A bioassessment of the impact of livestock restriction on benthic macroinvertebrate communities in the Grand River watershed in Ontario, Canada

by

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A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Environmental Studies
in
Environment and Resource Studies

Waterloo, Ontario, Canada, 2015

Author’s Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Livestock can have negative impacts on water quality – they can trample and disturb aquatic habitats, their urine and feces can increase nutrient levels in the water, and they also deteriorate riparian vegetation. In Ontario, Canada, regional bodies known as conservation authorities are partially responsible for (improving) water quality; one prominent example is the Rural Water Quality Program (RWQP) that was developed by the Grand River Conservation Authority (GRCA). One major project from the RWQP involves building fences along streams to restrict livestock access, reduce livestock excreta, and improve water quality passively. It was conjectured that reduced excreta loads might spur passive ecological restoration of the benthic invertebrates and perhaps the larger aquatic community if there was response to reduced nutrient loads in the reaches where fences were built. The actual success at improving both water quality and spurring passive ecological restoration is unclear because there were insufficient funds to monitor the projects after implementation. My thesis focused on whether there is evidence of passive ecological restoration in a watershed as large as the Grand River, using benthic macroinvertebrates as indicators of water quality because they respond to land use changes. My specific objectives were to: (1) test whether there were simple block effects (location-based) in mixed-level aggregations of benthic macroinvertebrates at different streams that have been fenced, and (2) test two different lengths and ages of fences categorized as short (less than 400 m) or long (greater than 800m), and young (built after 2007) or old (built before 2003) for differences in impacts on benthic macroinvertebrate assemblages. In 2014, 11 streams were sampled once each month between May and August, using a standard method - the Ontario Benthos Biomonitoring Network (OBBN) protocol. Site 11 was removed from the study because of the sparse number of invertebrates present at the site. Samples were collected downstream, in the middle, and upstream of each fenced location. Insects were identified to family, and all other invertebrates were identified to the 27 groups outlined in the OBBN protocol. I compared the data using taxa richness, the proportion of benthic macroinvebrates that were a) Ephemeroptera-Plecoptera-Trichoptera, b) Chironomidae and c) Oligochaeta, Simpson's Index, and the Shannon-Weiner Index. Hilsenhoff's Family Biotic Index was also calculated and used as a proxy to test for water quality. ANCOVA tests using fence age and length as covariates for data that was normally distributed and homogeneous, and Kruskal-Wallis tests for data that was not

normally distributed or homogeneous, were used to compare each index between the different sampling locations. The third objective of my thesis was to use historical and current data, to test if benthic macroinvertebrate communities have changed over a 7-year time scale in areas where fencing has been installed. To address this third goal, 7 locations that had been sampled in 2007 were re-sampled in 2014 using similar methodology. Insects were identified to family and all other invertebrates were identified to the 27 groups outlined in the OBBN protocol. I also calculated taxa richness, abundances of Ephemeroptera-Plecoptera-Trichoptera, Chironomidae, and Oligochaeta, Simpson's Index, Shannon-Weiner Index, Hilsenhoff's Family Biotic Index, and used paired t-tests or Whitney Mann-U tests to compare indices between the two sets of data. Jaccard's coefficient was also calculated to compare differences in the types of taxa present in 2007 and 2014. Overall, I hypothesized that locations with longer and older fences would have higher water quality than locations with the other types of fences and that there would be an increase in invertebrate diversity between 2007 and 2014 as a result of passive restoration.

The data analysis revealed minimal significant differences between downstream, midstream, and upstream invertebrate assemblages, regardless of fence age or length, and the differences were not consistent. I found no significant differences in the ANCOVA tests; however, the Kruskal-Wallis test identified some significant differences. Old fences had a higher Simpson's Index than new ones in May ($p=0.002$) and August ($p=0.026$). Taxa richness was higher in old fences than new fences June ($p=0.012$) and July ($p=0.027$). Abundance of Oligochaeta was higher for young fences in June and July ($p=0.031$ and $p=0.024$ respectively). There were also significant differences between fence length and Simpson's Index and Shannon-Wiener Index in July where the shorter fences had higher index values ($p=0.013$ and $p=0.012$ respectively). Lastly, abundance of Chironomidae was higher in short fences than long fences in August ($p=0.008$). Considering that the differences are not consistent with expectations, this suggests that fence age and fence length do not have strong influences on the benthic macroinvertebrate assemblages. This is congruent with results that the sampling sites range from fairly poor, to poor, according to comparing Hilsenhoff's water quality bioassessment.

Corroborating these results, there were also few significant differences between the benthic macroinvertebrate communities between 2007 and 2014. Of the 7 different indices calculated, only the Shannon-Wiener Index and Hilsenhoff's Family Biotic Index were significantly different. The Shannon-Wiener Index indicated lower diversity between 2007 and

2014 (p=0.024) and Hilsenhoff's Family Biotic Index revealed that water quality has decreased from 2007 to 2014 (p=0.037). The Jaccard's Coefficient values also complemented HFBI because it implied that there were changes in taxa compositions between the 2007 and 2014 samples. Even after 7 years, there has been minimal passive ecological restoration of benthic macroinvertebrate community - a critical part of the aquatic riverine ecosystem.

I interpret these results, as indicating that fencing of a few reaches are likely not effective at fostering passive ecological restoration or improving water quality in a watershed as large as the Grand River. This does not mean it is not effective in smaller watercourses nor does it mean that it cannot ever be effective if many more reaches were fenced or if livestock were kept penned in feedlots well away from the watercourse. It means that the cumulative impact of limited fencing is not sufficient to achieve ambitious objectives.

Acknowledgements

This thesis would not exist without the help and support of many wonderful people who I have had the pleasure to work with in the past two years.

Thank you to Tracey Ryan and Anne Loeffleur from the Grand River Conservation Authority for helping me to arrange my field sites and providing me with historical data.

Thank you to my supervisor Stephen Murphy for putting up with my frantic late night emails, for all the non-alcoholic beverages at the Grad House, for forcing me to have a work-life balance, and for providing me with guidance throughout my entire masters journey.

Thank you to my committee member Simon Courtenay for being the third brain in this operation. Thank you for all of your advice, insight, and all of your help with the statistical analysis.

Thank you Chris Jones and Sarah Sinclair for your help with OBBN, and for your help with invertebrate identification.

Thank you to the folks at the Ecology Lab at the University of Waterloo - Anne Grant, Bev Raimbault, Ian Blainey, Cheryl Reyes, Hayley Rogers, and Jakob Sricha for providing me with equipment I needed for my fieldwork. Thank you for also not grey listing me because I always returned my equipment late.

Thank you to my field assistants Emma Buckrell, Steven Buckrell, and my fieldwork partner in crime, Dianne Watkins for getting stuck in mud, crawling under fences and through culverts, and being attacked by flies with me. Tough Mudder ain't got nothing on us.

Thank you to my lab assistants Emily Trendos and Jaimie Bortolotti for helping me pick through mud, decaying plants and other questionable unidentified organic matter to find invertebrates.

Thank you Gabriel Tan-Chen for being my bugcatching-equipment carrying-computer fixing-cheerleading-chauffeur, and all the things in-between. Who would have thought that, that girl who regretted enrolling into forensic entomology in undergrad would end up doing a master's thesis about bugs?

Lastly thank you to you, yes you – the person reading this. Thank you for taking the time to read my thesis. This entire masters degree has been quite an adventure and I am so grateful to have been able to experience it!

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1. Prologue

The structure of this thesis follows the manuscript style, beginning with a literature review in Chapter 1.[1] Chapter 2 provides a description of the Grand River watershed, as well as a description of the study sites. An explanation of the Ontario Benthic Biomonitoring Network protocol, and descriptions of the indices I used in my data analysis are provided in Chapter 2. Chapter 3 provides an overall summary of the methodology of the study.

In Chapter 4, I describe the two different studies I conducted – a spatial, and a temporal study. In the spatial study, I sampled benthic macroinvertebrates once each month in May, June, July and August of 2014 at 11 locations across the Grand River watershed that had fences built to prevent livestock access into the creek. The purpose was to determine if fence length, or fence age facilitated passive restoration, which would influence the benthic macroinvertebrate communities downstream, within the fenced area, and upstream of the fenced portions of the creeks. For the temporal study, I sampled benthic macroinvertebrates in locations in the Grand River watershed that had been sampled in a 2007 study to determine if there were any changes in benthic macroinvertebrate assemblages after these areas had been given 7 years to undergo passive restoration.

In Chapter 5 I provide an in-depth discussion about the results from the two studies. I also provide suggestions to help improve the Rural Water Quality Program, and the overall impact livestock restriction has as a best management practice. Lastly, I identify areas that require further research and uncertainties that need to be further explored.

[1] As a result of this thesis being written in manuscript style, there will be some repetition between some of the chapters.

2. Introduction

2.1 Freshwater Ecosystems

Freshwater ecosystems are interactions amongst hydrogeomorphological processes and the biota in aquatic habitats with low concentrations of salt. Although freshwater ecosystems make up only a small percentage of all of the world's water (0.01% of it), they sustain almost 6% of all the species in the world and are valued for providing ecosystem services and natural resources as well as cultural/recreational benefits (Dudgeon *et al.*, 2006). Ecosystem services provided by aquatic environments include sources of water for conversion for potable use, irrigation, waste management, sources of food (often for subsistence or supplements to diets), carbon sequestration, and microclimate stabilization (Baren *et al.*, 2002; Korsgaard & Schou, 2010).

A healthy aquatic ecosystem needs variable flow patterns, sufficient sediment and organic matter inputs, natural changes in heat and light amounts, clean water, and a diverse community of flora and fauna. If any of these requirements are not met, then services provided by the aquatic ecosystem are impacted (Baren *et al.*, 2002). A healthy ecosystem is also resilient – they are able to recover from a disturbance and maintain their functions, structure, identity, and feedbacks (Folke *et al.*, 2004). Although freshwater ecosystems provide a variety of ecosystem services, they are rapidly being degraded (Sala *et al.*, 2000).

2.2 Impacts of Agriculture on Water Quality

Along with other land uses (e.g. urbanization, direct extraction), agriculture can be a source of over-exploitation of resources, water pollution, habitat degradation, vectors for invasive species, and flow modification. These impacts may reduce freshwater biodiversity and broader aquatic ecosystem resilience (Dudgeon *et al.*, 2006). Savannas, grasslands, steppes, forests and woodlands have undergone the most extensive conversions into land for agriculture in North America (Ramankutty & Foley, 1999). Currently, 7.5% of the land in Canada is used for agricultural purposes (The World Bank, 2012). Globally, 30% of the land is used for agriculture (Secretariat of the Convention on Biological Diversity, 2010). Agricultural land use can impact streams as a result of an increase of nonpoint sources of pollutants from increased inputs of sediment, fertilizers, nutrients, and pesticides (Allan, 2004). Eutrophication increases the amount of plant and algal growth in the streams, impairing the ability of periphyton to grow because less light is able to reach the benthic layer. Subsequently, this disrupts habitats and food availability for organisms living in the streambeds (Smith *et al.*, 1999). Dams and irrigation diversion canals are also created to divert water and alter in-stream habitats; therefore disrupting migration patterns and energy flows (Allan, 2004; Poff *et al.*, 2011). Additionally, channelization of the streams leads to changes in the dimensions and shapes of the streams, which degrades the heterogeneous habitats in the streams (Kristensen *et al.*, 2013). Tile drainage is often used to create more arable land; however, this reduces the water holding capacity of riparian systems and can create reduced capacity for water cycling (Fucik *et al.*, 2015).

Deterioration of riparian vegetation, and the adjacent water bodies, has also occurred because of agricultural activities (Allan, 2004). Riparian vegetation plays an important role in filtering sediment and nutrients before entering a water body (Schlosser & Karr, 1981; Lowrance *et al.*, 1984; Cooper *et al.*, 1987). Bare ground represents less vegetation, and with less vegetation, there is a higher probability of wind and water erosion occurring (Miller *et al.*, 2010). The loss of riparian vegetation can lead to erosion of the stream banks and can disrupt substrates that armour the bottom of the channels. As a result, riffles and pools begin to disappear, water levels decrease, and riparian vegetation communities change from hydric species, to ones that are mesic (Poff *et al.*, 2011). Large woody debris and litter from riparian vegetation that end up in the water bodies are also important for providing fish with productive habitats, but are lost with degraded riparian vegetation. The woody debris and litter also influence the physical, chemical, and biotic characteristic of riparian and in-stream ecosystems (Naiman *et al*., 1992).

Livestock can contribute to riparian vegetation damage. Livestock can impact riparian vegetation through soil compaction, removal of plants through grazing, and damage to plants through trampling (Kauffman & Kruger, 1984). Livestock grazing has been observed to impact native perennial cover, increase the number of exotic plants present, decrease litter cover, increase erosion, change the concentrations of nutrients present in the soil, damage the structure of surface soil, and decrease the rate of water infiltration through the soil (Yates *et al.*, 2000). Additionally, when livestock have access to water bodies, they can impact a stream’s channel morphology, bank stability, and increase concentrations of nutrients in the water via direct fecal and urine inputs into the stream (Scrimgeour & Kendall, 2003). Trampling also re-suspends sediments, disrupting habitats, increasing turbidity, and light reaching the benthic layer (Strand & Merritt, 1999; Quinn, 2000; Miller *et al.*, 2010). Weeds can also be introduced into the water from livestock activity. Weeds that grow on gravel bars can outcompete the native vegetation, reducing evapotranspiration and bar stability (Hancock, 2003).

2.3 Changes in Aquatic Ecosystems

Riparian zones are ecologically resilient; they can maintain current/historical norms even when faced with disturbances. Healthy riparian ecosystems are able to protect water bodies from intense nutrient inputs as well as from natural disturbances such as rainfall and wind (Kauffman *et al.*, 1997; Vaughan *et al.*, 2010). Anthropogenic impacts can cause the resilience of an ecosystem to decline. Declines in resilient abilities increase vulnerability and susceptibility to regime shifts, which are changes in an ecosystem that has caused the system and its services to be less desirable, and less productive (Folke *et al.*, 2004). Climate change, land use changes and the invasion of non-native species are all contributing to the rapid changes in the composition and function of ecosystems (Hobbs *et al.*, 2009).

When thresholds of resilience are crossed, there are shifts to alternate states of an ecosystem, where feedbacks in ecosystems change (Folke *et al.*, 2004). Biotic thresholds can be crossed when atypical relationships occur between species and functional groups (Hobbs *et al.*, 2006). Abiotic thresholds can be crossed when there are dramatic changes to the physical components of an ecosystem, such as changes in the composition of soil (Hobbs *et al.*, 2006). As biotic and abiotic conditions continue to change in an ecosystem, thresholds are crossed where it is no longer possible to restore these ecosystems to historical conditions (Hobbs *et al.*, 2009).

There have been many shifts in ecosystems that have been documented in which plant communities have completely changed, clear waters become highly turbid, and food webs have been altered (Folke *et al.*, 2004). Typically, the goal of restoration has been to restore biota to its original state; however, the question now is whether or not it is practical and possible to restore an ecosystem to its original state, considering the rapid extinction of flora and fauna (Hobbs *et al.*, 2009). Some impacts are irreversible, therefore making it impossible to restore an ecosystem to its original state (Hobbs & Harris, 2001). These changed ecosystems, coined novel ecosystems, can no longer be conserved and restored in the same manner as the past (Hobbs *et al.*, 2009). Global trade has facilitated the movement of species to parts of the world where they would have never reached normally (Hobbs *et al.,* 2006). Sometimes it is impossible to completely remove a non-native species. Sometimes a non-native species has established itself in a community and other organisms begin to depend on it. The integration of these non-native species into these ecosystems makes it challenging to restore these ecosystems to their historic states (Hobbs *et al.,* 2009).

Agricultural activity stresses ecological thresholds. It is questionable whether these ecosystems are still resilient in an environment where not only eutrophication has happened, but where climate change, land use, and changes in biodiversity have impacted the ecosystems (Steffen *et al.,* 2011). Yates *et al.* (2000) suggest that it is not sufficient to simply remove cattle to restore water quality because thresholds have been crossed and now require restoration plans that involve actively capturing resources and developing microclimates. For example, if the abiotic components of an ecosystem have been degraded, it is not possible for the biotic components to recover until the abiotic components have been restored (i.e. if soil has been degraded, plants cannot grow) (Hobbs & Harris, 2001).

Squires & Dube (2013) argue that there are still too many unknowns that exist regarding aquatic ecosystems to be able to define clear limits. Aquatic ecosystems are dynamic and complex; however, benchmarks do exist that are able to measure how much a system has changed over time. Squires & Dube (2013) suggest that assigning a threshold to aquatic ecosystems assumes that there is sufficient knowledge about the capacity of ecosystems to be able to adapt and be resilient to stresses before the point of undesirable and mostly likely irreversible deterioration occurs. Although there are unknowns, it is important to apply the precautionary principle, which argues that even if there is a lack of scientific knowledge, it is still important to act to protect the environment to prevent further degradation (Martuzzi & Tickner, 2004).

2.4 Restoring Rural Water Quality Through Passive Ecological Restoration

Ecological restoration is the rebuilding of processes, functions, and associated biological, chemical, and physical interactions between aquatic and riparian ecosystems. Ecological restoration attempts to reverse the negative impacts humans have had on the environment (Kaufman *et al.,* 1997). There are two types of restoration, passive and active. Passive restoration stops the anthropogenic impacts that are damaging the ecosystems and preventing them from recovering (Kaufman *et al.,* 1997). Passive restoration relies on the resilience of ecosystems to be able to recover after a disturbance (Kaufman *et al.*, 1997). Active restoration includes the reintroduction of flora and fauna, and in-stream changes such as structural alterations to stream flow and modifications to the substrate. Active restoration requires more resources and effort; therefore it is not always the most practical method of restoration (Kaufman *et al.,* 1997).

Livestock fencing is a type of passive restoration that has been implemented along streams all across the world. Livestock restriction allows for increases in bank stability, and increases in riparian and in-stream vegetation biomass. It also prevents livestock from directly adding pollutants into the water (Scrimgeour and Kendall, 2003). The restriction of livestock reduces the amount of grazing of riparian vegetation, therefore it allows for riparian vegetation to grow, it allows for a decrease in the amount of bare ground exposed, and allows native species of grasses and sedges to recolonize (Miller *et al.,* 2010). In an area where water had been constantly withdrawn for agricultural and municipal purposes, paired with intensive cattle grazing on the riparian vegetation, the cessation of these activities allowed 24% of the damaged riparian area to recover within 4 years of restoration, even though that area had been impacted for 50 years (Kaufman *et al.*, 1997). Furthermore, Miller *et al.* (2009) and Line *et al.* (2000) analysed stream-bank fencing (800m and 335m respectively) and observed decreased pollutant concentrations downstream of the fenced areas. It is important to note however, that these studies were conducted in small watersheds (550 hectares and 56 hectares, respectively) in comparison to my watershed of study – the Grand River watershed.

2.5 An example of using livestock fencing to improve water quality and promote passive ecological restoration of aquatic ecosystems: The Grand River Conservation Authority's Rural Water Quality Program

The Grand River watershed is one of the largest watersheds in Ontario (Figure 2.1; GRCA, 2015a). Approximately 93% of the watershed is considered rural and 78% of the land is used for agriculture (GRCA, 2015a). The Grand River empties into Lake Erie, a lake that has been impacted for decades from phosphorous and other nutrient loadings from agricultural activities (International Joint Commission, 2014). There is a large Mennonite community in the rural areas of the Grand River watershed that practice traditional agricultural techniques and have not embraced newer farming methods or technology (Parks Canada, 2015).

In 1997, The Grand River Conservation Authority (GRCA) established the Rural Water Quality Program (RWQP) to mitigate the problems of agricultural pollution in the water systems (Dupont, 2010). The RWQP is a cost-sharing program that provides farmers with incentives for implementing best management practices on their farms. The farmers apply to be a part of the program, and develop an environmental farm plan for their farm in collaboration with the GRCA (Dupont, 2010).

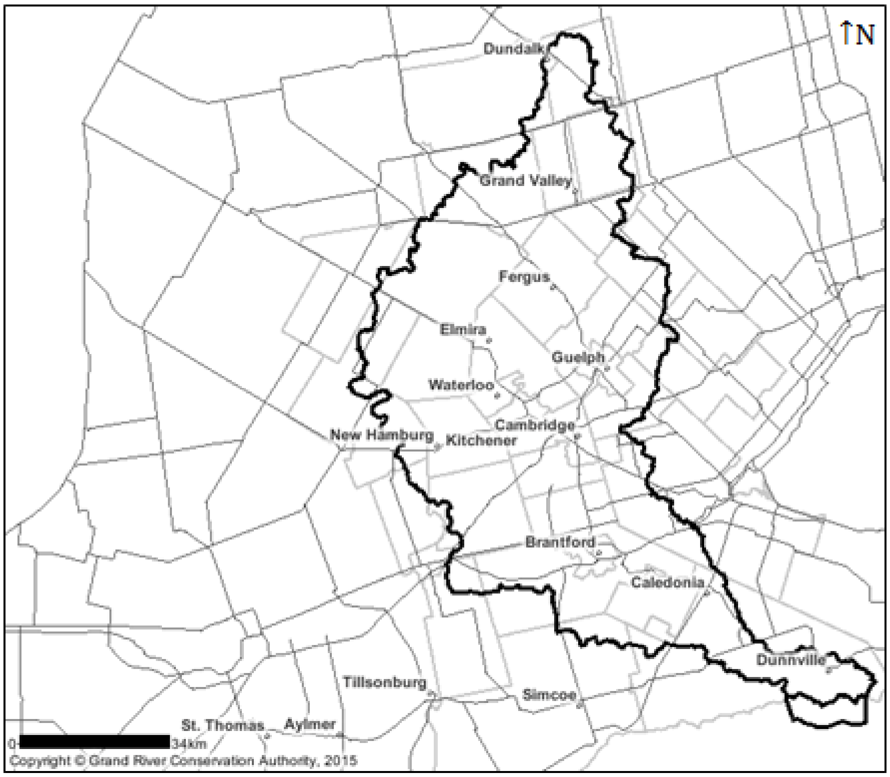

Figure 2.1: Map of the Grand River watershed located in southern Ontario, Canada (GRCA, 2015).

Funding for the RWQP comes from the GRCA and their government and non-government organization partnerships (Dupont, 2010). Best management practices that have been implemented to improve water quality include the building of manure storage facilities, riparian vegetation planting, plugging/upgrading wells, and livestock restriction through fencing (GRCA, 2015b). Funding for the projects ranges from 50%-100% of the total costs of the projects. The funding for these projects is from the RWQP as well as municipal partnerships (Dupont, 2010).

Over 2190 projects have been implemented since the creation of the program (GRCA, 2015b). Since its establishment, the RWQP has fenced 190 of the 23000km of stream bank in the

Grand River watershed (GRCA, 2010). Over 10000 livestock (cows and horses) have been restricted from stream access and have been prevented from disturbing stream morphology, grazing on riparian vegetation, and urinating, and defecating into the streams (GRCA, 2015a).

2.6 Assessing water quality and passive ecological restoration by using benthic macroinvertebrates

Biotic indicators are often used to assess the health of an ecosystem (Resh *et al.*, 1995; Bazenet *et al.* 2010; Pander & Geist, 2013). Benthic macroinvertebrates have often been used to evaluate the degree of anthropogenic impacts to aquatic ecosystems (Wallace and Webster, 1996; Braccia and Voshell, 2007; Herbst *et al.* 2007). Taxa richness of invertebrates has been observed to decrease in the presence of disturbances; therefore assessment of the invertebrates can be used to measure the impact of disturbances (Death & Winterbourn, 1995; Erman & Erman, 1995; Hoopes, 1974; Kristensen *et al.* 2012; Quinn, 2011)**.**

Invertebrates have important roles in freshwater ecosystems, impacting rates of primary production, decomposition, water clarity, thermal stratification, and nutrient cycling in lakes, streams and rivers (Strayer, 2006). Benthic macroinvertebrates are organisms without spines that are visible to the naked eye and dwell in the streambeds. Benthic macroinvertebrates are impacted at both bottom-up and top-down disturbances - eutrophication can impact sources of food for invertebrates, and overfishing can remove invertebrate predators (Gray, 1993; Wallace & Webster, 1996; Ruetz *et al.,* 2003). Because macroinvertebrates have different tolerances to pollution, they can be used to determine if restoration efforts are affecting the community assemblages, and ultimately water quality (Hilsenhoff, 1987; Krieger, 1984; Carr & Hiltunen, 1965). Weigal *et al.* (2000) observed that benthic macroinvertebrate assemblages changed in streams within 100-300m of changes in riparian vegetation. Invertebrates can also respond to habitat heterogeneity. There tends to be a higher taxa richness of invertebrates in areas where the substrates are composed of a variety of components such as leaves, gravel or cobble, wood, moss, and macrophytes, compared to areas were the substrate is simpler, i.e. fine sediment (Benke *et al.,* 1984; Hawkins, 1984; Angradi, 1996; Vinson & Hawkins, 1998). Areas that have had livestock access tend to have more fine sediment in the substrate as a result of erosion from the adjacent banks (Allen, 2004).

Local invertebrate communities are not only impacted by local impacts, but they are also impacted by the conditions of the larger basin. Increases in taxa richness require a source of colonists for re-colonization (Parkyn *et al.,* 2003). It is the larger basin or regional pool that dictates the maximum taxa richness that could be present. Invertebrates have the potential to move from location to location within the larger basin or regional pool; however, it is the local chemical and physical conditions that control if an invertebrate is able to inhabit that area or not (Palmer *et al.* 1996; Ricklefs, 1987). Moreover, stream flow can impact the movement of invertebrates. Invertebrates from upstream areas can be washed downstream and deposited (Allan, 1975). However, impoundments, and other alterations to stream flow such as culverts and levees impact invertebrates' abilities to migrate in response to disturbances (Strayer, 2006).

After a best management practice has been completed through the Rural Water Quality Program, the Grand River Conservation Authority inspects the project to ensure that it has been correctly implemented, and then provides the farmer remuneration for the project. No monitoring of water quality is performed by the GRCA after this time so, as is common in many ecological studies (Dupont, 2010), evaluation of outcomes is absent.

3. Methodology

3.1 Site Descriptions

For the spatial study, 11 sites were chosen from the 284 fencing projects that had been implemented as of February 2014. The 11 sites span the Grand River watershed and permission was obtained from all the property owners in order to access the streams (Figure 3.1). Site selection was based on fences that were similar in length, and similar in year of implementation. To assess effects of fence age and length on the benthic macroinvertebrates, the longest and the shortest, and oldest and newest fences were selected. Because it was difficult to find projects that had fences similar in length and age, it was not possible to also consider the order of the stream, the size of the riparian vegetation buffers, channel morphology, substrate, or canopy for site selection. Livestock includes cows, horses, and sheep; however, the majority of the livestock that has been fenced through the RWQP is cows.

For the temporal study, I attempted to re-sample 13 locations that had been sampled for macro-invertebrates in 2007 (Neary, 2008; Figure 2.1). However, of the 13 original sites that were resampled in 2014, four sites were dry, one site was no longer used as pasture for livestock, and I was unable to contact the property owner of one site, leaving seven sites. I sampled as close as possible to where the sites were sampled in 2007 when faced with missing historical location data.

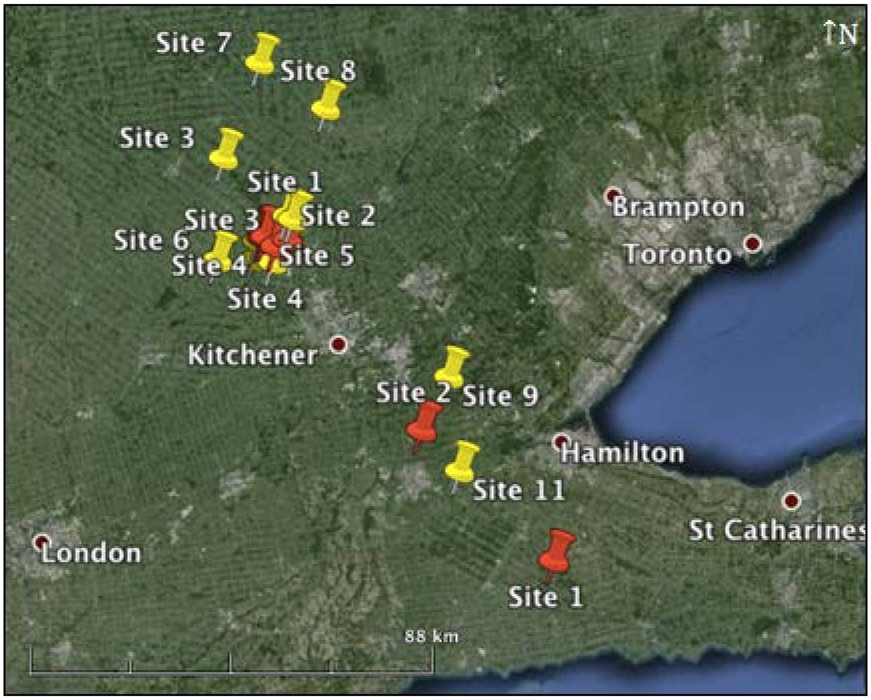

Figure 3.1: Map of sampling sites throughout the Grand River watershed. The yellow pins are the sampling sites of the spatial study, and the red pins are the locations of the temporal study (Modified from Google Earth, 2015).

3.2 Benthic Macroinvertebrate Sampling

The Ontario Benthic Biomonitoring Network (OBBN) protocol was used to collect the benthic macroinvertebrates using the travelling kick and sweep method (Jones, 2007). Samples were collected at all microhabitats in an area, and the degree of sampling effort was proportional to the dominance of each microhabitat.

Taxonomic sufficiency is the concept of being able to balance the information revealed through bioassessments, and the amount of effort applied to identify the organisms. Taxonomic sufficiency is the ability to identify organisms to the coarsest taxonomic level possible without jeopardizing ecological detail (Ellis, 1985). Bowman and Bailey (1997) determined that family-level data was as informative as species-level data after using the data from various studies to make comparisons. Subtle differences amongst samples are harder to detect as a result of higher taxonomic classification (Yates & Bailey, 2011). Natural variability such as water depth and sediment size may impact a community more at the species, than family, level because of specific adaptations evident at the species level. Therefore, higher taxonomic level assessments are able to give a better depiction of overall changes in benthic communities in response to human impacts (Bowman & Bailey, 1997). Mueller *et al.* (2013) suggests that order level identification is the critical threshold of taxonomic resolution before the explanatory power of biological indices greatly decreased. However, because of the challenge of identifying certain taxa to more specific levels, the risk of incorrect identification, and the known life histories and behaviour of invertebrates that are more challenging to identify, all insects were identified to family, and all other invertebrates were identified to a mixture of phylums, classes, and orders - Turbellaria, Nematoda, Oligochaeta, Hirudinea, Isopoda, Bivalvia, Amphipoda, Decapoda, Hydrachnidia, and Gastropoda. Several studies have used mixed taxa aggregates when identifying invertebrates (Parsons *et al.*, 2010; Linke *et al.* 1999; Armanini *et al.* 2014).

3.3 Biological Metrics

Biologic metrics are used to calculate the diversity and distribution of organisms (Wihlm, 1967). The following calculations were used to assess the benthic invertebrate assemblages: taxa richness, the abundances of Ephemeroptera-Plecoptera-Trichoptera (EPT), Chironomidae, and Oligochaeta, the Shannon-Wiener Index, Simpson's Index, Hilsenhoff's Family Biotic Index, and for the temporal study, Jaccard's Coefficient was also calculated.

Taxa richness has been observed to decrease when an area experiences anthropogenic activities. In areas of higher pollution, invertebrates intolerant to pollution cannot survive, therefore it is expected that areas that have lower water quality will have lower taxa diversity (Lenat, 1984; Death & Winterbourn, 1995; Erman & Erman, 1995; Hoopes, 1974). Because approximately 100 invertebrates were subsampled from each sample in the spatial study (as per the OBBN protocol), it is possible to compare diversity amongst samples without having to account for differences in the number of organisms within each sample.

Percent abundance calculations are important because different benthic macroinvertebrates have different tolerances to pollution (Hilsenhoff, 1987). Percent abundance calculations have been observed to be able to detect differences in community assemblages between sites that have little human impact (Yates & Bailey, 2001). To calculate percent abundance, the total number of organisms of the taxa of interest is divided by the total number of organisms in the sample, and multiplied by 100. In my analysis, we chose to calculate the percent abundances of Oligochaeta, Chironomidae and of EPT. Oligochaeta are tolerant to high levels of organic pollution, and have been observed to replace other benthic macroinvertebrates that cannot tolerate high concentrations of nutrients (Schenkova & Helesic, 2006; Lin & Yo, 2008). Chironomids are a fairly ubiquitous macroinvertebrate because of their tolerance levels to pollution; however, they can still be used to pinpoint localized areas of pollution (Saether, 1979). Lastly, Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) are three orders of aquatic insects that are found in stony-cobble streams and are sensitive to environmental disturbances. EPT can only thrive in clean, highly oxygenated aquatic ecosystems and are the most sensitive groups to agricultural runoff and decline as eutrophication increases (Lenat, 1984); a high percentage of EPT in a sample indicates good water quality (Rosenberg & Resh, 1993).

The Shannon-Wiener Index is a calculation that not only considers taxa diversity, but also indicates the evenness of the population (Shannon, 1948). The higher the value is, the higher the diversity and evenness of the population (Barton, 1991). In a polluted area, it is expected that the population would be less evenly distributed. It is important to keep in mind however that an increase in the Shannon-Wiener index value may not be because there was a positive impact on a community, but simply that there were changes in evenness as a result of a decrease in organisms that are typically more abundant (Perkins, 1983). Although there is no actual upper bound for the value, the highest value is typically 5 (Washington, 1984). The Shannon-Wiener Index is calculated using the following formula:

$$H' = -\Sigma p_i ln p_i$$

Where: p_i is the proportion of individuals found in species i

Simpson’s Index is a dominance index, meaning that it is influenced by the abundance of the most common taxa (Simpson, 1949; Wilhm, 1967). Ravera (2001) observed that Simpson’s index was most effectively able to differentiate the diversities between sampling sites, and between months (Ravera, 2001). Simpson’s Index (D) ranges from 0 to 1. Zero represents infinite diversity, and 1 represents no diversity (Simpson, 1949). Simpson’s Index is often represented by its complement, Simpson’s Index of Diversity, 1-D. These values still range from 0 to 1, but in this case, 1 represents infinite diversity and 0 indicates no diversity. Simpson’s Index of Diversity is calculated using the following formula:

$$1 - D = \Sigma {p_i}^2$$

Where: p_i is the proportion of individuals found in species i

Hilsenhoff's Family Biotic Index (HFBI) is a weighted tolerance average of organic pollution (Hilsenhoff, 1987) calculated from the proportion of each group present and its tolerance value. The tolerance values range from 0 to 10, with 0 being the most intolerant organisms to pollution, and 10 being the most tolerant taxa to pollution. The HFBI value corresponds with a particular water quality classification, ranging from very poor, to excellent, and it also describes the degree of organic pollution present in the sample (Table 3.1). Lower values indicate cleaner water, while higher values indicate waters polluted with organic pollutants. For this thesis, the tolerance values used were from Bouchard (2004)'s *Guide to Aquatic Invertebrates of the Upper Midwest* which uses the tolerance values from Hilsenhoff (1988) and uses Barbour *et al.* (1999) for the tolerance values that were not included in Hilsenhoff (1988).

Hilsenhoff's Family Biotic Index serves as a proxy for water quality. A chemical analysis of water samples from the sites could reveal the chemicals present in the water. However, the presence of chemicals in the samples is partially dependent on whether the samples captured pulse-type chemical disturbances moving through the streams (Griffth *et al.*, 2005). Instead of having to take multiple water samples at each sampling point in attempts to determine the range of chemicals present in a water body, benthic macroinvertebrates can be used to indicate the water conditions because they respond to long-term chemical impacts. The tolerance values for Hilsenhoff's Family Biotic Index were based on assessing 53 polluted, and non-polluted streams in Wisconsin in which physical and chemical characteristics had been measured and invertebrates were identified (Hilsenhoff, 1977). The formula for Hilsenhoff's Family Biotic Index is:

$$FBI = \sum n_i t_i$$

Where: i is a particular taxa group

n is the number of organisms in i

t is the tolerance value of taxa i

Table 3.1: Hilsenhoff's Family Biotic Index values, with its relation to water quality, and the degree of organic pollution (Adapted from Hilsenhoff, 1988).

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very Good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly Poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very Poor	Severe organic pollution likely

Jaccard's Coefficient is an absence/presence calculation that determines if taxa compositions differ between samples. Presence/absence calculations have been observed to be able to detect differences between sites that have been largely impacted by humans (Yates & Bailey, 2011). The coefficient uses binary variables; therefore, it is only the presence or the absence of a taxa group that is significant. The value of the coefficient ranges from 0 to 1, 0 means that the two sites are completely dissimilar, and 1 means that the two sites contain the same taxonomic groups. It is important to note however that Jaccard's Coefficient does not consider the abundance of organisms in each taxa (Scheiner & Gurevitch, 2001). The formula for Jaccard's Coefficient of is:

$$J = \frac{s_c}{s_a + s_b + s_c}$$

Where: s_c is the number of taxa common in both samples a and b
s_a is the number of unique taxa only in sample a
s_b is the number of unique taxa only in sample b

Different metrics range in their responses depending on the type of stresses that have occurred (i.e. presence/absence data successfully detecting differences between areas of major impact vs. percent abundance calculations detecting differences between areas of minimal impacts (Yates & Bailey, 2001). A variety of indices should be used to assess a range of stressors and cross-compare the appropriateness of each index (Yuan and Norton 2003). This is illustrated by Brown *et al.* (1997) who found that although the Shannon-Wiener Index indicated that two sites had similar levels of diversity, Jaccard's Coefficient of Community revealed that the similarities between the two sites was low. One sample could be all pollution tolerant invertebrates and the other sample could be all pollution intolerant invertebrates, but assessing only the Shannon-Wiener Index would not reveal this information.

4. Are there indirect influences of livestock exclusion on benthic macroinvertebrates on a spatial and temporal scale?

Outline

When an ecosystem has been disturbed, passive restoration can occur when the impact is stopped and the ecosystem is able to naturally recover. Restricting livestock from accessing a water body via livestock enclosures is one method of passive restoration. When livestock are not restricted from water bodies, they can overgraze riparian vegetation, increase nutrient inputs into the water, disrupt streambeds, disturb habitats, and decrease overall water quality. The Grand River Conservation Authority (GRCA) has created the Rural Water Quality Program to promote best management strategies in the Grand River watershed in Ontario, Canada. One of the best management practices that the GRCA has implemented is the fencing off of streams to prevent livestock access. The purpose of this study was to assess if there is an indirect impact of fences on the diversity of benthic macroinvertebrates. Benthic macroinvertebrates were chosen because they can respond to disturbances and land use changes. They are also sensitive indicators of water quality. The lengths of the fences, and the time since fencing were considered in the spatial study, and benthic invertebrate data from 7 sites in 2007 was compared to data collected in 2014 for the temporal study. For the spatial study, 11 sites in 2014 were sampled for benthic macroinvertebrates downstream, at the fenced area, and upstream of the fencing each month between May and August. Taxa richness, abundance of Ephemeroptera-Plecoptera-Trichoptera, Chironomidae, and Oligochaeta, Shannon-Wiener Index, Simpson's Index, and Hilsenhoff's Family Biotic Index were all calculated. The same indices were calculated for 2007 and 2014 invertebrate data. If passive restoration had occurred, it would be expected that sites with longer and older fences would have the greatest diversity of benthic macroinvertebrates because of longer buffers, and a longer time to recover. Furthermore, it would be expected that downstream communities would have similar or more diverse community assemblages than upstream of the same site because the fences were preventing livestock from impairing water quality, which in turn would increase benthos diversity. Lastly, if passive restoration had occurred between 2007 and 2014, there would be an increase in invertebrate diversity, and sensitive taxa. Statistical analyses (ANCOVAs, Kruskal-Wallis tests, and t-tests) indicated that there were minimal differences between the biological indices calculated within sites, or between sites each month in

2014. There were also minimal differences between 2007 and 2014 data. Hilsenhoff's Family Biotic Index also characterized the streams as characteristic of eutrophication, regardless of fence age or fence length. To date, livestock fencing has not led to detectable improvements in water quality or ecological restoration based on the diversity of benthic macroinvertebrate communities. Therefore, other best management practices also need to be implemented at a larger scale in order to improve water quality in the Grand River watershed**.**

Introduction

Ecological restoration is a process that facilitates the recovery of abiotic and biotic components of an ecosystem after they have been disturbed (SER, 2004). Ecosystems are naturally resilient to disturbances; however, continual anthropogenic disturbances on ecosystems reduce the likelihood of recovery (Folke *et al.*, 2004). A variety of organizations have created programs to help improve the state of disturbed aquatic ecosystems in rural areas as a result of runoff, fertilizers, livestock, etc. (Dupont, 2010). Ecological restoration can be done passively or actively. Passive restoration is based on the principle of resilient ecosystems and their ability to recover after disturbance (Folke *et al.*, 2004). Passive restoration involves the cessation of disturbance, and then allowing the vegetation and animals to naturally recolonize, which would also benefit the abiotic components of the ecosystem (Kaufman *et al.*, 1997). Active restoration takes a more aggressive approach to accelerate recovery in an ecosystem by directly changing the disturbed ecosystem so that it mimics historical norms (Watanbe *et al.*, 2005). Examples of active restoration include riparian vegetation planting, streambed alterations, channel morphology changes, and reintroduction of animals (Kaufman *et al.*, 1997). Because of the high costs of active restoration, passive restoration is preferred (Kirstensen *et al*, 2013).

The Grand River Conservation Authority (GRCA) in Ontario, Canada, created the Rural Water Quality Program in 1997 to help curtail impacts of agriculture on rural water quality in the Grand River watershed (GRCA, 2015b). The Rural Water Quality Program is a cost-sharing incentive to encourage farmers to engage in best management practices to help restore aquatic ecosystems (Dupont, 2010). Ninety-three percent of the land in the Grand River watershed is considered rural, though water quality is impacted by both rural and urban activities (GRCA, 2015a).

Livestock have been known to impact local aquatic ecosystems. Riparian vegetation typically act as buffers that filter nutrients and other pollutants out before they enter the water body. However, the grazing of riparian vegetation has created areas of bare ground, which have escalated the degree of erosion from wind and water (Miller *et al.*, 2010). The lack of plants in riparian zones as a result of grazing has therefore contributed to increases in nutrients and fine sediments in the water (Schlosser & Karr, 1981; Lowrance *et al.*, 1984; Cooper *et al.*, 1987). Moreover, when livestock are able to enter the water systems, they can trample and disturb aquatic habitats, urinate and defecate into the water and re-suspend sediments (Kauffman & Kruger, 1984; Yates *et al.*, 2000).

Stream bank fencing, which facilitates passive restoration, can help to improve riparian vegetation cover by preventing livestock grazing. As riparian vegetation recovers, improvements to water quality, in terms of chemical and biological components, are expected (Kauffman *et al.*, 1997; Miller *et al.,* 2010). Livestock restriction is one best management practice implemented through the Rural Water Quality Program. Over 284 fencing projects have fenced off approximately 190 km of stream bank (GRCA, 2015b). Because of budget constraints, there has been little to no monitoring done by the GRCA to assess the effectiveness of the projects in improving water quality (Dupont, 2010).

My project was designed to test whether fences have resulted in detectable improvements in water quality, measured by using benthic macroinvertebrates (e.g. Weigal et al. 2000), and whether this can be inferred to have instigated passive ecological restoration of the aquatic benthic macroinvertebrate community. Galeone (2000) had observed increases in taxa richness between downstream of fenced areas when compared to upstream samples. My study consisted of two components – a spatial component to determine if fence length and/or fence age influenced passive restoration, and a temporal study to determine if there were detectable changes in benthic macroinvertebrate communities within fenced areas from 2007 to 2014. For the spatial study, 11 creeks across the Grand River watershed were sampled in 2014. The 11 creeks belonged to 4 different categories according to the fence length, and age of fence – long and old, long and young, short and old, short and young. Sampling occurred once each month from May to August, using the travelling kick and sweep method, as part of the Ontario Benthos Biomonitoring Network (OBBN) protocol (Jones *et al.*, 2007). Samples were collected downstream, midstream, and upstream of each fenced location. The following biological indices were calculated; taxa richness, Shannon-Weiner Index, Simpson's Index, Hilsenhoff's Family Biotic Index, and the abundances (%) of Ephemeroptera-Plecoptera-Trichoptera, Chironomidae, and Oligochaeta in the samples. ANCOVAs and Kruksal-Wallis tests were used to statistically analyze the data. It was expected that if passive restoration had occurred, there would be improvements in benthic invertebrate communities in terms of increases in taxa richness, a higher diversity of invertebrates intolerant to pollution, and a lower diversity of invertebrates tolerant to pollution. It is also expected that longer fences that are older would have the highest water quality because the longer buffers would more effectively decrease the amounts of pollutants in the water, and having the fencing installed for a longer period of time gives the ecosystem more time to recover. However, in studies conducted in smaller watersheds than the Grand River, minimal water quality improvements were observed through livestock exclusion (i.e. Miller *et al.*, 2009; Line *et al.*, 2000). Similarly Walsh (2007) analyzed a case where 2.4 km of fencing, bank stabilization projects, and stream crossings were also installed. After 3-5 years post livestock exclusion, there were no changes in the diversity of the benthic macroinvertebrates (Carline & Walsh, 2007).

For the temporal study, 7 sites were resampled in 2014 that were originally sampled in 2007 (Neary, 2008). The biological indices of taxa richness, Shannon-Weiner Index, Simpson's Index, abundances of Ephemeroptera-Plecoptera-Trichoptera, Chironomidae, and Oligochaeta, Hilsenhoff's Family Biotic Index and Jaccard's Coefficient were calculated to compare the two sets of data. It was expected that if passive restoration had been occurring, the benthic invertebrate communities in 2014 would be more diverse, have higher abundances of invertebrates that are more sensitive to pollution, and ultimately indicate that water quality had been improving. However, if livestock exclusion does not seem to be facilitating passive restoration, other methods of restoration may need to be considered in order to improve water quality.

Spatial Study Methodology

Study Area and Site Selection

The Grand River watershed is one of the largest watersheds in Ontario draining 6965km^2 of area, and ultimately draining into Lake Erie. Approximately 93% of the watershed is considered rural and 78% of the land is used for agriculture (GRCA, 2015a). The Grand River watershed has a complex geography, consisting of till and clay plains and moraines, 18 subwatersheds, and four main tributaries – the Speed, Nith, Conestogo, and Eramosa rivers. All of the rivers, streams, and creeks in the Grand River watershed have a total length of about 11650 km (GRCA, 2010).

From the February 2014 inventory of 284 fencing projects the Rural Water Quality Program, each fence was categorized into different groups based on fence length and the year the fence was installed. Long fences were 800m or longer, short fences were 400m or shorter, and medium fences were between 400m and 800m. Old fences were considered to be fences installed in 2004 or earlier, fences that were considered young were built from 2008 to 2014, and middle fences were built between 2004 and 2008. The lengths and ages were categorized in this manner by looking at all the sites overall, and then dividing all the sites into the three age and length categories. I chose fences that were at the extremes of fence age and length (old or young fences, and long or short fences), so that any differences in benthic macroinvertebrate community diversity would have been more likely to be detected, if they existed.

Based on the attributes of the fences available, 11 sites were chosen for the study that were categorized into 4 groups – long and old fences, long and young fences, short and old fences, and short and young fences. The fences that were chosen for each group were chosen because they had the most similar fence lengths, and were installed during similar times (Figure 4.1; Figure 4.2; Table 4.1; Table 4.2).

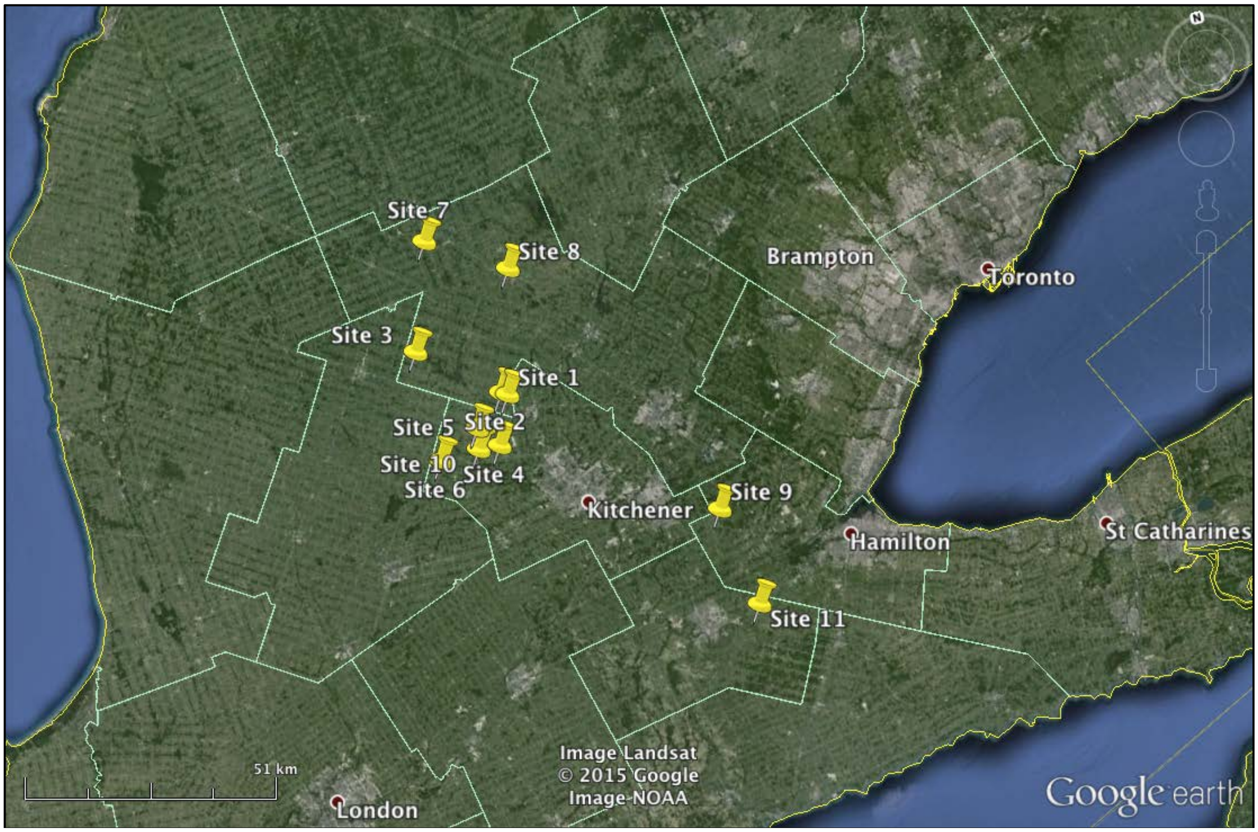

Figure 4.1: Map of the 11 original sites sampled across the Grand River watershed in 2014 (Google Earth, 2015)


a) Site 1: June 9, 2014.


b) Site 2: June 9, 2014.


c) Site 3: June 13, 2014.


d) Site 4: June 14, 2014.


e) Site 5: July 14, 2014.


f) Site 6: June 10, 2014.

g) Site 7: June 9, 2014.

h) Site 8: June 9, 2014.

i) Site 9: August 13, 2014.

j) Site 10: June 14, 2014.

Figure 4.2: Images of the 10 field sites sampled throughout May-August 2014 in the Grand River watershed.

Table 4.1: Description of sampling locations according to fence lengths and time since installation.

Site	Fence Length (m)	Length Category	Fence Year	Age Category
1	1051	Long	2009	Young
2	810	Long	2009	Young
3	1040	Long	2010	Young
4	156	Short	2009	Young
5	945	Long	2002	Old
6	1000	Long	2002	Old
7	152	Short	2002	Old
8	155	Short	2002	Old
9	96	Short	2002	Old
10	214	Short	2008	Young
11	1170	Long	2002	Old

Table 4.2: Coordinates of all 33 stations sampled for benthic macroinvertebrates (A – Downstream, B – Fenced Area, C – Upstream).

Station	Coordinates	
1A	N 43°36.172'	W 080°38.517'
1B	N 43°36.191'	W 080°38.351'
1C	N 43°36.285'	W 080°38.383'
2A	N 43°37.579'	W 080°39.944'
2B	N 43°37. 551'	W 080°40.015'
2C	N 43°37.544'	W 080°40.079'
3A	N 43°42.407	W 080°50.503'
3B	N 43°42.478'	W 080°50.439'
3C	N 43°42.549'	W 080°50.376'
4A	N 43°30.821'	W 080°40.712'
4B	N 43°30.815'	W 080°40.725'
4C	N 43°30.796'	W 080°40.758'
5A	N 43°33.111'	W 080°44.062'
5B	N 43°33.118'	W 080°44.166'
5C	N 42°33.087'	W 080°44.238'
6A	N 43°31.306'	W 080°49.833'
6B	N 43°31.385'	W 080°49.702'
6C	N 43°31.349'	W 080°49.590'
7A	N 43°53.931'	W 080°45.607'
7B	N 43°31.350'	W 080°49.587'
7C	N 43°53.893'	W 080°45.518'
8A	N 43°49.334'	W 080°34.104'
8B	N 43°49.311'	W 080°34.084'

8C	N 43°49.263'	W 080°34.050'
9A	N 43°18.956'	W 080°11.336'
9B	N 43°18.968	W 080°11.359
9C	N 43°18.985'	W 080°11.370'
10A	N 43°30.749'	W 080°44.199'
10B	N 43°30.755'	W 080°44.149'
10C	N 43°30.772'	W 080°44.122'
11A	N 43°08.217	W 080°08.962'
11B	N 43°08.118	W 080°09.093'
11C	N 43°08.305	W 080°09.321'

Benthic Macroinvertebrate Sampling

At each of the 11 sampling sites, three benthic macroinvertebrate samples were collected – downstream (A), midstream (B), and upstream (C). The upstream sampling station acted as the control, while midstream and downstream samples were considered the locations that could be impacted by livestock. The downstream sampling location at each site was either where the stream was no longer fenced, or where a different farm property began. Upstream sampling locations at each site were located immediately upstream of where the fencing began. The samples collected in the fenced areas were approximately midway between the downstream and upstream sampling locations.

Benthic macroinvertebrates were collected using the travelling kick and sweep method outlined in the Ontario Benthic Biomonitoring Network (OBBN) Protocol using a 500µm D-net (Jones *et al.*, 2007**)**. One person kicked the disturbed the sediment while another person stood downstream and swept the net through the re-suspended sediment to capture the invertebrates that had been dislodged from the benthic layer. However, instead of collecting invertebrates in two riffles and a pool, invertebrates were collected through bank-to-bank transects that went through the varying microhabitats present. This is because many streams sampled were very shallow and therefore did not possess riffle-pool-riffle sequences. The goal was to collect at least 100 invertebrates from each sample because 100 invertebrates would be subsampled during sample processing (Jones *et al.*, 2007). The sampling effort at each microhabitat was related to the proportion of area that each microhabitat took up in each transect. When approximately 100 invertebrates had been collected through at least one transect, or three minutes of kicking and sweeping had occurred, large rocks and sticks were removed from the sample, and the sample was placed in a 1L mason jar and preserved with 70% ethanol.

Sample Processing

In the lab, each sample was drained of the ethanol, and the sample was rinsed with water. The sample was then randomized using a Marchant box (Marchant, 1989). A random number generator was used to select cells, and the entire contents of the chosen cell was removed and all of the invertebrates were picked and preserved in 70% ethanol until the cell containing the 100th invertebrate was thoroughly picked through to find of the all the invertebrates (Figure 4.3). A five-hour limit for invertebrate picking was established for practical reasons because some samples had low densities of invertebrates. Using a dissecting microscope, all insects were identified to family level, and all other invertebrates were identified to the taxa levels outlined in the OBBN Protocol (Jones *et al.* 2007). For invertebrates where identification was uncertain, identification was verified with benthic biomonitoring scientists at the Dorest Enviornmental Science Centre in Dorset, Ontario.

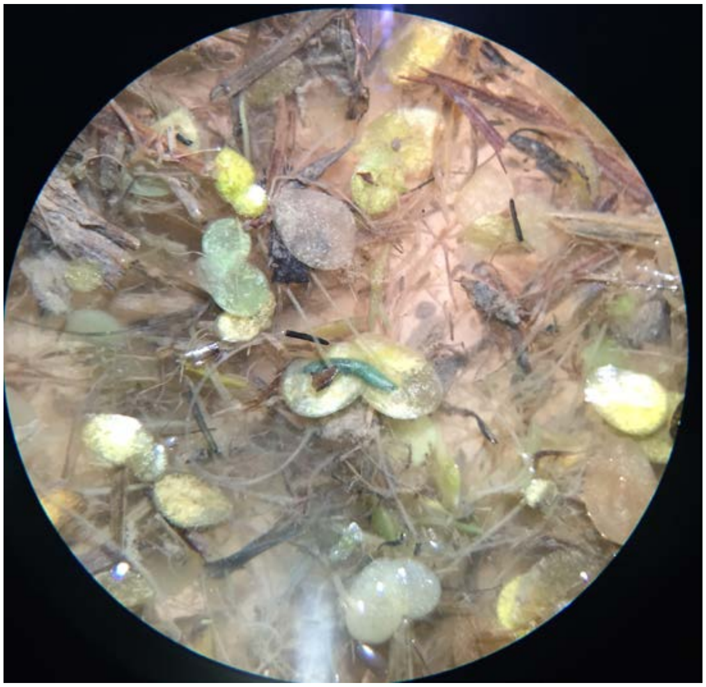

Figure 4.3: Sample picture of the typical contents in one cell of the Marchant box. Each cell usually consisted of plant matter, sediment, and invertebrates.

Data Analysis

The data were summarized using several biological indices: taxa richness, Shannon-Wiener Index and Simpon's Index, abundances of Ephemeroptera-Plecoptera-Trichoptera, Oligochaeta and Chironomidae were also calculated. Hilsenhoff's Family Biotic Index (HFBI) was used to assess the amount of organic pollution present in the water (Table 4.3). The tolerance values used were from Bouchard (2004)'s *Guide to Aquatic Invertebrates of the Upper Midwest* which uses the tolerance values from Hilsenhoff (1988) and uses Barbour *et al.* (1999) for the tolerance values that were not included in Hilsenhoff (1988).

Table 4.3: Hilsenhoff's Family Biotic Index values, with its relation to water quality, and the degree of organic pollution (Adapted from Hilsenhoff, 1988).

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very Good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly Poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very Poor	Severe organic pollution likely

Prior to analysis, all variables were tested for the assumption of normality (Shapiro-Wilk Test) and homoscedasticity (Levene's Test). Several variables were normally distributed and had homogeneous variances: May: taxa richness, % Chironomidae, Shannon-Wiener Index, FBI; June: Simpson's Index, Shannon-Wiener Index, FBI; July: % Chironomidae, HFBI; August: taxa richness, Shannon-Wiener Index, HFBI. For these, general linear models were used to test the homogeneity of regression between stations (A, B or C) and its interaction with fence length or fence age. If the homogeneity of regression assumption was not rejected, then an ANCOVA was run, using the number of years between when the fence had been built and 2014 (fence age), and the actual lengths (in metres) of the fence (fence length) as covariates ($\alpha = 0.05$). There were several variables that were non normal and/or had variances that were non homogenous: May: %EPT, %Oligochaeta, Simpson's Index; June: taxa richness, % EPT, %Chironomidae, %Oligochaeta; July: taxa richness, %Oligochaeta, Simpson's Index, Shannon-Wiener Index; August: %EPT, %Chironomidae, %Oligochaeta, Simpson's Index. For these, a Kruskal-Wallis Test was used to compare the biological indices between sites (A vs. B vs. C), between fence lengths (categorized as long or short), and between fence ages (categorized as new or old) separately, with an α of 0.05. The statistical analyses were conducted using Systat 13.0.

Temporal Study Methodology

Study Area and Sampling Sites

Of the 13 sites that were originally sampled in 2007 across the Grand River watershed, I was unable to contact 4 property owners, one site was now being used for crops rather than livestock, and one location was dry. Therefore, 7 sites were used for the comparison of benthic macroinvertebrates (Figure 4.4; Figure 4.5; Table 4.4).

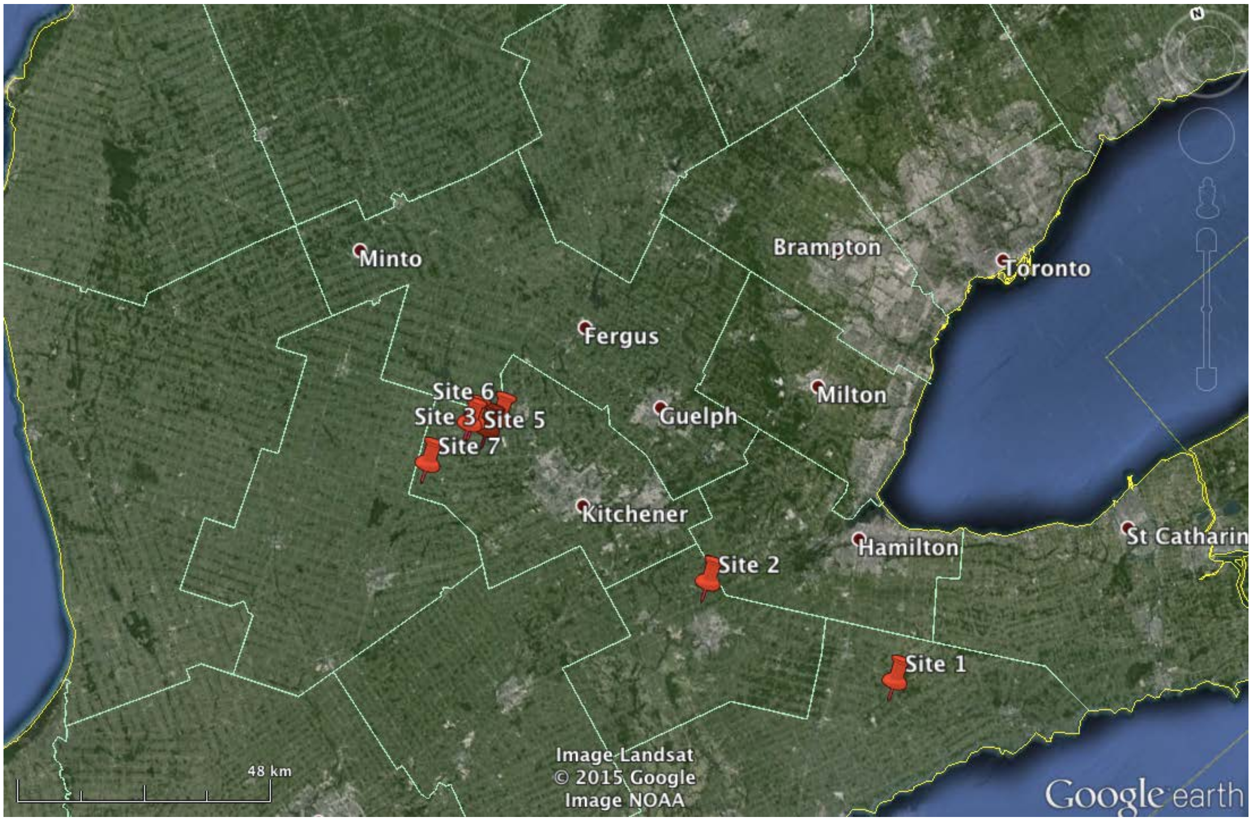

Figure 4.4: Map of sites sampled in 2014. Site 4 is not visible, but is in the cluster of Site 3, 5, 6 and 7.


a) Site 1


b) Site 2


c) Site 3


d) Site 4


e) Site 5


f) Site 6

g) Site 7
Figure 4.5: Images of the 7 sites in the Grand River watershed sampled in 2014.

Table 4.4: List of coordinates for all sites visited.

Site	Coordinates	
1	N 42°58.203'	W 079°53.937'
2	N 43°12.435'	W 080°15.346'
3	N 43°32.963	W 080°38.906'
4	N 43°32.965'	W 080°38.876'
5	N 43°33.412'	W 080°42.512'
6	N 42°33.529'	W 080°42.871'
7	N 43°33.478'	W 080°42.908'

Benthic Macroinvertebrate Sampling

The sampling dates chosen were as close to the 2007 conditions as possible. In 2007, samples were collected between August 2 and 28. In 2014, samples were collected on August 19 and 20. The 2007 locations were re-sampled in 2014 in as close proximity to the original sites as possible. The protocol used to sample benthic macroinvertebrates in 2014 was the same as the 2007 protocol (Neary, 2008). At each site, a 500µm mesh kick-net was used. Beginning in the middle of the channel and walking upstream, one person kicked and disturbed the substrate while one person stood immediately downstream of the kicker and used the kick-net to collect the disturbed sediment and the invertebrates that had been dislodged. This continued for 1 minute and the speed travelled upstream was dependent on the variety of microhabitats present in the reach. Once 1 minute had elapsed, the contents of the net were transferred into a glass Mason jar, and 70% ethanol was poured into the jar to immediately preserve the sample. Sites 1, 2, 3, 5 and 7 were sampled in one location, and sites 4 and 6 were sampled in 3 different locations as per the sampling method from the 2007 study (Neary, 2008). In the lab, all invertebrates were picked out of the samples using a dissecting microscope and identified and counted. All insects were identified to family level, and all other invertebrates were identified to the mixed taxonomic level classification in the Ontario Benthic Biomonitoring Network protocol (Jones *et al.* 2007). The average abundance of invertebrates in the 3 samples for site 4 and 6 were calculated and used for analysis. All invertebrates were re-preserved in vials of 70% ethanol for future reference.

Data Analysis

The data were summarized for each sample using the following biological indices: taxa richness, Shannon-Wiener Index and Simpson's Index, abundance (%) Ephemeroptera-Plecoptera-Trichoptera, Oligochaeta, and Chironomids. The Family-Level Hilsenhoff Biotic Index was used to assess the amount of organic pollution present in the water. The tolerance values used were from Bouchard (2004)'s *Guide to Aquatic Invertebrates of the Upper Midwest* which uses the tolerance values from Hilsenhoff (1988) and uses Barbour *et al.* (1999) for the tolerance values that were not included in Hilsenhoff (1988) (Table 3.3). Lastly, Jaccard's Coefficient was calculated to compare the differences in the presence/absence of invertebrate taxa between sites.

Prior to analysis, all variables were tested for the assumption of normality (Shapiro-Wilk Test) and homoscedasticity (Levene's Test). For the variables that met these assumptions (% EPT, %Chironomidae, taxa richness, Simpson's Index, Shannon-Wiener Index, and Hilsenhoff's Family Biotic Index), a paired t-test was performed (α= 0.05). For the other variables (% Oligochaeta), a Mann-Whitney U-test was performed (α= 0.05). Systat 13.0 was used to conduct all statistical analyses.

Spatial Results

The streams at several of the sampling stations (1C, 9A, 9B, 9C) dried up during the sampling season so benthic macroinvertebrates could not be collected there. Site 11 had too small a sample size (there were few invertebrates) and was excluded from the rest of the study.

Abundance of Invertebrates

Overall 109 samples were collected across all the sampling sites from May to August, and a total of 11394 invertebrates were subsampled from the samples. The most abundant invertebrates collected were Chironomidae (32.6%), Isopoda (20.9%), and Oligochaeta (14.8%) (Table 4.5).

Table 4.5: Abundances of invertebrates at each sampling station (A, B and C) at all 10 sites sampled in the Grand River watershed in 2014.

Taxa	Abundance (%)
Turbellaria	1.439
Nematoda	4.520
Oligochaeta	14.850
Hirudinea	0.843
Isopoda	20.915
Bivalvia	3.318
Amphipoda	0.606
Decapoda	0.219
Gastropoda	3.256
Hydrachnidia	0.246
Coenagrionidae	0.079
Lestidae	0.009
Perlodidae	1.106
Perlidae	0.035
Caenidae	0.377
Ephemerellidae	0.035
Heptagenaidae	0.298
Baetidae	0.562
Gerridae	0.009
Veliidae	0.035
Corixidae	0.834
Brachycentridae	0.097
Hydropsychidae	2.238
Hydroptilidae	0.430
Odontoceridae	0.026
Philoptamidae	0.009
Polycentropodidae	0.009
Heliopyschidae	0.044
Rhycophilidae	0.009
Limnephilidae	0.009
Unoeidae	0.132
Pyralidae	0.070
Dytiscidae	0.579

Elmidae	6.398
Haliplidae	0.123
Hydrophilidae	0.272
Gyrinidae	0.035
Psephenidae	0.070
Athericidae	0.158
Psychodidae	0.026
Ceratapagonidae	1.641
Chironomidae	32.605
Empididae	0.500
Culicidae	0.009
Ephyridae	0.474
Simuliidae	0.272
Tabanidae	0.088
Tipulidae	0.088

Water Quality of Sites

Based on Hilsenhoff’s Family Biotic Index, all samples were characterized as having fair to very poor water quality (terms defined in Table 4.3). There was only one sample (7A in June) that was considered to have good water quality (Figure 4.6). The most frequent type of water quality at the stations in May was fairly poor (43.3%), fairly poor in June (51.9%), poor in July (36%), and poor in August (37.5%) (Figure 4.6). Fairly poor water quality indicates substantial organic pollution likely, and poor water quality indicates that there is substantial eutrophication, presumably from agricultural run-off and other sources (Figure 4.6).

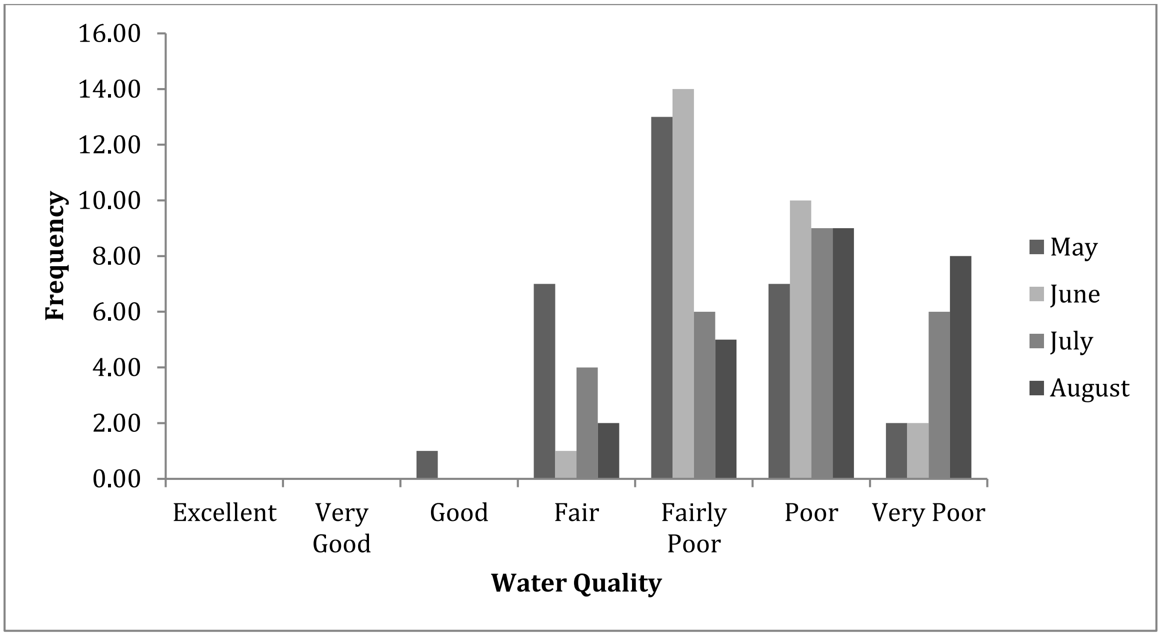

Figure 4.6: Changes in water quality of all sampling stations based on Hilsenhoff's Family Biotic Index of 10 sites across the Grand River watershed between May, June, July, and August of 2014.

Biological Indices Analysis

There were no significant differences between the stations (upstream vs. fenced area vs. downstream), the length of the fence, or the age since fencing - as per ANCOVAs used to test differences between sites in regards to taxa richness, % EPT, % Chironomidae, % Oligochetae, Simpson's Index, Shannon-Wiener Index or HFBI (Table 4.6; Table 4.7).

Table 4.6: ANCOVA p-values of normally distributed and homogeneous data used to assess the effects that fence age and length (as covariates) had on upstream, midstream and downstream macroinvertebrate assemblages by calculating biological indices from May-August, 2014.

	May				June			July		August		
Covariate	Taxa	%Chironomidae	Shannon	HFBI	Simpson	Shannon	HFBI	%Chironomidae	HFBI	Taxa	Shannon	HFBI
Length	0.904	0.71	0.845	0.515	0.445	0.582	0.378	0.769	0.938	0.305	0.531	0.688
Age	0.865	0.761	0.773	0.605	0.425	0.551	0.387	0.716	0.932	0.284	0.459	0.773

Table 4.7: ANCOVA F-values (with degrees of freedom) of normally distributed and homogeneous data used to assess the effects that fence age and length (as covariates) had on upstream, midstream and downstream macroinvertebrate assemblages by calculating biological indices from May-August, 2014.

	May				June		
Covariate	Taxa	%Chironomidae	Shannon	HFBI	Simpson	Shannon	HFBI
Length	(2,26) = 0.101	(2, 26) = 0.347	(2, 26) = 0.169	(2, 26) = 0.472	(2, 24) = 0.611	(2, 24) = 0.553	(2, 24) = 1.014
Age	(2, 26) = 0.146	(2, 26) = 0.277	(2, 26) = 0.260	(2, 26) = 0.512	(2, 24) = 0.888	(2, 24 = 0.611	(2, 24) = 0.988

July		August		
%Chironomidae	HFBI	Taxa	Shannon	HFBI
(2, 22) = 0.266	(2, 22) = 0.065	(2, 21) = 1.258	(2, 21) = 0.653	(2, 21) = 0.381
(2, 22) = 0.339	(2, 22) = 0.071	(2, 21) = 1.336	(2, 21) = 0.807	(2, 21) = 0.261

Kruskal-Wallis tests indicated significant differences between Simpson's Index and fence age in May (p=0.002) with older fences being associated with a higher Simpson's Index value. Additionally, taxa richness and fence age in June (p=0.012) and taxa richness and fence age in July (p=0.027) both had increased richness in areas with older fences, % Oligochaeta and fence age in June (p=0.031) and July (p=0.024) with younger fences having more Oligochaeta present in both months, Shannon-Wiener Index and fence age in July (p=0.037) with old fences having a higher value, and Simpson's Index and fence age in August (p=0.026) with old fences having a higher value. Where there were significant effects of fence age, Simpson's Index showed higher values with shorter fences (July, p=0.013), the Shannon Wiener Index showed higher values with shorter fences (July, p=0.12), and the percent of Chironomids was higher in shorter fences than longer fences (August, p=0.008). There were no significant differences among the upstream, fenced area, and downstream stations in any index during any month (Table 4.8; Table 4.9).

Table 4.8: Kruskal-Wallis p-values for data that was not normally distributed and/or non-homogeneous from May to August 2014 to compare the impacts upstream, midstream, and downstream locations; fence length; and fence ages had on different biological indices calculated based on benthic macroinvertebrate assemblages.

	May			June			
	%EPT	%Oligochaeta	Simpson	Taxa	%EPT	%Chironomidae	%Oligochaeta
Station	0.904	0.173	0.75	0.598	0.929	0.7	0.432
Length	0.949	0.917	0.772	0.563	0.74	0.073	0.232
Age	0.134	0.709	0.002	0.012	0.406	0.854	0.031

	July					August			
	Taxa	%EPT	%Oligochaeta	Simpson	Shannon	%EPT	%Chironomidae	%Oligochaeta	Simpson
Station	0.791	0.979	0.076	0.701	0.716	0.895	0.415	0.139	0.557
Length	0.069	0.16	0.959	0.013	0.012	0.377	0.008	0.173	0.283
Age	0.027	0.054	0.024	0.05	0.037	0.053	0.314	0.828	0.026

Table 4.9: Kruskal-Wallis (H) and Mann-Whitney U-Test (U) values for data that was not normally distributed and/or non-homogeneous from May to August 2014 to compare the impacts upstream, midstream, and downstream locations; fence length; and fence ages had on different biological indices calculated based on benthic macroinvertebrate assemblages. Kruskal-Wallis tests provided Mann-Whitney U-Test values for tests run with nominal variables that had only two values (e.g. fence length, and fence age).

	May			June			
	%EPT	%Oligochaeta	Simpson	Taxa	%EPT	%Chironomidae	%Oligochaeta
Station H(2)	0.201	3.51	0.576	1.02	0.146	0.713	1.678
Length U(1)	0.949	110	119.5	110	104	59	72
Age U(1)	0.134	103.5	37	43.5	83	94	145

	July					August			
	Taxa	%EPT	%Oligochaeta	Simpson	Shannon	%EPT	%Chironomidae	%Oligochaeta	Simpson
Station H(2)	0.468	0.043	5.166	0.712	0.669	0.223	1.759	3.942	1.171
Length U(1)	49	40	85	36	35	57	26	95	53
Age U(1)	41.5	49	128	46	43.5	44.5	59.5	74	37

Temporal Study Results

The most abundant invertebrates from 2007 were Hydropsychidae (25.1%), Elmidae (14.2%), and Amphipoda (12.8%). The most abundant invertebrates from 2014 were Chironimidae (27.2%), Isopoda (28.5%), and Elmidae (12.5%) (Table 4.10).

Table 4.10: Comparison of abundances of taxa between 7 different sampling locations sampled in 2007 and in 2014 in the Grand River watershed.

Taxa	Abundance (%)	
	2007	2014
Turbellaria	0.90	2.57
Nematoda	0.00	0.07
Oligochaeta	0.78	1.89
Hirudinea	1.67	0.54
Isopoda	5.55	28.47
Bivalvia	5.17	1.47
Amphipoda	12.83	0.59
Decapoda	2.62	0.25
Gastropoda	3.88	0.31
Hydrachnidia	0.42	0.25
Gomphidae	0.06	0.02
Libellulidae	0.36	0.00
Coenagrionidae	0.24	0.78
Caenidae	1.33	11.68
Ephemerellidae	0.06	0.02
Heptagenaidae	0.00	3.87
Baetidae	1.92	1.21
Ephemeridae	0.12	0.00
Leptohyphidae	0.12	0.19
Corixidae	4.33	0.10
Belostomatidae	0.12	0.00
Sialidae	0.48	0.10
Corudalidae	0.12	0.00
Hydropsychidae	25.09	2.75
Hydroptilidae	0.36	1.12
Polycentropodidae	0.00	0.05
Heliopyschidae	0.06	0.10
Unoeidae	0.00	0.07
Philopotamidae	1.13	0.00
Elmidae	14.23	12.53
Hydrophilidae	1.52	0.22
Dryopidae	0.06	0.00

Dytiscidae	0.51	0.00
Haliplidae	1.37	0.00
Psephenidae	0.30	0.11
Ceratapagonidae	0.24	0.05
Chironomidae	10.78	27.18
Empididae	0.06	0.07
Simuliidae	0.42	1.18
Tabanidae	0.42	0.14
Tipulidae	0.39	0.05

Taxa richness, Simpson's Index, % EPT, % Chironomidae, and % Oligochaeta were not significantly different between 2007 and 2014 ($p>0.05$). There was however a significant difference between the benthic macroinvertebrate assemblages between 2007 and 2014 for the Shannon-Wiener Index ($p=0.024$) where the values were higher on average in 2007 than 2014 (Table 4.11 & Table 4.12).

Table 4.11: Biological indices calculated from surveying benthic macroinvertebrates in August 2007.

Location	%EPT	%Chironomidae	%Oligochaeta	Taxa	Simpson's Index	Shannon-Wiener	Tolerance Value	Water Quality
1	19.83	14.88	0.00	16	0.81	1.86	5.00	Good
2	2.04	28.57	8.16	12	0.79	1.82	6.86	Poor
3	64.00	6.67	0.27	17	0.62	1.51	4.57	Good
4	12.93	25.86	0.86	17	0.88	2.40	6.08	Fairly Poor
4	26.69	22.31	0.40	19	0.86	2.24	5.58	Fair
4	6.21	8.70	0.00	14	0.58	1.43	7.66	Very Poor
5	13.04	10.43	0.00	22	0.92	2.72	5.65	Fairly Poor
6	43.75	4.17	1.25	14	0.75	1.78	5.64	Fair
6	50.32	11.08	0.00	14	0.70	1.57	4.78	Good
6	15.52	2.30	0.00	18	0.84	2.26	5.28	Fair
7	22.44	8.52	0.00	24	0.83	2.20	5.21	Fair

Table 4.12: Biological indices calculated from surveying benthic macroinvertebrates in August 2014.

Location	%EPT	%Chironomidae	%Oligochaeta	Taxa	Simpson's Index	Shannon-Wiener	Tolerance Value	Water Quality
1	0.43	46.38	3.83	11	0.63	1.29	5.77	Fairly poor
2	0.00	5.26	57.89	4	0.56	0.99	8.53	Very Poor
3	25.27	28.28	0.43	24	0.78	1.84	6.46	Fairly Poor
4	11.43	46.53	13.06	15	0.72	1.64	6.13	Fairly Poor
4	21.45	55.81	5.92	23	0.64	1.51	6.35	Fairly Poor
4	18.18	61.21	1.41	11	0.59	1.29	5.74	Fair
5	30.32	25.53	3.19	15	0.79	1.92	5.34	Fair
6	60.50	17.02	2.52	22	0.64	1.60	6.68	Poor
6	38.33	35.33	7.00	16	0.73	1.66	6.65	Poor
6	11.63	30.81	6.40	11	0.79	1.81	7.02	Poor
7	8.97	6.96	0.00	16	0.63	1.47	6.73	Poor

The differences between Hilsenhoff's Family Biotic Index values between 2007 and 2014 were also statistically significant (p=0.037). Based on Hilsenhoff's Family Biotic Index as a proxy for water quality and degree of organic pollution, 5 sites decreased in water quality (sites 1, 2, 3, 6 and 7), one site remained the same (site 4), and one site had an improvement in water quality (site 5) (Figure 4.7). Moreover, according to HFBI, there has been an overall decrease in water quality, with a likely increase in organic pollution (Table 4.3, Table 4.13). Congruently, Jaccard's Coefficient revealed that there have been changes in the types of taxa present between the two sampling periods. Site 2 had the biggest change in taxa composition between 2007 and 2014; and site 3 had the most similar composition of taxa between 2007 and 2014 (Table 4.14).

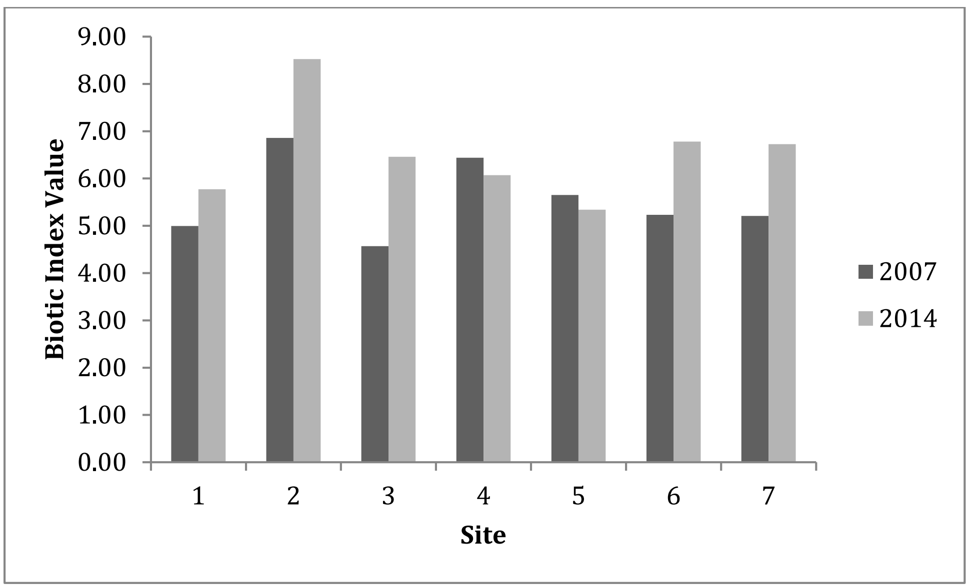

Figure 4.7: Comparison of Hilsenhoff's FBI between samples collected in 2007 and 2014 at 7 different sites in the Grand River watershed.

Table 4.13: Comparison of water quality between sites sampled in 2007 and in 2014 using Hilsenhoff's Family Biotic Index.

Site	2007	2014
1.00	Good	Fairly Poor
2.00	Poor	Very Poor
3.00	Good	Fairly Poor
4.00	Fairly Poor	Fairly poor
5.00	Fairly Poor	Fair
6.00	Fair	Poor
7.00	Fair	Poor

Table 4.14: Jaccard's Coefficient values comparing the presence and absence of taxa between 2007 and 2014 of each of the 7 sites sampled in the Grand River watershed.

Site	Value
1	0.25
2	0.23
3	0.60
4	0.52
5	0.44
6	0.57
7	0.50

Discussion

Spatial Study

Overall, livestock exclusion does not seem to be having a statistically detectable impact on benthic macroinvertebrates. The most abundant macroinvertebrates present in the samples were Chironomidae, Oligochaeta, and Isopoda, all of which are tolerant to a wide variety of pollutants. Oligochaetes in particular are common indicators of organic pollution and are tolerant to a variety of trophic ranges (Chapman *et al.*, 1982; Saether, 1979). Isopoda can be present in lower numbers in relatively clean water (Adcock, 1979); however, it is more common to observe increased abundance in Isopoda where there is increased nutrient loading (Aston & Brown, 1975). Chironomidae are highly diverse taxa, and also have a wide variety of tolerances to different environmental conditions; therefore, they are usually the most abundant taxa in a sample (Pinder, 1986).

The benthic macroinvertebrate community data are congruent with the interpretation of the state of the ecosystem based on Hilsenhoff's Family Biotic Index. HFBI indicated that all of the streams were still subject to eutrophication. Based on Hilsenhoff's Family Biotic Index, water quality progressively degraded from May to August. However, this may have been a natural issue because of the decreased stream flow typical in the spring to summer transition in temperate climates. Decreased stream flow entails decreased habitat availability for invertebrates, which leads to decreases in taxa richness (Cazaubon & Giudicellim, 1999). When the flow rate decreases, there is also less dissolved oxygen, killing sensitive macroinvertebrates and sparing ones more tolerant to such stress (Cortes *et al.* 2002).

There were no significant differences when comparing indices downstream, upstream and fenced area samples, indicating that the fences have not changed the benthic invertebrate compositions. However, there were some indices (though not many when considering how many tests were conducted) that appear to be impacted by fence age and length. Most benthic macroinvertebrate indices showed no effect from fence age but the ones that were significantly different supported my expectations. Fences that were old were associated with higher diversities of taxa in the streams, had higher Simpson Index and Shannon-Wiener Index values, and had lower abundances of Oligochaeta than the newer fences. The data did not consistently support the expectations about the impact fence lengths have on the indices. Shorter fences had higher abundances in Chironomidae than longer fences, which were expected because shorter fences have shorter buffer areas to prevent nutrient inputs into the water. However, based on the data, shorter fences had higher Simpson's Index and Shannon-Wiener Index values than longer fences, which was not expected because higher index values indicate evenness amongst taxa abundances and higher taxa diversity, which I expected longer fences to have compared to shorter fences.

Temporal Study

Hydropsychidae and Elmidae, two of the three most abundant taxa in 2007, are indicators of high eutrophication. Because of their tolerance to organic pollutants, Hydropsychidae can become abundant under eutrophic conditions (Geraci *et al.*, 2010). Elmidae are similarly unaffected by factors such as agricultural runoff and thrive in eutrophic environments (Lenat, 1984). The third abundant family in 2007, Amphipoda, is considered to be medially tolerant to eutrophication and their abundance is less clear as an indicator (Bouchard, 2004). In 2014, the most abundant taxa were Chironomidae, Isopoda, and Elmidae, which are all fairly tolerant organisms, suggesting the presence of pollutants in the water. The Chironomidae family have a wide variety of tolerances to different environmental conditions; therefore, they are usually the most abundant taxa in a sample (Pinder, 1986). Additionally, Isopoda abundances increase in the presence of eutrophication (Aston & Brown, 1975). Although there are differences in the most abundant taxa between 2007 and 2014, the abundant taxa are all still considered to be tolerant to pollution.

The Shannon-Wiener Index, Jaccard's Coefficient, and Hilsenhoff's FBI reveal that there are some differences between 2007 and 2014. The significant decrease in the Shannon-Wiener

Index from 2007 and 2014 indicates that there were decreases in community diversity and evenness. Jaccard's Coefficient calculation suggested that the community compositions based on the presence and absence of different taxa between samples of each site were different. The significant difference of the Shannon-Wiener values, in tandem with Jaccard's Coefficient values that signify that the 2007 and 2014 taxa compositions were different, support the significant difference in Hilsenhoff's Family Biotic Index between 2007 and 2014. On average, the HFBI values increased from 2007 to 2014, which implies that water quality has decreased, suggesting that the fences have not facilitated in improving water quality and may have actually decreased water quality. However, the lack of statistically significant differences between 2007 and 2014 invertebrates in terms of taxa richness, Simpson's Index, and abundances of EPT, Chironomidae, or Oligochaeta imply that livestock exclusion may not have had a large impact on the benthic macroinvertebrates overall.

Conclusion

Because of the lack of significant differences in the statistical analysis, it is difficult to conclude if livestock exclusion has influenced passive restoration or not. My results suggest that local efforts of stream fencing do not appear to be effective because there were not large improvements to the diversity of invertebrates in the streams. The lack of positive results may indicate that a larger watershed-wide restoration approach might be more effective at addressing the issues of water quality. Herbst *et al*. (2011) assessed ecological restoration performed at the reach scale; and they found increases in taxa richness between areas where livestock had been removed from larger areas, and areas where livestock grazing was still happening. In contrast, when upstream and fenced areas were sampled at small scales, there was no noticeable change in benthic macroinvertebrate communities. Their interpretation was that upstream livestock influence downstream water quality; and that livestock exclusion may be improving water quality at a small scale, but influences upstream may have a bigger impact on overall water quality. Though I did not examine this factor, the amount of livestock present can also impact the invertebrate communities; for example, Braccia & Voshell (2007) determined that higher intensities of grazing had higher impacts on the benthic invertebrates. It is possible that if more of the 284 fencing projects were sampled, there may be some clearer improvements detected. An effect may have been detected if the sample size was larger. A power analysis was not conducted because the number of sites available to be sampled that were similar in fence age and length was already a limiting factor. Using the different sites as random factors may also be a good alternative to potentially detect statistical differences, but because of the research question and the nature of the data collected, it was not plausible to also consider the sites as random factors. Another option would have been to use a repeated measures test however the effects of the varying stream conditions and the reduction of stream flow between May and August may influence the results.

Considering the inconclusive results of the study, what then is the benefit of the Rural Water Quality Program? This question will require further study but I would argue that the intent was to build community support for changes in farm management. For example, the RWQP encourages farmers to create Environmental Farm Plans for best economic and ecological practices. The GRCA and farmers acknowledge that it takes a lot of time and cumulative effort to have a detectable and significant positive ecological impact on a watershed, particularly a watershed as large as the Grand River. Because localized restoration efforts do not seem to be working, a larger scale project should be considered. All upstream influences impact downstream water bodies; therefore, it is important to address the fundamental problems causing water degradation at the headwaters, and then progressively restore downstream ecosystems. Active restoration practices such as changes to stream morphology, and direct riparian vegetation planting probably need to be implemented because allowing a stream to passively restore itself does not seem to be occurring within a reasonable timeframe. Restoration that requires an extended amount of time to occur may lead to repercussions in the future. My study indicates that the work is far from complete and perhaps one strategy might be to start with (a) headwaters, to deal with upstream influences, and (b) shift from simple fencing to more active restoration and nutrient management plans that not only curtail nutrient inputs into the water, but also address the problem of the elevated nutrient levels that already exist in the water.

5. Moving from wishes (fencing, passive restoration, historical fidelity) to action (testing active ecological restoration across ecotones and at subwatershed scales in novel ecosystems)

Our two studies together suggest that passive restoration is not occurring (at least at a detectable rate) where fencing has been implemented. The most abundant invertebrates collected in both studies were invertebrates that are tolerant to a wide range of pollutants. The invertebrates that are sensitive to pollutants were sparse or completely absent in many of the samples. Regardless of the lengths and the ages of the fences, there were no differences in benthic invertebrate assemblages between downstream, at the fenced areas, and upstream of the fences. Similarly, there were no consistent differences between invertebrate communities sampled in 2007 and 2014. The streams are all characterized as impacted because of the level of organic pollution present according to Hilsenhoff's Family Biotic Index.

Scale of Restoration

Many other studies are congruent with my own study that suggests restoration needs to happen on a larger scale – at least at the catchment scale, but watershed scale is most ideal (i.e. Jahnig *et al.* 2010; Mueller *et al.* 2014). Ranganath *et al.* (2009) did not find a significant difference in the macroinvertebrate communities of streams with and without adjacent livestock grazing. They suggested that short sections of stream-bank fencing to restrict livestock access are not effective at improving biological integrity. The scale of restoration also depends on the goals. The purpose of the Rural Water Quality Program is to improve the water quality in the Grand River watershed that has been deteriorated by agricultural activity. Water quality is impacted by both physical and biological components and it is a combination of physical stream restoration and in-stream and adjacent land vegetation recovery that will restore water quality.

Importance of Riparian Buffers

Passive restoration should not be completely discredited. Although stream bank fencing may not have had a large indirect impact on the benthic macroinvertebrates in this study, it still did prevent livestock from grazing on riparian vegetation, allowing the riparian vegetation to grow. Riparian vegetation seems to be highly resilient because of its ability to recolonize after severe degradation. Kauffman *et al.* (1997) observed that even after 50 years of intensive agricultural disturbances of cattle grazing and water diversions on the riparian buffers, 24% of the lost riparian vegetation was able to re-establish within a few years. Considering that riparian vegetation is the first line of defense in protecting streams from nutrient inputs, the ability of riparian vegetation to regrow after fencing has been installed makes livestock restriction an important tool for conservation and restoration.

Longer riparian buffers along the stream banks may be necessary to improve benthic macroinvertebrate communities and water quality. When Parkyn *et al.* (2003) assessed riparian buffer zones and their impacts on benthic macroinvertebrates; they noticed that the most significant changes in benthic macroinvertebrate assemblages were a result of cooler water temperatures. Longer buffers that have canopy shading would facilitate in decreasing water temperatures. Having forested buffers along streams would be beneficial for water quality. Forested reaches of streams typically have cooler temperatures, wider channels, less suspended sediments, and high diversity of benthic macroinvertebrates (Sweeney, 1993; Storey & Cowley, 1997; Abell & Allan, 2002). Nevertheless, farmers are often opposed to having woody vegetation in the buffers because of the buildup of woody debris that would decrease pasture space (Carline & Walsh, 2007). It is important to note, however, that woody vegetation planted 10m from the stream would not contribute to the shading because it is too far from the stream (Watanbe *et al.,* 2005). Sovell *et al.* (2000) observed that grass buffers in rural streams of low stream orders are often sufficient for cooling the water because the tall grasses can shade the entire width of a low order stream. Therefore, a variety of riparian buffer types could help improve water quality, depending on stream size.

Rutherford *et al.* (1999) modeled streams in New Zealand and determined that buffers ranging from 1-20 km, depending on the stream order, and with 75% shading, would decrease the water temperature by 5°C. Complete shading is not desirable because it would decrease the primary productivity of macrophytes and periphyton, which would impact nutrient filtering and food availability for invertebrates (Rutherford et al. 1999).

In-Stream Modifications for Restoration

Livestock restriction and improvements to riparian vegetation can help facilitate passive restoration. However, considering the lack of progress with these passive restoration efforts, a more active approach may be needed. In-stream modifications therefore may be required to help increase taxa richness of benthic macroinvertebrates, and improve water quality.

Because unrestricted livestock, and the lack/destruction of riparian vegetation buffers have increased sedimentation in the water, the habitats in the streambeds have become more homogeneous and support a lower diversity of invertebrates. Wagenhoff *et al.* (2012) argue that if fine sediment covers over 5% of the streambed, taxa richness of invertebrates begins to be impacted. Mueller *et al.*, (2014) saw increases in invertebrate taxa richness when the streambed was raked and gravel was introduced. Additionally, a meta-analysis performed by Miller *et al*. (2010) on studies that had assessed effects of in-stream habitat restoration on benthic macroinvertebrates observed that there was a significant increase in taxa richness as a result of increased physical habitat heterogeneity. Re-meandering a stream that has been channelized may help to improve habitat heterogeneity. If left alone, higher order streams can re-meander themselves naturally. Low order streams however may not be able to return to natural or "least disturbed condition" streams because of their low stream power (low slopes and discharge rates) that is not effective at altering the sinuosity of streams, or the substrate compositions (Krisensen *et al.*, 2013). Heavy machinery would need to be used to change the shape of the stream.

Simply changing the composition of the streambed may not be sufficient in long-term restoration however. Brooks *et al*. (2002) monitored invertebrates at riffles prior to disturbance, after a disturbance, and when the sites had been restored by adding different sizes of streambed particles to change habitat heterogeneity and stream flow, they did not see a difference in re-colonization patterns amongst invertebrate communities between different restoration methods.

The pollutants present in the stream also need to be considered when trying to improve water quality. Even if nutrient inputs are curtailed, the nutrients that are embedded in the sediment can still be washed downstream. Nutrient retention and uptake is therefore important in preventing large nutrient loads downstream (Hall *et al.* 2002; Weigelhofer *et al.* 2012). Bukaveckas (2007) and Craig *et al.* (2008) suggest that re-meandering a stream, or adding woody debris would help increase nutrient retention in the sediment because of the increases in the occurrence of pools and longer meander sequences of streams which would allow for more time for nutrients to be absorbed by biota in the stream, or by adjacent riparian vegetation. It is clearly the combination of interactions between the physical and biological components of a stream that can help improve water quality. Hence, both components must be considered for restoration projects to be successful.

Adaptively Managing the Novel Ecosystems

The Rural Water Quality Program is a voluntary program for farmers that require them to invest money and time into the projects they decide to implement. Davies *et al.* (2008) argue that with the limited resources available for biodiversity protection, it is important to allocate the resources to areas that would benefit the most. The Environmental Stewardship is an English agri-environmental initiative that operates similarly to the Rural Water Quality Program of the GRCA. The majority of the buffer strips along farms in south England are three times narrower than required to be effective. Davies *et al.* (2008) suggest that it would therefore be more beneficial to have used those resources at targeted areas that would have benefitted the most from being protected.

The streams in the Grand River watershed are still considered impacted because of a variety of influences from nutrient inputs from tile drains, submerged livestock crossings, upstream areas where livestock are not fenced off from the stream, pesticides, and other nutrients that enter the streams through runoff, etc. However, it is not only agricultural activities that are polluting the streams. Urban impacts through construction, storm water runoff, sewage overflows, and lawn maintenance are also influencing water quality (IJC, 2014). The water quality of Lake Erie has been a large concern for decades, because of intensive nutrient inputs from non-point sources upstream (IJC, 2014). The GRCA is in the early stages of developing soil and nutrient management programs to improve water quality by identifying areas of highest concern (GRCA, 2014). However, a larger scale program must be established that considers the synergism of both rural and urban influences on the water quality of the Grand River watershed because these novel ecosystems respond differently to restoration. The multiple stressors on the streams require a more complex method of restoration. A joint nutrient management plan amongst rural and urban areas needs to be developed in the future.

Considerations for Future Research

Because of the site selection restrictions based on fence age and length, streambed substrates, stream order, channel morphology, canopy cover, riparian buffer widths and geographic location were not assessed. If these other variables were accommodated for, the results may have been different. Other biological indicators, such as fish, periphyton, and macrophytes were also not assessed though so I do not know if they benefitted from the stream-fencing program. Different biological indicators can respond to restoration differently. Restoration success of a particular area may be indicated by one group of organisms, but not for another group of organisms (Mueller *et al.*, 2014). The amount of effort used for biomonitoring however comes down to practicality, and resource availability. There have been a variety of studies that have observed changes in benthic macroinvertebrates in response to changes in land use and habitat (Mueller *et al.*, 2014; Galeone, 2000; Herbst *et al*., 2011), but also studies that have not seen any differences when riparian buffer conditions change (Ranganath *et al.*, 2009; Carline & Walsh, 2007). In the present study I focussed on benthic macroinvertebrates so that I could characterize a variety of streams across the entire Grand River watershed in an attempt to understand what is going on at a watershed scale.

The next steps to restore water quality in the Grand River watershed are to determine which ecosystem services have been impaired or lost, and determine which restoration techniques would be the most effective and efficient at restoring water quality and ecosystem functions8 in these agricultural settings that are also impacted by urbanization. Nutrient inputs to the streams are still ongoing, and that is the first issue. Best management practices of when to apply fertilizer, where to store manure, how far away livestock pens should be from streams, etc. need to be enforced. Once nutrient inputs are curtailed, in-stream restoration techniques can be implemented. The challenge to curtailing nutrient inputs and stream degradation is more of a social one, especially in the Grand River watershed, where traditional farming techniques are still practiced. Strong relationships must continue to be built between the GRCA and the Mennonite community so that cultural traditions can be respected while accommodating best management practices that are acceptable and beneficial to both parties and able to restore the Grand River to its historic condition.

Recommendations

I therefore recommend that right now, with their limited budget, the Rural Water Quality Program focus their efforts on protecting and restoring the headwaters of the Grand River watershed, including all the local headwaters in the subwatersheds first before they allocate more money to restoring small tributaries further downstream. The efforts should be a combination of passive and active restoration that contributes to improving riparian zones, stream structure and water quality. The restoration projects would have to be maintained by the farmers, therefore it is important to collaborate and have positive relations with the farmers. The cost-share incentives should continue and the benefits to the farmers (aesthetics, increases in fish populations, upgrades to old infrastructure, etc.) need to be emphasized because everyone benefits from these best management practices – the farmers, the environment, the people downstream who use the water in the Grand River watershed for recreation, as well as the people who use Lake Erie as a source of drinking water.

Best management practices will continue to evolve as people discover more effective and efficient ways to conserve and restore ecosystems. It is important that people are willing to adapt as these findings are made because ecosystems are dynamic, therefore management plants need to be too.

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Appendix

Table A1: Biological indices calculated using benthic macroinvertebrate assemblage data collected in May 2014.

Site[2]	Taxa Richness	%EPT	%Chironomidae	%Oligochaeta	Simpson's Index	Shannon-Wiener	Family Biotic Index	Water Quality[3]
1-1-A	6	0.00	72.62	8.33	0.45	0.95	6.04	Fairly Poor
1-1-B	6	0.00	15.94	52.17	0.67	1.40	7.25	Poor
1-1-C	5	0.00	0.00	93.16	0.13	0.33	7.92	Very Poor
1-2-A	9	19.09	4.55	0.91	0.64	1.37	6.52	Poor
1-2-B	11	18.63	0.98	4.90	0.49	1.16	7.05	Poor
1-2-C	10	45.00	9.00	4.00	0.81	1.90	5.28	Fair
1-3-A	7	0.00	0.00	21.25	0.73	1.47	6.64	Poor
1-3-B	3	0.00	0.00	2.86	0.07	0.18	5.10	Fair
1-3-C	6	0.00	12.00	34.00	0.62	1.14	6.26	Fairly Poor
1-4-A	3	0.00	98.00	1.00	0.04	0.11	6.01	Fairly Poor
1-4-B	4	0.00	78.43	19.61	0.35	0.60	6.34	Fairly Poor
1-4-C	3	0.00	24.53	74.53	0.38	0.61	7.48	Very Poor
1-5-A	11	6.00	10.00	22.00	0.69	1.54	5.79	Fairly Poor
1-5-B	11	7.00	38.00	26.00	0.76	1.74	6.69	Poor
1-5-C	10	36.00	20.00	15.00	0.79	1.83	5.34	Fair
1-6-A	8	3.00	62.00	11.00	0.58	1.31	6.05	Fairly Poor
1-6-B	13	10.48	43.81	3.81	0.75	1.81	5.78	Fairly Poor
1-6-C	11	8.33	21.67	4.17	0.74	1.70	5.65	Fair
1-7-A	19	35.85	18.87	4.72	0.85	2.27	4.94	Good
1-7-B	14	23.58	14.15	1.89	0.73	1.79	5.11	Fair
1-7-C	14	10.89	31.68	31.68	0.78	1.86	6.46	Fairly Poor
1-8-A	12	17.82	15.84	28.71	0.84	2.06	5.92	Fairly Poor
1-8-B	11	9.80	46.08	3.92	0.72	1.64	5.85	Fairly Poor
1-8-C	9	9.30	41.09	34.88	0.70	1.48	6.48	Fairly Poor
1-9-A	5	0.00	46.75	7.79	0.59	1.03	5.74	Fair
1-9-B	5	0.00	40.86	54.84	0.53	0.88	7.12	Poor
1-9-C	6	0.00	53.92	19.61	0.65	1.35	6.57	Poor

[2] The first digit is the month sampled (1-May, 2-June, 3-July, 4-August), the second digit is the sampling site, and the letter is station (A-downstream, B-midstream. C-upstream)
[3] Water quality characterization was based on the Hilsenhoff Family Biotic Index (Table 3.3).

1-10-A	10	1.00	46.00	17.00	0.72	1.59	6.29	Fairly Poor
1-10-B	8	13.08	71.96	2.80	0.46	1.04	5.56	Fair
1-10-C	8	11.43	70.48	7.62	0.48	1.07	5.76	Fairly Poor

Table A2: Biological indices calculated using benthic macroinvertebrate assemblage data collected in June 2014.

Site	Taxa Richness	%EPT	%Chironomidae	%Oligochaeta	Simpson's Index	Shannon-Wiener	Family Biotic Index	Water Quality
2-1-A	7	0.00	74.79	4.20	0.43	0.98	5.97	Fairly Poor
2-1-B	9	0.00	3.92	3.92	0.33	0.84	7.67	Very Poor
2-1-C	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
2-2-A	10	7.21	33.33	6.31	0.74	1.60	6.65	Poor
2-2-B	10	5.94	24.75	19.80	0.72	1.53	7.08	Poor
2-2-C	12	14.00	39.00	4.00	0.71	1.61	6.50	Fairly Poor
2-3-A	8	0.00	6.93	0.99	0.35	0.82	5.43	Fair
2-3-B	8	0.00	35.29	29.41	0.73	1.48	6.45	Fairly Poor
2-3-C	3	0.00	1.94	97.09	0.06	0.15	7.96	Very Poor
2-4-A	4	0.00	70.48	20.00	0.46	0.84	6.51	Poor
2-4-B	6	0.00	55.45	16.83	0.62	1.18	6.49	Fairly Poor
2-4-C	6	0.00	50.00	18.63	0.68	1.39	6.47	Fairly Poor
2-5-A	10	0.00	26.21	0.97	0.68	1.48	6.84	Poor
2-5-B	9	0.00	31.25	2.68	0.61	1.25	7.22	Poor
2-5-C	10	0.00	31.00	28.00	0.72	1.44	7.24	Poor
2-6-A	5	1.60	63.20	0.00	0.49	0.83	6.58	Poor
2-6-B	10	2.91	52.43	0.00	0.67	1.46	5.99	Fairly Poor
2-6-C	12	0.00	64.71	0.98	0.56	1.34	6.00	Fairly Poor
2-7-A	15	21.01	47.06	10.08	0.74	1.88	5.72	Fairly Poor
2-7-B	11	5.22	58.26	0.00	0.62	1.42	5.96	Fairly Poor
2-7-C	14	5.47	75.00	3.13	0.43	1.14	6.01	Fairly Poor
2-8-A	11	0.00	72.73	10.00	0.46	1.12	6.26	Fairly Poor
2-8-B	8	2.94	60.78	1.96	0.56	1.13	6.37	Fairly Poor
2-8-C	11	0.00	16.22	27.93	0.79	1.76	6.74	Poor
2-9-A	6	0.00	0.93	8.41	0.58	1.04	7.37	Very Poor
2-9-B	8	0.00	30.69	12.87	0.75	1.50	7.14	Poor
2-9-C	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
2-10-A	4	0.00	72.16	16.49	0.44	0.83	6.24	Fairly Poor
2-10-B	8	0.00	52.00	12.00	0.67	1.40	6.70	Poor
2-10-C	5	0.00	70.30	8.91	0.47	0.89	6.39	Fairly Poor

Table A3: Biological indices calculated using benthic macroinvertebrate assemblage data collected in July 2014.

Site	Taxa Richness	%EPT	%Chironomidae	%Oligochaeta	Simpson's Index	Shannon-Wiener	Family Biotic Index	Water Quality
3-1-A	12	0.00	25.00	1.00	0.70	1.62	5.74	Fair
3-1-B	6	0.00	3.00	40.00	0.56	0.98	7.93	Very Poor
3-1-C	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
3-2-A	7	4.24	9.32	5.08	0.39	0.88	7.53	Very Poor
3-2-B	6	1.83	7.34	3.67	0.26	0.61	7.72	Very Poor
3-2-C	7	2.70	25.23	4.50	0.51	0.99	7.24	Poor
3-3-A	7	0.00	1.00	1.00	0.71	1.39	7.22	Poor
3-3-B	7	0.00	62.00	4.00	0.57	1.22	6.35	Fairly Poor
3-3-C	1	0.00	0.00	100.00	0.00	0.00	8.00	Very Poor
3-4-A	9	0.00	56.00	29.00	0.60	1.23	6.59	Fairly Poor
3-4-B	10	0.00	38.46	42.31	0.66	1.39	7.01	Poor
3-4-C	6	0.00	28.00	34.00	0.74	1.47	6.95	Poor
3-5-A	7	0.00	5.04	3.36	0.26	0.64	7.74	Very Poor
3-5-B	8	0.00	15.09	8.49	0.56	1.18	7.39	Very Poor
3-5-C	10	0.00	46.53	36.63	0.64	1.33	6.78	Poor
3-6-A	10	0.00	10.78	1.96	0.67	1.48	6.58	Poor
3-6-B	8	1.92	3.85	1.92	0.69	1.42	5.52	Fair
3-6-C	14	1.94	0.97	12.62	0.77	1.82	6.12	Fairly Poor
3-7-A	9	30.91	19.09	0.91	0.75	1.58	5.26	Fair
3-7-B	11	15.53	22.33	0.00	0.79	1.84	5.45	Fair
3-7-C	11	20.69	36.21	3.45	0.77	1.74	5.52	Fair
3-8-A	9	3.77	40.57	1.89	0.63	1.28	6.79	Poor
3-8-B	10	6.86	36.27	3.92	0.74	1.62	6.39	Fairly Poor
3-B-C	9	7.77	51.46	2.91	0.70	1.61	6.26	Fairly Poor
3-9-A	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
3-9-B	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
3-9-C	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
3-10-A	11	4.08	60.20	12.24	0.61	1.46	6.20	Fairly Poor
3-10-B	9	6.00	32.00	12.00	0.69	1.44	6.83	Poor
3-10-C	8	0.00	49.50	15.84	0.69	1.46	6.83	Poor

Table A4: Biological indices calculated using benthic macroinvertebrate assemblage data collected in August 2014.

Site	Taxa Richness	%EPT	%Chironomidae	%Oligochaeta	Simpson's Index	Shannon-Wiener	Family Biotic Index	Water Quality
4-1-A	6	0.00	0.00	0.00	0.29	0.64	5.55	Fair
4-1-B	5	0.00	2.00	10.00	0.25	0.53	7.95	Very Poor
4-1-C	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
4-2-A	6	29.52	9.52	0.95	0.59	1.16	6.60	Poor
4-2-B	8	3.90	3.25	5.19	0.38	0.94	7.49	Very Poor
4-2-C	7	3.74	19.63	4.67	0.50	1.04	7.33	Very Poor
4-3-A	8	0.00	1.00	7.00	0.75	1.64	7.32	Very Poor
4-3-B	6	0.00	5.17	49.14	0.70	1.49	7.28	Poor
4-3-C	10	0.00	15.00	1.00	0.69	1.50	6.32	Fairly Poor
4-4-A	9	0.00	79.63	1.85	0.35	0.83	6.18	Fairly Poor
4-4-B	5	0.00	78.22	6.93	0.37	0.79	6.24	Fairly Poor
4-4-C	7	0.00	58.00	11.00	0.61	1.24	6.53	Poor
4-5-A	5	0.00	0.00	8.74	0.23	0.50	7.91	Very Poor
4-5-B	6	0.00	58.25	36.89	0.52	0.89	6.76	Poor
4-5-C	10	1.00	14.00	52.00	0.68	1.56	7.28	Very Poor
4-6-A	11	7.50	9.17	0.83	0.60	1.41	6.83	Poor
4-6-B	8	2.00	27.00	7.00	0.72	1.52	6.61	Poor
4-6-C	10	0.00	38.24	20.59	0.77	1.73	6.52	Poor
4-7-A	15	18.00	34.00	5.00	0.83	2.17	5.78	Fairly Poor
4-7-B	10	23.01	29.20	0.00	0.82	1.94	5.50	Fair
4-7-C	11	15.45	62.60	5.69	0.58	1.39	5.77	Fairly Poor
4-8-A	13	2.22	32.33	4.44	0.72	1.64	6.99	Poor
4-8-B	11	2.74	26.03	2.74	0.76	1.76	6.82	Poor
4-8-C	16	3.76	5.26	3.76	0.76	1.93	7.40	Very Poor
4-9-A	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
4-9-B	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
4-9-C	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
4-10-A[4]	-	-	-	-	-	-	-	-
4-10-B	9	1.96	13.73	2.94	0.46	1.05	7.50	Very Poor
4-10-C	6	0.00	30.69	28.71	0.72	1.38	7.32	Very Poor

[4] Benthic macroinvertebrates were sparse in this location, therefore this sample was excluded from further analysis.

Table A5: Raw counts of benthic macroinvertebrates sampled from all locations upstream, midstream, and downstream of the 10 sites.

Taxa	1-1-A	1-1-B	1-1-C	1-2-A	1-2-B	1-2-C	1-3-A	1-3-B	1-3-C	1-4-A	1-4-B	1-4-C	1-5-A	1-5-B	1-5-C
Turbellaria														1	
Nematoda	9	8					26	101	50			1	50	3	2
Oligochaeta	7	36	109	1	5	4	17	3	34	1	20	79	22	26	15
Hirudinea					1		1		2				1	5	7
Isopoda	1	8	2	61	71	32	3						3	13	7
Bivalvia		4					27	1					4	4	10
Amphipoda											1		1	1	1
Decapoda				1	1										
Gastropoda		2	4				5		1						
Hydrachnidia													1		
Zygoptera															
Coenagrionidae														1	
Lestidae															
Plecoptera															
Perlodidae													6	7	35
Perlidae															
Ephemeroptera															
Caenidae															
Ephemerellidae					2	2									
Heptagenaidae				2	2	2									
Baetidae															
Hemiptera															
Gerridae															
Veliidae															
Corixidae															
Trichoptera															
Brachycentridae						10									
Hydropsychidae				15	14	23									1
Hydroptilidae															
Odontoceridae															
Philoptamidae															
Polycentropodidae															
Heliopyschidae															
Rhycophilidae															

Limnephilidae															
Unoeidae				4	1	8									
Lepidoptera															
Pyralidae				1	1	1									
Coleopetera															
Dytiscidae															
Elmidae			1	20	3	9							1	1	2
Haliplidae							1								
Hydrophilidae									1	1					
Gyrinidae															
Psephenidae															
Diptera															
Athericidae	1														
Psychodidae															
Ceratapagonidae	5		1												
Chironomidae	61	11		5	1	9			12	98	80	26	10	38	20
Empididae															
Culicidae															
Ephyridae															
Simuliidae													1		
Tabanidae															
Tipulidae											1				
Sum	84	69	117	110	102	100	80	105	100	100	102	106	100	100	100

Taxa	1-6-A	1-6-B	1-6-C	1-7-A	1-7-B	1-7-C	1-8-A	1-8-B	1-8-C	1-9-A	1-9-B	1-9-C	1-10-A	1-10-B	1-10-C
Turbellaria	2	1		1											
Nematoda					1	6	5	3	2	33	1	4	14		
Oligochaeta	11	4	5	5	2	32	29	4	45	6	51	20	17	3	8
Hirudinea						1	2						1		
Isopoda	6	11	14	4	3		4	17	9	1	2	11	1	3	1
Bivalvia			4	1	7		6	1					6	5	7
Amphipoda				1	1		1	1				4	1	2	1
Decapoda		1						1							
Gastropoda												8			
Hydrachnidia	4	3	4	1		1	2								
Zygoptera															
Coenagrionidae															
Lestidae															
Plecoptera															
Perlodidae		1		9	6	2	10	6	8					14	11
Perlidae															
Ephemeroptera															
Caenidae				2	2	6									
Ephemerellidae															
Heptagenaidae				8	1	1									
Baetidae		1		3	3	1							1		1
Hemiptera															
Gerridae															
Veliidae															
Corixidae						2			1						
Trichoptera															
Brachycentridae							1								
Hydropsychidae		3		12	12	1	7	3	4						
Hydroptilidae	3	6	10	1											
Odontoceridae															
Philoptamidae															
Polycentropodidae				1											
Heliopyschidae				1	1										
Rhycophilidae								1							
Limnephilidae															

Unoeidae				1											
Lepidoptera															
Pyralidae															
Coleopetera															
Dytiscidae						1			1					2	2
Elmidae	11	21	52	31	51	11	18	18	6	1	1				
Haliplidae													1		
Hydrophilidae			1												
Gyrinidae														1	
Psephenidae															
Diptera															
Athericidae															
Psychodidae															
Ceratapagonidae	1												12		
Chironomidae	62	46	26	20	15	32	16	47	53	36	38	55	46	77	74
Empididae		2	1	2	1	4									
Culicidae															
Ephyridae															
Simuliidae		5	2												
Tabanidae															
Tipulidae			1	2											
Sum	100	105	120	106	106	101	101	102	129	77	93	102	100	107	105

Taxa	2-1-A	2-1-B	2-2-A	2-2-B	2-2-C	2-3-A	2-3-B	2-3-C	2-4-A	2-4-B	2-4-C	2-5-A	2-5-B	2-5-C
Turbellaria					2									
Nematoda	9			2	1	81	25					3	2	2
Oligochaeta	5	4	7	20	4	1	30	100	21	17	19	1	3	28
Hirudinea			1			1						3	4	1
Isopoda	3	83	39	43	36	4	4	1	2			50	60	33
Bivalvia		4		1		5	2				8			1
Amphipoda		2										6		1
Decapoda														
Gastropoda		2				1	1		8	22	14			
Hydrachnidia												1		
Zygoptera														
Coenagrionidae														1
Lestidae														
Plecoptera														
Perlodidae														
Perlidae														
Ephemeroptera														
Caenidae			3		5									
Ephemerellidae														
Heptagenaidae				1	5									
Baetidae				1	1									
Hemiptera														
Gerridae										1				
Veliidae														
Corixidae						1							1	
Trichoptera														
Brachycentridae														
Hydropsychidae			4	2										
Hydroptilidae				2										
Odontoceridae					2									
Philoptamidae					1									
Polycentropodidae														
Heliopyschidae														
Rhycophilidae														

Limnephilidae														
Unoeidae			1											
Lepidoptera														
Pyralidae														
Coleopetera														
Dytiscidae	8	1									9		1	1
Elmidae		1	16	4	3							10	5	1
Haliplidae														
Hydrophilidae	3									1				
Gyrinidae														
Psephenidae														
Diptera														
Athericidae		1												
Psychodidae														
Ceratapagonidae	2				1									
Chironomidae	89	4	37	25	39	7	36	2	74	56	51	27	35	31
Empididae												1		
Culicidae														
Ephyridae			1				2					1	1	
Simuliidae			2				2			4				
Tabanidae														
Tipulidae											1			
Sum	119	102	111	101	100	101	102	103	105	101	102	103	112	100

Taxa	2-6-A	2-6-B	2-6-C	2-7-A	2-7-B	2-7-C	2-8-A	2-8-B	2-8-C	2-9-A	2-9-B	2-10-A	2-10-B
Turbellaria	1	1											
Nematoda						2	1		1		1	9	
Oligochaeta			1	12		4	11	2	31	9	13	16	12
Hirudinea							2		7				
Isopoda	41	14	5	1	13	5	3	26	15	37	32		18
Bivalvia		1	1	1					1				12
Amphipoda							1	1	3	1			
Decapoda				3		1	1						
Gastropoda					1		4	1		58	21		3
Hydrachnidia	2	1	3				2						
Zygoptera													
Coenagrionidae											1		
Lestidae											1		
Plecoptera													
Perlodidae				1				2					
Perlidae				1	2	1							
Ephemeroptera													
Caenidae				4	2	2							
Ephemerellidae													
Heptagenaidae				4									
Baetidae		2		6	1	3							
Hemiptera													
Gerridae													
Veliidae													
Corixidae			3			1							
Trichoptera													
Brachycentridae													
Hydropsychidae		1		5	1			1					
Hydroptilidae	2			3		1							
Odontoceridae													
Philoptamidae													
Polycentropodidae													
Heliopyschidae													
Rhycophilidae													
Limnephilidae				1									

Unoeidae													
Lepidoptera													
Pyralidae													
Coleopetera													
Dytiscidae		2	2		1	1	4			1			1
Elmidae		18	14	17	15	7		7	32				
Haliplidae			1			1							
Hydrophilidae			1								1		1
Gyrinidae													
Psephenidae					2								
Diptera													
Athericidae													
Psychodidae													
Ceratapagonidae			4				1		1				
Chironomidae	79	54	66	56	67	96	80	62	18	1	31	70	52
Empididae				4	10	3							
Culicidae													
Ephyridae									1				
Simuliidae		9	1										
Tabanidae									1			2	
Tipulidae													1
Sum	125	103	102	119	115	128	110	102	111	107	101	97	100

Taxa	2-11-C	3-1-A	3-1-B	3-2-A	3-2-B	3-2-C	3-3-A	3-3-B	3-3-C	3-4-A	3-4-B	3-4-C	3-5-A	3-5-B	3-5-C
Turbellaria					1	3							1		
Nematoda		1					12	4		2	1				6
Oligochaeta	9	1	40	6	4	5	1	4	110	29	44	34	4	9	37
Hirudinea			2				15						1	1	1
Isopoda	2	1	53	91	93	72	1	3					102	67	2
Bivalvia	18						43	18		2	2	23			3
Amphipoda													2		
Decapoda															
Gastropoda		3					27	8		6	8	5			2
Hydrachnidia				1											
Zygoptera															
Coenagrionidae		1													
Lestidae															
Plecoptera															
Perlodidae															
Perlidae															
Ephemeroptera															
Caenidae				1											
Ephemerellidae															
Heptagenaidae						1									
Baetidae															
Hemiptera															
Gerridae															
Veliidae															
Corixidae															
Trichoptera															
Brachycentridae															
Hydropsychidae				4	2	1									
Hydroptilidae															
Odontoceridae						1									
Philoptamidae															
Polycentropodidae															
Heliopyschidae															
Rhycophilidae															
Limnephilidae															

Unoeidae															
Lepidoptera															
Pyralidae		2									1				1
Coleopetera															
Dytiscidae	1	5										1			
Elmidae		4	1	4				1		2			3	10	1
Haliplidae															
Hydrophilidae		4			1					1	1			1	
Gyrinidae										1	1				
Psephenidae															
Diptera															
Athericidae		5													
Psychodidae											3				
Ceratapagonidae		48	1								3	9			1
Chironomidae	71	25	3	11	8	28	1	62		56	40	28	6	16	47
Empididae														1	
Culicidae														1	
Ephyridae															
Simuliidae										1					
Tabanidae															
Tipulidae															
Sum	101	100	100	118	109	111	100	100	110	100	104	100	119	106	101

Taxa	3-6-A	3-6-B	3-6-C	3-7-A	3-7-B	3-7-C	3-8-A	3-8-B	3-8-C	3-10-A	3-10-B	3-10-C	4-1-A	4-1-B
Turbellaria	19	46	33		3	4								
Nematoda				1								1	2	
Oligochaeta	2	2	13	1		4	2	4	3	12	12	16		10
Hirudinea	1		2			1				1		2		
Isopoda	54	29	33	10	8	4	47	30	2		43	16		86
Bivalvia	1	2	1	4	3				2		3	12		
Amphipoda	8		2			3			4			1		
Decapoda				1						5				
Gastropoda	1							1	11	7		3		
Hydrachnidia			1											
Zygoptera														
Coenagrionidae														
Lestidae														
Plecoptera														
Perlodidae											6			
Perlidae														
Ephemeroptera														
Caenidae					2			1						
Ephemerellidae														
Heptagenaidae														
Baetidae			1	2	1		1		8	4				
Hemiptera														
Gerridae														
Veliidae														
Corixidae						4			12				1	1
Trichoptera														
Brachycentridae														
Hydropsychidae		2	1	32	13	24	3	6						
Hydroptilidae														
Odontoceridae														
Philoptamidae														
Polycentropodidae														
Heliopyschidae														
Rhycophilidae														

Limnephilidae														
Unoeidae														
Lepidoptera														
Pyralidae											1			
Coleopetera														
Dytiscidae	3		6				1				1		1	
Elmidae	2	18	6	38	37	27	5	18	8	4				
Haliplidae			1			1				2				
Hydrophilidae			2							2	1		2	
Gyrinidae														
Psephenidae					2									
Diptera														
Athericidae													11	
Psychodidae														
Ceratapagonidae										1			85	1
Chironomidae	11	4	1	21	23	42	43	37	53	59	32	50		2
Empididae					10	2	2	2						
Culicidae														
Ephyridae														
Simuliidae		1						1						
Tabanidae							2	2						
Tipulidae					1					1	1			
Sum	102	104	103	110	103	116	106	102	103	98	100	101	102	100

Taxa	4-2-A	4-2-B	4-2-C	4-3-A	4-3-B	4-3-C	4-4-A	4-4-B	4-4-C	4-5-A	4-5-B	4-5-C	4-6-A	4-6-B	4-6-C
Turbellaria		7		9									10	11	2
Nematoda					15	3	1				1	7			
Oligochaeta	1	8	5	7	57	1	2	7	11	9	38	52	1	7	21
Hirudinea				17		1						3			2
Isopoda	62	120	72	6	11	1				90	1		73	42	13
Bivalvia		4		18	16	4	5	1	18	2	2	14			2
Amphipoda										1			2		2
Decapoda			1												
Gastropoda				41	11	23	10	9	10			3	1		
Hydrachnidia															
Zygoptera															
Coenagrionidae						1						2			
Lestidae															
Plecoptera															
Perlodidae															
Perlidae															
Ephemeroptera															
Caenidae													2		
Ephemerellidae															
Heptagenaidae													1		
Baetidae												1		1	
Hemiptera															
Gerridae															
Veliidae															
Corixidae							1		1						
Trichoptera															
Brachycentridae															
Hydropsychidae	22	3	3												
Hydroptilidae	9	3	1										6	1	
Odontoceridae															
Philoptamidae															
Polycentropodidae															
Heliopyschidae															
Rhycophilidae															

Limnephilidae															
Unoeidae															
Lepidoptera															
Pyralidae															
Coleopetera															
Dytiscidae						3	1		1				1	1	
Elmidae	1	4	4							1	1	3	12	10	16
Haliplidae									1			1			
Hydrophilidae							1								1
Gyrinidae															
Psephenidae															
Diptera															
Athericidae															
Psychodidae															
Ceratapagonidae								5							4
Chironomidae	10	5	21	1	6	15	86	79	58		60	14	11	27	39
Empididae															
Culicidae															
Ephyridae						48									
Simuliidae							1								
Tabanidae															
Tipulidae				1											
Sum	105	154	107	100	116	100	108	101	100	103	103	100	120	100	102

Taxa	4-7-A	4-7-B	4-7-C	4-8-A	4-8-B	4-8-C	4-10-B	4-10-C
Turbellaria	2	3	1					
Nematoda								
Oligochaeta	5		7	4	2	5	3	29
Hirudinea						3	2	1
Isopoda	9	7	1	37	28	1	73	31
Bivalvia	2	13	3				5	8
Amphipoda	1					12		
Decapoda	3			3	2	1		
Gastropoda				7		17		1
Hydrachnidia					1			
Zygoptera								
Coenagrionidae						2		
Lestidae								
Plecoptera								
Perlodidae							2	
Perlidae								
Ephemeroptera								
Caenidae	5	1	2	1		2		
Ephemerellidae								
Heptagenaidae	3		1	1	1			
Baetidae	1	10	7			3		
Hemiptera								
Gerridae								
Veliidae					3	1		
Corixidae				1	5	60		
Trichoptera								
Brachycentridae								
Hydropsychidae	8	12	9		1			
Hydroptilidae	1							
Odontoceridae								
Philoptamidae								
Polycentropodidae								
Heliopyschidae		3						

Rhycophilidae								
Limnephilidae								
Unoeidae								
Lepidoptera								
Pyralidae								
Coleopetera								
Dytiscidae						1	1	
Elmidae	17	26	13	3	9	12		
Haliplidae				1		3		
Hydrophilidae						3	1	
Gyrinidae							1	
Psephenidae	4							
Diptera								
Athericidae								
Psychodidae								
Ceratapagonidae				1				
Chironomidae	34	33	77	29	19	7	14	31
Empididae	5	5	2					
Culicidae								
Ephyridae								
Simuliidae				1				
Tabanidae				1	2			
Tipulidae								
Sum	100	113	123	90	73	133	102	101

Table A6: Biological indices calculated from surveying benthic macroinvertebrates in August 2007.

Location	%EPT	%Chironomidae	%Oligochaeta	Taxa	Simpson's Index	Shannon-Wiener	Tolerance Value	Water Quality
1	19.83	14.88	0.00	16	0.81	1.86	5.00	Good
2	2.04	28.57	8.16	12	0.79	1.82	6.86	Poor
3	64.00	6.67	0.27	17	0.62	1.51	4.57	Good
4	12.93	25.86	0.86	17	0.88	2.40	6.08	Fairly Poor
4	26.69	22.31	0.40	19	0.86	2.24	5.58	Fair
4	6.21	8.70	0.00	14	0.58	1.43	7.66	Very Poor
5	13.04	10.43	0.00	22	0.92	2.72	5.65	Fairly Poor
6	43.75	4.17	1.25	14	0.75	1.78	5.64	Fair
6	50.32	11.08	0.00	14	0.70	1.57	4.78	Good
6	15.52	2.30	0.00	18	0.84	2.26	5.28	Fair
7	22.44	8.52	0.00	24	0.83	2.20	5.21	Fair

Table A7: Biological indices calculated from surveying benthic macroinvertebrates in August 2014.

Location	%EPT	%Chironomidae	%Oligochaeta	Taxa	Simpson's Index	Shannon-Wiener	Tolerance Value	Water Quality
1	0.43	46.38	3.83	11	0.63	1.29	5.77	Fairly poor
2	0.00	5.26	57.89	4	0.56	0.99	8.53	Very Poor
3	25.27	28.28	0.43	24	0.78	1.84	6.46	Fairly Poor
4	11.43	46.53	13.06	15	0.72	1.64	6.13	Fairly Poor
4	21.45	55.81	5.92	23	0.64	1.51	6.35	Fairly Poor
4	18.18	61.21	1.41	11	0.59	1.29	5.74	Fair
5	30.32	25.53	3.19	15	0.79	1.92	5.34	Fair
6	60.50	17.02	2.52	22	0.64	1.60	6.68	Poor
6	38.33	35.33	7.00	16	0.73	1.66	6.65	Poor
6	11.63	30.81	6.40	11	0.79	1.81	7.02	Poor
7	8.97	6.96	0.00	16	0.63	1.47	6.73	Poor

Table A8: Raw benthic macroinvertebrate counts of samples from 2007 and 2014 in 7 sites across the Grand River watershed.

	1-2007	1-2014	2-2007	2-2014	3-2007	3-2014	4-2007	4 -2014	5-2007	5-2014	6-2007	6-2014	7-2007	7-2014
Turbellaria						38	12	2	1	1	1	1	1	64
Nematoda						1	0	0			0	1		1
Oligochaeta		9	8	11	1	8	1	30		6	3	15		
Hirudinea			1	6	1	1	4	4	1	2	11	9	10	1
Isopoda	23			1		591	10	18	5	4	2	13	53	552
Bivalvia	2	7	30		16	32	5	14	1		29	6	4	2
Amphipoda	50	5			1	4	6	1	13		27	13	118	1
Decapoda	3	1	5		14	5	7	1	10	1	5	2		
Gastropoda					9	6	19	0	10	1	9	2	18	4
Hydrachnidia	1					1	2	2	1		2	7	1	1
Odonata														
Gomphidae							0	1			0	0	1	
Libellulidae							5	0			0	0	1	
Zygoptera														
Coenagrionidae		5	1		1		0	0	2		0	28		
Ephemeroptera														
Caenidae					4	270	7	71	7	10	4	133		
Ephemerellidae							0	1			0	0	1	
Heptagenaidae						136	0	7		12	0	6		
Baetidae	1		1		8	20	2	2	2	16	8	2	11	11
Ephemeridae							2	0			0	0		
Leptohyphidae					1	2	0	1		5	0	0	1	
Hemiptera														
Corixidae	1		16			1	52	0	2		2	3		
Belostomatidae							0	0	2		0	0		
Megaloptera														
Sialidae	1				3	3	1	1	2		0	0	1	
Corudalidae	2						0	0			0	0		
Trichoptera														
Hydropsychidae	46		1		220	16	20	27	3	13	85	0	46	58
Hydroptilidae		1			3	20	2	4	1	1	0	5		16

Polycentropodidae						2	0	0			0	0		
Heliopyschidae						4	0	0			1	0		
Unoeidae							0	2			0	1		
Philopotamidae							0	0			0	0	19	
Coleoptera														
Elmidae	70	91	3		61	168	24	58	17	67	47	15	17	120
Hydrophilidae						3	9	4	9		3	1	5	1
Dryopidae							0	0			0	0	1	
Dytiscidae							3	0	4		1	0	1	
Haliplidae							2	0	8		5	0	8	
Psephenidae					3	2	0	1			2	2		
Diptera														
Ceratapagonidae		1	3				1	0			0	1		
Chironomidae	36	109	28	1	25	526	33	296	12	48	16	80	30	66
Empididae		1					0	1		1	1	0		
Simuliidae	2						1	0			4	0		49
Tabanidae	1	5	1				1	0			3	1	1	
Tipulidae	2						2	1			1	0	2	1
Sum	241	235	98	19	371	1860	230	550	113	188	271	343	351	948