80	25
CuyAwt04	0.009085	0	0	11.75	8.35	6.35	10.0	55.0	50.0	75.0	0.0
CuyAwt05	0	0.603334	1.2587	10.4	7.75	8.5	10.0	80.0	55.0	75.0	25.0
CuyDwt04	0.0077	0	0	12.4	7.6	6.05	15.0	85.0	30.0	95.0	37.5
CuyDwt05	0	0.6098	1.301	10.4	8	8	10.0	60.0	45.0	95.0	37.5
CHwt04	0	0	0	12.7	7.45	6.1	0.0	55.0	65.0	65.0	75.0
CHwt05	0	0	0	12.15	7	5.9	30.0	100.0	35.0	100.0	62.5
SGwt04	0	0	0	13.5	7.6	8	4.0	95.0	40.0	65.0	75.0
SGwt05	0	1.5874	0.01287	13.8	7.5	8.1	28.0	100.0	35.0	100.0	75.0
Grwt04	0	0	0	11.65	8.2	7.2	25	95	50	60	50
Grwt05	0	0	0	10.2	8.3	6.1	28	100	30	60	62.5

4.3.2.4 ANOVA and Functional Feeding Groups

One-way ANOVA was used to compare functional feeding groups (FFG) in the macroinvertebrate community between sites. Two FFG, collector-gatherers (p=0.032) and scrapers (p=0.050), (Table XV; figures IV.5 and IV.6) were significantly different between the sites following a one-way ANOVA, Tukey's honestly significant difference (HSD), and Bonferroni post hoc tests (See Table XVI). The analysis revealed that there was a statistically significantly difference between collector-gathers in the Rocky River and Cuyahoga CU2, as well as Cuyahoga CU2 and the Grand River. Scrapers were significantly different between Rocky River and Stebbins Gulch.

Collector-gatherers feed on fine particulate organic matter (FPOM) that passes by in flowing water or is found within bottom sediments. FPOM is organic material of 0.5µm – 1mm in size. It is mostly composed of feces, algae, plant and animal fragments, and contains different types of bacteria. While collector-gatherers are dominantly omnivores, scrapers are mainly herbivores. They remove algae, bacteria and fungus growing on the surface of rocks, twigs and leaf debris, with specialized mouthparts that scrape the surface of rocks and other sediment. Many of these organisms are flattened to better attach to rocks while they feed in strong currents typical to headwater and low order streams.

Stream order has a major influence on the distribution of aquatic macroinvertebrates. According to the River Continuum Concept (Vannote, 1980), stream order will influence FFG densities collected at each site. In theory, low and very high order streams have more consumers than primary producers, while middle order streams have a larger percentage of producers. These characteristics will in turn affect the type of FFGs present. All streams within this study are categorized as low to middle order streams.

Summer samples from the Rocky River and spring samples from the Grand River, stand out for the large number of collector-gatherers identified at each site. In the Rocky River, a total of 479 collector-gathers were collected, 338 in the genus *Hydropsyche.* Within the Rocky River, summer had the highest total number of collector gathers, with 255 specimens. In the Grand River, a total of 488 collector-gatherers were identified. The highest number of specimens (n = 199) were collected in spring, most of which were collected in spring 2005 (n = 172). Similar to the Rocky River, the Grand River had a large number of *Hydropsyche* (n= 246) collected in both years. Cuyahoga site CU2 had the lowest numbers of collector-gatherers throughout the two year collection period. While total numbers were lower, Cuyahoga site CU2 had a higher diversity of collector-gatherers. In addition to *Hydropsyche*, *Procloeon*, and Chironomidae, other collector-gatherers identified included *Capnidae* (order Plecoptera), and *Culex* (order Diptera). This fits the River Continuum Concept (RCC) which states that lower order streams may have lower numbers of individual taxa, but higher taxa diversity. As a headwater stream, the Cuyahoga River site CU2 is smaller and aquatic insects need to adapt to the harsh conditions of colder temperatures, narrower channel widths, and swift currents. Headwaters may freeze over during the winter and even dry up in the summer. These conditions result in fewer individuals per taxa. Furthermore, in headwaters like Cuyahoga CU2, FPOM is limited and provides fewer resources for large populations of collector-gatherers, while mid-order streams like the Grand and Rocky Rivers have large quantities of FPOM available and can support larger populations like *Hydropsyche.*

Surrounding land use is another factor determining available food resources and thus the type of functional feeding groups found in these macroinvertebrate communities. As previously discussed in chapter 3, all collecting locations are currently within protected lands (i.e. Holden Arboretum, Cuyahoga Valley National Park, and Cleveland Metroparks). However, as noted by Allan (2004) and the discussion in Chapter 3, not all agricultural landscapes are identical. Historically, the Rocky River, Cuyahoga River and the Grand River were predominately adjacent to row crop agriculture, while the Chagrin River was historically surrounded by pastoral agriculture. Row crops tend to have more negative effects on stream ecosystems than pastoral agriculture, but the type, amount, and frequency of sediment load, nutrient input, riparian structure and size, and land use modifications will influence the stream and be reflected by the macroinvertebrate community. In streams impacted by agriculture, there would be a shift in functional feeding groups. Filter –feeders and grazers increase in numbers in agricultural land cover due to increase in nutrient input and loss of canopy cover. However, the loss of canopy cover and other riparian vegetation leads to a decrease in shredders and collector-gathers within the macroinvertebrate community.

The second functional feeding group of significance was scrapers. Scrapers, like collector-gatherers also respond to change in stream orders and the environmental shifts associated with it. Since scrapers feed on algae, bacteria, and fungi that grow on bottom substrates, their presence is related to available stream depth, current velocity, and they prosper best in mid-order streams. In low order streams, the narrow channel width, fast current velocity, and low light penetration, provides few resources, while in higher order streams, stream velocity and canopy cover decreases, but less light makes it to the stream bed. Mid-order streams have optimal conditions for scrapers, with ideal stream velocity, depth, and canopy cover and abundant food resources. Stebbins Gulch, a first order stream with dense canopy cover, narrow channel width, and low light penetration, provides few resources to scrapers. However Rocky River a third order stream was more open and lower percent canopy cover that allowed more light, and thus more food resources available for scrapers.

Most scrapers were collected in the Rocky River during the summer while the greatest numbers in Stebbins Gulch were found in spring when canopy cover is low. The dominant scraper collected in the Rocky River was *Stenelmis* (order Coleoptera) (n= 151), followed by three genera in the order Ephemeroptera: *Baetis* (n= 16), *Ephemerella* (n =4) and *Paraleptophlebia* (n=2). The total number of specimens collected for the remaining three seasons during the two year sample collection period were much lower – 79 scrapers in spring, 48 in fall, and 32 scrapers in winter. There were 90 scrapers collected in Stebbins Gulch during the spring season with the dominant scrapers being *Psephenus* (order Coleoptera; n = 38), *Baetis* (n=29), and *Stenelmis* (n=17).

Table XV Total Number taxa analyzed for Functional Feeding Groups analyzed in the ANOVA analysis.

Rocky

Collector-Gathers

RR_SP04	RR_SP05	RR_SU04	RR_SU05	RR_FAL04	RR_FAL05	RR_WT04	RR_WT05
0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0
1	0	1	0	6	0	0	0
0	0	0	0	0	0	2	1
0	28	0	0	0	0	2	0
0	0	2	0	0	0	2	0
0	59	21	210	20	17	2	9
12	7	20	0	16	21	10	5
0	1	0	1	1	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0
13	95	44	211	43	38	20	15

Cuyahoga CU1

Collector-gathers

CU1_SP04	CU1_SP05	CU1_SU04	CU1_SU05	CU1_FAL0	CU1_FAL0	CU1_WT04	CU1_WT05
0	0	2	1	1	2	2	0
0	0	0	0	0	0	0	1
0	0	1	1	4	0	0	0
0	0	5	4	1	7	4	3
0	0	0	0	0	0	0	1
0	0	6	0	3	0	1	0
0	0	0	0	0	0	1	1
1	4	9	20	25	24	23	30
10	31	20	24	20	44	113	22
0	0	2	1	1	1	14	6
1	0	0	0	0	0	0	0
0	0	0	0	0	0	2	0
2	1	5	4	6	6	5	7
0	0	0	0	0	0	0	0
14	36	50	55	61	84	165	71

Collector-filters

RR_SP04	RR_SP05	RR_SU04	RR_SU05	RR_FAL04	RR_FAL05	RR_WT04	RR_WT05
0	0	0	0	0	0	1	0
0	0	1	0	1	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	1	0	0	0
0	3	14	0	10	14	0	4
0	3	16	0	12	14	1	4

Collector-Filters

CU1_SP04	CU1_SP05	CU1_SU04	CU1_SU05	CU1_FAL0	CU1_FAL0	CU1_WT04	CU1_WT05
0	3	0	0	0	0	0	0
0	0	0	1	0	0	2	1
0	0	0	0	1	0	1	0
1	0	1	0	0	0	0	0
0	1	3	13	14	11	4	15
1	4	4	14	15	11	7	16

Scrapers

RR_SP04	RR_SP05	RR_SU04	RR_SU05	RR_FAL04	RR_FAL05	RR_WT04	RR_WT05
2	0	0	0	0	0	0	0
2	2	12	4	4	2	1	7
0	0	0	0	2	0	0	0
8	0	4	0	6	0	0	0
1	0	0	0	0	0	0	0
0	1	2	0	0	0	0	0
7	0	0	0	8	0	2	1
3	1	0	2	12	4	0	5
16	36	6	145	10	0	16	0
0	0	0	0	0	0	1	0
39	40	24	151	42	6	20	13

Scrapers

CU1_SP04	CU1_SP05	CU1_SU04	CU1_SU05	CU1_FAL0	CU1_FAL0	CU1_WT04	CU1_WT05
0	0	0	0	0	0	1	0
2	0	9	6	8	11	11	12
0	0	7	0	0	3	0	0
0	0	2	10	11	2	5	5
0	0	0	1	1	0	1	6
0	0	0	0	0	2	0	0
0	0	0	0	0	0	7	1
0	0	14	17	10	15	10	16
0	0	15	20	16	20	15	25
0	0	0	0	0	0	0	0
2	0	47	54	46	53	50	65

Shredders

RR_SP04	RR_SP05	RR_SU04	RR_SU05	RR_FAL04	RR_FAL05	RR_WT04	RR_WT05
4	0	0	0	1	0	0	2
1	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0
0	0	0	0	0	0	0	0
2	0	0	0	2	3	0	0
0	0	0	0	0	0	0	0
0	0	0	0	5	0	0	1
0	0	0	0	0	0	0	0
4	3	9	14	3	0	0	1
11	3	11	14	12	3	0	4

Shredders

CU1_SP04	CU1_SP05	CU1_SU04	CU1_SU05	CU1_FAL0	CU1_FAL0	CU1_WT04	CU1_WT05
0	2	0	3	2	2	2	1
0	0	0	1	1	1	0	1
0	1	0	0	3	0	2	0
0	0	0	0	0	0	2	0
0	0	0	1	2	0	1	1
0	0	0	1	3	1	1	1
0	0	0	0	1	0	1	5
0	0	0	0	0	0	0	0
0	0	1	4	2	2	1	0
0	0	0	0	0	0	0	0
0	4	5	4	5	5	6	6
0	7	6	14	19	11	16	15

Predators

RR_SP04	RR_SP05	RR_SU04	RR_SU05	RR_FAL04	RR_FAL05	RR_WT04	RR_WT05
0	2	0	0	0	0	0	0
0	0	0	0	3	0	0	0
0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0
12	1	6	1	4	1	0	1
4	0	0	0	1	0	19	1
1	0	2	2	2	0	0	0
0	0	1	2	1	0	0	0
0	0	1	4	1	1	0	1
2	0	1	1	4	0	0	0
0	1	4	0	1	0	0	0
0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0
0	2	0	0	1	0	2	0
0	0	0	0	4	0	0	0
0	1	0	0	0	0	0	0
0	0	0	0	0	0	2	0
0	0	0	0	0	0	0	0
5	0	0	0	2	0	12	0
0	0	0	0	0	0	1	0
24	7	15	12	25	2	36	3

Predators

CU1_SP04	CU1_SP05	CU1_SU04	CU1_SU05	CU1_FAL0	CU1_FAL0	CU1_WT04	CU1_WT05
0	0	2	1	0	1	1	0
0	0	0	0	1	1	0	1
0	0	0	2	3	0	1	0
0	0	2	0	2	0	0	0
0	0	6	8	9	14	6	10
0	6	0	0	0	0	0	1
0	0	2	0	2	0	1	5
0	0	3	0	5	1	1	0
0	0	0	3	0	0	0	0
0	0	1	1	1	3	1	0
0	0	1	1	1	0	0	1
0	0	1	0	1	1	1	0
0	0	0	0	5	2	2	1
0	0	0	0	0	0	0	0
0	0	0	1	0	0	2	0
0	0	0	0	1	1	0	0
0	0	2	3	4	4	3	4
0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
0	10	0	0	0	0	4	0
0	1	0	3	5	1	1	2
0	0	0	0	0	0	0	0
6	17	20	23	40	29	24	25

Cuyahoga CU2

Collector-gathers

CU2_SP04	CU2_SP05	CU2_SU04	CU2_SU05	CU2_FAL0	CU2_FAL0	CU2_WT04	CU2_WT05
0	0	0	0	0	1	1	0
0	1	0	1	0	0	0	0
0	3	0	0	0	0	0	0
4	1	1	5	2	2	2	2
0	0	0	1	1	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1
17	11	14	11	18	22	21	19
14	13	15	11	15	20	19	11
0	2	0	2	2	1	2	1
0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0
4	0	4	2	8	1	4	4
0	0	0	0	1	0	0	0
39	31	34	33	47	48	49	38

EB Chagrin

Collector-gather

CH_SP04	CH_SPO5	CH_SU04	CH_SU05	CH_FAL04	CH_FAL05	CH_WT04	CH_WT05
0	0	0	1	0	1	0	0
0	9	0	0	1	0	1	2
0	0	1	1	0	0	3	0
0	0	1	0	0	1	1	0
0	0	1	0	0	0	0	0
0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	22	20	12	18	24	4	0
5	19	21	0	10	14	26	15
0	3	2	0	1	1	2	0
0	0	0	0	0	0	0	0
0	0	0	0	0	5	0	0
0	0	5	1	3	0	6	0
1	0	0	0	0	0	0	0
6	54	51	15	33	46	43	17

Collector-Filters

CU2_SP04	CU2_SP05	CU2_SU04	CU2_SU05	CU2_FAL0	CU2_FAL0	CU2_WT04	CU2_WT05
0	0	0	0	0	0	0	0
0	0	0	0	0	0	3	0
0	1	0	2	0	0	0	0
0	0	0	0	0	0	0	0
10	11	17	18	15	12	15	15
10	12	17	20	15	12	18	15

Collector-Filter

CH_SP04	CH_SPO5	CH_SU04	CH_SU05	CH_FAL04	CH_FAL05	CH_WT04	CH_WT05
0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	2	0
0	0	20	0	11	10	19	29
0	0	20	0	11	11	21	29

Scrapers

CU2_SP04	CU2_SP05	CU2_SU04	CU2_SU05	CU2_FAL0	CU2_FAL0	CU2_WT04	CU2_WT05
0	0	0	2	0	1	0	1
11	12	11	11	10	13	16	14
0	3	0	1	0	1	0	0
3	2	1	5	3	5	9	1
7	0	1	1	0	0	1	5
0	0	0	1	1	0	0	0
0	0	5	0	1	0	1	0
11	14	11	15	11	10	11	14
9	12	4	20	10	15	20	20
0	0	1	1	2	0	0	6
41	43	34	57	38	45	58	61

Scraper

CH_SP04	CH_SPO5	CH_SU04	CH_SU05	CH_FAL04	CH_FAL05	CH_WT04	CH_WT05
0	0	0	0	0	0	0	0
0	0	15	0	14	2	18	1
0	0	0	0	4	2	0	0
0	0	4	0	0	3	8	0
0	0	7	0	0	5	0	0
0	0	0	0	0	0	0	0
1	7	1	0	1	1	1	0
0	1	12	0	10	15	5	0
0	11	5	3	11	21	11	0
0	0	0	0	0	0	0	0
1	19	44	3	40	49	43	1

Shredders

CU2_SP04	CU2_SP05	CU2_SU04	CU2_SU05	CU2_FAL0	CU2_FAL0	CU2_WT04	CU2_WT05
1	7	1	2	1	1	1	1
0	1	0	1	0	1	0	1
0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	1
0	1	0	1	0	1	1	2
3	1	0	0	4	2	5	1
0	0	0	0	0	1	1	0
0	1	2	1	0	0	0	4
0	0	5	0	0	0	1	0
2	5	3	6	2	4	7	7
6	18	12	11	7	10	16	17

Shredder

CH_SP04	CH_SPO5	CH_SU04	CH_SU05	CH_FAL04	CH_FAL05	CH_WT04	CH_WT05
3	4	7	0	1	2	3	7
6	0	0	0	0	0	0	0
0	0	1	0	1	0	7	0
0	1	0	0	0	1	0	0
0	0	0	0	1	0	1	0
0	0	0	0	1	0	1	0
0	0	2	0	1	1	0	0
0	0	0	0	0	0	0	0
0	0	1	0	2	0	1	0
0	0	0	0	1	0	0	0
0	0	5	2	4	1	4	0
9	5	16	2	12	5	17	7

Predators

CU2_SP04	CU2_SP05	CU2_SU04	CU2_SU05	CU2_FAL0	CU2_FAL0	CU2_WT04	CU2_WT05
2	1	2	1	0	1	0	0
1	2	0	0	0	1	1	1
0	1	1	0	1	0	0	0
0	0	1	1	0	1	0	0
1	14	10	15	7	10	6	8
3	0	0	2	0	0	1	1
0	2	1	1	0	1	1	0
0	0	1	0	0	0	3	0
0	0	0	1	2	1	1	2
0	3	1	0	1	2	0	1
2	0	0	1	0	0	0	1
4	1	1	1	1	0	1	1
0	2	0	3	0	1	0	0
0	0	0	0	0	1	0	0
0	0	2	0	0	0	0	1
0	1	1	0	2	1	1	1
2	2	1	4	1	1	2	5
3	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0
0	2	0	0	0	0	0	0
3	3	1	4	3	4	3	3
0	0	0	0	0	0	1	1
21	34	23	34	18	25	21	27

Predator

CH_SP04	CH_SPO5	CH_SU04	CH_SU05	CH_FAL04	CH_FAL05	CH_WT04	CH_WT05
0	0	2	0	0	1	1	0
0	0	1	1	2	0	0	0
0	0	2	0	4	1	1	0
0	0	0	0	0	0	0	0
0	0	6	1	7	4	4	3
2	1	0	0	0	0	0	0
0	0	2	0	2	4	2	0
0	0	3	0	0	0	0	0
0	0	0	0	1	1	0	1
0	0	1	0	1	0	1	0
0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	0
0	0	2	0	2	4	2	0
0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0
0	1	3	5	0	1	2	1
0	2	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0
0	0	11	0	4	5	1	0
3	0	0	0	0	0	0	0
5	5	34	7	24	22	16	5

Stebbins Gulch

Collector-gather

SG_SP04	SG_SP05	SG_SU04	SG_SU05	SG_FAL04	SG_FAL05	SG_WT04	SG_WT05
0	0	0	0	0	0	0	0
0	33	0	0	0	0	2	0
0	0	31	3	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1
2	23	1	5	2	0	0	0
0	0	0	0	0	0	1	0
31	38	2	13	15	11	22	20
0	24	20	1	0	8	21	6
1	0	0	0	5	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0
2	0	0	0	0	0	0	0
36	118	54	23	22	19	46	27

Collector-Filter

SG_SP04	SG_SP05	SG_SU04	SG_SU05	SG_FAL04	SG_FAL05	SG_WT04	SG_WT05
0	4	0	0	0	0	5	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0
2	8	0	0	26	23	196	35
2	12	0	0	26	23	202	35

Scraper

SG_SP04	SG_SP05	SG_SU04	SG_SU05	SG_FAL04	SG_FAL05	SG_WT04	SG_WT05
0	0	0	0	0	0	0	0
19	10	3	0	0	5	0	5
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
2	0	11	0	0	0	0	0
0	0	0	0	0	0	0	0
0	4	0	0	0	0	7	2
6	32	9	8	2	7	1	9
6	11	6	12	0	0	1	0
0	0	0	0	0	0	0	0
33	57	29	20	2	12	9	16

Shredder

SG_SP04	SG_SP05	SG_SU04	SG_SU05	SG_FAL04	SG_FAL05	SG_WT04	SG_WT05
11	105	0	0	0	3	18	10
5	0	0	0	0	0	0	0
0	0	0	0	0	0	26	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
5	0	0	0	6	0	0	0
4	0	1	0	0	1	0	0
0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
4	1	1	0	1	0	2	8
29	106	2	1	7	4	46	18

Predator

SG_SP04	SG_SP05	SG_SU04	SG_SU05	SG_FAL04	SG_FAL05	SG_WT04	SG_WT05
0	3	0	1	0	0	0	0
0	0	0	1	0	0	0	0
0	0	1	0	0	0	0	0
0	0	2	0	0	0	0	0
9	1	0	0	1	9	0	1
0	13	0	0	0	0	14	2
0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0
1	1	1	0	0	1	0	0
0	0	0	1	0	0	1	0
0	1	0	0	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	4	1	0	1	18	0
0	0	0	1	0	0	0	0
40	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0
0	3	0	0	0	0	0	0
4	0	0	0	0	0	3	0
54	24	9	7	1	11	36	3

Grand River

Collector-gather

GR_SP04	GR_SP05	GR_SU04	GR_SU05	GR_FAL04	GR_FAL05	GR_WT04	GR_WT05
0	0	0	0	0	0	1	0
0	25	0	0	0	0	0	0
0	0	0	19	0	0	1	0
2	0	0	0	0	0	2	0
0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0
1	1	0	3	0	0	0	1
20	90	50	35	5	6	26	14
2	47	44	3	15	17	14	18
2	9	0	0	0	0	2	0
0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0
3	1	0	1	0	0	6	0
0	0	0	0	0	0	0	0
30	173	94	61	20	23	53	34

Collector-filter

GR_SP04	GR_SP05	GR_SU04	GR_SU05	GR_FAL04	GR_FAL05	GR_WT04	GR_WT05
0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0
0	0	0	0	0	0	1	0
4	6	0	0	0	5	13	1
4	7	0	0	0	5	15	1

Scraper

GR_SP04	GR_SP05	GR_SU04	GR_SU05	GR_FAL04	GR_FAL05	GR_WT04	GR_WT05
0	0	0	0	0	0	0	0
6	3	15	5	8	10	12	3
0	0	0	0	0	0	1	0
3	0	0	0	0	0	5	0
2	0	4	0	0	0	0	0
0	0	0	0	0	0	0	0
5	12	0	0	10	4	0	0
8	5	1	38	3	2	14	5
11	2	0	0	0	0	20	0
0	0	0	0	0	0	0	0
35	22	20	43	21	16	52	8

Shredder

GR_SP04	GR_SP05	GR_SU04	GR_SU05	GR_FAL04	GR_FAL05	GR_WT04	GR_WT05
1	54	0	0	0	0	1	1
0	0	11	0	0	0	1	0
4	0	3	0	0	0	0	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	0
0	0	0	0	1	0	0	0
0	0	0	0	0	0	1	0
0	0	0	0	0	0	1	0
0	0	0	0	2	2	5	2
5	54	14	0	3	3	11	3

Predator

GR_SP04	GR_SP05	GR_SU04	GR_SU05	GR_FAL04	GR_FAL05	GR_WT04	GR_WT05
0	0	0	0	0	0	1	0
1	0	1	0	0	0	0	0
0	0	0	0	0	0	1	0
0	0	0	0	1	2	0	0
15	0	3	3	1	3	7	5
0	3	0	0	0	0	0	1
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
1	0	1	0	1	1	5	2
0	0	0	1	0	0	1	0
0	1	0	0	0	0	0	0
0	0	0	0	0	0	1	0
2	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0
0	31	0	8	1	0	1	1
0	0	0	0	0	0	2	0
0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0
0	3	0	0	0	0	0	0
6	0	0	0	0	0	5	0
0	0	0	0	0	0	1	0
25	38	5	12	4	6	27	9

Table XVI ANOVA descriptive data between seasonal variation and collecting sites for functional feeding groups. **Collector-gathers and Scrapers were significantly different among the six collecting sites.

		Sum of Squares	df	Mean Square	F	Sig.
collector-gather	Between Groups	192.880	5	38.576	2.737	.031
	Within Groups	591.958	42	14.094		
	Total	784.838	47			
collector-filter	Between Groups	13.656	5	2.731	1.211	.321
	Within Groups	94.687	42	2.254		
	Total	108.343	47			
scraper	Between Groups	58.153	5	11.631	2.424	.051
	Within Groups	201.539	42	4.799		
	Total	259.692	47			
shredder	Between Groups	6.396	5	1.279	1.042	.406
	Within Groups	51.540	42	1.227		
	Total	57.936	47			
predator	Between Groups	5.442	5	1.088	1.358	.259
	Within Groups	33.659	42	.801		
	Total	39.100	47			

Figure IV.5 ANOVA Analysis Collector-gathers. Based on results from the ANOVA analysis collector-gathers (c-g) had a statistically significant difference both between Rocky River and Cuyahoga site CU2) as well as Cuyahoga siteCU2 and Grand River.

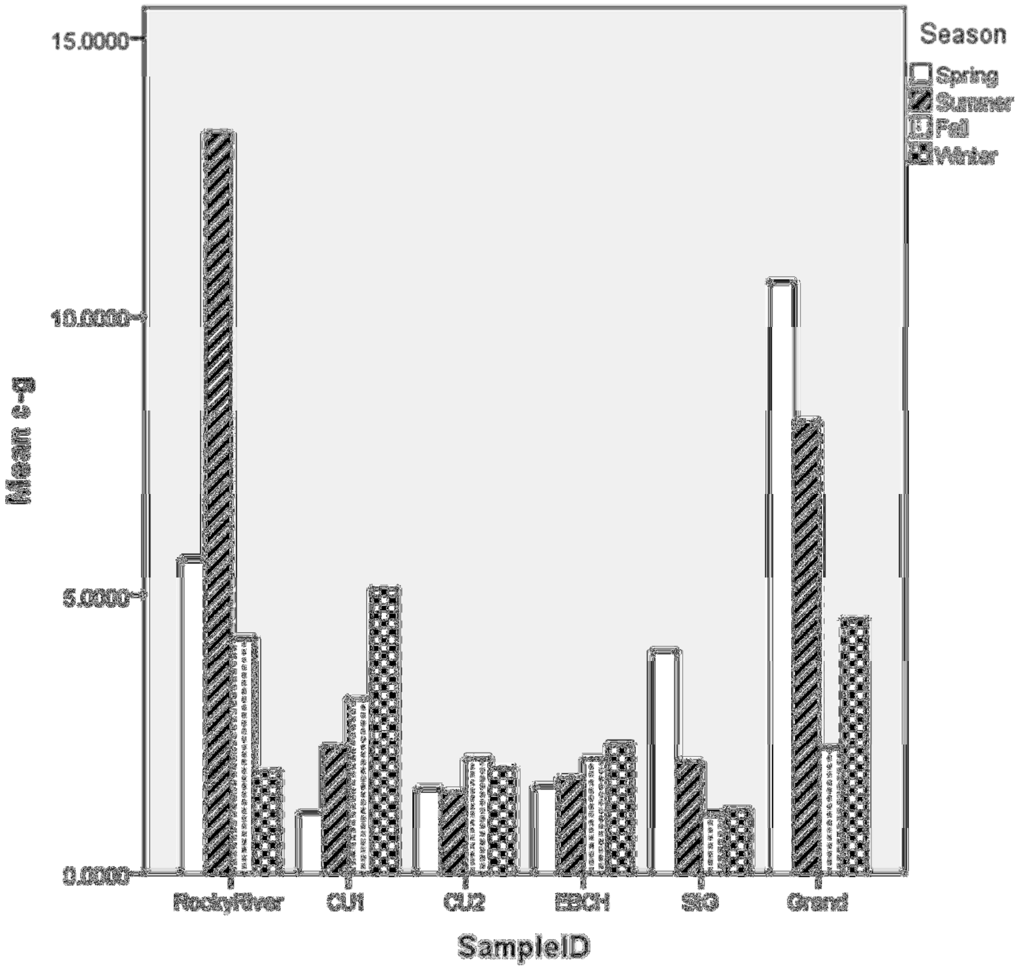
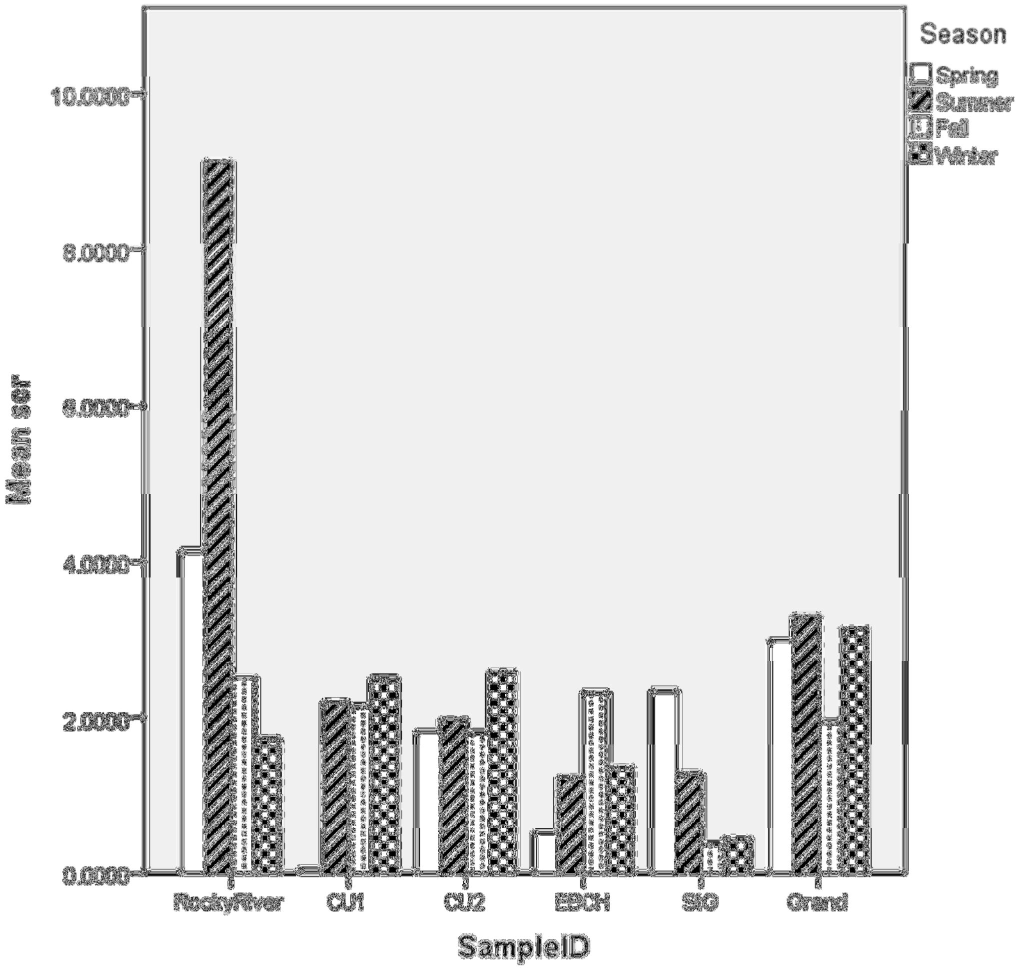


Figure IV.6ANOVA Analysis of Scrapers. Based on results from the ANOVA analysis scrapers (scr) were found to be statistically significantly different between Rocky River and Stebbins Gulch (StG)



4.4 <u>Synthesis</u>

4.4.1 Seasonal Perspective

Seasonal variation in macroinvertebrate communities result from varied life history differences in growth, development, and reproduction. Many macroinvertebrate communities exhibit seasonal life cycles that are timed to take advantage of optimal environmental conditions or to avoid sub-optimal conditions (Wise, 1980; Beche et al., 2006; and Johnson et al., 2012). Biotic variables are often affected by abiotic factors including water temperature, water velocity, food availability, dissolved oxygen concentrations and competition which in turn affect population structure and size, (Hilsenhoff, 1988; Stark and Phillip 2009). The interaction of macroinvertebrate communities is dynamic and displays differently from season to season, resulting in a wide range of life history strategies. Therefore, year round macroinvertebrate sampling occurring in the same stream and in the same reach often reveals substantial variation in the type and abundance of taxa.

As shown through the year-round sampling, seasonal variation in biological and physical variables can be a major confounding factor affecting macroinvertebrate assessment data. Throughout the course of this study, samples collected from one season to another appeared to contradict each other due to dramatic changes in community composition which was not always due to observable changes in the environment.

Most comparative seasonal studies have been conducted during dry periods and/or periods of increased hydrologic inputs, such as increased precipitation or urban-based runoff. Few studies have addressed temporal variations between all seasons (spring, summer, winter, and fall) in a humid continental climate (Koppen Climate Classification Dfa., 2013). Two studies that have looked at seasonal differences are Reece et al., 2001 and Zhang et al. 2012, both of which used all four seasons and found a statistically significant relationship between taxa diversity and community structure at different times of year. In addition, both studies concluded that the fall season is the time of the year with the richest diversity of taxa.

As with Reece et al. 2001 and Zhang et al. 2012, the results of this research revealed seasonal variation; with some seasons being more diverse than others. However, the diversity was not consistent from site to site. An examination of seasonal diversity on a site-by-site basis revealed that for two of the six collection sites, spring was the most diverse season, two other collection sites revealed that fall was the most diverse season and finally, in the remaining two sites, winter was the most diverse season. As a result, summer – the season in which most macroinvertebrate studies are conducted in northeast Ohio – was the only season that did not have the greatest seasonal diversity among collection sites.

One possible explanation for inconsistencies in seasonal results is the lack of a predictive flow regime from season to season. This is common in lower order streams (most streams sites within this study are located in headwaters or low order streams), especially in the spring and summer, when stream velocity can be very fast and forceful as a result of increased runoff from precipitation and snow melt, along with sudden, high precipitation, spring storm events. High rates of stream flow often cause an increase in the downstream migration of macroinvertebrates; making it very difficult to estimate true population size. This makes accurate population estimation even more problematic as certain taxa remove themselves from the water column and move into the hyporehic zone – the region beneath and adjacent to the streambed where ground water and surface water mix. A final phenomenon that affects community estimates is the fact that some macroinvertebrate taxa avoid irregular stream flow altogether by either going through diapause or emergence as terrestrial adults.

4.4.2 ***Land Use Perspective***

Landscape perspective is also important in understanding the distribution of macroinvertebrates. Biogeographers have formalized reasons for macroinvertebrate distribution by using two approaches, ecological distribution and/or historical distribution

(Bonada et al., 2009). Ecological distribution focuses on contemporary environmental factors and small spatial scales while historical distribution is centered upon historical environmental factors and their impact on a larger scale (Wiens and Donoghue, 2004; Bonada et al., 2009). Although few studies have addressed both perspectives together, there is considerable evidence for the contribution of each to current spatial patterns of organisms and the evolutionary processes that have occurred over distinct time-scales (Vargas et al., 1998; Qian, 2008).

Current biodiversity and organism distribution is the result of both contemporary and historic environmental conditions. Muto et al. (2011) suggested that in order to maintain diversity among macroinvertebrate communities, diversity must also be maintained among riparian vegetation. Thus, the greater riparian zone vegetation variation, the greater variation of environmental factors. This simplified but significant factor is an important consideration for riparian management, particularly in areas of reforestation and forested wetland restoration. Several federal, state, and regional organizations that have executed riparian management and restoration plans have found themselves hindered by budget restrictions and political issues, leading to single (or very limited) species plantings. While the effort to return these regions to pre-disturbance conditions is a positive step, the lack of variation limits the diversity of macroinvertebrates capable of thriving in the stream system.

The comparison of land use data, particularly historical versus contemporary data, excludes larger spatial factors in exchange for static temporal data – only providing a snapshot of points in time. The evaluation of these data carries an assumption that locations which differ in land use are similar in all other respects and change is commonly viewed as progressive over time. This methodology ignores the immediate impact that a transition in land use can have, such as conversion from natural to developed land (Herlihy et al., 1998; Allan, 2004). Investigators are increasingly recognizing that human actions at the landscape scale are a principle threat to the ecological integrity of river ecosystems, impacting habitat, water quality, and biota via numerous, and complex, pathways. In addition to direct influences, land use interacts with other anthropogenic stressors that affect the health of stream ecosystems; such as climate change and invasive species. The increase in studies on relationships between land use and stream condition have been driven by several developments. First is the widespread recognition of the extent and significance of change in land use and land cover over a greater area and in a number of different regions worldwide. Secondly, conceptual and methodological advances in landscape ecology, combined with readily available land use/land cover data, has changed the way aquatic ecosystems are studied. Finally, the use of stream health indicators to assess status and trends in rivers (Allan, 2004) has become more prevalent.

Whereas these advances are important, interpreting a particular land use variable as the primary driver of stream condition must be used with caution (Herlihy et al., 1998). It is well known that streams draining agricultural lands support less diverse insect populations, fewer fish taxa, and fewer pollution intolerant species. Researchers have found that row crops and other forms of intensive cultivation strongly impact stream conditions, but the influence of pasture agriculture may be less intense than previously thought (Meador and Goldstein, 2003; Allan 2004). Overland flow commonly occurs in agricultural lands during extreme storm events due to enhanced drainage ditches, limited subsurface drainage, decrease bank stability, loss of riparian zone, and wetland areas. High flows can eliminate stream taxa if it occurs during vulnerable times in the life cycle or with a frequency that selects for resistant and rapidly dispersing species.

4.4.2.1 Past Land use Evaluation

The National Land Cover Database (NLCD) provides spatial reference and descriptive data for characteristics of the land surface. Using the most recent data available for this study, 2001 data, and the dominant land cover for all six collecting sites was characterized by deciduous forest. In the Rocky River, the dominant land cover was deciduous forest along with forested wetlands, however, low to medium intensity human development and cultivated crops were also present around the stream collection site. Land cover for the two collection sites in the Cuyahoga River, site CU1 and site CU2, changed little from 1992 to 2001 but, there was an increase in low intensity development and developed open space, i.e. parking lots and playgrounds. The Chagrin River East Branch site remained partially deciduous forest but 2001 data revealed small patches of evergreen trees, medium density levels of development, and much larger areas of pasture and hay fields than those present in the early 1990s. Land use surrounding Stebbins Gulch is similar to that of the Chagrin River East Branch. However, human populations are lower, there are more pasture and hayfields, and more open land not used for anthropogenic purposes. The final collection site, the Grand River, revealed a distinct transition from predominantly deciduous forest to pasture and hayfields, along with cultivated crops.

Studies of stream assemblage recovery after short-term catastrophic disturbances (e.g. logging, construction, flooding, and point-source pollution) have often shown relatively rapid recovery of biotic communities. However, high impact or sustained anthropogenic disturbance, such as agriculture, may profoundly alter biotic communities; the effects of which may be persistent over time. These effects, termed legacy land use effects, are the consequence of disturbance that continues to influence ecological systems long after the initial disturbance (Harding et al. 1998, Allan 2004). Legacy land use is one explanation for why currently forested streams have macroinvertebrate assemblages that are more similar to agricultural regions than those of forested areas (Harding et al. 1998).

Harding et al. (1998) found that large-scale and long-term agriculture disturbances in a watershed limit the recovery of macroinvertebrate diversity many decades later. The authors compared two streams that were both forested streams at the time of the research,. However, one of the two streams had only been forested since 1950, (i.e. previously agriculture) while the other, according to historical documentation, had never been used for any other purpose. Their research found that the reforested stream had a macroinvertebrate assemblage similar to those in current agricultural streams and were dominated by pollution tolerant taxa even though the stream had been free of agriculture for over forty years. Additionally, the recovery time for any associated geomorphic alterations is especially long, particularly when compared to changes in land use. As a result, stream habitat and channel shape may never reach equilibrium with ongoing development (Brierley et al. 1999). Although all collecting sites within this study were under some form of federal, state, or regional protection, no site can be considered pristine. Major storm events were observed at several sites during the collecting years (i.e. Cuyahoga River site A and Grand River) which caused changes in hydrology and substrate that devastated the macroinvertebrate communities at those sites. Had the streams remained in pristine conditions and not gone through transitions of agriculture in their past, the overall affects may not have brought about such dramatic changes to the biota.

Maloney et al. (2008), using small heterotrophic streams, suggested that anthropogenic effects may influence in-stream conditions for centuries to millennia, much long in the smaller, lower order streams than in higher order streams, because heterotrophic streams, are more dependent on allochthonous material. Thus for lower order streams, not only is complete recovery dependent on direct in-stream interactions and riparian zone vegetation, but also age and decomposition rate of the vegetation. Maloney et al. (2008) illustrated the significance of in-stream coarse woody debris and how it helps to stabilizes stream channels (especially important in low order streams) and provide a habitat for macroinvertebrate communities. However, coarse woody debris results from inputs by surrounding vegetation decades to centuries old. Thus the researchers suggest that before complete stream recovery success should be acknowledge, not only should the vegetation present be accounted for, but also the rate at which the vegetation decomposes and becomes an available food resource (Maloney et al. 2008; Entrekin et al., 2009).

Anthropogenic activities in and around watersheds in northeast Ohio consistently are changing the landscape and the habitat of the streams within them. Sedimentation, hydrologic alteration, nutrient enrichment, contamination, and forest clear-cutting, among other activities, alter stream ecosystems and their biotic dynamics. Often the relationship between anthropogenic land use and the ecological integrity of streams are complicated by co-variation between anthropogenic and natural gradients and uncertainties concerning the importance of legacies and thresholds. Furthermore, land use, in addition to flight ability and emergence success, has the potential to affect the overall community structure of macroinvertebrates (i.e. *Allocapnia recta*) at the collection sites. If macroinvertebrate communities become isolated due to the aforementioned effects, gene flow could be slowed or halted completely due to isolation, leading to biotic homogeneity (Olden, 2004).

With so much variation between stream sites, and the complicated relationship of innumerable variables within sites, developing a complete data set necessarily requires consistent sampling over an extended period of time. Traditionally, the summer season is thought to be the best time for optimizing time, space, and money to monitor stream health and macroinvertebrates. While this spatially and temporally constrained methodology has been thought adequate for many decades, the prevailing wisdom is beginning to change. Several recent studies have shown that the autumn or fall season is the best time of year for accurately estimating population size (Zhang et al., 2012). Other studies that may best answer the scientific question(s) being studied by winter collection are not conducted due to less than hospitable weather, semester intercession, lack of student assistance, fear of personal safety around iced-over streams, etc. A new way of thinking in methodological development must occur, as this study has revealed, and implement year-round sampling over an extended period of time to effectively track macroinvertebrate community trends.

Furthermore, incorporating a legacy land use perspective into ecological studies may help to elucidate potential mechanisms explaining outlier data. Such a perspective might provide insight into subtle biological interactions and their associations with regional environmental conditions, as well as aid in identification of reference conditions for studies of biotic integrity and restoration. Without quantitatively rigorous approaches designed to assess the potential influence of historical disturbance on contemporary measures, one can only offer hypothetical explanations for high levels of habitat alteration in certain streams, and underestimate the legacy effects on contemporary biological data (Maloney et al., 2008). Fortunately, even though most studies today investigate biotic integrity and restoration success, disturbance levels typically are based on contemporary land use and watershed conditions, however, in some cases it may not be too difficult to go back in time with historic records to reconsider current stream conditions based on prior land use which could manifest as a measurable legacy effect.

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Chapter V Extended Comprehensive Summaries

CHAPTER V

CONCLUSION AND SYNTHESIS

5.1 Generalization

The worldwide loss of biodiversity, coupled with both a scientific and sociocultural need to prevent continuing losses, has made biodiversity a "hot topic" for researchers. A combined methodological integration of entomology, genetics, hydrology, and the collective results of my work have led to a better, holistic understanding of four stream systems in Northeastern Ohio; successfully demonstrating the importance of approaching ecology from a multidisciplinary perspective.

Rivers are an integral part of ecosystems, providing food, energy, habitat, organismal transportation, and drinking water. In addition, they serve a valuable role in human economic growth, commerce, transportation, irrigation, and waste disposal. It comes as no surprise that the interrelationship of humans and riverine systems has resulted in long and intense impacts. Under the influence of humans, rivers have been channelized, poisoned, fed with sewage and non-native fish, dammed, and drawn from to the point of extinction. However, because of their rapid turnover and resilience, rivers have, in some cases, the capacity for recovery and renewal.

5.2 Summary of Plecoptera Dispersal and Species Comparison

Earlier chapters of my research revealed how adult terrestrial emergence period and flight capabilities have significant effects over the current population size and genetic differentiation of *Allocapnia recta* versus *Leuctra tenuis* between the Chagrin and the Grand Rivers. The two species of plecopteran were chose for this research because of their differences in wing structure and opposing seasonal emergence as terrestrial adults. Differences in wing structure and terrestrial emergence periods were designations made for analyzing the potential genetic dispersal of macroinvertebrates. The culmination of the research revealed that specimens of *A. recta* were not likely to fly from one watershed to another due to their poorly developed wing structure and winter emergence as terrestrial adults. In contrast, *L. tenuis* is a strong flying stonefly with well-developed wings, and a summertime emergence. For their comparison the results revealed statistically significant genetic differences between *A. recta* populations in the Chagrin River compared to the Grand River, while there was no statistically significant difference between the *L. tenuis* populations in the same rivers.

Four unique *A. recta* haplotypes were identified in the Chagrin River and three unique haplotypes were collected in the Grand River. The two most common haplotypes, haplotypes 1 and 2, were collected in both the Chagrin River and Grand River. The presence of the haplotypes was significant and indicated that although these streams were once connected, there has been sufficient time and land cover change– both natural and anthropogenic, for the two populations to become isolated, succumbing to different environmental factors, and mutate into distinctly different haplotypes.

Leuctra tenuis showed insignificant genetic differences among the populations in the Chagrin River and the Grand River. Two haplotypes were collected in the Chagrin River and three haplotypes were collected in the Grand River. The two most common haplotypes, haplotypes 1 and 2, were collected in both watersheds. The results indicate that *L. tenuis* samples are not genetically isolated between the two watersheds and, as such, are able to migrate back and forth between the two watersheds.

Expanding my study on flight capability and genetic differentiation, *A. recta* samples were further employed to investigate dispersal patterns. By adding two additional watersheds, the Rocky River and the Cuyahoga River, to the previously studied Chagrin and Grand Rivers, and utilizing a larger sample size, enabled me to further investigate larger and farther populations of *A. recta* from each other in Northeast Ohio. Distance was hypothesized to be the driving force in haplotype differences between sites. Sites that were geographically closer to each other would have similar haplotypes, and sites with greater distance between them would share little to no haplotypes between them. However, this hypothesis was proven to be false; overland distance between the watersheds was not a significant contributor to genetic differences in *A. recta* populations. Data analysis revealed 19 different haplotypes among the sites, with haplotypes 1, 2, 3, 4, and 19 being the most common among all sites; with haplotypes 3, 4, and 19 being the most abundant. Most of the remaining fourteen haplotypes were either unique to a particular watershed or limited to one or two examples of each. Even between the most common haplotypes, no haplotype was identified in all four watersheds. These findings led to recognizing alternative reasons for the current genetic distribution of *A. recta* populations. Some of the alternative factors affecting genetic diversity and isolation, as discussed in chapter three are a combination of post-glacial migration, land fragmentation, and immediate anthropogenic effects.

5.3 Macroinvertebrate Community Structure

Chapter 4 summarized the seasonal collection of macroinvertebrates at six sites in Northeast Ohio, within the aforementioned four watersheds. Each site was analyzed by using both physical and biological factors for a complete analysis of both the lotic system and the macroinvertebrate community structure from January 2004 until December 2005. Seasonal variation in aquatic macroinvertebrate communities result from a myriad of life cycle differences among the community's constituent taxa, including growth, development, and voltinism. Macroinvertebrate populations exhibit seasonal life cycles that are timed to take advantage of optimal environmental conditions or avoid unfavorable environmental variables like temperature, hydrological cycle, and food availability (Johnson et al. 2012).A complete analysis of seasonality was performed when evaluating the current macroinvertebrate distribution in streams. No single season could be defined as the most diverse season for all sites and both collection years. However, in reviewing the totality of the results, certain conclusions can be drawn. To begin, although the most diverse season differed from site to site, year 2004 collections experienced greater fluctuations than year 2005. Five out of eight times, 2004 collections were the most diverse and, was the least diverse year three out of eight times, illustrating the dramatic dynamics that occur within a year of a macroinvertebrate. One of the most remarkable seasonal dynamics is the 100-year storm event in the Cuyahoga site CU1 during the summer of 2003, which still had an overall effect of macroinvertebrate community structure three to six months later. . While dynamic in its own right, 2005 maintained moderate diversity throughout the collection period; however, the Grand River was the one exception. During the summer of 2005, the Grand River experienced a 50-year storm event during the summer, that like Cuyahoga site CU1, changed the stream substrate, and species diversity was lowered compared to the previous collecting periods. Looking at season specific summary data, the fall and winter seasons were the most diverse two of eight seasonal sampling periods, collectively, while spring and summer seasons were the most diverse only once each between the seasonal sampling periods.

Legacy land use was also reviewed within chapter 4 to better understand not only the anthropogenic effects of land use, but how long those effects endure. All six collecting sites included in this research are currently under some form of land use protection and management; governed by agencies such as the Cleveland Metroparks, the Cuyahoga National Forest, and Holden Arboretum. However, land management practices have not always been employed at the sites. Information gathered from United States Geological Survey (USGS) maps and landholder survey records revealed that, historically, most sites were agricultural. The agricultural activities ranged from pastoral to row crops, both of which are known to negatively impact nearby streams, and the macroinvertebrate community structure reveals continued negative impacts by these lingering legacy land use effects.

5.4 Education

Throughout the course of any research project, many lessons will be learned that cause the researcher(s) to think differently about scientific phenomenon. Some of these lessons become reasonable suggestions that should be shared toward creating dialogue and more efficient research in the future. This research is no exception. Perhaps the most seminal lesson learned during the course of this research is that not every detriment to a stream is human related, rather, a combination of anthropogenic and natural phenomenon (i.e., storm events and climate change).

While it is understood that not every case of polluted land and water is anthropogenic in nature, we tend to assume that if there is corruption in nature it must be due to humans. This research was started with that very assumption in mind and it was quickly withdrawn after a 100-year storm event in 2003. This work set out to collect plecopteran samples in four watersheds and analyze their genetic distribution within and between adjacent watersheds. To get a general idea of their numbers and distribution, plecopterans were collected at Cuyahoga River CU1, as well as the other sites in this work, during the winter and summer of 2003 to determine if adequate sample sizes were present. Based on the data collected at the sites, *Leuctra tenuis* and *Allocapnia recta* numbers were both sufficient to proceed with this work. However, as previously mentioned the 100-year storm event that occurred in August of 2003 had a major impact on Cuyahoga River CU1. An upstream foot bridge was pushed downstream destroying large sections of the stream bank vegetation and deposited large volumes of clay over the gravel and cobble substrate. Based on continued collections at Cuyahoga River Site CU1 following the storm event, the *A. recta* and especially, *L. tenuis* population sizes decreased significantly and had not yet recovered by the end of the collecting period for this research (i.e. December 2005). Without sufficient sample sizes for the remainder of the research, the scope and direction of the work changed significantly. The storm served as a valuable reminder of the strength and fortitude of natural impacts on stream systems, and that humans are not always the primary source of land and water disturbance.

A second important lesson is how invaluable year round sampling of macroinvertebrates is for assessing stream health. Traditionally, summer is the most common time of year for collecting macroinvertebrate samples. While convenient and hospitable during the summer months, this research demonstrates that summer is not the best season for assessing the population structure of macroinvertebrates in a lotic system. In fact, scientists that only collect once a year are clearly underestimating the population size. As indicated by the results of chapter 4 (and partially chapter 2, and Chapter 3), summer sampling data alone restricts measures of diversity and community structure; particularly with fall and winter samplings yielding greater sample numbers and indicating much greater diversity. Not only should stream ecologists design long-term projects that cover a span of several years, but they should also sample macroinvertebrates seasonally in temperate forests.

A final lesson, building on year-round collecting, is the particular importance of winter sampling. It is often difficult for researchers in a temperate continental climate to find the self-motivation, and student assistants, to collect during the less than hospitable winter season. In addition, the safety of researchers and assistants during the icy winter season is a valid and important concern. However, if stream ecologists and government agencies like the USEPA are to collect an accurate and robust data set, they need to be trained on technique and safety in sampling macroinvertebrates during the winter months. In extreme conditions that may freeze over part of the stream, macroinvertebrates are not inactive. Many macroinvertebrates are in a dormant stage (or overwintering stage) to avoid the harshness of winter within an aquatic environment, often residing in the hyporheic zone, or may become terrestrial adults to avoid the stream altogether. Thus, even in extreme cold conditions, macroinvertebrates are still major contributors to the energy and nutrient cycling in the stream.

5.5 <u>Averting Methodological Error</u>

Too often in the recovery of aquatic ecosystems, there is a misplaced assumption that post-disturbed ecosystems should return to pre-disturbance conditions. Recovery from past events in a variety of environmental conditions is not easy to characterize and, as a result, it may require human intervention and decades of time to restore habitats and reintroduce lost species (Power 1999; Rupprecht 2009). There are numerous examples of stream restoration projects, world-wide, in which immeasurable amounts of time and money have been expended for research and promotion of site recovery. Unfortunately, very few researchers continue to study and/or follow-up on the biological and physical dynamics of these projects over the long-term, with most monitoring lasting only five years. The absence of continued oversight on the part of the researcher has led to projects deemed unsuccessful immediately due to the disturbance of biological and physical variables. These projects over time go on to reach equilibrium, positive growth, and a full recovery. Likewise, other projects have immediately been deemed a success, only to experience a dramatic decline in overall health with the first major storm event or upstream development. Some researchers, as cited in Palmer (1997), call this false, positive declaration a *Field of Dreams Hypothesis* - if you build it they will come. The foundation of this “field of dreams” is the continuous misconception among environmental managers that once areas have gone through reconstruction and “restored” to previous conditions the organisms that were lost or displaced will return.

A second issue in attempts to restore streams to their previously pristine condition is the erroneous use of laboratory results as a predictor for real life restoration. Rupprecht (2009) attempted to reintroduce five species of plecopteran into several third order streams in Hessen, Germany. All streams in the study had been previously affected by poor wastewater management and had lost most of their macroinvertebrate communities; particularly pollution intolerant species. Following the installation of several purification plants over a thirty year period that was put in place in what was believed would dramatically improve the water quality, many orders of pollution sensitive macroinvertebrates had returned to the sites on their own. However, not all taxon did, and one of those were stoneflies. Stoneflies did not successfully reintroduced themselves, thus Rupprecht and his team began to physically add stonefly eggs and larvae to the streams. Over a two year period, 2,000 eggs and over 500 larvae were introduced into the four brooks in and around Hessen. Following ten years of oversight, the team of researchers only found a single larva in the brook. Based on findings from laboratory results, the ten year time period should have yielded a much larger population size of plecopterans. Given the extensiveness of the project, coupled with the laboratory results guiding the study, the researchers concluded that there is too much unpredictability in the biological and environmental aspects of a natural environment. The level of unpredictability, regardless of the streams former conditions, prevented any foreseeable results. Although the study attempted to restore populations it illustrated instead that real life results are not identical to laboratory results and a lot of energy is placed into remediation efforts that may not actually work. In fact, based on a meta-analysis of similar studies, it is more likely that restoration projects will fail to attain their previous conditions than they are to succeed.

Finally, it is also important to set standardized criteria to acknowledge when recovery has occurred. The longer the evaluation process occurs the better the data reflects the successes and failures of recovery, and the more likely confounding events can affect the recovery trajectory. Macroinvertebrate community diversity at any site is influenced by a variety of factors such as the degree that restoration overcomes altered water quality, flow regime, food sources, habitat, and dispersal pathways. Drought events, weather patterns, water chemistry, and flooding can all have profound effects on stream systems (Power 1999; Galic et al. 2013). In addition, many of these aforementioned factors are not acting alone but as co-variables to each other (Palmer et al. 2010 and Parkyn and Smith 2011). Stream ecologists should view aquatic ecosystems as complex, nonlinear dynamic systems in which specific endpoints (i.e. macroinvertebrate biodiversity, abiotic factors) are not guaranteed to return to pre-disturbance values in the post-disturbance period (Power 1999; Ward and Tockner 2001).

5.6 Connectivity and Dispersal

Macroinvertebrates are mobile organisms and due to this fact, macroinvertebrates use streams as their main corridors or highway for dispersal as both aquatic and/or aerial adults. Streams act as corridors by increasing connectivity, population size, movement between island habitats, and enabling gene flow among the aquatic species (Parkyn and Smith 2011). Despite the fact that it is almost impossible to ever restore land back to its original pristine condition, there are positive efforts that can be made towards effective restoration. For example, restoration of smaller but continuous habitats of land, as opposed to restoring a large area of land in patches, has been shown to have a greater level of restorative success. The ability of any organism to move from region to region is essential not only as the movement of the organism, but also the genes of that organism as well. . Limiting connectivity of a species limits its genetic variability and increases the chances of a monoculture, or biological homogeneity (Olden and Rooney2006). Biological homogenous communities are unstable groups of genetically similar organisms that have been cut off from other similar species either though a loss of reproduction or the loss of mobility from patches of land. Loss of genetic variability could cause a single catastrophic event to wipe out the entire population. Species isolation or loss may be accelerated by the fact that some species of macroinvertebrates are already poor dispersers. If those macroinvertebrates are cut off from direct connections between viable habitats, one will be creating even more devastating effects on macroinvertebrate communities.

5.7 Conclusion

As a final point for discussion, evidence of climate change effects on biodiversity at a global scale is now unequivocal in many habitats, and aquatic ecosystems are exception (Li et al. 2012). Available long-term environmental data has already illustrated significant warming trends in many rivers over large geographical areas (Floury et al. 2013). On a consistent basis, predictive models on the effects of global climate change on aquatic ecosystems indicate increasing seasonality effects on hydrological patterns, including increased discharge, flooding and drought events occurring with greater frequency and severity. The result is both thermal and hydrological changes in rivers that have major ecological consequences. Water temperatures play fundamental roles on organismal survival, metabolism, growth, reproduction, and behavior in biotic interaction. Temperature also impacts primary production and leaf litter decomposition, modifying river energy and chemical fluxes along the entire river continuum (Vannote et al. 1980). In turn, river flooding and drought variations have, and will continue to have, a fundamental ecological effect on macroinvertebrate community structure.

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