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**DEVELOPMENT, TESTING, AND APPLICATION OF STRESSOR
GRADIENTS IN RURAL, HEADWATER STREAMS IN
SOUTHWESTERN ONTARIO**

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**DEVELOPMENT, TESTING, AND APPLICATION OF STRESSOR
GRADIENTS IN RURAL, HEADWATER STREAMS IN
SOUTHWESTERN ONTARIO**

(Spine Title: Development, Testing, and Application of Stressor Gradients)

(Thesis Format: Integrated Article)

By

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Graduate Program
in
Biology

A thesis submitted in partial fulfillment of the requirements for the degree
of
Doctor of Philosophy

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Abstract and Keywords

Abstract:

Effective biological monitoring requires a conceptual model of how human activity varies and how this activity affects resident biota. This model can then be used to generate appropriate hypotheses and study designs in response to bioassessment needs. Stressor gradients have the potential to improve this process, but questions about the development and effectiveness of stressor gradients must be addressed before they can be widely applied to biological monitoring.

With the aim of developing the most effective and efficient stressor gradient, four gradients were calculated from stressor information differing in level of detail and spatial explicitness for 479 rural, headwater basins. Fine detail gradients also described substantially more variation in the stressor environment than those using coarse detail data. Data that described the location of the stressors within the basin resulted in only minimal improvements to the description of the stressor environment.

The responsiveness of aquatic biota to stressor gradients was determined using surveys of aquatic assemblages in 160 small, rural, streams. Canonical correspondence analysis indicated that fish and macroinvertebrates responded to stressor gradients through compositional shifts from intolerant to tolerant taxa as human activity intensified. This response was confounded by a similar compositional shift in response to a gradient of surface geology. Partial Mantel's tests controlling for the effect of natural gradients indicated that aquatic assemblages are associated to gradients in the human environment.

A stressor gradient was applied in the development of an objective method for selecting environmentally stratified, regional reference sites for the purpose of assessing ecological condition in freshwater ecosystems. This method groups potential sites based on their natural environments prior to establishing the degree of human activities occurring at each site within each group. Sites exhibiting the least amount of human activity are then selected to act as reference sites for each group.

In addition to having immediate impact on how biological monitoring is conducted in the Southwestern Ontario region, the results of this study can be conceptually applied to bioassessments worldwide. Furthermore, this study can act as the

foundation for using stressor gradients for the development of predictive models that will aid in planning and management of future activities that may affect aquatic ecosystems.

Keywords: stressor gradient, bioassessment, fish, benthic macroinvertebrates, small streams, rural environments, southwestern Ontario

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Chapter 1. General Introduction

1.1. INTRODUCTION

The advent of agriculture more than 10,000 years ago signified the beginning of what has been the greatest cause of land use change globally and perhaps the single most important factor in subsequent human-caused environmental changes. Today, nearly 40% of the Earth's land is being used for agricultural purposes (Wood et al. 2000). However, in many regions this proportion is actually much greater. One such area is southwestern Ontario. In this region, the history of intensive agricultural land use began between 1800 and 1840 when virtually all the forest cover was removed and the land converted to agriculture (Langman 1971). Today, the southwestern Ontario landscape is still dominated by agriculture, although the proportion of land cover being farmed is diminishing as urban areas rapidly sprawl into the surrounding countryside. Despite the reduction in agricultural lands, agricultural production in the region has actually increased over the past 50 years because of increasing densities of livestock (Figure 1.1) and increased use of inorganic fertilizers and herbicides (Statistics Canada 2001).

Although early agriculture caused widespread destruction and alteration of local ecosystems, the shift towards more intensive agriculture, accompanied by increased environmental awareness in society and more value placed on the natural environment, has generated significant public concern regarding the effects that modern agriculture may be having on the environment. Aquatic ecosystems are particularly at risk of degradation from agriculture because they aggregate materials, including toxins and nutrients from their watersheds. Small streams are often the most noticeably degraded aquatic ecosystems because they are regularly in close proximity to prime agricultural lands and can be more easily affected than larger aquatic systems. A variety of agricultural activities can lead to the degradation of the physical and chemical environment of stream ecosystems relied on by the resident aquatic biota (Figure 1.2). Specifically, agriculture reduces diversity of stream biological communities through alterations of stream flow regimes (Poff et al. 1997), increases sediment and nutrient loads (Lenat 1984; Johnson et al. 1997), and simplifies riparian and instream habitats (Richards et al. 1996, Wang et al. 1997).

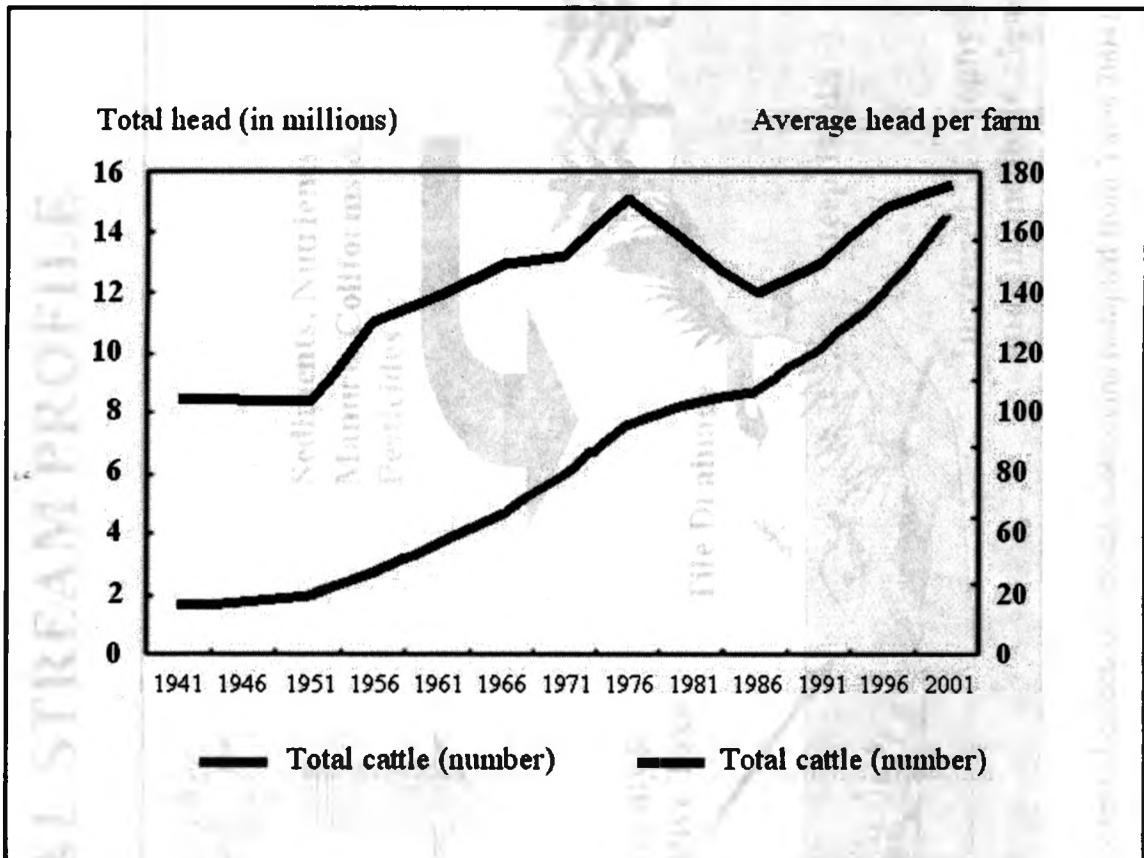


Figure 1.1. Change in total number of cattle and average number of cattle per farm between 1941 and 2001 for Canada (Modified from: Statistics Canada, 2001).

AGRICULTURAL STREAM PROFILE

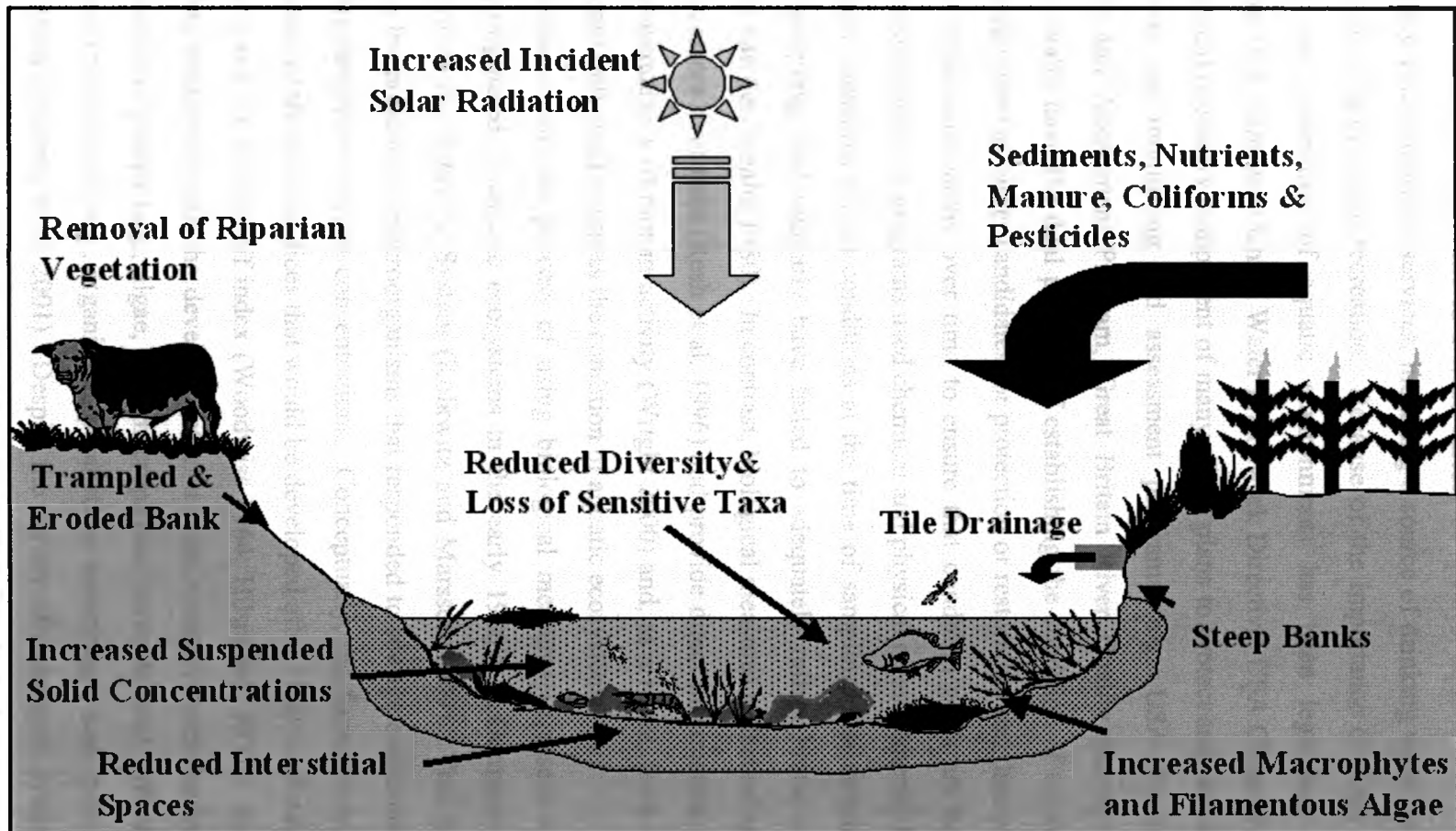


Figure 1.2. Common agricultural stressors and their potential effects on stream ecosystems (adapted from Yates 2004).

In addition to being home to a wide variety of species, aquatic ecosystems also provide many vital ecosystem services, including a source of drinking water for humans and livestock, irrigation and recreation. Because of the importance of these uses, the protection and restoration of aquatic environments has been legislated in many jurisdictions (e.g., European Union Water Framework Directive, USA Clean Water Act). A common and necessary component of many of the plans to protect and restore aquatic environments are monitoring and assessment programs (e.g., USA Environmental Monitoring and Assessment Program, Great Britain River Quality Survey). These programs usually have the dual purpose of establishing the condition of ecosystems in order to assign them as either candidates for protection or restoration and then continuing to monitor these ecosystems over time to ensure that condition goals are being met. Initially, most assessment programs used chemical and physical measurements. However, because these measures reflect conditions at the time of sampling, they require almost continual sampling, and regularly have failed to adequately reflect biotic conditions (Cairns and van der Schalie 1980). In contrast, biological measures reflect an aggregated signal of stressors over time (Resh et al. 1996) and provide direct information about the ultimate consequences of human activity (Wright 2000) and, as a result, these measures are now commonly used to assess the condition of aquatic ecosystems.

Bioassessment, the process of using biological measures to assess ecosystem condition, originated in aquatic ecosystems in the early 1900's in Germany with the development of the Saprobic System (Kolkwitz and Marsson 1909). This index was based upon the presence of microorganisms that responded to sewage contamination and the resulting decline in oxygen concentrations. Conceptually, the Saprobic system was the foundation of the many indices that would be developed after 1960, such as the Trent Biotic Index and the Hilsenhoff index (Woodwiss 1964; Hilsenhoff 1977). Since then, the field has broadened with the development of a wide variety of techniques utilizing several taxonomic groups (e.g., algae, fish, macrophytes, invertebrates), different levels of biological organization (e.g., organismal, population, assemblage) and, most recently, biological traits (Statzner et al. 2001). Despite the variety of techniques available most stream bioassessments use either multimetric or multivariate techniques to assess the condition of one or more biotic assemblages.

The multimetric approach uses a variety of metrics that each describe a component of the biota (e.g., richness, proportion of pollution tolerant species), and together give an indication of biological condition (Barbour et al. 1995). In contrast, the multivariate approach uses multivariate statistical analysis of relative abundance data to develop models that predict the taxa that should be present if the ecosystem was undisturbed and evaluate condition based on the departure between the observed taxa versus the predicted expected taxa. While proponents of both methods have criticized the alternative approach (e.g., Gerritsen 1995 *versus* Norris 1995) these two methods do have many similarities including the fact that they both require the establishment of a biological reference condition (Bailey et al. 2006).

Reference condition has been defined as “the condition that is representative of a group of minimally disturbed sites organized by selected physical, chemical, and biological characteristics” (Reynoldson et al. 1997). Multivariate and multimetric approaches use this condition as the standard against which to compare individual test sites (i.e., sites that have been exposed to human disturbance). Both these methods are limited, however, by a rather idiosyncratic definition of what constitutes a reference site (Bailey et al. 2006). Adequate characterization of human activity at the landscape scale, what is sometimes known as building a stressor gradient, can overcome this problem.

The term “stressor” has been widely and variably used in the aquatic ecosystem assessment literature. Most commonly, stressors have been defined as the physical (e.g., temperature), chemical (e.g., phosphorus concentration), and biological (e.g., predators) factors that affect biota (Stevenson et al. 2004). The premise of this definition is that stressors are the effects of human activities (sometimes known as “drivers”), and that stressors in turn cause a given response in the biota. Although this model is an accurate depiction of the pathways and processes through which human activities affect biota, using it to build bioassessment-based ecological management models can be problematic. The goal of ecological planning and management is often to maintain or restore some desirable state of ecological condition. Because this goal can only be achieved through management of human actions, predictive models that link the cause of impairment (or restoration) to the final outcome (i.e. a change in ecological condition) are needed. To build these models using information about the processes requires substantial monitoring

and associated expenditure of resources to describe the processes operating in even one ecosystem. As a result, generating regional predictive models for a given type of ecosystem would not be feasible. In contrast, treating the processes as a “black box” and directly relating measures of human activity to ecological condition does not require a large expenditure of resources for data collection and can therefore provide correlative evidence of the effects of a given human activity on ecological condition for even very large regions.

Based on the above rationale, my approach is to define stressor gradients that describe variation in the types and extents of human activity (e.g. livestock farming and livestock density), and do not include information about the effects (e.g. nitrogen loading) of those activities. As a result, throughout this dissertation, a “stressor gradient” will refer to a set of ecosystems (i.e. headwater basins) that vary in the nature and intensity of human activity occurring within those ecosystems. A stressor gradient is a comprehensive description of the activities and variation in those activities that may be causing changes in ecosystem condition and as a result forms the foundation of any well designed freshwater monitoring and assessment program (Bailey et al. 2006). Stressor gradients can be used to choose objectively defined reference sites (see Chapter 4), stratify test sites (Danz et al 2005; Yates and Bailey 2006), and select relevant ecological indicators (e.g. Fore et al 1996; Johnson et al 2006). Stressor gradients will also be instrumental in allowing freshwater assessments to diagnose the cause of impairment and in building predictive models that could be used to inform planners and managers of the effects of future development and restoration projects.

1.2. RESEARCH GOAL AND OBJECTIVES

Goal:

To generate, test, and apply multivariate stressor gradients in rural southwestern Ontario headwater stream ecosystems as a tool for bioassessment.

Objectives:

1. Determine what information about human activity in rural southwestern Ontario generates the most efficient and informative stressor gradient. (Chapter 2)
2. Determine if and how fish and aquatic macroinvertebrate assemblages covary with both the stressor gradient and gradients of natural environmental features. (Chapter 3)
3. Use a stressor gradient developed for rural southwestern Ontario to identify the ecosystems with the least amount of human activity as candidate reference basins in a regional monitoring program. (Chapter 4)

1.3. STUDY AREA

Southwestern Ontario is located in the heart of the Great Lakes Basin (Figure 1.3) in the southernmost part of Canada. The region is comprised of two ecoregions that divide the region into northern and southern halves (Wickware 1989). The northern ecoregion, Manitoulin-Lake Simcoe, is slightly wetter (annual precipitation 700 - 1000 mm) and cooler (mean summer and winter temperatures, 16.5 °C and -4.5 °C) than the more southern Lake Erie Lowlands (annual precipitation 750 - 900 mm, mean summer and winter temperatures, 18 °C and -2.5 °C) (Wickware 1989). Both ecoregions are dominated by a wide variety of glacial deposits overlying calcium-carbonate rich Paleozoic bedrock. The natural vegetation of the region is predominately temperate deciduous forest, however, most of this forest was removed following European settlement and only small, remnant patches remain or have been allowed to regenerate.

Despite a regional population of close to two million people, agriculture is by far the dominant land use, comprising upwards of 90% of the land cover in some areas. Agriculture in the region tends to be a mixture of row crop agriculture (i.e. corn and soybeans) and high density livestock farms (i.e. beef, dairy, pork, and poultry). However, specialty crops (e.g. vegetables and tobacco) are commonly grown in the Norfolk sand plain (Statistics Canada 2001). Regional agriculture development has led to substantial alteration of the region's hydrology. In addition to the removal of the natural forest cover, the majority of the regions wetlands have been drained and ditched (Snell 1987), while many of the regions headwater streams have been entrenched and channelized to increase drainage rates (Rudy 2004). Further hydrologic manipulation has occurred through the addition of field tiles that reduce soil water retention times and lower the local water table (Poff et al. 1997).

All field sampling for this study was conducted in small, wadeable streams with drainage areas of 600 to 3000 hectares. The majority of these streams were part of either the Thames or Grand River systems. These two rivers are the largest rivers in southwestern Ontario, draining approximately 7000 km² each. The remaining study streams were located in either the Lake Ontario drainage basin of Spencer Creek or in one of four smaller watersheds (South Otter Creek, Otter Creek, Big Creek, and Detrick Creek) that flow into Lake Erie. The Spencer Creek stream drains approximately 200

km², while the four Long Point Region streams combine to drain approximately 1650 km².



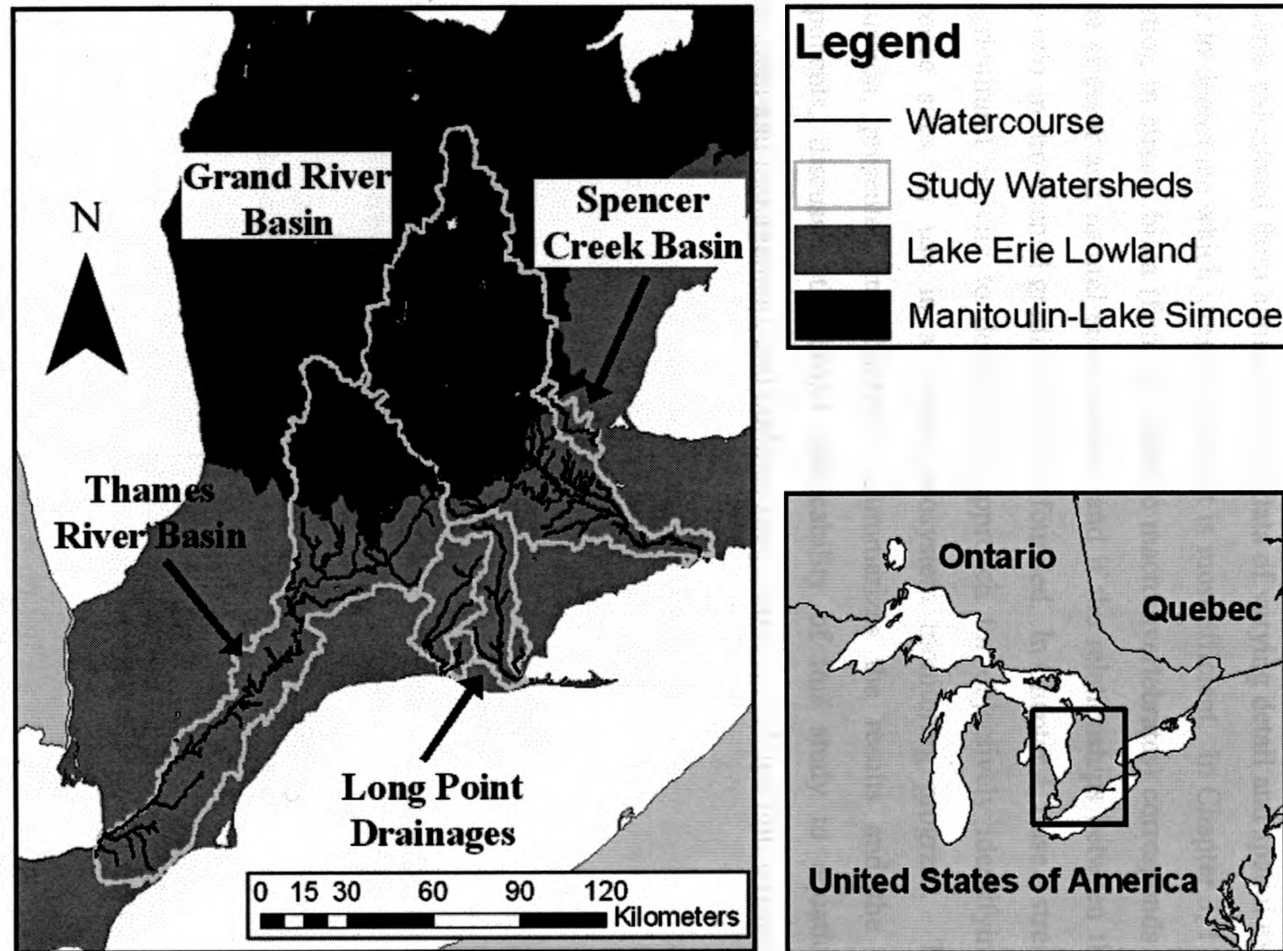


Figure 1.3. Location of study area in Great Lakes Region (bottom right) and position of study watersheds relative to the two southwestern Ontario ecoregions (main).

1.4. SCOPE OF DISSERTATION

This dissertation is composed of five chapters including this General Introduction. In Chapter 2, I generate and compare the descriptive power of four landscape scale stressor gradients calculated from human activity data of varying detail and spatial explicitness in order to determine which stressor gradient is most efficient. In Chapter 3, I determine if variation in stream biota (fish and benthic macroinvertebrates) corresponds to gradients in the stressor and natural environment, and if the relationships between the biota and these two environmental gradients are confounded. In Chapter 4, I use a stressor gradient of agricultural activity to develop an approach for objectively identifying candidate reference sites for use in a stream ecosystem monitoring program. The General Discussion, presented in Chapter 5, summarizes the results and the three study components, discusses the broad applicability of this study to aquatic ecosystem assessment and management, and outlines suggestions for related future research.

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Chapter 2. Improving the description of the stressor environment: evidence supporting the use of detailed stressor information

2.1. INTRODUCTION

Increasing impairment of freshwater ecosystems worldwide, accompanied by growing recognition of the need to conserve and maintain these valued ecosystems, has initiated a dramatic increase in freshwater monitoring and assessment programs. Although these programs vary considerably in terms of goals, indicators, data collection, and analytical procedures, successful monitoring and assessment of freshwater ecosystems always requires a conceptual model that describes the pathways by which human activities may be affecting them (Stevenson et al. 2004). Prior to generating this conceptual model a comprehensive description of the human activities present, and how they vary across the region, must be completed. This task can often be accomplished through the development of a stressor gradient (Bailey et al. 2006).

The term stressor is often used as “catch all” for human activities (e.g., agriculture, forestry, and channelization) and their associated direct effects (e.g., changes in nutrient concentration, sediment loads, habitat degradation) on receiving ecosystems. Using such an all encompassing definition can, however, lead to confusion of causes and effects. Therefore, I will use the term stressor to refer only to human activities that have the potential to alter the receiving environment.

A stressor gradient is a set of ecosystems (e.g., reaches, basins, or other geographical units of interest) that vary in their exposure to human activities. Stressor gradients are widely used in assessment studies to stratify variables in a sampling design (Danz et al. 2005; Yates and Bailey 2006), nominate reference sites more objectively (Bailey et al. 2007), or select ecological metrics responsive to stressors of interest (e.g., Fore et al. 1996; Johnson et al. 2006). Although a stressor gradient can serve many purposes, the data from which they are derived have generally been the same, as nearly all studies strictly use stressor information based on land cover data aggregated at the basin scale.

The popularity of land cover information for characterizing stressors can be attributed to the wide availability of regional land cover data, which provides a good

summary of human activities occurring at the landscape scale. One potential drawback, however, is the relatively coarse classification of the landscape that leads to many stressors being aggregated into a small number of very broad classes (e.g., % agriculture). Incorporating additional information that better resolves differences within land use classes (e.g., number of livestock farms) could significantly change the nature of the stressor gradient. Yet, with the notable exception of the studies by Bryce et al. (1999), Brown and Vivas (2005), and Danz et al. (2005), information beyond the level provided by regional land cover layers has rarely been incorporated into stressor gradients. More importantly, it has not been established if the addition of more detailed stressor information substantially improves characterization of the stressor environment. The potential importance of spatially explicit data in stressor gradients has received more recognition, in large part due to the substantial body of work (e.g., Roth et al. 1996; Allan et al. 1997; Johnson et al. 1997; Wang et al. 2001) aimed at identifying the relationship between spatial scale and instream measures (e.g., water chemistry and biota). However, the effect of space on the nature of the stressor gradient has received less attention, although Brown and Vivas (2005) determined that spatial variability and scale had little effect on their gradient of Landscape Development Intensity.

Because the stressor gradient is the foundation of many stream monitoring programs, understanding the importance of detail and space on the characterization of stressor gradients is critical to ensuring that the goals of the monitoring program can be achieved. Furthermore, as most of these programs have limited budgets, an understanding of the effects of the choice of data on stressor gradients is vital to ensuring that the balance between improved discrimination of variation in the stressor environment and the expenditure required to achieve that discrimination is as efficient as possible. This study tests the effects of incorporating detailed and spatially explicit stressor data on the nature of the resulting stressor gradient. Specifically, four stressor gradients based on the same 479 rural, headwater basins using data of varying detail and spatial explicitness were compared to determine how the data used to derive a stressor gradient affects the description of the stressor environment.

2.2. METHODS

2.2.1. Study Area

To test the effects of location and information on stressor gradients, I collected data on human activity in 479 rural, headwater basins (600 to 3000 ha) in southwestern Ontario, Canada (Figure 2.1, Appendix 1). These basins were predominantly within two drainages, the Grand River and Thames River watersheds (~ 7000 km² each). Smaller numbers of basins were located in four small watersheds that comprise the Long Point Drainage Area (~ 1650 km²) and an additional five basins were located in the small watershed of Spencer Creek (~ 215 km²).

The geology of southwestern Ontario is typical of a recently glaciated landscape, with till deposits dominating in the northern headwater portions of the watersheds and glacial lacustrine deposits of sand and clay more common in the south where these basins drain into the Great Lakes. Land cover in these watersheds is generally characterized by high levels of agriculture, consisting primarily of a matrix of intensive row crop agriculture and high density livestock operations with small, isolated forest patches interspersed.

2.2.2 Data

Headwater basin boundaries were delineated using a 10 m digital elevation model (DEM) and a stream layer generated at the 1:10000 scale by the Ontario Ministry of Natural Resources (OMNR). All basins were delineated using the ArcHydro 9.1 extension for ArcGIS 9.1 (ESRI 2005) to generate flow direction and accumulation layers. Small (< 600 ha) and large (> 3000 ha) basins were removed from this set, and the remaining were inspected for urban land cover using 2006 orthoimages taken and rectified by the OMNR. Any basins with urban land cover were removed from further analysis, resulting in a set of 479 rural, headwater basins.

The effect of location and information detail on the nature of the stressor gradient was tested by generating four different stressor gradients, hereafter defined as Coarse Aspatial (CA), Coarse Spatial (CS), Fine Aspatial (FA) and Fine Spatial (FS). The CA gradient was calculated using the proportion of each land cover type summed for the entire basin. The CS gradient also used the regional land cover data, but summed the proportion of each cover type for four distinct spatial zones based on proximity to a

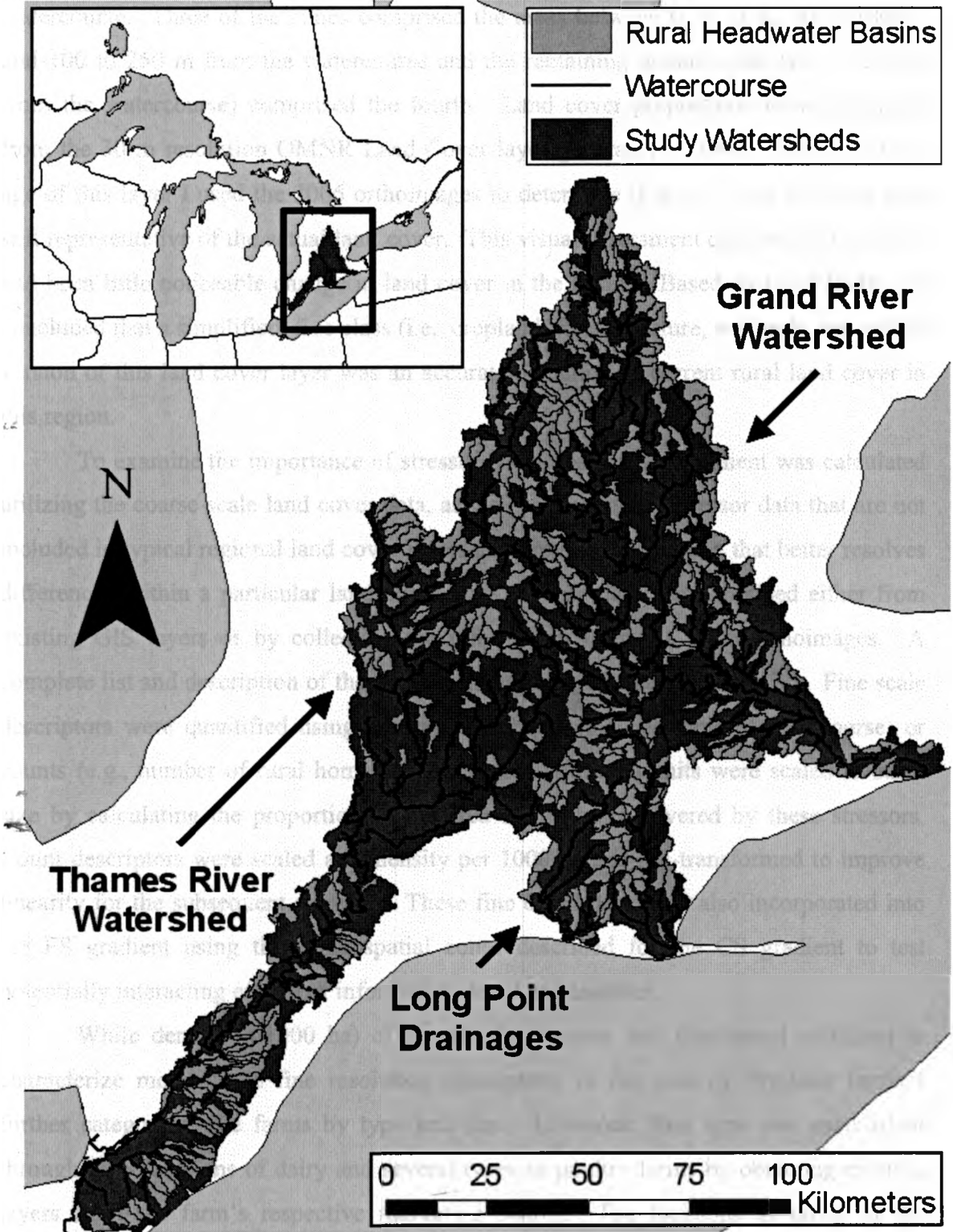


Figure 2.1. Location of study region in the Great Lakes Region, Ontario, Canada (inset) and the distribution of the 479 rural, headwater basins within this region.

watercourse. Three of the zones comprised the areas between 0 to 30 m, 30 to 100 m, and 100 to 250 m from the watercourse and the remaining upland areas (i.e., > 250 m from the watercourse) comprised the fourth. Land cover proportions were calculated from the 30 m resolution OMNR Land Cover layer generated in 1992. Because of the age of this layer I used the 2006 orthoimages to determine if this 15 year old layer was still representative of the actual land cover. This visual assessment determined that there had been little noticeable change in land cover in the basins. Based on these findings I concluded that a simplified, five class (i.e., cropland, forest, pasture, wetlands, and water) version of this land cover layer was an accurate depiction of current rural land cover in this region.

To examine the importance of stressor resolution, the FA gradient was calculated utilizing the coarse scale land cover data, as well as finer detail stressor data that are not included in typical regional land cover layers and provide information that better resolves differences within a particular land cover class. These data were obtained either from existing GIS layers or by collecting the information from the 2006 orthoimages. A complete list and description of the data layers used is presented in Table 2.1. Fine scale descriptors were quantified using area measurements (e.g., hectares of golf course) or counts (e.g., number of rural homes). Descriptors with area units were scaled to basin size by calculating the proportion of the area of the basin covered by these stressors. Count descriptors were scaled as a density per 1000 ha and log-transformed to improve linearity for the subsequent analyses. These fine detail data were also incorporated into the FS gradient using the same spatial zones described for the CS gradient to test potentially interacting effects of information detail and location.

While density (#/1000 ha) of a particular stressor was considered sufficient to characterize most of the fine resolution descriptors, in the case of livestock farms I further categorized the farms by type and size. Livestock farm type was established through GPS locations of dairy and several types of poultry farms by obtaining existing layers from the farm's respective marketing boards. The locations of farms of the remaining farm types (i.e. hogs, sheep, and horses) were identified using the high resolution 2006 orthoimages. Because it was not possible to obtain exact numbers of livestock at each farm, I estimated the number of animals present based on the area of

Table 2.1. Fine detail stressor descriptors and groups of descriptors used in the initial PCA of the stressor gradient analyses.

| Group | Descriptor | Source | Description |
|----------------------------------|-----------------------|---|---|
| Field Tile Drainage | No Field Tiles | Ontario Ministry of Agriculture and Rural Affairs | Indicates area of land that is tile drained and if the drainage is systematic (i.e., tiles are evenly spaced across an entire field) or random (i.e., tiles are laid selectively in poorly drained areas) |
| | Random | | |
| | Systematic | | |
| Non-Agricultural Land Use | Pits and Quarries | Ontario Ministry of Natural Resources | Location and area of open pits and quarries |
| | Golf Courses | Generated from 2006 Orthoimages | Location and area of golf courses |
| | Campgrounds | Generated from 2006 Orthoimages | Location and area of campgrounds and trailer parks |
| | Landfills | Ontario Ministry of the Environment | Location and area of municipal sanitary landfills |
| Transportation | Road Network | Ontario Ministry of Natural Resources | Layers indicate the location and length of transportation networks |
| | Rail Network | Canadian Department of Natural Resources | |
| Septic Systems | Business | Generated from 2006 Orthoimages | Indicates the location of private septic systems and the use of the buildings which are serviced (i.e., home, business, industry) |
| | Home | | |
| | Industry | | |
| Livestock Farms | Beef | Generated from 2006 Orthoimages | Layers indicate the location, type, and size of livestock farms |
| | Dairy | Dairy Farmers of Ontario | |
| | Horse | Generated from 2006 Orthoimages | |
| | Poultry | Egg Farmers of Ontario, Turkey Farmers of Ontario, Ontario Broiler Hatching Egg & Chick Commission, Generated from 2006 Orthoimages | |
| | Sheep | Generated from 2006 Orthoimages | |
| | Swine | Generated from 2006 Orthoimages | |
| Water Withdrawal | Permits to Take Water | Ontario Ministry of the Environment | Indicates location of permit holders and source (ground, surface, or both ground and surface waters) of the water being withdrawn |

the livestock housing facilities and average housing densities. Housing densities were based upon recommended codes of practice established by the Canadian Agri-Food Research Council (1995, 1998, 2003a,b) and the Canadian Federation of Humane Societies (1990, 1991, 1993). Areas were calculated using GIS and the high resolution orthoimages mentioned previously. Using natural break points in the distributions of the estimated number of animals present, four size classes (i.e., small, medium, large, and very large) were established. Each farm was assigned to the appropriate class. The number of farms of each livestock type in a given class were then summed for each basin. The resulting density of farms was multiplied by the median number of livestock associated with each class, providing an estimate of the number of livestock in each basin. Because the same number of livestock at farms of different types are not necessarily equal in the degree of potential stress (e.g., 1000 cows do not equal 1000 chickens), I converted the density of all livestock types into nutrient units per basin. Nutrient units were calculated using the Ontario Ministry of Agricultural and Rural Affairs Nutrient Unit Conversion Factors (OMAFRA 2007). These factors scale all livestock types based on the amount of nutrients they produce per animal (e.g., 1 nutrient unit = 1 horse = 58 turkeys). Dividing the median density of livestock in each size class by the nutrient factor and then summing the nutrient units for each livestock type resulted in comparable, area scaled, measures of the stressor extent of the major livestock farm types.

2.2.4. Data Analysis

The CA and CS stressor gradients were each derived from a single Principal Component Analysis (PCA) of the covariance matrix of land cover variables. For the CS gradient, the proportion of each land cover type in each of the four zones was entered as a separate variable (example for percent forest cover: 0 – 30 m forest, 30 – 100 m forest, 100 – 250 m forest, Upland Forest). For the FA and FS stressor gradients, a two-step PCA approach was used. The stressor descriptors were grouped according to the nature of the stressor (Table 2.1), and each group was analyzed in a separate PCA of its covariance matrix. The factor scores from the first component of each of these initial PCAs were then subjected to a second PCA of the correlation matrix. The factor scores from the important component(s) of this second PCA were used to form the stressor

gradient(s). Despite the somewhat increased complexity in the two-step PCA, it enabled us to efficiently summarize functionally similar stressors (e.g., Livestock Farms) before analyzing overall patterns of stressors among basins.

To test if the use of detailed or spatially explicit stressor data improved characterization of the stressor gradients, Pearson correlations were calculated between gradients derived from data with different resolution (i.e., CA vs FA, CS vs FS) or spatially explicit versus aspatial data (i.e., CA vs CS, FA vs FS).

2.3. RESULTS

The stressor descriptors varied substantially in both percent occurrence and magnitude in the basins studied (Table 2.2). Many stressors, particularly those in the non-agricultural land use group (e.g., % Golf Course, % Landfill), appeared only infrequently. Other stressors, such as pasture and roads, were nearly ubiquitous, but did not exhibit substantial variation in magnitude across the basins. The effects of scaling livestock operations into nutrient units (OMAFRA 2007) was also demonstrated as the farm types that were most common (i.e., horse and beef) actually contributed little to the overall variation in total nutrient units.

2.3.1. Stressor Gradients

Results of the PCA to derive the CA stressor gradient indicated a single important component that explained 94% of the total variation in land cover, and varied most strongly with the proportion of cropland in the basin (Table 2.3.). Similarly, percent cropland, particularly for the 30m zone, was the primary driver for the CS gradient which explained 84% of the total variation in the CS descriptors (Table 2.3). In general, CS descriptors of a particular land cover type were loaded to the same end of the gradient regardless of spatial zone. There was, however, a tendency for the strength of loadings to increase the closer the zone was to a stream. Overall, both the CA and CS gradients were considered to be reflective of the degree of agricultural intensity in the basins.

For the fine detail data, the initial PCAs of groups of descriptors (as defined in Table 2.1) described the variability in the extent and nature of human activity among the basins. For the transportation group, the first component explained 84% of the variation in road and rail density, but most of the variability in the component was due to the density of rails (loadings: rail = 0.51, road = 0.06). The first component of the septic system group explained 69% of the variation in septic systems associated with rural homes, businesses, and industry (loadings: businesses = 0.69, homes = 0.54, industry = 0.25). Hog, dairy, and poultry farming contributed most to the first component, which explained 43% of the variation in livestock farming. The generally less intensive beef farms played a smaller role in defining the gradient, whereas the usually small horse and infrequently occurring sheep farms played an insignificant role in defining the component (loadings: swine = 2.39, dairy = 1.82, poultry = 1.11, beef = 0.49, horse = -0.03, and

Table 2.2. The distribution and abundance of stressors in 479 rural, headwater basins in southwestern Ontario, Canada.

| Descriptor | % Occurrence | Minimum | Maximum | Median | Std. Dev. |
|----------------------------------|--------------|---------|---------|--------|-----------|
| Land Cover | | | | | |
| % Cropland | 100 | 15.3 | 100.0 | 84.9 | 16.7 |
| % Forest | 100 | 0 | 65.7 | 11.8 | 12.7 |
| % Pasture | 95 | 0 | 28.3 | 1.5 | 5.7 |
| % Water | 48 | 0 | 8.1 | 0 | 0.6 |
| % Wetland | 5 | 0 | 2.4 | 0 | 0.2 |
| Field Tile Drainage | | | | | |
| % No Tile Drainage | 100 | 11.5 | 100.0 | 79.1 | 26.9 |
| % Random Tile Drainage | 71 | 0 | 84.1 | 4.3 | 13.7 |
| % Systematic Tile Drainage | 76 | 0 | 80.1 | 11.0 | 19.9 |
| Non-Agricultural Land Use | | | | | |
| % Campground | 6 | 0 | 2.1 | 0 | 0.2 |
| % Golf Course | 6 | 0 | 13.2 | 0 | 0.9 |
| % Landfill | 2 | 0 | 0.4 | 0 | 0.1 |
| % Pits and Quarries | 36 | 0 | 24.0 | 0 | 2.6 |
| Transportation | | | | | |
| Roads (km/1000 ha) | 100 | 3.3 | 29.3 | 10.9 | 2.8 |
| Rails (km/1000 ha) | 28 | 0 | 6.3 | 0 | 1.1 |
| Septic Systems | | | | | |
| Businesses (#/1000 ha) | 56 | 0 | 60.7 | 0.8 | 4.1 |
| Homes (#/1000 ha) | 100 | 0 | 325.5 | 37.0 | 38.4 |
| Industries (#/1000 ha) | 22 | 0 | 30.9 | 0 | 2.2 |
| Livestock Farms | | | | | |
| Beef Farms (#/1000 ha) | 70 | 0 | 15.6 | 1.2 | 2.4 |
| (NU/1000 ha) | | 0 | 181.0 | 12.3 | 28.0 |
| Dairy Farms (#/1000 ha) | 47 | 0 | 15.3 | 0 | 1.8 |
| (NU/1000 ha) | | 0 | 859.6 | 0 | 133.9 |
| Horse Farms (#/1000 ha) | 85 | 0 | 16.0 | 2.3 | 2.5 |
| (NU/1000 ha) | | 0 | 117.2 | 7.7 | 14.2 |
| Poultry Farms (#/1000 ha) | 43 | 0 | 7.5 | 0 | 1.2 |
| (NU/1000 ha) | | 0 | 927.8 | 0 | 106.3 |
| Sheep Farms (#/1000 ha) | 8 | 0 | 2.8 | 0 | 0.3 |
| (NU/1000 ha) | | 0 | 21.1 | 0 | 0.3 |
| Swine Farms (#/1000 ha) | 38 | 0 | 12.4 | 0 | 1.2 |
| (NU/1000 ha) | | 0 | 3255.7 | 0 | 386.6 |
| Permits to Take Water | | | | | |
| Both | 8 | 0 | 7.6 | 0 | 0.8 |
| Ground | 18 | 0 | 31.3 | 0 | 3.8 |
| Surface | 14 | 0 | 12.3 | 0 | 1.8 |

Table 2.3. Principal Component Analysis axis 1 loadings for descriptors used to calculate the coarse aspatial and coarse spatial stressor gradients.

| Stressor Descriptor | Aspatial | Spatial |
|----------------------------|-----------------|----------------|
| Cropland (Whole Basin) | 16.68 | |
| Cropland (30 m Zone) | | 21.93 |
| Cropland (30-100 m Zone) | | 19.24 |
| Cropland (100-250 m Zone) | | 15.83 |
| Cropland (Upland Zone) | | 13.93 |
| Forest (Whole Basin) | -12.37 | |
| Forest (30 m Zone) | | -18.12 |
| Forest (30-100 m Zone) | | -15.50 |
| Forest (100-250 m Zone) | | -11.81 |
| Forest (Upland Zone) | | -9.99 |
| Pasture (Whole Basin) | -4.00 | |
| Pasture (30 m Zone) | | -3.39 |
| Pasture (30-100 m Zone) | | -3.45 |
| Pasture (100-250 m Zone) | | -3.77 |
| Pasture (Upland Zone) | | -3.70 |
| Wetlands (Whole Basin) | -0.04 | |
| Wetlands (30 m Zone) | | -0.02 |
| Wetlands (30-100 m Zone) | | -0.02 |
| Wetlands (100-250 m Zone) | | -0.02 |
| Wetlands (Upland Zone) | | -0.03 |
| Water (Whole Basin) | -0.08 | |
| Water (30 m Zone) | | -0.27 |
| Water (30-100 m Zone) | | -0.17 |
| Water (100-250 m Zone) | | -0.06 |
| Water (Upland Zone) | | -0.03 |

sheep = -0.01). The water withdrawal descriptors resulted in a first component explaining 82% of the variation in the amount and source of water withdrawals. Water withdrawals from groundwater were the most strongly loaded, almost twice as strongly as withdrawals from surface waters. The loading for water withdrawals from both ground and surface was small in comparison (ground = 0.74, surface = 0.46, both = 0.19). For the non-agricultural land use group, only the pits and quarries descriptor significantly contributed to the first component, which explained 89% of the variation in these stressors (loadings: pits & quarries = 2.59, golf courses = 0.10, campground = 0.02, landfill = 0.01). In contrast, loadings for the field tile drainage component (85% of variation explained) loaded the "no field tiles" descriptor opposite to the different extents of field tiling (loadings: no field tiles = -26.85, systematic field tiles = 18.12, random field tiles = 8.72).

The second-step PCA of the aspatial, fine detail data (FA) resulted in a single important component explaining 30% of the variation in the stressor descriptors. This component indicated a general separation of agricultural stressors (i.e., cropland, field tiles, and livestock) from non-agricultural stressors (i.e., septic systems and water withdrawals), with the non-agricultural stressors being more prevalent in areas with less developed landscapes (i.e., more forested areas). Based on the component loadings (Table 2.4) transportation and non-agricultural land use components had negligible correspondence with the stressor gradient, whereas land cover, tile drainage, and livestock farms were strongly related to the gradient. Inspection of basins along the gradient revealed that forested basins with few human-added stressors of any kind had the lowest scores. Basins with a greater extent of non-agricultural stressors and a smaller extent of agricultural stressors were generally lower on the stressor gradient than the reverse situation.

Results for the fine detail, spatially explicit analysis (FS) were similar to the FA analysis. The FS results also mirrored the CS gradient in that there was a tendency for particular spatial zones to weigh more heavily in the final derivation of the stressor gradient, although the more heavily weighted zone varied among the groups of stressor descriptors. The land cover and septic system stressor groups tended to have the highest

Table 2.4. Principal Component Analysis axis 1 loadings for stressor groups used to calculate the fine aspatial and fine spatial stressor gradients.

| Stressor Group | Aspatial | Spatial |
|---------------------------|-----------------|----------------|
| Land Cover | 0.73 | 0.74 |
| Field Tile Drainage | 0.83 | 0.84 |
| Non-Agricultural Land Use | -0.05 | -0.06 |
| Transportation | 0.02 | -0.01 |
| Septic Systems | -0.25 | -0.24 |
| Livestock Farms | 0.74 | 0.72 |
| Water Withdrawals | -0.53 | -0.52 |

weightings in the near stream zones, whereas the transportation, non-agricultural land use, and livestock groups tended to have the highest weights in the upland zone. Mid-distance zones were most heavily weighted for the tile drainage group. Weightings in the water withdrawal group were more specific to the source of the water being withdrawn as weightings for withdrawals from groundwater were consistent across all zones but when the water came from either a surface water source or from both ground and surface water weightings were substantially higher in the near stream zone. Despite these differences in zone weightings, there was virtually no difference between the overall nature of the stressor gradient calculated from the FS data compared to that based on the FA data.

2.3.3. Gradient Comparisons

Plotting the four stressor gradients (CA, CS, FA, FS) against one another revealed strong differences between the effects of information detail and space on the description of the stressor environment (Figure 2.2). For the plots comparing stressor gradients of different details, but the same spatial explicitness (i.e., CA vs FA and CS vs FS), the use of fine detail data resulted in substantial differences in the scoring and relative ranking of individual basins. Using Pearson's correlation coefficient it was determined that the addition of fine detailed stressor data was responsible for the description of greater than 25% more variation in the stressor environment than could be described by the coarse detailed stressor gradients alone. In contrast, plotting the stressor gradients that had the same level of information but differed in their spatial explicitness (i.e., CA vs CS and FA vs FS) against each other (Figure 2.2) revealed only slight differences in the scoring and relative rankings of individual basins. Accordingly, Pearson's correlation coefficients indicated that less than 5% of the variation in the CS or FS gradient was unexplained by the CA or FA gradients respectively. Examination of the distribution of basins along the gradient for the CS vs CA and FS vs FA plots did, however, demonstrate differences in how the basins were distributed along the stressor gradients. Basins tended to be much more evenly distributed along the fine detail stressor gradients than the coarse detail gradients, where a substantial proportion of the basins were clumped at the high stressor extent end of the gradient.

Examination of the relative rankings of individual basins also revealed substantial

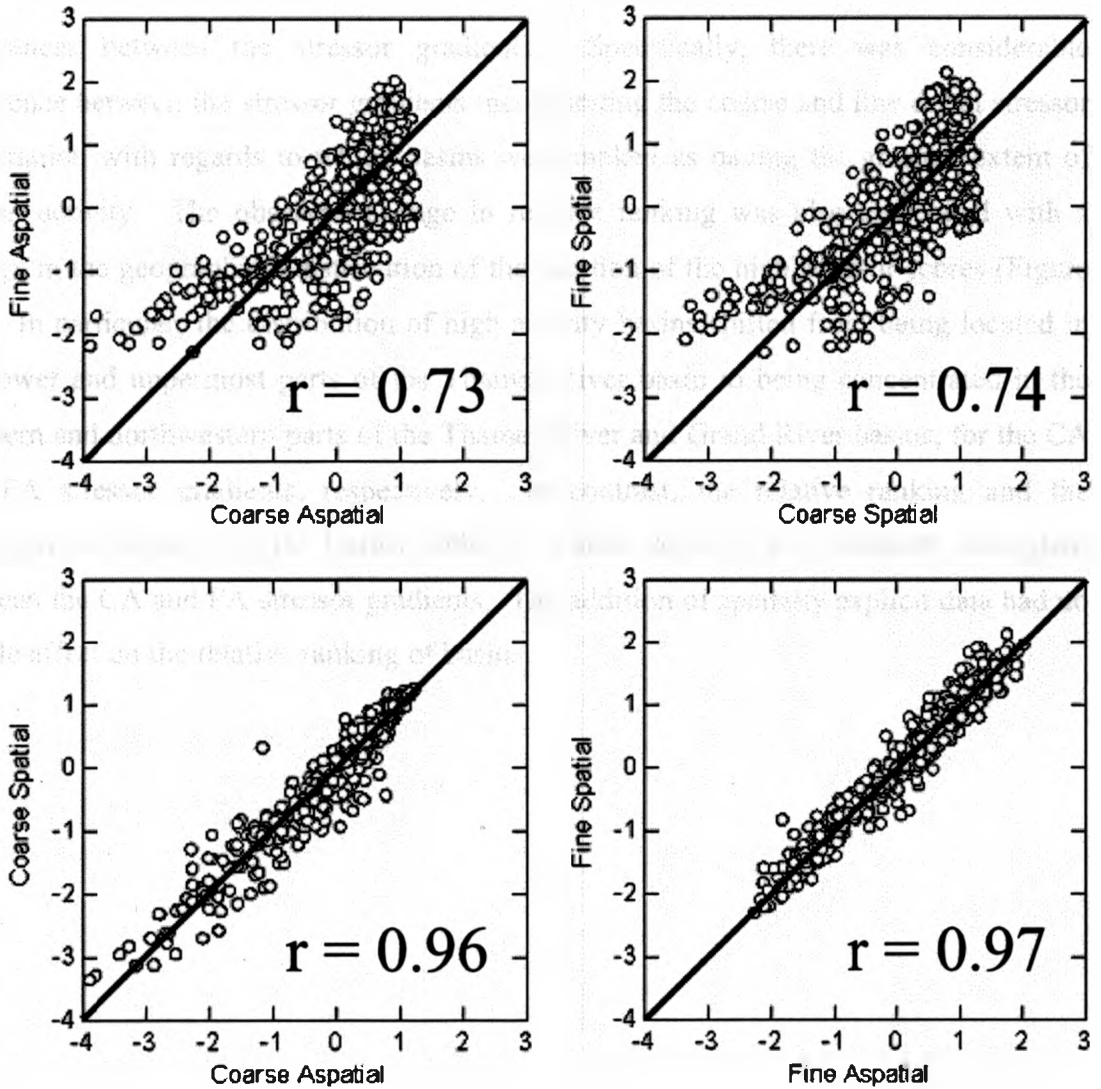


Figure 2.2. Plots of basin stressor gradient scores calculated using data varying in the levels of detail (fine and coarse) and spatial explicitness (aspatial and spatial). Basins with the same score for both stressor gradients would fall along the lines of equality.

differences between the stressor gradients. Specifically, there was considerable difference between the stressor gradients incorporating the coarse and fine detail stressor information with regards to which basins were ranked as having the greatest extent of human activity. The observed change in relative ranking was also associated with a change in the geographical distribution of the location of the high stressor scores (Figure 2.3). In particular, the distribution of high activity basins shifted from being located in the lower and uppermost parts of the Thames River basin to being concentrated in the northern and northwestern parts of the Thames River and Grand River basins, for the CA and FA stressor gradients, respectively. In contrast, the relative ranking and the geographical location of the basins with low human activity was relatively consistent between the CA and FA stressor gradients. The addition of spatially explicit data had no visible affect on the relative ranking of basins.

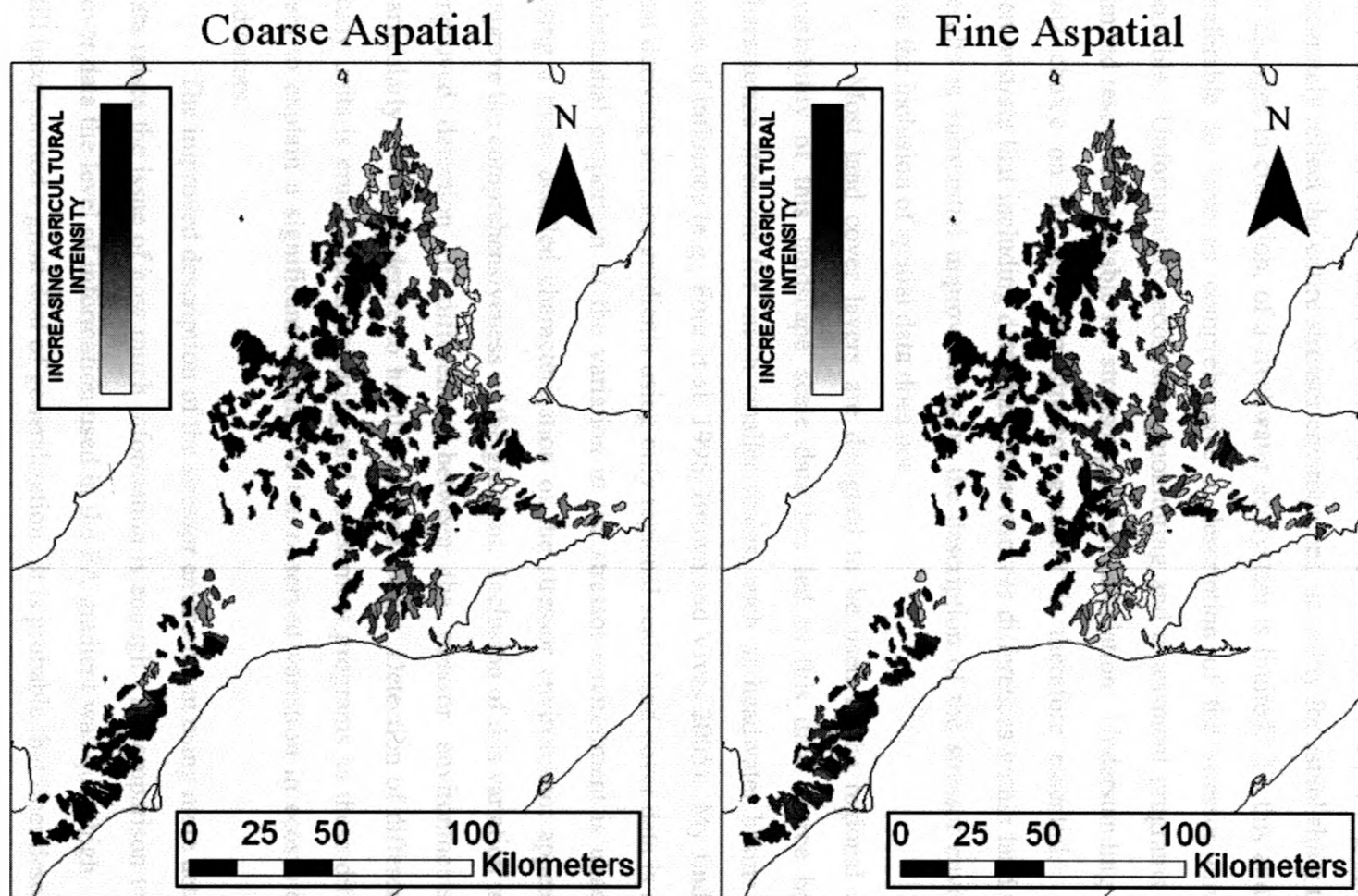


Figure 2.3. Geographical distribution of 479 rural headwater basins in southwestern Ontario, Canada. Shading describes relative extent of agricultural intensity in each basin based on stressor gradients calculated using coarse (left) and fine (right) detail information.

2.4. DISCUSSION

Stressor gradients are an effective method of describing variation in the stressor environment of ecosystems (Bailey et al. 2006). If, however, the gradient does not accurately reflect the entire stressor environment, its utility for establishing the condition, or changes in condition, of a freshwater ecosystem is limited. To this end, it is always preferable to have as comprehensive a description of the stressor environment as possible. Unfortunately, ecosystem monitoring and assessment programs usually have limited resources available for stressor gradient calculation. Understanding the effects of data choice on the resulting stressor gradient is therefore essential. My findings demonstrate that including data that better resolves differences within land cover classes results in substantial improvements in the description of the stressor environment, but that the inclusion of spatial data does not.

Most land cover layers are designed to be used at the regional scale. Wide availability of this landscape scale data has led to this data source being used to characterize stressors for much smaller scales, such as headwater basins and wetland zones of influence (e.g., Fore et al. 1996, Brown and Vivas 2005). My findings indicate that deriving stressor gradients using only regional scale land cover data is not ideal as a substantial proportion of the variation in the stressor environment is unaccounted for. Using a more detailed characterization of the stressor environment appears to greatly improve the comprehensiveness of the gradient. Inclusion of this variation translated into improved detection of differences between the stressor environments of basins, particularly in the moderate to highly stressed range. Detection of differences between study units is crucial for monitoring and assessment programs as these differences will help to explain a significant amount of the observed variation in ecosystem condition indicators.

The improved description of the stressor environment using more detailed stressor data raises the issue of how much information is enough. In comparison to typical land cover data the level of information used in the FA gradient was quite high. Yet this data still incorporated a great deal of generalization. It is probable that finer description of the stressor data, perhaps by including management practices, would result in an even more realistic depiction of the stressor environment. In theory it would be ideal to continue

increasing the detail of the stressor information used until only minimal amounts of additional stressor variation were being described. Attaining this level of information, however, is unlikely to be practical under most circumstances. Indeed, even reaching the level of detail used in this study required significant expenditure of resources not normally available for most monitoring and assessments programs. Attaining an even finer level of stressor information that is even less available and more transient over time could well be infeasible. This problem is likely to be alleviated to some extent as more and better electronic data sources become available, many programs and agencies may currently be in the difficult position of deciding between using their resources to sample more ecosystems or to derive a more accurate stressor gradient. I would argue that the importance of the stressor gradient is paramount because it forms the foundation for sampling site selection, detection of impairment of ecosystem condition, and ultimately determines whether the goals of the program can be met. This is particularly true if the purpose of the assessment and monitoring is to determine the cause of impairment and inform future land use planning decisions, purposes that are considered the future of freshwater ecosystem assessment (Allan 2004, Bailey et al. 2007).

My findings indicate that including spatially explicit data does not substantially improve the description of the stressor environment for this region. Brown and Vivas (2005), the only other study I know of to test this hypothesis, also found that incorporating spatially explicit data did not significantly improve their stressor gradient. These results are most likely attributable to the strong covariation that exists between surface geology and many types of human stressors that has been demonstrated in many regions (e.g., Iverson 1988, Burgi and Turner 2002, Jobin et al. 2003), including the region this study was conducted in (Yates and Bailey 2006). In highly developed regions this covariation leads to a homogeneous distribution of stressors across the geological units that enable these types of activities. The fact that both my study and Brown and Vivas (2005) were conducted at fairly small scales (i.e., headwater basins and wetlands) where only minimal variation in geology can be expected would further reduce the likelihood of patterns of spatial variability within a stressor. Space might therefore be more important to descriptions of stressors across larger scales (e.g., large river basins) where there is likely to be more spatial variability in geology. It is also important to

recognize that although study indicates that finer detailed information substantially improves stressor description, but spatially explicit data does not, this result pertains to the question of what is the most effective way to describe the stressor environment. The question of which source of variation in the stressor gradient (i.e., resolution or space) is best at explaining differences in ecosystem condition, such as biotic community structure or composition, can only be addressed by sampling the ecosystem. It is possible that the small amount of variation due to spatial variation of stressors may explain a large amount of variation in ecosystem condition.

For stressor gradients to be an effective tool for monitoring and assessment of freshwater ecosystems it is imperative that the effects of data choice on the quality of the stressor gradient be well understood. My study demonstrates that when generating a stressor gradient using data that incorporates fine details regarding stressors adds substantially more variation to the description of the stressor environment than spatially explicit data. Based on this finding I recommend that resources for stressor gradient creation first be used to better resolve the stressor environment and then, if resources are still available, the spatial configuration of the stressor environment be incorporated.

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Chapter 3. Response of small stream communities to natural and stressor gradients in southwestern Ontario, Canada

3.1. INTRODUCTION

The structure of aquatic communities is a reflection of both natural and human-generated environmental conditions. Bioassessment protocols depend on these relationships in two ways. First, that aquatic biota can be classified based upon their relationship with the natural environment, and second, that aquatic biota respond to human perturbations of the environment in a predictable manner (Bailey et al. 2004). It is therefore essential that the relationships between aquatic biota and their surrounding natural and human environments be described prior to the development of any regional bioassessment program (Stevenson et al. 2004).

Numerous studies have demonstrated associations between human activity and aquatic biota (see review by Allan 2004). Common biotic responses to increased human activity include loss of diversity (e.g., Resh et al. 1995), increased dominance of tolerant taxa (e.g., Lenat 1984), and changes in species traits (e.g., Richards et al. 1997). Although many different types of land use, including forestry (Nislow and Lowe 2006), urban development (Wang et al. 2001), and mining (Bruns 1995) have been shown to alter the condition of aquatic ecosystems, the effects of agriculture on aquatic ecosystems have been particularly well studied. Differences in aquatic communities across gradients of agricultural activity have regularly been detected (e.g., Richards et al. 1993; Roth et al. 1996; Wang et al. 1997). One very important conclusion that has resulted from these studies is that the effect of agriculture on aquatic ecosystems is strongly dependent upon the type and extent of agriculture that is being practiced (Allan 2004). For example, Strayer et al. (2003) found that low intensity pasture agriculture had no discernible impact on stream assemblages, whereas most studies looking at catchments dominated by intensive row crop agriculture have found dramatic changes in stream biota compared to reference streams (e.g., Meador and Goldstein 2003).

The distribution of aquatic biota has also been correlated with a wide variety of natural environmental features. Ecoregions, areas of relatively homogeneous geology, soils, and potential vegetation (Omernik 1987), have often been used to classify aquatic

communities. Indeed, many studies have found variation in fish and invertebrate assemblages to be associated with ecoregion boundaries (e.g., Lyons 1993; Van Sickle & Hughes 2000; Sandin & Johnson 2000), however, many other studies have not (e.g., Hawkins & Vinson 2000; McCormick et al 2000; Herlihy et al 2005). The discrepancy between results has been hypothesized to be due to environmental gradients within ecoregions. In response, a nested approach that considers factors such as altitude, stream size, and catchment characteristics has been proposed (Sandin & Johnson 2000). Many of these ecosystem features have also been shown to be correlated with the community structure of various groups of aquatic biota. Ferriera et al. (2007) found fish assemblages corresponded to changes in altitude and climate in Portugal. Fish assemblages have also been found to correspond to river catchment, geology, and soils in Kansas (Hawkes et al. 1986). Geology and altitude have been shown to structure macroinvertebrate communities (Chaves et al. 2005), and surface geology in particular was found to be an important determinant of macroinvertebrate communities in Michigan (Richards et al. 1997).

In addition to structuring biological communities, the natural environment may be strongly correlated with the human-generated stressor environment. Agricultural activities in particular have been shown to be strongly constrained by surface geology (Iverson 1988; Jobin et al. 2003; Richards et al. 1997; Yates & Bailey 2006). In some instances covariation between the natural and stressor environments has made it difficult for researchers to determine the independent effect of human activities on aquatic communities (Fitzpatrick et al. 2001) and some studies have found agricultural effects to be completely masked (Richards et al. 1997).

In this study, I used multivariate gradient analysis to establish how two aquatic assemblages, fish and benthic macroinvertebrates (BMI), varied across natural and stressor gradients in southwestern Ontario streams. I also determined the degree of covariation between the natural and human environments and partitioned this variation according to how it is affecting the stream biota. Identifying these relationships will result in a better understanding of how natural and human features interact to structure aquatic communities. This knowledge will be useful for the generation of more effective bioassessment protocols for the region.

3.2 METHODS

3.2.1. *Study Area*

Southwestern Ontario is located in the Great Lakes Basin and is bordered by Lake Huron to the north and west, Lake Erie to the south, and Lake Ontario to the east (Figure 3.1.). This region is characterized by glacial landforms that were predominantly forested prior to European settlement. More than 75% of the forest has since been cleared and the region is now dominated by agricultural lands. The intensity of agriculture in the region is strongly related to geographical gradients that correspond with the region's two ecoregions, the Lake Erie Lowlands and the Manitoulin-Lake Simcoe regions. Intensive row crop agriculture (primarily corn and soybeans) and livestock farming predominate in the fertile soils of the more southern Lake Erie Lowlands region. Although still intensive, particularly in the southern part of the Manitoulin-Lake Simcoe region, shallower soils in much of this region restricts agriculture to lower intensity pasture agriculture. Across both regions the agricultural mosaic is interspersed with numerous small towns, several urban centres, and remnant forest patches.

Within these two ecoregions, I sampled 160 headwater streams predominantly in the Grand River and Thames River watersheds (Appendix 2). Streams were also sampled within four small watersheds that comprise the drainage of the Long Point (Lake Erie) region. These streams were selected from the 479 rural headwater basins defined by Yates and Bailey (in review). Streams were selected so that they encompassed the range of variation in both natural and human-generated environments.

3.2.2. *Data Collection*

Landscape data for each of the 160 basins was collected using ArcGIS (ESRI 2005). Following examination of several descriptors of the natural environment, I determined that although surface geology varied substantially across the study basins, climate, topography, latitude, and altitude did not. Based on these findings, it was decided that surface geology alone would be used to describe the natural environment. Surface geology was described by determining the proportion of each basin that was characterized by a particular texture of parent material (e.g., sand) as defined by the Ontario Geological Survey (2003). The human-generated (stressor) environment was described at both the local and landscape scale. I defined the stressor environment as

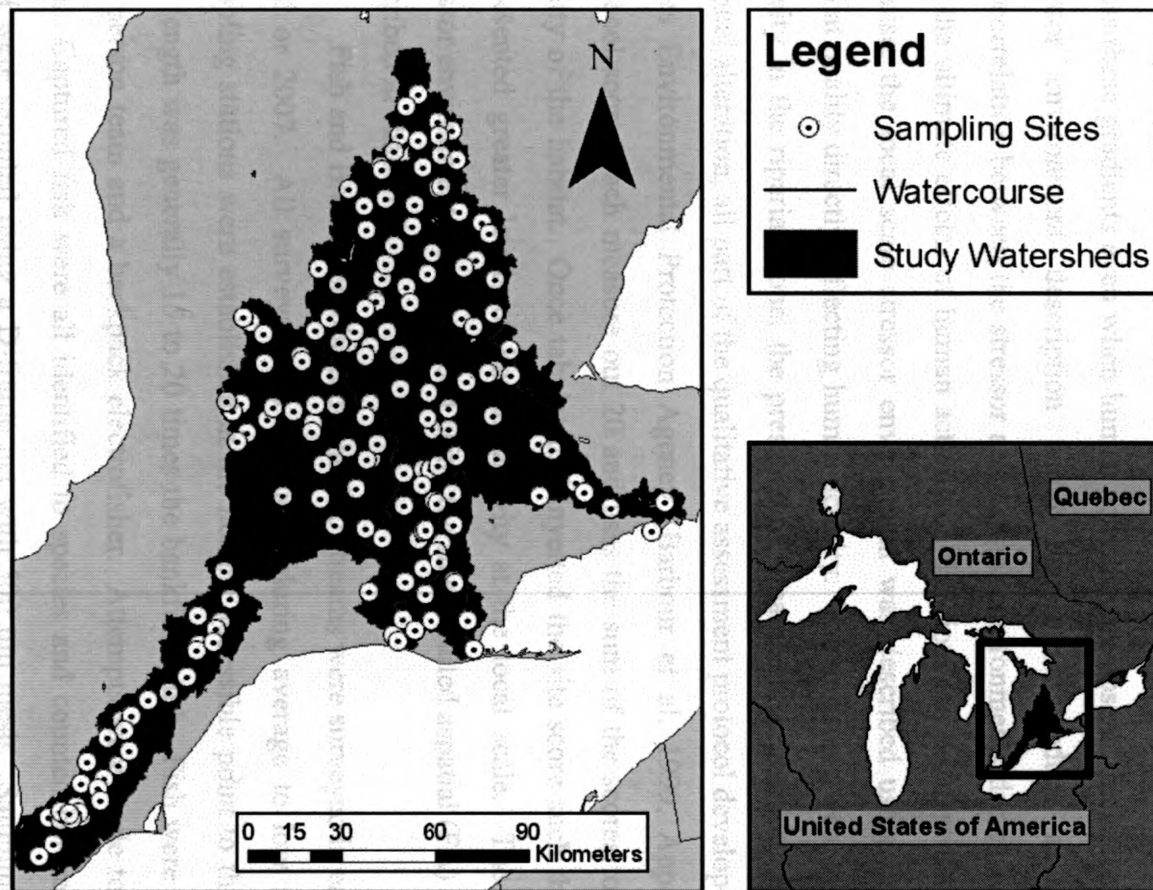


Figure 1. Location of study region in the Great Lakes Basin (bottom right) and location of 160 sampling sites across the two southwestern Ontario ecoregions (Main).

the types and magnitudes of human activities (e.g., density of livestock, removal of riparian vegetation) present rather than the effects of those activities (e.g., sedimentation, increased nutrient loadings). This definition was appropriate because the purpose of this study was to determine the response of the biota to the stressor environment across natural gradients and many of the measures of effects (e.g., nitrate concentration) vary across these gradients even when human activities are absent. Inclusion of effects in the stressor environment description could therefore introduce a high degree of autocorrelation between the stressor and natural environments decreasing the likelihood that the ultimate effects of human activity on the biota can be identified. Based on this premise, the local scale stressor environment was described using three measures of habitat quality directly reflecting human activity. The measures were the level of human activity in the riparian zone, the presence of streambank vegetation, and the degree of channel alteration, all part of the qualitative assessment protocol developed by the United States Environmental Protection Agency (Barbour et al. 1999, Appendix 3). This protocol scores each measure out 20 and uses the sum of the scores to rate the overall quality of the habitat. Once tabulated, I inverted the site score such that higher scores represented greater levels of human activity at the local scale. The landscape scale stressor environment was described using the fine detailed aspatial (FA) stressor gradient described in chapter 2.

Fish and BMI assemblages in the 160 streams were surveyed in early fall of either 2006 or 2007. All surveys were conducted during average to low flow conditions. Sampling stations were established at the nearest accessible point to the basin outflow. Site length was generally 15 to 20 times the bankfull width. Fish were sampled using a two person team and a backpack electrofisher. Attempts were made to capture all fish seen. Captured fish were all identified to species and counted before being released. BMI were sampled using a D-frame net with 500 μm mesh. Sampling consisted of a three minute kick and sweep across all habitats present in the site in proportion to their relative abundance. All captured BMI were preserved in 95% ethanol and taken to the lab for processing. In the lab, samples were washed and large debris removed using a 500 μm sieve. Samples were then spread evenly across a gridded pan. Grid cells were randomly selected and all the BMI in the cell removed using a dissecting scope. This

subsampling process was repeated until a minimum of 300 individuals had been removed from the sample. Subsampled BMI were then identified to lowest taxonomic level possible.

3.2.3. Data Analysis

To prepare data for analysis I generated four matrices. Two matrices consisted of environmental data. The total habitat quality score and calculated values for the FA stressor gradient made up the stressor environment matrix, while the seven types of surface geology comprised the natural environment matrix. The remaining two matrices used biological data and described fish and BMI assemblages for each of the 160 sites. To reduce the effect of rare taxa, only taxa that were present at 5% or more of the total number of sites (corresponding to a minimum of eight sites) were included in the matrices. Because of varying rates of catchability among fish species, I built the fish assemblage matrix using only presence/absence (rather than less reliable abundance) data. In contrast, the matrix of macroinvertebrate assemblage used percent composition, predominately at the family level (exceptions included midges, crayfish, worms, and leeches which were included at the subfamily, order, class, and class levels, respectively). Family level resolution was used because in many cases only one or two genera of a particular family were present, and this degree of taxonomic resolution has been deemed sufficient for bioassessment purposes (Bailey et al. 2001).

PC-ORD 4.17 (McCune and Mefford 1999) was used to conduct separate canonical correspondence analyses (CCA) of the natural and human matrices against the fish and BMI assemblages to determine the level of correspondence between the biota and the different environmental components. A fifth CCA was conducted to determine the level of correspondence between the natural and human environments. In order to separate the effects of the natural and stressor environments on the fish and BMI assemblages a second set of matrices were created by calculating the distances between sites using a Mantel's Test. Based on the nature of the datasets, I used Jaccard's Distance for the fish presence-absence data, Bray-Curtis Distance for the BMI relative abundances, and Relative Euclidean Distance for the stressor and natural environment matrices. These matrices were entered into the R-package (Casgrain and Legendre 2001) where two partial Mantel's tests were conducted to determine if significant relationships existed

between the stressor environment and the two assemblages when the affect of the natural environment was controlled.

3.3. RESULTS

Across the 160 sampled sites I collected a total of 22,011 fish comprising 53 species, however, only 33 of these species were collected at 5% or more of the sites (Appendix 4). Fish species richness at a site varied from a maximum of 16 species to a minimum of one species, and averaged 7.4 species, with a standard deviation of 3.3. BMI sampling resulted in the collection of 97 taxonomic groups, with 59 of the groups being found at a minimum of eight of the sampled sites (Appendix 5). BMI richness at a site varied from a maximum of 36 to a minimum of 6 with an average of 18.7 and a standard deviation of 5.6.

Results of the CCA of the fish assemblage versus the stressor environment indicated that composition of fish communities varied considerably with changes in the nature of the stressor environment. Based upon a Monte Carlo test (permutations = 999), Axes 1 and 2 were significant ($p = 0.001$). Axis 1 had a species-environment correlation of $r = 0.629$ and was negatively correlated with human activity occurring at both the landscape and local scales, however, the association was much stronger for the landscape compared to the local scale (Table 1). Sites with a high axis one score tended to be exposed to lower extents of human activity. This variation in the stressor environment corresponded to compositional shifts in the fish assemblages present. In general, cold water species (e.g., rainbow trout, brown trout, and sculpin spp.) that are generally intolerant of human disturbance scored highest on Axis 1, whereas more tolerant, warm water species (e.g., common shiner, least darter, and central stoneroller) had lower scores. Axis 2 had a more moderate species-environment correlation ($r = 0.486$), but only the local scale was strongly associated to the axis (Table 1). In general, Axis 2 separated those fish species that are more specific in their habitat requirements for spawning (e.g., hornyhead chub, brook trout) from the more generalist species (e.g., central mudminnow and common carp). However, Axis 2 also predicted which of the tolerant species would present in the basins with low Axis 1 scores (i.e., high levels of human activity) according to local conditions. Specifically Axis 2 divided the tolerant species into three groups: generalists present under all local conditions (e.g., common shiner and bluntnose minnow), habitat specialists present only when local conditions are of high quality (e.g.,

Table 3.1. Intra-set correlations between environmental descriptors and canonical correlation axes for 160 small streams in rural, southwestern Ontario.

| Matrix 1 | Matrix 2 | Descriptor | Axis 1 | Axis 2 | Axis 3 |
|----------|----------|------------|---------|---------|---------|
| Fish | Stressor | Landscape | - 0.954 | - 0.299 | N/A |
| | | Local | - 0.529 | 0.849 | N/A |
| Fish | Natural | Bedrock | 0.086 | 0.055 | 0.380 |
| | | Clay | 0.024 | - 0.648 | 0.706 |
| | | Gravel | 0.243 | 0.643 | 0.176 |
| | | Organic | 0.110 | 0.510 | 0.565 |
| | | Sand | 0.840 | - 0.277 | - 0.396 |
| | | Silt | - 0.606 | - 0.045 | - 0.281 |
| | | Till | - 0.615 | 0.243 | 0.010 |
| Benthos | Stressor | Landscape | - 0.885 | 0.465 | N/A |
| | | Local | - 0.666 | - 0.746 | N/A |
| Benthos | Natural | Bedrock | 0.091 | - 0.968 | - 0.165 |
| | | Clay | 0.014 | - 0.293 | 0.211 |
| | | Gravel | 0.119 | 0.248 | - 0.797 |
| | | Organic | - 0.054 | - 0.140 | - 0.348 |
| | | Sand | 0.921 | 0.169 | 0.252 |
| | | Silt | - 0.419 | - 0.003 | 0.461 |
| | | Till | - 0.720 | 0.131 | - 0.246 |
| Natural | Stressor | Landscape | - 0.947 | 0.323 | N/A |
| | | Local | - 0.543 | - 0.840 | N/A |

fantail darter and hornyhead chub), and highly tolerant ones present when the local environment has been highly disturbed (e.g., least darter and green sunfish).

The CCA testing the correspondence of the BMI to the stressor environment resulted in two significant axes ($p = 0.001$). Axis 1 had a species-environment correlation of $r = 0.625$ and was highly correlated with the level of human activity occurring at the landscape scale and moderately correlated with the local scale; both the landscape and local scales were negatively associated with Axis 1 (Table 1). Based on these results the axis was defined as a gradient of the extent of human activity. Similar to the fish assemblages, the response of the BMI assemblages was a shift from assemblages dominated by a relatively small number of tolerant taxa (e.g., Hydrophilidae, Unionicolidae, and Coenagrionidae) in basins with high levels of human activity to a more diverse assemblage with increased numbers of intolerant taxa, particularly the orders Ephemeroptera, Plecoptera, and Trichoptera, (e.g., Taeniopterygidae, Philopotamidae, and Psychomyiidae), in basins with lower levels of human activity. Axis 2 had a species-environment correlation of $r = 0.492$ and was strongly and positively associated with the local scale, but moderately and negatively associated with the landscape scale (Table 1). As a result, Axis 2 was defined in the same fashion as Axis 2 for the fish. Although the majority of taxa were found in a band about the point of origin, the taxa that scored low on this axis that were on the tolerant side of the Axis 1 were predominantly taxa which preferred slow moving waters and were tolerant of fine substrates (e.g., Hirudinea, Lymnaeidae, and Leptohiphidae). Taxa that scored high on Axis 1, but low on Axis 2, tended to be filter feeders (i.e., Simuliidae, Psychomyiidae, and Philopotamidae). Only two taxa scored notably high on Axis 2, Decapoda and Helicopsychidae.

The CCA of the fish assemblages versus the natural environment resulted in three significant axes ($p = 0.001$) based upon the Monte Carlo analysis. Axis 1 had a species-environment correlation of $r = 0.634$ and represented a gradient differentiating basins dominated by till and silt deposits from those characterized by sand textured deposits (Table 1). Similar to axis one of the stressor environment CCA, fish that scored most highly on this axis tended to be intolerant, cold water species, particularly brown and brook trout, whereas tolerant, warm water, benthic species, such as the central stoneroller

and fantail darter, scored lowest on this axis. The second axis had a species-environment correlation of $r = 0.562$ and varied across a gradient characterizing the proportion of clay versus gravel deposits in the basin. Fish that scored high on the gradient (i.e., those that were most often collected in basins with a high proportion of gravel deposits) tended to be invertivores that required gravel substrates for spawning (e.g., longnose dace, Iowa darter, and brook trout). In contrast, species collected more frequently in clay dominated basins were water column feeders, not requiring gravel substrate for spawning (e.g., common carp and spotfin shiner). Axis 3 was again strongly associated with the proportion of clay deposits in the basin, as well as the proportion of organic deposits (Table 1). Species that scored high on this axis were either species regularly found in cold to cool streams running through organic materials (e.g., central mudminnow, Iowa darter, and brook trout) or more pool oriented species (e.g., largemouth bass, striped shiner, and spotfin shiner). Species with low scores on this axis were rainbow and brown trout, both species that were only found in basins dominated by sand deposits.

Only one significant axis resulted from the CCA between the BMI and the natural environment. This axis was Axis 2 and had a species-environment correlation of $r = 0.725$ ($p = 0.005$), Axes 1 and 3, despite species-environment correlations of $r = 0.653$ and $r = 0.493$ respectively, were not significant based upon the Monte Carlo Tests ($p = 0.13$ and $p = 0.09$). Axis 2 was strongly associated with the proportion of the basin that had bedrock at or near the surface (Table 1). The vast majority of taxa scored positively on this axis indicating they tended to be present in basins where bedrock did not make up a substantial proportion of the surface geology. Only a small number of taxa (e.g., Simuliidae, Corydalidae, and Planorbiidae) were most likely to be collected when bedrock made up a substantial proportion of the surface geology. Axis 1, despite not statistically significant at $p = 0.05$, was noteworthy as it appeared to be the primary gradient across which the BMI assemblage varied. Much like the first axis for the fish, this axis corresponded to a change from till to sand dominated basins (Table 1). Taxa tended to be situated along this axis according to tolerance, with most of the EPT and other intolerant taxa being found in basins with higher proportions of sand. The highest scoring species, however, tended to be coldwater species, preferring sand or large woody debris substrate (e.g., Gammaridae, Pyschomyiidae and Taeniopterygidae) whereas the

lowest were taxa that preferred slower moving waters and often fed on plant material (e.g., Caenidae, Valvatidae, and Haliplidae). Although not statistically significant, Axis 3 also exhibited an ecologically realistic pattern. This axis was associated with the amount of gravel deposits in the basin (Table 1) and had ordinated taxa on the basis of habitat guilds. Taxa that were common when the proportion of gravel was high tended to be clingers and/or taxa that preferred gravel or cobble substrate (e.g., Psephenidae, Ephemerellidae and Corydalidae). Species scoring at the opposite end of this gradient tended to be swimmers (e.g., Hirudinea and Gammaridae) or burrowers (e.g., Pyschodidae).

The human-generated and natural environments were strongly correlated. Axis 1 had a human-natural correlation of $r = 0.786$ and a significance of $p = 0.001$ based on the Monte Carlo test. Once again human activity at the landscape and local scales were negatively associated with Axis 1, although the association was much stronger with the landscape than local scale (Table 1). This axis of human activity corresponded to changes in surface geology where sites with high amounts of human activity tended to be in areas where silt and till deposits dominated, while sand and bedrock landforms were most common in areas where human activity was of low to moderate levels. Axis 2, while statistically significant ($p = 0.001$) explained little additional variation to Axis 1 ($< 1\%$) and had a weak human-natural correlation ($r = 0.249$). This axis appeared to separate surface geology types that were common (i.e., till, sand, and gravel) from those that were comparatively rare among the basins (i.e., organic, clay, silt, and bedrock). This nonsensical relationship was interpreted as an artifact of the test and not indicative of any meaningful relationship between human activity and surface geology.

Results of the partial Mantel's test indicated that once the natural environment was accounted for, a significant association with the stressor environment could be seen in the BMI assemblage ($r_s = -0.10$, $p = 0.04$), but not in the fish assemblage ($r_s = 0.07$, $p = 0.10$). In general, correlations between the assemblages and the environments were low when using the distance matrices and a Mantel's test, although all were statistically significant ($p < 0.05$). The strongest correlation was between the fish and the natural environment, but it was only $r_s = 0.12$. All the other correlations also had r_s -values about 0.1.

3.4. DISCUSSION

The results of this study clearly indicated that in the agricultural areas of southern Ontario, as the intensity of human activity increases, intolerant fish and benthic macroinvertebrate taxa are lost and replaced by more tolerant taxa. Loss of intolerant species is a common response of aquatic biota to agricultural activity (e.g., Lenat 1984), and as a result several tolerance based indices have been developed and widely applied to agricultural streams (e.g., Hilsenhoff biotic index [Hilsenhoff 1987]). Based on my findings, it is likely that these tolerance metrics would be useful in assessing the condition of streams in the southwestern Ontario region as well.

Fish and BMI taxa were also found to be organized by a general gradient of tolerance in their respective correlations with the main surface geology gradient. This response is partly due to the high degree of covariation between this region's natural and stressor environments, but also a reflection of the way that tolerance levels are assigned. Tolerance incorporates a wide range of ecological characteristics that determine whether or not a species can live and reproduce in the presence of stressors (Bressler et al. 2006). Availability of appropriate habitat is one of the many factors that determine whether a species can survive in a given location. In addition to responding to human activity, habitat also covaries with regional surface geology (Richards et al. 1997). In this study many of the individual fish and BMI taxa were grouped according to habitat preferences and associated species traits (i.e., reproduction, feeding, and movement) within the overall tolerance trend. Given that surface geology has been shown to influence aquatic biota through control over habitat features (Richards et al. 1996; Richards et al. 1997; McRae et al. 2004), it is likely that these more subtle differences in composition are due to surface geology related differences in habitat.

My results indicate that variations in fish and BMI assemblages were better predicted by human activities occurring at the landscape as opposed to the local scale. This result is consistent with other studies (e.g., Roth et al. 1996, Richards et al. 1996) that found the catchment to be the best predictor of stream biota. As was pointed out by Allan et al. (1997), this may be in part due to the fact that my replication was at the basin scale, not the reach scale, as studies conducted at the reach scale within a small number of basins have often found reach scale variation to be the better predictor of community

composition (e.g., Lammert and Allan 1999). However, in a southwestern Ontario study examining a hierarchy of spatial scales, variation in BMI community structure was mostly at the among stream scale indicating that a single sample per catchment is appropriate for establishing the condition of stream ecosystems (Ciesielka and Bailey 2007). The results of Ciesielka and Bailey (2007), accompanied by the fact that my study encompassed substantial variation in human activity at both local and landscape scales, makes us confident that my results were not an effect of study design. A more likely explanation for why landscape scale variation was most important than local variation is that surface geology, and hence human activity, is quite variable among my study basins but fairly homogeneous within them. Sites in basins where human activity was high at the landscape scale, therefore, also had a tendency to have greater activity at the local scale. This result is consistent with the finding that an overall gradient of human disturbance best corresponded with variation in both fish and BMI assemblages. Another contributing factor may have been that my definition of stressors did not include the effects of human activity. As a result, many of the local predictors (e.g., % fines) that have been used in past research that concluded that the local scale is the better predictor (e.g., Richards et al. 1997) were not included in this study. Although there is great value in measuring the relationship between the effects of human activity and aquatic biota, I consider my study to be a fairer test of the relative importance of the landscape and local scale because it avoids the problem of indirect effects. For example, variation in percent fines among two sites could be due to activities occurring at the landscape scale, the local scale, or due to differences in geology.

Although responses between fish and BMI were similar in terms of the shift in dominance of intolerant species to tolerant ones, the size of the pool of taxa that were likely to be present as human activity in the basins increased differed. The BMI assemblages exhibited the typical response of biota to increased human activity; a declining number of potential taxa as human activity increased (Rosenberg and Resh 1996). In contrast, a relatively small number of coldwater fish species were present in the basins with low levels of human activity, whereas a much larger number of warm water species tended to be found in basins with high levels of human activity. This result implies that as human activity increases, conditions in these small streams change from

those that favour a few stenotherms to those that favour a much more specious set of eurytherms. Such a hypothesis is consistent with what is known about coldwater streams (Hughes et al. 2004). Indeed, Marshall et al. (2008) found that Wisconsin streams that had once been coldwater prior to agricultural impacts experienced declines in species richness and the number of eurytherms when agricultural effects were reduced through a conservation program.

My study is consistent with the findings of a growing number of researchers (e.g., Fitzpatrick et al. 2001; Richards et al. 1997) who are finding it difficult to attribute variation in aquatic biota to a particular influence because of significant covariation in the natural and stressor environments. In my study, variation in human activity mirrored the main surface geology gradient. This covariation is to be expected because variation in the natural environment is the template by which land use decisions are made. This covariation does, however, have important implications for bioassessment. In particular, it makes the identification of reference condition very difficult for highly developed regions like southwestern Ontario, because virtually all of the basins that exhibit the natural characteristics that are suitable for intensive human activity are highly disturbed. As a result, when assessing gradients of human activity, I see relationships like the one in this study, where the different levels of human activity correspond to a particular natural feature. Associations between community composition and the stressor gradients like those described in this research could be useful in overcoming this problem by helping to establish a simulated reference condition as has been done in other parts of Ontario (Kilgour and Stanfield 2006). However, broad application of these associations is risky because of the confounding effect of surface geology on these assemblages. For instance, in this study the fact that low and moderate levels of human activity were generally associated with basins dominated by glacial deposits of sand, whereas high levels of activity were primarily restricted to tills and silts raises the question of whether stream assemblages in these different landforms would have been similar prior to agricultural development. Streams in coarse glacial outwash of sand and gravel regularly have large influxes of groundwater and are thus likely to support coldwater taxa. In contrast, old lake bed deposits of silt and clay tend to have streams dominated by surface flows and are more likely to contain warm water taxa. Therefore, simulating reference

condition in streams flowing through old lake beds using taxa representative of cold water streams may be inappropriate. Although more work is required to better separate the relative influences of natural and stressor gradients, it appears that using relationships between biota and the environment to predict reference condition is a promising area that is worthy of more attention.

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Chapter 4. Selecting Objectively Defined, Environmentally Stratified, Regional Reference Sites for Bioassessment Programs

4.1. INTRODUCTION

An ecosystem in “reference condition” has been defined as one that has had minimal exposure to stressors of concern (Bailey et al. 2004). The reference condition approach to bioassessment uses relationships between biota at sites in reference condition and their natural environment to predict what biota would be expected if a “test” site is in reference condition (Bailey et al. 2004). This approach has become the method of choice for virtually all assessment programs seeking to monitor ecological condition in many freshwater ecosystems (e.g., Barbour et al. 1995, Davies 2000, Rosenberg et al. 2000, Wright 2000). In my experience, however, program managers are often unsure as to how to go about selecting reference sites because they do not know what minimally disturbed sites “look like” in their region. I believe that much of this confusion stems from the fact that regional reference sites have in the past been selected mainly using professional judgment to determine what amount and type of human activity (e.g., < 5% agriculture in the catchment) is considered “minimally disturbed”. Development of a method for objectively establishing what constitutes the least disturbed sites in a region would help managers reduce this uncertainty and decrease the likelihood that inappropriate reference sites are used to establish reference conditions. The purpose of this study was to develop and demonstrate a procedure that can objectively and efficiently identify candidate reference sites that are stratified across regional gradients of natural environmental variation. This method is demonstrated by presenting results from a case study in which candidate headwater stream reference sites were identified for the region of Southwestern Ontario.

4.2. CASE STUDY BACKGROUND

Southwestern Ontario is a highly disturbed region that comprises the southernmost part of Canada. Historically, the region's glaciated landscape was dominated by temperate deciduous forests. European settlement, however, resulted in the removal of virtually all the original forest cover and only small, isolated patches of largely secondary growth forest remain today. The region's contemporary landscape is dominated by agriculture, comprising upwards of 75% of the region's land cover. Agriculture in the region is characterized by cash crop (predominantly corn and soybeans) and livestock (including dairy, beef, hog, and poultry) operations and is particularly intensive in the southern portion of the region.

Regional increases in the intensity of agriculture and the fact that much of the agriculture occurs in close proximity to small, headwater streams has resulted in growing concern about the impacts of agriculture and other rural land uses on small lotic ecosystems. In response to these concerns, agencies responsible for the protection of water resources are increasing monitoring of these important watercourses. Because freshwater monitoring in Ontario and Canada regularly use a Reference Condition Approach (RCA), reference sites need to be established against which more intensively farmed basins can be compared. Regionally, agriculture is most intensive in the southern half of southwestern Ontario, so it was decided that a project would be initiated to identify candidate headwater reference basins across three major drainages (the Thames and Grand River basins, and the Long Point Drainage area) in this area (Figure 4.1, Appendix 1). The Thames and Grand River basins both drain approximately 7000 km² of largely agricultural land while the Long Point drainage area drains approximately 1650 km² between the four small drainages that comprise the region.

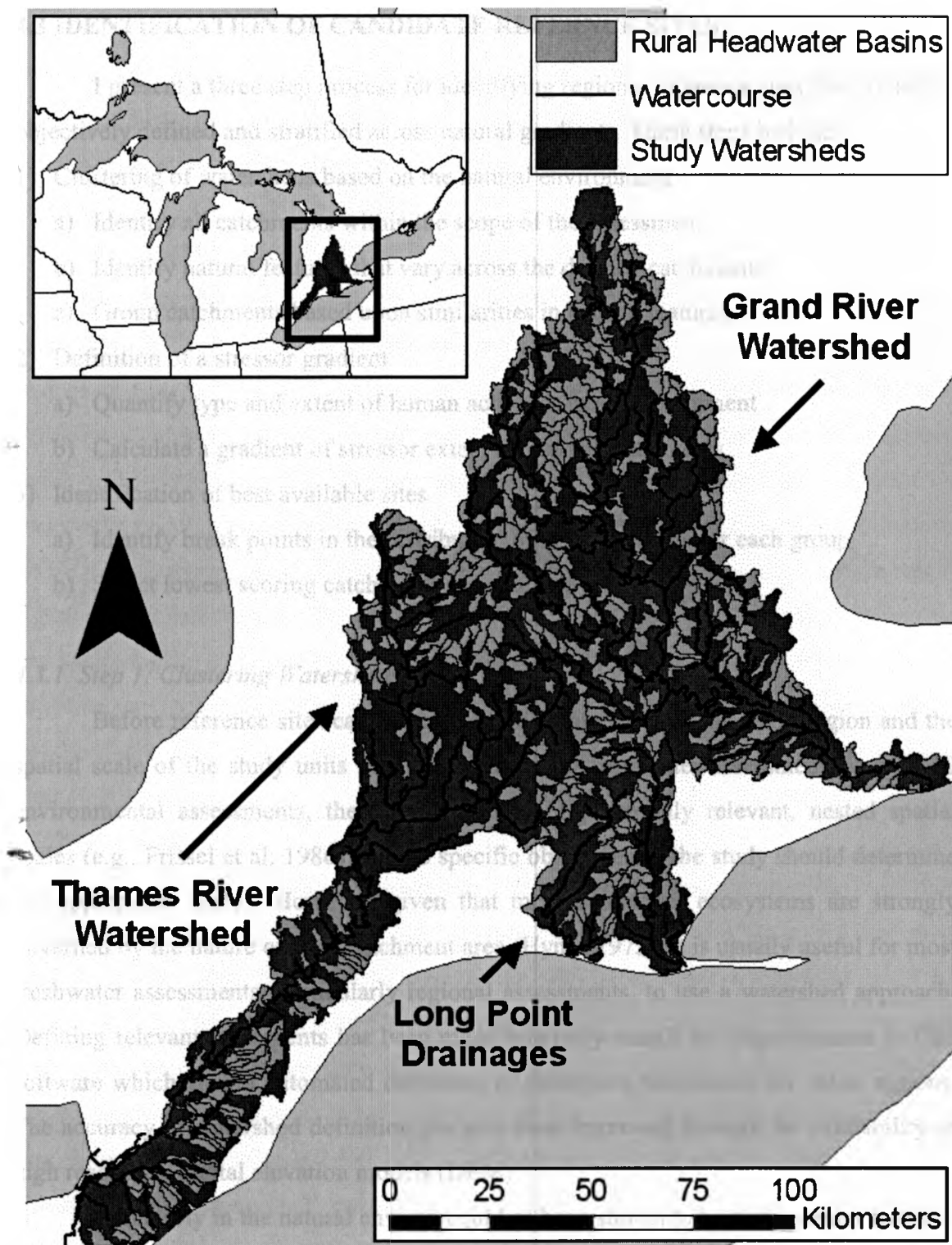


Figure 4.1. Location of the 479 rural, headwater study basins and their associated drainages in Southwestern Ontario, Canada.

4.3 IDENTIFICATION OF CANDIDATE REFERENCE SITES

I present a three step process for identifying regional reference sites that are both objectively defined and stratified across natural gradients. These steps include:

- 1) Clustering of watersheds based on the natural environment
 - a) Identify all catchments within the scope of the assessment
 - b) Identify natural features that vary across the defined catchments
 - c) Group catchments based upon similarities in natural features
- 2) Definition of a stressor gradient
 - a) Quantify type and extent of human activities in each catchment
 - b) Calculate a gradient of stressor extent across the catchments
- 3) Identification of best available sites
 - a) Identify break points in the distribution of stressor scores for each group
 - b) Select lowest scoring catchments as candidate reference sites

4.3.1. Step 1: Clustering Watersheds

Before reference sites can be selected, the boundaries of the study region and the spatial scale of the study units must be defined. In freshwater assessment, as with all environmental assessments, there are a variety of potentially relevant, nested spatial scales (e.g., Frissel et al. 1986) and the specific objectives of the study should determine the appropriate scale. However, given that most freshwater ecosystems are strongly governed by the nature of their catchment area (Hynes 1975), it is usually useful for most freshwater assessments, particularly regional assessments, to use a watershed approach. Defining relevant catchments has been made relatively simple by improvements in GIS software which enable automated definition of catchment boundaries for entire regions. The accuracy of watershed definition has also been improved through the availability of high resolution digital elevation models (DEM).

Variability in the natural environment has been shown to be an important factor in structuring freshwater communities (Corkum 1989). This variation has also been demonstrated to confound the relationship between human activities and the associated effects on biological assemblages (Allan 2004). As a result, it is essential that any monitoring program utilizing biological assemblages account for variability in the natural

environment across the region of interest. This variability is best accounted for by ensuring that candidate reference sites are stratified across the existing range of natural conditions (Reynoldson & Wright 2000). Stratification of sites across environmental conditions also maximizes the probability that all distinct biological assemblages will be sampled.

Because the purpose of this procedure is to identify candidate regional reference sites, environmental descriptors affected by human activities, such as water temperature, should be avoided. Natural landscape scale features that are known to structure biological communities in streams, such as geology and latitude (Richards et al. 1997; Sandin and Johnson 2001), are most useful. Landscape scale features can be described using widely available GIS layers, precluding the need for costly data collection in previously understudied regions. Using methods such as principal component analysis (PCA), the environmental features that exhibit significant variation across the study region can be determined. The identified features can then be used to group catchments that are similar (e.g., large vs small catchments, sand vs clay soils). Cluster analysis is an objective method of generating such groups. Grouping the region's catchments improves the likelihood that catchments within a group are comparable in terms of the resident biological communities.

4.3.2. Case Study

The objective of the southwestern Ontario project was to identify candidate reference sites for rural headwater basins. Only basins of 600 to 3000ha with no urban land cover were identified. Basins meeting the area criterion were identified using the ArcHydro 9.1 extension for ArcGIS 9.1 (ESRI 2007), a 5 m resolution DEM and a layer depicting the region's stream network. A 1992 regional land cover layer and 2006 colour orthoimages were then used to select and remove basins containing urban areas. This process resulted in 479 candidate headwater basins across the region (Figure 4.1). GIS layers describing the natural environment for each of the basins were then analyzed to determine important gradients of variation across the region. Analysis found the region to be relatively homogeneous in terms of climate and topography, but showed significant variation in surface geology among the basins. Because stream size had been homogenized by the basin size criterion, I classified the basins using only surface

geology. Surface geology was represented by proportions of the total area of each basin that was comprised by a particular texture of landform (e.g., % sand). Clustering was accomplished using a K-means analysis based upon Euclidean distance measures. K-means analysis was conducted for two through eight groups. Discriminant function analysis (DFA) was then used to establish the relative separation of different numbers of classification groups. This analysis indicated that two groups resulted in the most separated groups ($F = 300.9$). However, the second group was based on which surface geology types were not present, rather than what deposit types were present. This commonality in absence resulted in basins that would have very different natural conditions and thus, potentially, very different biological communities being grouped together. Therefore it was decided that this classification was unsuitable. The second strongest classification, and the one that was selected, contained six groups ($F = 267.2$). This classification grouped basins with texturally similar deposits (Table 4.1). Geographically, there were some strong regional patterns in where basins of each group were located (Figure 4.2). The clay and sand groups in particular were largely confined to the lower Grand and the Long Point Drainage Area, respectively, and all groups demonstrated a tendency to dominate a particular part of the study region. The geographical grouping pattern accompanied by the fact that texture of surface geology deposits has been associated with variation in stream communities (e.g., Richards et al. 1997), increases the likelihood that the classification is a true reflection of differences in the environment and also that it is ecologically relevant.

4.3.3. Step 2: Defining a Stressor Gradient

In order to identify which ecosystems are least disturbed by human activities, it must first be determined how human activities vary across the landscape. The level of disturbance can be determined by quantifying human activities occurring within the study units in a comprehensive manner. Because the goal of this procedure is to eventually differentiate between the extent of human activity among basins, the various human activities should be described in as much detail as possible. Increasing the detail to which stressors are described has been demonstrated to significantly increase the amount of variation in the stressor environment that is described and result in improved differentiation of catchments (see Chapter 2).

Table 4.1. Dominant deposit texture(s) for each of the six identified surface geology groups and the number of rural, headwater basins that were assigned to each group.

| Group | Dominant Texture(s) | # of Basins Assigned |
|-------|---------------------|----------------------|
| 1 | Till | 214 |
| 2 | Sand | 72 |
| 3 | Silt | 55 |
| 4 | Sand & Till | 65 |
| 5 | Gravel & Till | 35 |
| 6 | Clay | 38 |

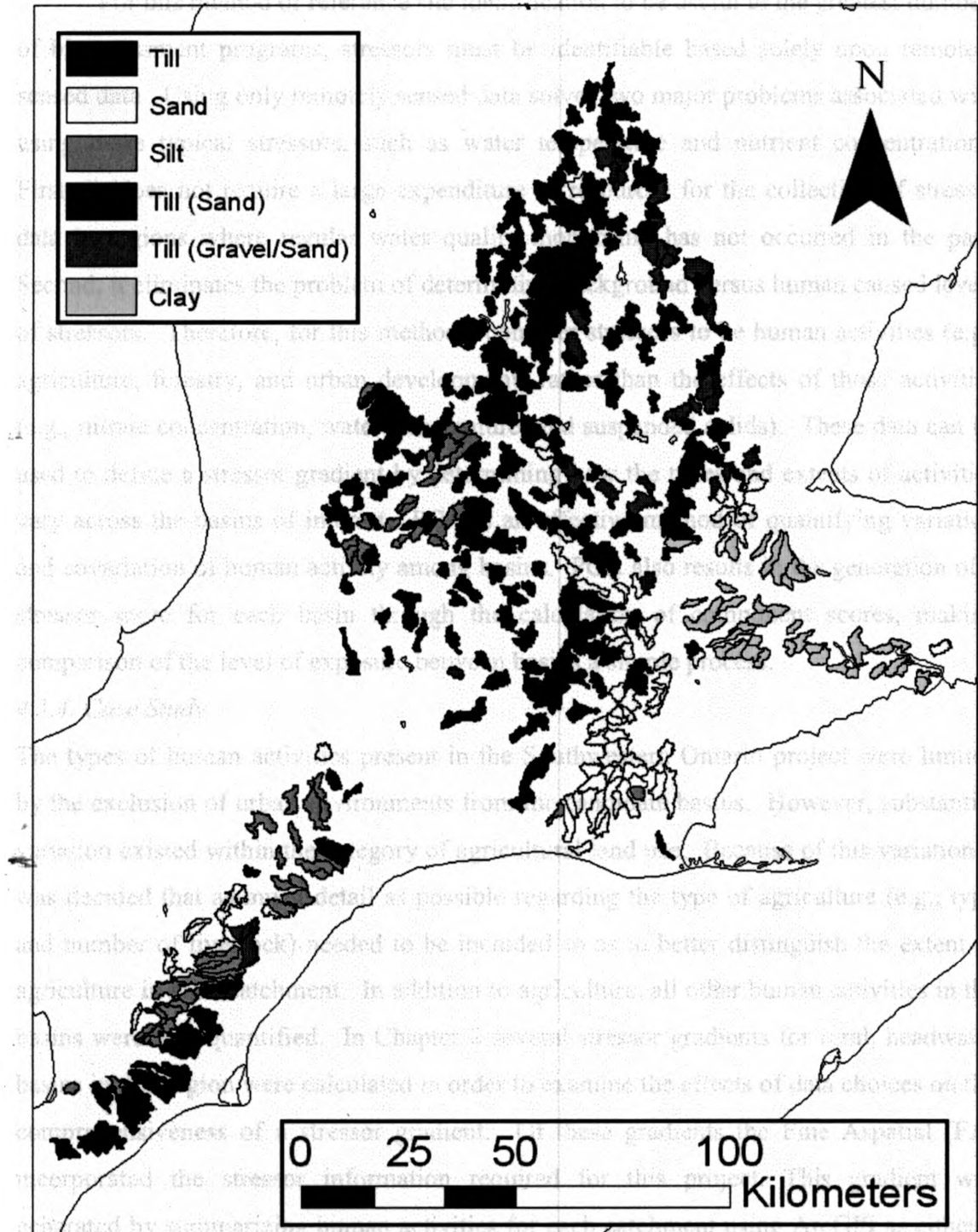


Figure 4.2. Geographical distribution of surface geology groups across the Grand and Thames River Basins and Long Point Drainage Area in southwestern Ontario, Canada.

For this method of reference site identification to be useful to the greatest number of bioassessment programs, stressors must be identifiable based solely upon remotely sensed data. Using only remotely sensed data solves two major problems associated with using more typical stressors, such as water temperature and nutrient concentrations. First, it does not require a large expenditure of resources for the collection of stressor data in regions where regular water quality monitoring has not occurred in the past. Second, it eliminates the problem of determining background versus human caused levels of stressors. Therefore, for this method I consider stressors to be human activities (e.g., agriculture, forestry, and urban development) rather than the effects of those activities (e.g., nitrate concentration, water temperature, and suspended solids). These data can be used to define a stressor gradient by determining how the types and extents of activities vary across the basins of interest. PCA is an effective method of quantifying variation and covariation of human activity among basins. PCA also results in the generation of a stressor score for each basin through the calculation of component scores, making comparison of the level of exposure between basins a simple process.

4.3.4. Case Study

The types of human activities present in the Southwestern Ontario project were limited by the exclusion of urban environments from the candidate basins. However, substantial variation existed within the category of agricultural land use. Because of this variation it was decided that as much detail as possible regarding the type of agriculture (e.g., type and number of livestock) needed to be included so as to better distinguish the extent of agriculture in each catchment. In addition to agriculture, all other human activities in the basins were also quantified. In Chapter 2 several stressor gradients for rural, headwater basins in this region were calculated in order to examine the effects of data choices on the comprehensiveness of a stressor gradient. Of these gradients the Fine Aspatial (FA) incorporated the stressor information required for this project. This gradient was generated by summarizing human activities for each catchment using ArcGIS as either a percent of area coverage (e.g., % pasture) or a density per unit area (e.g., # of rural homes per 100 ha). Due to the substantial variety of stressors that were quantified, descriptors were then grouped according to activity type and a separate PCA run for each group. The resulting first component scores for each group were then entered into a second PCA to

calculate the final stressor score for each basin. This process resulted in a single stressor gradient that described the extent of human activity present in each of the 479 basins. The nature of this gradient was such that basins with the lowest stressor scores tended to have moderate amounts of agriculture and forest cover and were largely found in the Long Point Drainage area and along the northeastern boundary of the Grand River Basin (Figure 4.3). In contrast, basins with very high stressor scores tended to have almost exclusively agricultural land cover with high densities of livestock and were primarily located in the north part of the Thames River Basin and the northwest section of the Grand River Basin.

The geographical distribution of the stressor scores also demonstrated a strong correspondence to the basin groups generated from the surface geology characteristics. High intensity agriculture tended to be restricted to the till areas while lower intensity agriculture tended to occur on the sand and coarse till areas. This mirroring of distributions demonstrates the importance of stratifying reference sites according to gradients in the natural environment as selection of reference basins from the stressor gradient alone would have resulted in fundamental differences in the natural characteristics of many of the reference and test basins.

4.3.5. Step 3: Identification of Best Available Conditions

Ideally, reference condition is defined as an ecosystem that has not been exposed to human activity. In practice, however, the notion of reference condition is really one of "best available" condition (Reynoldson et al. 1997). This alters the search for reference condition from attempting to find the absence of human activity to quantifying the extent of human activity. A comprehensive stressor gradient is, therefore, a valuable tool in the search for the best available condition because it indicates the relative difference in stressor extent among catchments. The distribution of the candidate catchments and their associated stressor scores can then be used to indicate the basins in each natural grouping with the least amount of human activity present.

The point on a stressor gradient which is deemed to be the boundary between reference and test sites is dependent upon the number of sites that are needed to meet the level of precision required to meet the goals of the project (Bailey et al. 2004). Balanced against this optimal number of sites, however, must be an assurance that the pool of

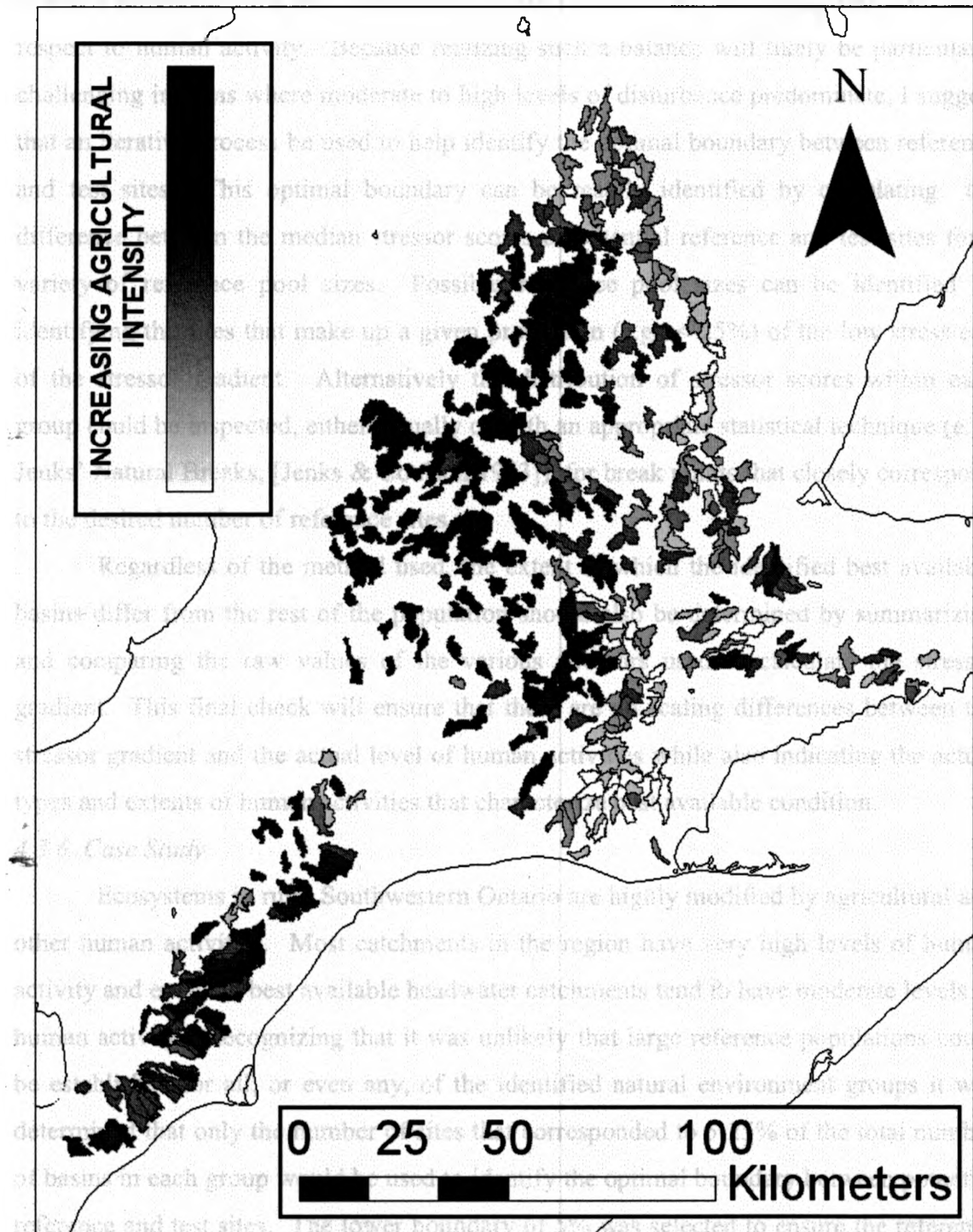


Figure 4.3. Geographical distribution of agricultural intensity across the Grand and Thames River Basins and Long Point Drainage Area in southwestern Ontario, Canada. Agriculture intensity levels based on calculated basin stressor scores.

selected reference sites are substantively different from the remaining test sites with respect to human activity. Because realizing such a balance will likely be particularly challenging in areas where moderate to high levels of disturbance predominate, I suggest that an iterative process be used to help identify the optimal boundary between reference and test sites. This optimal boundary can be readily identified by calculating the difference between the median stressor scores of potential reference and test sites for a variety of reference pool sizes. Possible reference pool sizes can be identified by identifying the sites that make up a given proportion (e.g., < 25%) of the low stress end of the stressor gradient. Alternatively the distribution of stressor scores within each group could be inspected, either visually or with an appropriate statistical technique (e.g., Jenks' Natural Breaks, [Jenks & Coulson 1963]), for break points that closely correspond to the desired number of reference sites.

Regardless of the method used, the extent to which the identified best available basins differ from the rest of the population should also be determined by summarizing and comparing the raw values of the various stressors used to calculate the stressor gradient. This final check will ensure that there are no scaling differences between the stressor gradient and the actual level of human activities while also indicating the actual types and extents of human activities that characterize best available condition.

4.3.6. Case Study

Ecosystems in rural Southwestern Ontario are highly modified by agricultural and other human activities. Most catchments in the region have very high levels of human activity and even the best available headwater catchments tend to have moderate levels of human activity. Recognizing that it was unlikely that large reference populations could be established for all, or even any, of the identified natural environment groups it was determined that only the number of sites that corresponded to 5-25% of the total number of basins in each group would be used to identify the optimal boundary between potential reference and test sites. The lower boundary of 5% was selected to ensure the reference population would contain enough reference sites to meet the statistical requirements of RCA models (Bailey et al. 2004). The upper boundary was arbitrarily deemed to be a cutoff point above which there would be too much activity to merit designation as a reference site.

The proportion of sites corresponding to the optimal boundary was determined by calculating the difference between the median stressor score for the potential reference and test site populations. Calculated differences were then plotted against the proportion of sites that were used to determine the reference median for that difference calculation (Figure 4.4). The optimal boundary was considered to be at the proportion for which the greatest difference between reference and test site medians occurred. Only the Sand and Gravel & Till groups, however, actually exhibited a difference peak. For the remaining four groups the absolute difference continued to rise as fewer and fewer sites were included in the potential reference pool. Thus, for these groups it was decided that the proportion at which the greatest change in the difference between reference and test medians occurred would constitute the boundary. The above two criteria resulted in the optimal boundary including 7, 9, 6, 8, 14, and 13 % of the total pool of sites being the proportion selected as best available for the Till, Sand, Silt, Till (Sand), Till (Sand/Gravel), and Clay groups, respectively.

To ensure that the selected best available basins substantively differed in the extent of human activity in the catchment area medians were calculated from raw stressor values for three human activities that were identified as most important to variation in the regions stressor environment (i.e., percent cropland, percent tiled, # of livestock nutrient units per 1000 ha) for both the selected reference sites and the remaining test sites. For all natural groupings the extent of most of the three human activities in the identified best available basins did substantially differ from the rest of the basins in the group (Table 4.2). However, the difference in the median amounts of percent cropland were not nearly as large for Groups 2 and 3 as they were for the rest of the groups. This comparison also demonstrated the substantial variation between the groups as to the extent of human activity which constituted best available condition. In general, the level of human activity in the potential reference sites would at best be considered moderate. For the selected reference pool of Group 3, however, the level of human activity, with the notable exception of the number of livestock units, could only be considered high, an indication of how widespread human activities like agriculture can be when natural conditions are favourable. The ability of the selected sites in Group 3 to act as reference sites may therefore be limited.

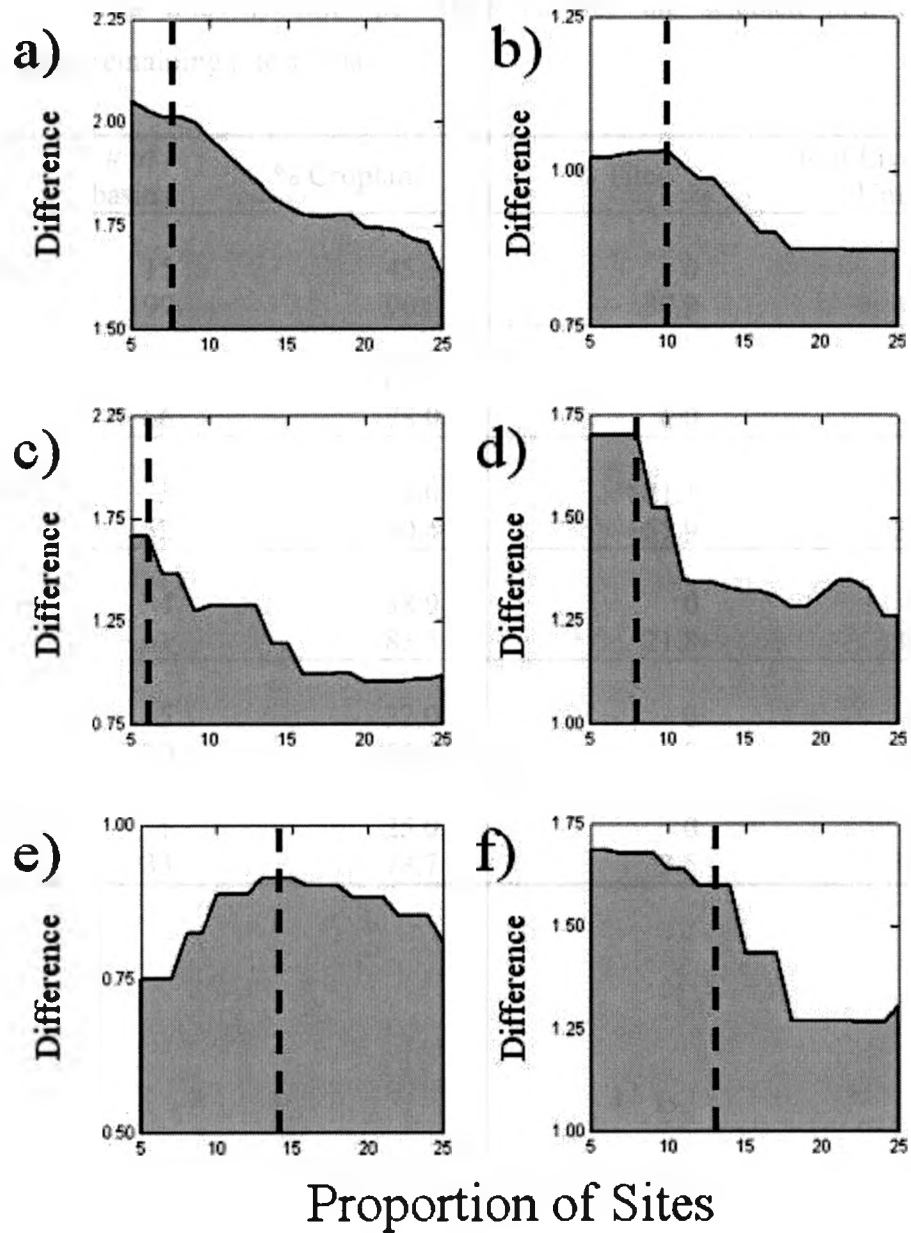


Figure 4.4. Differences between median stressor scores of potential best available and remaining sites when best available sites make up 5 to 25 percent of the total number of basins in a group. Where a) is till group, b) is sand group, c) is silt group, d) is sand till group, e) is gravel till group, and f) is clay group. Dashed bar represents the proportion selected to be the boundary between reference (left side) and test sites (right side).

Table 4.2. Median values of three stressors, percent cropland, percent of field tiled, and number of livestock nutrient units per 1000 ha, for the selected best available ("reference") and remaining ("test") basins.

| | # of basins | % Cropland | % Tiled | # of Livestock Units |
|----------------|-------------|------------|---------|----------------------|
| Group 1 | | | | |
| Reference | 15 | 45.3 | 0 | 44.3 |
| Test | 199 | 90.8 | 37.9 | 428.0 |
| Group 2 | | | | |
| Reference | 6 | 64.9 | 3.7 | 7.2 |
| Test | 66 | 78.0 | 6.0 | 29.8 |
| Group 3 | | | | |
| Reference | 3 | 74.0 | 21.1 | 7.8 |
| Test | 52 | 90.4 | 51.9 | 252.9 |
| Group 4 | | | | |
| Reference | 4 | 38.9 | 0 | 29.1 |
| Test | 61 | 83.5 | 21.8 | 338.8 |
| Group 5 | | | | |
| Reference | 5 | 32.0 | 0 | 46.3 |
| Test | 30 | 66.9 | 0 | 152.0 |
| Group 6 | | | | |
| Reference | 5 | 25.0 | 0 | 11.9 |
| Test | 33 | 78.7 | 3.5 | 168.2 |

4.4. FOLLOW-UP CONSIDERATIONS

It must be emphasized that this method is intended to be used to target initial reference site sampling by ensuring that the best available sites are being sampled across the complete range of existing natural variation. Reference site characterization, however, should not end with the completion of the steps outlined above. All identified candidate reference sites should be visited in order to confirm the basins best available status by ensuring that there are no stressors present that were not identified using the GIS and remotely sensed data. Furthermore, the biota in each basin should be sampled. These samples can be used to determine whether biological communities are comparable across natural environment groupings. If communities are not significantly different then natural groupings should be combined where appropriate and the stressor gradient revisited to identify the appropriate number of best available basins for this new group. Finally, and perhaps most importantly, if the located best available sites do not exhibit significantly less human activity than the rest of the stressor gradient managers should strongly consider expanding the scope of the study in order to identify sites that are similar in natural characteristics, but exhibit lower extents of human activity.

4.5. SUMMARY

Reference site selection is fundamental to the development of all freshwater biomonitoring programs seeking to use reference condition based benchmarks. This process has been hampered in the past, however, by an idiosyncratic definition of what regional reference sites should "look like". In this study I have developed and illustrated a procedure for identifying candidate regional reference sites that are both objectively defined and stratified across environmental gradients. Because this method uses only widely available landscape scale GIS data it enables rapid and cost effective identification of candidate reference sites, even for very large and previously understudied regions, and should thereby be applicable to all freshwater biomonitoring programs.

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Chapter 5. General Discussion

5.1. SUMMARY & DISCUSSION

A stressor gradient is an invaluable tool for bioassessment. As a description of the human activities occurring in an ecosystem, stressor gradients enable the development of conceptual models linking human activities to ecological endpoints. These models can then be used to develop testable hypotheses about the effects of human activity on biota (Stevenson et al. 2004). Because differences in the extent of stressors that ecosystems are exposed to is often quite small this process is best served if the stressor gradient is as descriptive as possible. Resources for stressor gradient calculation are, however, usually limited, and information regarding specific stressors is often not readily available. Establishing what types of stressor information provides the most information for the least amount of resources is therefore an important task.

In chapter 2 of this study the point of balance between stressor discrimination and efficiency was addressed by comparing the relative difference in the amount of stressor variation described by four different stressor gradients. These gradients varied in the level to which the type and extent of a stressor was resolved and the degree to which its spatial position was described. As expected, results from this study indicated that stressor gradients generated from data that highly resolved differences between the type and extent of stressor best discriminated differences in the stressor environment. Contrary to expectation was the finding that spatial explicitness had little impact on the description of the stressor environment as stressor data aggregated at the basin scale performed essentially the same as stressor data described based on its proximity to a water course.

The findings of this study should greatly affect the manner in which many bioassessment studies and protocols describe the stressor environment. Currently most stressor gradients based on human activity are generated from regional scaled land use layers. Most of these gradients, however, are applied to much smaller ecosystem scales such as wadeable streams and wetlands (e.g. Fore et al. 1996, Brown and Divas 2005). Based on the results of the study presented here, incorporating such high levels of generalization into the stressor gradient results in a more clumped distribution of

ecosystems, particularly in the case of ecosystems exposed to a high degree of human activity. Using a coarsely resolved stressor gradient would therefore make it difficult to detect biological responses to all but the largest differences in human activity. In contrast, using a highly resolved stressor gradient enables the assessment of even relatively small differences in human activity. This is important because one of the current foci of bioassessment is how to move assessment beyond a statement of ecosystem condition to a point where assessments can also help establish the causes of impairment and build predictive models that can be used for land use planning (Bailey et al. 2006). As a result, it is strongly recommended that as much detail regarding the stressor environment be incorporated into the stressor gradient as possible.

Stressor gradients are essential for the selection of indicators of ecological condition. An ecological indicator is ideal if it is sensitive to the environmental effects of concern, yet exhibits low levels of variability in its response (Landres et al. 1988). Identification of such an indicator is best accomplished through the establishment of dose-response relationships between the stressor and the biota (Johnson et al. 2006). Generation of a stressor gradient is essential to this process as it stratifies ecosystems based on variability in the "dose" (i.e. the extent of human activity) of the stressor allowing sites to be selected that encompass the full range human activity. Sampling the biota at these sites and empirically relating the biota to the stressor gradient will then define the response. Taxa, species traits, or other descriptors of the biota that respond strongly to the stressor gradient can then be selected as indicators.

In chapter 3 of this study the relationships between a detailed stressor gradient and two aquatic assemblages (fish and benthic macroinvertebrates) were determined. Results of this study indicated that both assemblages did vary across the stressor gradient in an ecologically predictable pattern. In particular, tolerance to pollution, as well as thermal and habitat preferences of the taxa present shifted as human activity increased. Specifically, intolerant, cold water taxa requiring coarser substrates were replaced by tolerant, warm water taxa, with more general habitat requirements. These results indicate that metrics based on these characteristics would make useful indicators for these rural systems. These ecological characteristics have also been demonstrated to relate to human activities in other studies, especially those studying agricultural effects on aquatic biota

(e.g. Richards et al. 1997; Stewart et al. 2001), and has resulted in these characteristics commonly being used in multimetric indices (e.g. McCormick et al. 2001; Klemm et al. 2003).

Confounding the relationships between the biota and the stressor environment was a very strong association between the stressor and natural environment, in this case surface geology. Since the natural environment is the template upon which human activity is structured (Allan 2004), this result is not surprising. The strong correspondence between the biotic responses to the natural and stressor environments did however, raise the question of which environment was driving the changes in community composition, a finding common to many studies working in highly disturbed areas (e.g. Fitzpatrick et al. 2001; Richards et al. 1997). A partial mantels test helped to establish that the biota were indeed responding to both environments. Based on this finding it was concluded that the composition of fish and benthic macroinvertebrates vary with changes in both the natural and stressor environment.

The results of this study are applicable both locally and to the field of bioassessment as a whole. At the local level, this study has identified that aquatic biota respond to increasing intensity of human activities in the rural environment and that this activity results in an almost complete change in the composition of fish and benthic macroinvertebrate assemblages. This is an important result for the management of the rural landscape as it demonstrates the extent to which rural activities can change these ecosystems. From a planning perspective the results of this study also demonstrate that there are levels, relatively intensive levels, of human activity that can still support sensitive assemblage types. This result indicates that with proper planning accompanied by appropriate restoration activities there is potential to maintain fairly high levels of human activity without drastically altering the condition of these ecosystems. With regards to the field of bioassessment, the results of this study corroborate with three current areas of bioassessment research. First, the finding that both fish and benthic macroinvertebrate assemblages shifted along lines of expected tolerances demonstrates the utility of the tolerance concept and provides further evidence that tolerance to human activities can be empirically assessed at a regional scale (Bressler et al. 2006, Whittier et al. 2008). Second, the finding that multiple species traits were found to describe the

various ecological responses to the natural and stressor environments demonstrates the validity of using a variety of indices to assess ecological condition, as is done in the multimetric approach (e.g. Karr 1981). At the same time, however, because these results were found using a common multivariate approach, it once again raises the issue of whether metrics are even required in bioassessment. The most practical conclusion from this finding is that using a combined multimetric and multivariate approach will provide the most information regarding ecological condition. Third, this study further demonstrates the importance of the catchment scale to biota in association with the local scale. This is an increasingly common result (e.g. Roth et al. 1996; Allan et al. 1997) that has begun to change the scales at which bioassessment and biomonitoring are being conducted such that catchment features are now regularly included.

The Reference Condition Approach (RCA) is the idea that conditions in ecosystems unexposed to human activity can be used to determine if biological condition has been impaired in a similar ecosystem where human activity is occurring (Bailey et al. 2004). Using RCA addresses the problem that the condition of most ecosystems prior to exposure to human activity is generally unknown. Using reference sites that are similar in natural characteristics (e.g. geology, altitude) thereby gives a picture of what the characteristics of the biotic community likely were prior to being exposed. Ideally, the ecosystems used to define reference condition would be pristine. In reality, however, there is likely no pristine ecosystem left anywhere on the planet. As a result, it has generally been adopted that reference condition is the condition representative of a group of minimally disturbed sites (Reynoldson et al. 1997). Unfortunately, there is the problem of how to define what minimally disturbed is for a given region and ecosystem type. Stressor gradients are an objective manner by which this problem can be solved.

In chapter 4, a method for objectively establishing what sites were the best available (i.e. had the lowest amount of exposure to human activity) for a region was demonstrated. This method relied upon a detailed stressor gradient to describe the human environment and thereby establish which ecosystems contained the least human activity. In addition, this method demonstrated a procedure for ensuring that the selected best available sites were stratified across existing natural gradients.

The results of this study are immediately applicable to biomonitoring of the study region and it also has wide applicability to biomonitoring programs any where in the world. The use of widely available GIS data to rapidly and objectively identify the best available ecosystems stratified across the natural environment make it an essential first step in the initiation of any biomonitoring program. While this method is especially helpful in highly developed areas where identification of differences in human activity is most difficult, the ability to rapidly stratify sites across natural gradients also makes it useful in more remote areas where human activity is low. This method is also an improvement over previous methods of identifying best available conditions. First, it removes the subjectivity of professional judgment. Second, it relies completely upon remotely sensed data which eliminates the need for costly scoping sampling making it a practical method for biomonitoring of large and previously understudied areas such as the Canadian North. Third and finally, this method insists that sites be stratified using descriptors that are not affected by human activity, ensuring that environmental groups are not confounded with the presence of stressors increasing the likelihood that differences in reference communities are actually attributable to differences in the natural environment.

5.2. FUTURE RESEARCH

This thesis has demonstrated the utility of stressor gradients as a bioassessment tool. These results, however, are likely just scratching the surface of the full potential that stressor gradients possess. One area where stressor gradients possess tremendous potential is in enabling bioassessment to provide insight into the causes of impairment and predictive modeling of the probable outcomes of development and restoration activities. Extending bioassessment to include these objectives is made possible because stressor gradients score each site based on the stressors that are present. A change in stressor score thus corresponds to either the addition or removal of a particular stressor or the increase or decrease in the intensity of a stressor. Research that connects specific changes in aquatic biota to quantified changes in each human activity would allow assessments to establish probable cause of impairment or improvement in ecological condition. Better understanding these stressor-biota relationships would also allow predictive models to be generated that would allow *a priori* scenario testing of proposed development or restoration plans. Development of these models would allow planners and managers to determine the likely effects of a given type and extent of activity before approving the development. It would also enable the planners to adjust the proposed plan so that both development and ecological goals are met.

In addition to work on stressor gradients this thesis has identified covariation between natural and stressor gradients as an area requiring more research. The primary need for research is the establishment of reference communities in all natural environment types. Because the southwestern Ontario region, like many other regions, no longer has minimally disturbed basins representative of all, or even any, natural environments it is not possible to determine through sampling what the "pristine" reference condition was for the region. As a result, this work will have to be largely based upon modeling of the habitat requirements of taxa and the historical environmental conditions of the region. This establishment of a historical reference condition will allow planners and managers to set restoration objectives that are suitable for each unique natural environment. Once a historical reference condition is established it will also be possible to truly determine whether the compositional shift found in this study was due to human activity or the natural environment.

5.3. CONCLUSIONS

Together, the three body chapters of this dissertation provide an in depth study of the development, testing, and application of stressor gradients in a bioassessment context. Each of these chapters were designed to progressively enhance the state of knowledge regarding stressor gradients. The findings of chapter 2 set the stage for the use of stressor gradients by determining that the use of detailed information to generate the stressor environment is essential for ensuring a high quality description of the differences in human activity. This conclusion influenced both of the remaining chapters as the high detail stressor gradient was used to test the remaining hypotheses. In chapter 3 it was concluded that variation in biota does correspond to a highly detailed stressor gradient but that biota also vary with the changes in the natural environment. Based on these findings it was decided that a stressor gradient could be applied to the problem of objectively selecting reference sites for biomonitoring. Also relying on the results of chapter 3 the decision was made that there needed to be explicit regional stratification of sites based on the natural environment. Together, these two findings led to the development of the method for selecting objective, environmentally stratified, reference sites presented in chapter 4. Overall, this study has greatly improved the state knowledge regarding stressor gradients, how they are developed, their effectiveness, and their applicability to bioassessment and biomonitoring. These findings should help form the foundation of future research and application of stressor gradients.

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Appendix 1 - Watershed membership, Strahler order, area, and position of basin outflows in Universal Transverse Mercator Zone 17N (North American Datum 1983) of 479 rural basins in southwestern Ontario used to generate stressor gradient.

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|-------------|-------|-----------|--------------|---------------|
| GR0503 | Grand River | 3 | 1635 | 554624.44225 | 4881734.72541 |
| GR0533 | Grand River | 2 | 770 | 550009.72563 | 4882363.63131 |
| GR0559 | Grand River | 3 | 1038 | 551832.49362 | 4880736.78596 |
| GR0584 | Grand River | 3 | 1820 | 554681.47853 | 4874307.26334 |
| GR0585 | Grand River | 4 | 1054 | 560783.17223 | 4877784.91894 |
| GR0591 | Grand River | 2 | 699 | 560761.17218 | 4877792.34172 |
| GR0603 | Grand River | 3 | 1071 | 550029.25980 | 4878782.54359 |
| GR0627 | Grand River | 2 | 671 | 554669.17997 | 4874296.07225 |
| GR0645 | Grand River | 4 | 1944 | 546445.52216 | 4870719.04359 |
| GR0655 | Grand River | 2 | 1032 | 559832.34153 | 4872878.88844 |
| GR0657 | Grand River | 2 | 815 | 559883.55950 | 4872598.04038 |
| GR0660 | Grand River | 2 | 711 | 549327.72127 | 4873915.07597 |
| GR0668 | Grand River | 2 | 650 | 564281.14330 | 4869596.11660 |
| GR0673 | Grand River | 4 | 1659 | 564297.00697 | 4869589.21258 |
| GR0687 | Grand River | 3 | 2033 | 551936.80765 | 4868402.84169 |
| GR0696 | Grand River | 3 | 777 | 559636.52579 | 4868792.02957 |
| GR0707 | Grand River | 2 | 602 | 564654.62148 | 4870707.51992 |
| GR0710 | Grand River | 3 | 640 | 559625.49478 | 4868767.35311 |
| GR0711 | Grand River | 2 | 617 | 561302.81236 | 4868502.01903 |
| GR0747 | Grand River | 3 | 946 | 549959.11043 | 4867676.63372 |
| GR0754 | Grand River | 3 | 861 | 554131.21646 | 4865735.75729 |
| GR0757 | Grand River | 3 | 1568 | 559449.53624 | 4862776.99850 |
| GR0771 | Grand River | 3 | 645 | 553658.23964 | 4864472.03256 |
| GR0773 | Grand River | 3 | 880 | 555352.50322 | 4861806.47881 |
| GR0774 | Grand River | 3 | 1004 | 545946.70255 | 4866064.76223 |
| GR0775 | Grand River | 3 | 1043 | 564309.24237 | 4860838.97198 |
| GR0787 | Grand River | 3 | 1434 | 564310.14266 | 4860823.65543 |
| GR0788 | Grand River | 2 | 754 | 538942.73580 | 4863726.42926 |
| GR0792 | Grand River | 3 | 1438 | 538974.73668 | 4863705.36144 |
| GR0793 | Grand River | 4 | 1151 | 538499.30494 | 4862718.45355 |
| GR0802 | Grand River | 2 | 747 | 552296.09568 | 4860173.76511 |
| GR0814 | Grand River | 2 | 1314 | 552280.66857 | 4860168.26771 |
| GR0816 | Grand River | 3 | 2705 | 526064.95162 | 4857482.43018 |
| GR0828 | Grand River | 3 | 1020 | 561957.86429 | 4856138.99694 |
| GR0832 | Grand River | 3 | 1201 | 538543.73536 | 4861592.06920 |
| GR0833 | Grand River | 3 | 850 | 546847.92986 | 4857137.66793 |
| GR0838 | Grand River | 2 | 626 | 561957.90147 | 4856128.97608 |
| GR0844 | Grand River | 3 | 1026 | 546830.15096 | 4857122.66892 |
| GR0846 | Grand River | 4 | 1602 | 533193.65208 | 4854806.41175 |
| GR0852 | Grand River | 3 | 840 | 560056.42399 | 4854889.84712 |
| GR0855 | Grand River | 3 | 746 | 548966.07441 | 4855026.66764 |
| GR0856 | Grand River | 3 | 642 | 556514.31258 | 4854839.13881 |
| GR0860 | Grand River | 3 | 644 | 542151.53109 | 4855736.67720 |
| GR0861 | Grand River | 3 | 1424 | 556498.68726 | 4855743.01448 |
| GR0871 | Grand River | 3 | 1269 | 530627.03084 | 4853130.46994 |
| GR0876 | Grand River | 3 | 1108 | 557724.80837 | 4853848.32360 |
| GR0881 | Grand River | 3 | 1293 | 548833.19625 | 4852156.26802 |
| GR0896 | Grand River | 4 | 991 | 550924.75925 | 4849267.06839 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|-------------|-------|-----------|--------------|---------------|
| GR0900 | Grand River | 3 | 655 | 534186.03042 | 4853104.40674 |
| GR0904 | Grand River | 3 | 679 | 523227.62978 | 4854349.98681 |
| GR0916 | Grand River | 3 | 1577 | 547647.50576 | 4849719.11578 |
| GR0924 | Grand River | 4 | 2866 | 538249.48684 | 4852883.66222 |
| GR0926 | Grand River | 3 | 2253 | 532626.28391 | 4851958.77059 |
| GR0927 | Grand River | 3 | 698 | 547632.40105 | 4849713.15777 |
| GR0930 | Grand River | 3 | 1519 | 562153.41746 | 4845421.07163 |
| GR0931 | Grand River | 0 | 1028 | 562165.45908 | 4845422.86606 |
| GR0932 | Grand River | 3 | 1028 | 530466.19547 | 4851719.75972 |
| GR0934 | Grand River | 3 | 737 | 567430.54824 | 4845332.02164 |
| GR0938 | Grand River | 3 | 852 | 531799.63183 | 4851311.14895 |
| GR0944 | Grand River | 3 | 721 | 567417.88611 | 4845326.53072 |
| GR0947 | Grand River | 3 | 701 | 529208.38698 | 4849277.91371 |
| GR0948 | Grand River | 3 | 2038 | 520534.48267 | 4846887.39902 |
| GR0953 | Grand River | 3 | 949 | 530058.02806 | 4849763.10631 |
| GR0955 | Grand River | 3 | 2496 | 569498.61685 | 4841222.71459 |
| GR0957 | Grand River | 2 | 632 | 523562.97554 | 4850839.55208 |
| GR0968 | Grand River | 3 | 953 | 546953.16361 | 4844188.32529 |
| GR0975 | Grand River | 3 | 608 | 522296.66931 | 4848238.74557 |
| GR0978 | Grand River | 3 | 1432 | 563137.24924 | 4837704.27004 |
| GR0981 | Grand River | 3 | 1258 | 545649.21525 | 4843222.40438 |
| GR0983 | Grand River | 3 | 1091 | 571109.48086 | 4837936.63880 |
| GR0987 | Grand River | 3 | 1861 | 530161.81147 | 4844330.09690 |
| GR0993 | Grand River | 3 | 1583 | 544727.61014 | 4839425.74579 |
| GR0995 | Grand River | 2 | 1010 | 522195.99790 | 4843923.62784 |
| GR0997 | Grand River | 3 | 1826 | 538302.91783 | 4838696.38850 |
| GR1000 | Grand River | 4 | 1569 | 571155.25143 | 4837658.05693 |
| GR1008 | Grand River | 3 | 800 | 520932.32074 | 4844319.69037 |
| GR1012 | Grand River | 3 | 1174 | 530160.13634 | 4844320.81713 |
| GR1016 | Grand River | 4 | 1767 | 538289.51552 | 4838689.44991 |
| GR1022 | Grand River | 3 | 824 | 527569.58057 | 4842257.20834 |
| GR1026 | Grand River | 3 | 1026 | 569689.86377 | 4835299.28449 |
| GR1036 | Grand River | 3 | 2365 | 533704.07173 | 4832064.82661 |
| GR1044 | Grand River | 3 | 1050 | 550929.31386 | 4834518.24459 |
| GR1048 | Grand River | 3 | 663 | 544210.30921 | 4835518.13257 |
| GR1051 | Grand River | 3 | 940 | 573757.54634 | 4829791.55629 |
| GR1054 | Grand River | 2 | 770 | 525425.14950 | 4836597.57989 |
| GR1057 | Grand River | 3 | 1189 | 534846.32773 | 4833682.66380 |
| GR1059 | Grand River | 3 | 1276 | 542841.27907 | 4832875.30661 |
| GR1062 | Grand River | 3 | 2072 | 560989.09615 | 4829012.82602 |
| GR1064 | Grand River | 2 | 906 | 573781.44893 | 4829795.80082 |
| GR1068 | Grand River | 3 | 1450 | 565418.85142 | 4830598.27448 |
| GR1069 | Grand River | 3 | 2237 | 548049.42910 | 4828733.21997 |
| GR1072 | Grand River | 3 | 1207 | 511798.54102 | 4835240.76668 |
| GR1074 | Grand River | 3 | 2048 | 511898.67554 | 4834065.90799 |
| GR1075 | Grand River | 2 | 1330 | 511786.36431 | 4835232.96346 |
| GR1081 | Grand River | 2 | 895 | 526691.67502 | 4832753.14762 |
| GR1083 | Grand River | 3 | 1047 | 534835.85853 | 4833672.97951 |
| GR1091 | Grand River | 3 | 2063 | 540279.11590 | 4826028.31648 |
| GR1096 | Grand River | 3 | 1401 | 532603.83645 | 4829443.93213 |
| GR1102 | Grand River | 3 | 1568 | 558486.28583 | 4825138.48527 |
| GR1110 | Grand River | 3 | 1316 | 517896.77018 | 4830131.10646 |
| GR1112 | Grand River | 2 | 624 | 548037.05763 | 4828736.16933 |
| GR1135 | Grand River | 3 | 727 | 528631.46660 | 4829180.51656 |
| GR1136 | Grand River | 3 | 634 | 532595.07805 | 4829430.92928 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|---------------|-------|-----------|--------------|---------------|
| GR1137 | Grand River | 2 | 869 | 570754.57193 | 4823749.10797 |
| GR1146 | Grand River | 3 | 1359 | 517905.59582 | 4830120.35267 |
| GR1155 | Grand River | 2 | 855 | 570945.59860 | 4822456.57436 |
| GR1156 | Grand River | 4 | 839 | 570949.92198 | 4822414.75053 |
| GR1157 | Grand River | 3 | 930 | 540266.68153 | 4826017.49956 |
| GR1160 | Grand River | 3 | 1654 | 517395.82155 | 4829801.81645 |
| GR1161 | Grand River | 3 | 1352 | 528871.28554 | 4827954.85948 |
| GR1165 | Grand River | 3 | 1409 | 523184.01721 | 4827019.93320 |
| GR1184 | Grand River | 3 | 695 | 545036.66110 | 4824084.61693 |
| GR1194 | Grand River | 2 | 657 | 529550.84900 | 4823761.40005 |
| GR1199 | Grand River | 3 | 857 | 540260.12152 | 4822222.90565 |
| GR1211 | Grand River | 4 | 1544 | 529432.97102 | 4823075.19169 |
| GR1213 | Grand River | 2 | 1082 | 540628.49105 | 4821306.83199 |
| GR1217 | Grand River | 3 | 1102 | 513577.23649 | 4823801.66445 |
| GR1220 | Grand River | 2 | 1946 | 568636.44607 | 4813880.61027 |
| GR1221 | Grand River | 3 | 1911 | 568622.17607 | 4813881.18997 |
| GR1224 | Grand River | 2 | 960 | 508616.95855 | 4824568.97235 |
| GR1231 | Grand River | 3 | 816 | 523225.67531 | 4823010.29475 |
| GR1234 | Grand River | 2 | 704 | 508320.40862 | 4824150.71544 |
| GR1235 | Grand River | 4 | 2247 | 547564.42726 | 4816093.25245 |
| GR1248 | Grand River | 4 | 892 | 525393.36319 | 4821559.90106 |
| GR1259 | Grand River | 3 | 850 | 513005.19732 | 4820276.06550 |
| GR1264 | Grand River | 2 | 709 | 557503.19001 | 4814155.68531 |
| GR1272 | Grand River | 3 | 629 | 567713.29330 | 4811617.02105 |
| GR1276 | Grand River | 3 | 2772 | 513053.83453 | 4818756.43322 |
| GR1281 | Grand River | 2 | 608 | 561289.67195 | 4811132.25493 |
| GR1284 | Grand River | 2 | 1172 | 561301.20052 | 4811115.81080 |
| GR1288 | Grand River | 2 | 1280 | 520860.58050 | 4815148.52514 |
| GR1290 | Grand River | 3 | 660 | 533189.21297 | 4814377.07724 |
| GR1302 | Grand River | 3 | 1273 | 507432.58019 | 4817454.92591 |
| GR1304 | Grand River | 2 | 695 | 507444.35144 | 4817442.68268 |
| GR1308 | Grand River | 3 | 975 | 548432.28523 | 4810208.81806 |
| GR1309 | Grand River | 3 | 929 | 531654.27322 | 4813591.83577 |
| GR1310 | Spencer Creek | 3 | 2361 | 572082.77504 | 4802674.50947 |
| GR1312 | Grand River | 3 | 828 | 531673.33826 | 4813592.58350 |
| GR1314 | Grand River | 3 | 2717 | 525180.98723 | 4810363.82189 |
| GR1318 | Grand River | 3 | 640 | 564378.67024 | 4808768.52795 |
| GR1328 | Grand River | 2 | 669 | 553397.25311 | 4807298.71359 |
| GR1341 | Grand River | 3 | 1210 | 518037.52041 | 4812423.92545 |
| GR1342 | Grand River | 4 | 1935 | 514690.38819 | 4812732.20301 |
| GR1353 | Grand River | 3 | 807 | 525200.99047 | 4810359.82106 |
| GR1357 | Grand River | 3 | 1878 | 563422.96726 | 4796743.62016 |
| GR1362 | Grand River | 2 | 783 | 525784.15368 | 4809587.81526 |
| GR1373 | Grand River | 4 | 1192 | 567176.47813 | 4801751.91298 |
| GR1381 | Spencer Creek | 3 | 1292 | 577351.08895 | 4798332.34284 |
| GR1384 | Grand River | 3 | 1502 | 534511.75250 | 4806496.79337 |
| GR1386 | Grand River | 2 | 736 | 567187.27447 | 4801751.82969 |
| GR1392 | Grand River | 3 | 1717 | 523074.33717 | 4807514.05984 |
| GR1406 | Grand River | 3 | 843 | 565993.03890 | 4797144.09731 |
| GR1410 | Grand River | 4 | 2120 | 563409.54383 | 4796738.97243 |
| GR1414 | Grand River | 4 | 870 | 536318.51839 | 4802969.22016 |
| GR1418 | Grand River | 2 | 853 | 537252.10120 | 4801523.27560 |
| GR1419 | Grand River | 3 | 829 | 571154.05599 | 4794975.72131 |
| GR1423 | Thames River | 2 | 1326 | 506065.11111 | 4803121.53149 |
| GR1427 | Grand River | 2 | 791 | 549484.65028 | 4799339.18584 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|---------------|-------|-----------|--------------|---------------|
| GR1429 | Grand River | 3 | 1017 | 536398.80298 | 4802066.07712 |
| GR1431 | Grand River | 4 | 1794 | 529436.67143 | 4798466.67023 |
| GR1435 | Thames River | 2 | 734 | 509988.12331 | 4803688.50426 |
| GR1442 | Spencer Creek | 2 | 865 | 575662.81448 | 4793890.75392 |
| GR1443 | Grand River | 3 | 1216 | 546655.89937 | 4799177.04009 |
| GR1447 | Spencer Creek | 2 | 1272 | 575838.28907 | 4793787.62640 |
| GR1456 | Thames River | 3 | 1193 | 510128.00160 | 4803687.86077 |
| GR1461 | Grand River | 3 | 1608 | 542520.65922 | 4794158.21594 |
| GR1464 | Grand River | 3 | 931 | 555924.61352 | 4795333.84421 |
| GR1466 | Spencer Creek | 4 | 1851 | 575391.18967 | 4793051.31178 |
| GR1467 | Grand River | 3 | 825 | 566501.85457 | 4792861.13191 |
| GR1471 | Grand River | 3 | 1045 | 528595.39985 | 4798937.56063 |
| GR1476 | Grand River | 3 | 911 | 538193.98499 | 4795615.89231 |
| GR1480 | Grand River | 2 | 673 | 529826.40880 | 4796588.00976 |
| GR1486 | Grand River | 3 | 670 | 570759.24863 | 4792300.49293 |
| GR1489 | Grand River | 3 | 782 | 532913.15494 | 4796575.44226 |
| GR1500 | Grand River | 3 | 2108 | 545928.65066 | 4794611.40447 |
| GR1502 | Grand River | 4 | 1853 | 562673.29159 | 4791766.43363 |
| GR1503 | Grand River | 3 | 1382 | 521980.64179 | 4793571.78932 |
| GR1520 | Grand River | 4 | 1089 | 566043.21456 | 4789118.41363 |
| GR1521 | Grand River | 3 | 1062 | 575019.05960 | 4786883.00873 |
| GR1526 | Grand River | 3 | 2925 | 536193.70295 | 4793288.99633 |
| GR1536 | Grand River | 3 | 1401 | 521967.40303 | 4793561.22842 |
| GR1537 | Grand River | 3 | 669 | 574998.50659 | 4786897.40040 |
| GR1543 | Grand River | 3 | 1260 | 548303.92927 | 4788307.15807 |
| GR1551 | Grand River | 3 | 1106 | 524623.86483 | 4791979.83300 |
| GR1554 | Grand River | 3 | 1190 | 541950.04934 | 4787525.94060 |
| GR1559 | Grand River | 4 | 1254 | 561511.99623 | 4786398.69088 |
| GR1566 | Grand River | 4 | 1525 | 573891.49109 | 4781240.75097 |
| GR1569 | Grand River | 4 | 655 | 567428.63360 | 4784430.01224 |
| GR1571 | Grand River | 3 | 605 | 525902.92928 | 4790331.49800 |
| GR1582 | Grand River | 4 | 2656 | 562788.27452 | 4784088.57780 |
| GR1599 | Grand River | 4 | 1277 | 561825.93468 | 4783579.34385 |
| GR1600 | Grand River | 3 | 603 | 529757.17057 | 4788284.05416 |
| GR1607 | Grand River | 3 | 1680 | 540456.98372 | 4786597.81243 |
| GR1620 | Grand River | 3 | 954 | 566470.71475 | 4780234.59172 |
| GR1632 | Grand River | 3 | 1302 | 541819.94335 | 4783067.05806 |
| GR1635 | Grand River | 2 | 617 | 557796.34670 | 4782636.83369 |
| GR1649 | Grand River | 4 | 906 | 574244.50566 | 4778565.95850 |
| GR1653 | Grand River | 4 | 1810 | 576473.26856 | 4773811.46624 |
| GR1654 | Grand River | 3 | 1352 | 571069.25520 | 4778659.86640 |
| GR1658 | Grand River | 5 | 2970 | 580556.92537 | 4771627.69886 |
| GR1668 | Grand River | 2 | 714 | 547103.99300 | 4782398.10072 |
| GR1685 | Grand River | 4 | 1764 | 530912.43354 | 4783034.72716 |
| GR1699 | Grand River | 2 | 841 | 536984.65368 | 4779605.56112 |
| GR1708 | Grand River | 4 | 1543 | 587144.67212 | 4771968.74897 |
| GR1723 | Grand River | 3 | 707 | 540571.31794 | 4778350.26974 |
| GR1738 | Grand River | 3 | 655 | 587166.06742 | 4771973.73592 |
| GR1772 | Grand River | 4 | 1344 | 538411.98421 | 4776543.41424 |
| GR1774 | Grand River | 4 | 601 | 583016.44220 | 4770360.96064 |
| GR1776 | Grand River | 2 | 1228 | 548195.65437 | 4774107.15683 |
| GR1779 | Grand River | 2 | 903 | 546567.69110 | 4774398.80371 |
| GR1788 | Grand River | 3 | 623 | 569740.66138 | 4772068.86065 |
| GR1790 | Grand River | 4 | 2759 | 529800.38207 | 4771715.15344 |
| GR1809 | Grand River | 2 | 655 | 541673.12287 | 4773150.80137 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|-------------|-------|-----------|--------------|---------------|
| GR1814 | Grand River | 3 | 676 | 574954.85580 | 4770304.91405 |
| GR1820 | Grand River | 4 | 2821 | 565482.38514 | 4771398.87882 |
| GR1828 | Grand River | 2 | 601 | 561980.57568 | 4771569.40858 |
| GR1832 | Grand River | 3 | 1089 | 571544.75483 | 4769974.87595 |
| GR1840 | Grand River | 4 | 1996 | 541793.87514 | 4772322.47386 |
| GR1882 | Grand River | 3 | 803 | 536538.17668 | 4772003.17871 |
| GR1890 | Grand River | 3 | 951 | 581834.88328 | 4766465.12317 |
| GR1905 | Grand River | 3 | 1297 | 538605.11331 | 4771524.00639 |
| GR1926 | Grand River | 3 | 1069 | 529783.49587 | 4771694.08728 |
| GR1932 | Grand River | 3 | 1511 | 574566.30962 | 4763297.67120 |
| GR1947 | Grand River | 2 | 606 | 584213.29024 | 4763474.84339 |
| GR1961 | Grand River | 3 | 766 | 565601.83967 | 4765668.09816 |
| GR1988 | Grand River | 3 | 817 | 572337.58529 | 4762787.49440 |
| GR1995 | Grand River | 3 | 1118 | 587201.50944 | 4761030.91675 |
| GR2008 | Grand River | 2 | 1094 | 571926.21725 | 4762552.81880 |
| GR2025 | Grand River | 2 | 609 | 571722.84198 | 4762010.18160 |
| GR2041 | Grand River | 2 | 1075 | 569391.48292 | 4761372.93746 |
| GR2042 | Grand River | 3 | 1182 | 569402.13830 | 4761365.47300 |
| GR2043 | Grand River | 3 | 799 | 607735.81128 | 4753127.89007 |
| GR2045 | Grand River | 2 | 720 | 579470.65862 | 4759736.50277 |
| GR2054 | Grand River | 3 | 848 | 589186.61045 | 4757492.23706 |
| GR2055 | Grand River | 4 | 2071 | 597368.79362 | 4751901.93142 |
| GR2056 | Grand River | 3 | 1424 | 589209.18334 | 4757490.81872 |
| GR2063 | Grand River | 2 | 1011 | 615683.35528 | 4751265.74701 |
| GR2067 | Grand River | 3 | 1303 | 574079.65311 | 4758641.94138 |
| GR2068 | Grand River | 3 | 832 | 574090.15612 | 4758640.48537 |
| GR2071 | Grand River | 3 | 844 | 558763.11119 | 4760250.36703 |
| GR2074 | Grand River | 2 | 620 | 558752.87036 | 4760229.12362 |
| GR2076 | Grand River | 3 | 822 | 605506.58227 | 4753539.35601 |
| GR2100 | Grand River | 3 | 1207 | 609759.55316 | 4751990.64949 |
| GR2126 | Grand River | 3 | 884 | 594435.92555 | 4753188.23754 |
| GR2127 | Grand River | 3 | 602 | 610332.77321 | 4750940.68772 |
| GR2136 | Grand River | 3 | 752 | 602703.10225 | 4751462.88363 |
| GR2145 | Grand River | 3 | 625 | 596112.33955 | 4751800.97010 |
| GR2174 | Grand River | 3 | 648 | 616338.47786 | 4747078.83525 |
| GR2232 | Grand River | 2 | 784 | 609815.74811 | 4743355.48547 |
| LP0243 | Long Point | 3 | 1056 | 543697.59342 | 4765014.02963 |
| LP0266 | Long Point | 2 | 1769 | 545469.76966 | 4762652.14665 |
| LP0274 | Long Point | 3 | 1186 | 540812.62446 | 4766468.23263 |
| LP0298 | Long Point | 3 | 1660 | 535266.77756 | 4767408.72063 |
| LP0321 | Long Point | 3 | 968 | 541059.53471 | 4766225.80547 |
| LP0330 | Long Point | 3 | 2791 | 536762.61920 | 4766330.57035 |
| LP0344 | Long Point | 2 | 602 | 545024.08714 | 4763313.66781 |
| LP0378 | Long Point | 2 | 855 | 538294.07647 | 4762117.44283 |
| LP0382 | Long Point | 2 | 881 | 539390.53928 | 4761157.88784 |
| LP0388 | Long Point | 2 | 921 | 538274.39912 | 4762074.25077 |
| LP0397 | Long Point | 2 | 851 | 527867.38929 | 4762143.79118 |
| LP0427 | Long Point | 2 | 692 | 526201.93718 | 4761135.05590 |
| LP0430 | Long Point | 1 | 771 | 546142.02684 | 4757877.20052 |
| LP0445 | Long Point | 2 | 720 | 526192.10893 | 4761115.18069 |
| LP0449 | Long Point | 3 | 724 | 545908.81475 | 4757158.46920 |
| LP0482 | Long Point | 3 | 1263 | 543922.99850 | 4754083.41907 |
| LP0483 | Long Point | 2 | 869 | 545292.33723 | 4755207.94491 |
| LP0484 | Long Point | 1 | 1023 | 529557.65340 | 4755808.88592 |
| LP0500 | Long Point | 2 | 728 | 524141.47602 | 4757742.36173 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|--------------|-------|-----------|--------------|---------------|
| LP0501 | Long Point | 2 | 1262 | 524155.87571 | 4757728.20382 |
| LP0507 | Long Point | 2 | 1243 | 533199.50935 | 4753710.66143 |
| LP0508 | Long Point | 2 | 1302 | 543610.96468 | 4752770.48026 |
| LP0520 | Long Point | 1 | 1098 | 533848.36307 | 4754170.86688 |
| LP0555 | Long Point | 2 | 893 | 518938.78402 | 4753566.44164 |
| LP0558 | Long Point | 3 | 1496 | 538957.96055 | 4749513.05186 |
| LP0571 | Long Point | 2 | 819 | 542792.93740 | 4750462.50131 |
| LP0575 | Long Point | 1 | 641 | 518935.48029 | 4753571.17192 |
| LP0591 | Long Point | 2 | 1634 | 530897.84067 | 4751430.76761 |
| LP0593 | Long Point | 3 | 642 | 519747.89851 | 4751692.27697 |
| LP0596 | Long Point | 2 | 900 | 542185.47856 | 4748923.04048 |
| LP0600 | Long Point | 2 | 664 | 538943.49217 | 4749505.49961 |
| LP0611 | Long Point | 1 | 825 | 518008.19511 | 4752113.16844 |
| LP0612 | Long Point | 3 | 742 | 536744.25626 | 4747195.94397 |
| LP0624 | Long Point | 3 | 1293 | 530553.30019 | 4749001.31389 |
| LP0630 | Long Point | 3 | 648 | 536618.29405 | 4747062.14251 |
| LP0636 | Long Point | 2 | 624 | 515003.22202 | 4749434.52175 |
| LP0647 | Long Point | 2 | 994 | 514997.84370 | 4749424.28730 |
| LP0655 | Long Point | 3 | 1162 | 537172.85431 | 4743770.92455 |
| LP0675 | Long Point | 3 | 808 | 537193.85904 | 4743759.75147 |
| LP0677 | Long Point | 3 | 1179 | 530950.17376 | 4742388.98331 |
| LP0691 | Long Point | 3 | 2172 | 530965.87917 | 4742379.15987 |
| LP0700 | Long Point | 2 | 1608 | 541201.22370 | 4736510.05671 |
| LP0705 | Long Point | 3 | 2803 | 511821.65351 | 4738298.28675 |
| LP0712 | Long Point | 2 | 1267 | 540641.22340 | 4737343.28499 |
| LP0725 | Long Point | 3 | 1145 | 524341.18979 | 4739376.68833 |
| LP0731 | Long Point | 3 | 1521 | 540469.42943 | 4735325.17036 |
| LP0738 | Long Point | 3 | 725 | 530369.60482 | 4734915.84005 |
| LP0745 | Long Point | 2 | 702 | 530570.83924 | 4734614.97724 |
| LP0747 | Long Point | 2 | 688 | 511805.66409 | 4738289.49954 |
| LP0749 | Long Point | 3 | 1788 | 530361.16612 | 4734915.88232 |
| LP0751 | Long Point | 2 | 885 | 540058.21628 | 4732856.95129 |
| LP0752 | Long Point | 3 | 1456 | 540003.91172 | 4731449.20215 |
| LP0754 | Long Point | 3 | 2358 | 543723.11560 | 4725173.73096 |
| LP0769 | Long Point | 3 | 854 | 538422.59802 | 4729879.53484 |
| LP0771 | Long Point | 3 | 1468 | 511374.80231 | 4733172.15545 |
| LP0772 | Long Point | 3 | 2012 | 537199.89807 | 4727009.30397 |
| LP0778 | Long Point | 3 | 2072 | 524301.43667 | 4724520.89178 |
| LP0789 | Long Point | 3 | 833 | 521600.41804 | 4728524.86547 |
| LP0793 | Long Point | 3 | 900 | 521581.09070 | 4728537.09233 |
| LP0796 | Long Point | 2 | 961 | 530989.68683 | 4726792.82663 |
| LP0803 | Long Point | 3 | 769 | 514173.40356 | 4727475.28488 |
| LP0815 | Long Point | 3 | 1698 | 517379.71691 | 4724516.62097 |
| LP0823 | Long Point | 2 | 683 | 525030.53454 | 4724742.15852 |
| LP0841 | Long Point | 3 | 925 | 525028.85846 | 4724732.87842 |
| LP0895 | Long Point | 2 | 783 | 518576.69682 | 4722498.29764 |
| LP0925 | Long Point | 2 | 753 | 543927.59871 | 4716163.83270 |
| TR0327 | Thames River | 3 | 1832 | 484140.70648 | 4824821.97438 |
| TR0328 | Thames River | 3 | 796 | 483840.57043 | 4825555.48983 |
| TR0337 | Thames River | 3 | 2524 | 486247.70151 | 4822897.62307 |
| TR0367 | Thames River | 2 | 616 | 486248.89445 | 4822885.51379 |
| TR0369 | Thames River | 2 | 1783 | 485374.71699 | 4818907.73386 |
| TR0375 | Thames River | 3 | 2455 | 498658.37529 | 4816974.49235 |
| TR0380 | Thames River | 3 | 1755 | 489806.40540 | 4818680.16788 |
| TR0395 | Thames River | 2 | 1000 | 497511.98672 | 4817126.65758 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|--------------|-------|-----------|--------------|---------------|
| TR0415 | Thames River | 2 | 1081 | 489821.33806 | 4818672.11491 |
| TR0435 | Thames River | 2 | 2185 | 501425.34033 | 4809338.33706 |
| TR0455 | Thames River | 2 | 1343 | 486369.42198 | 4816017.35186 |
| TR0461 | Thames River | 3 | 1670 | 502563.87657 | 4812003.90644 |
| TR0474 | Thames River | 2 | 840 | 486370.32996 | 4816008.25721 |
| TR0475 | Thames River | 3 | 921 | 501295.45132 | 4810719.93762 |
| TR0477 | Thames River | 3 | 1105 | 492736.18885 | 4814118.59797 |
| TR0494 | Thames River | 2 | 1003 | 496753.93867 | 4808565.11163 |
| TR0520 | Thames River | 2 | 968 | 488910.87729 | 4810123.91688 |
| TR0533 | Thames River | 2 | 610 | 486145.98864 | 4809332.97000 |
| TR0538 | Thames River | 2 | 922 | 495411.14187 | 4805986.90776 |
| TR0544 | Thames River | 3 | 983 | 483082.30293 | 4807936.12307 |
| TR0547 | Thames River | 1 | 625 | 476505.06325 | 4808123.80465 |
| TR0564 | Thames River | 2 | 876 | 476619.53387 | 4806879.66734 |
| TR0600 | Thames River | 2 | 716 | 489047.86851 | 4801107.65903 |
| TR0605 | Thames River | 2 | 762 | 477753.57069 | 4803003.25245 |
| TR0606 | Thames River | 3 | 1983 | 510428.98248 | 4794822.82703 |
| TR0608 | Thames River | 3 | 2083 | 503415.66906 | 4795695.62158 |
| TR0613 | Thames River | 3 | 805 | 503431.78570 | 4797073.26184 |
| TR0614 | Thames River | 2 | 705 | 489045.85439 | 4801099.48124 |
| TR0615 | Thames River | 2 | 1476 | 474231.13824 | 4801116.22868 |
| TR0633 | Thames River | 1 | 876 | 474221.33346 | 4801122.44503 |
| TR0635 | Thames River | 2 | 937 | 503421.28427 | 4797074.74101 |
| TR0643 | Thames River | 2 | 1294 | 489344.14319 | 4797094.29682 |
| TR0644 | Thames River | 2 | 741 | 478826.15579 | 4799578.61067 |
| TR0647 | Thames River | 4 | 981 | 510422.98019 | 4794818.26117 |
| TR0655 | Thames River | 2 | 620 | 485188.66639 | 4798064.81419 |
| TR0660 | Thames River | 3 | 923 | 475434.56851 | 4798129.04946 |
| TR0662 | Thames River | 3 | 860 | 485399.86114 | 4797097.06187 |
| TR0663 | Thames River | 2 | 679 | 515917.37097 | 4792981.02633 |
| TR0664 | Thames River | 1 | 601 | 495932.65950 | 4795117.23881 |
| TR0671 | Thames River | 2 | 1459 | 495943.84253 | 4795117.53838 |
| TR0675 | Thames River | 4 | 1953 | 501974.42430 | 4790400.88830 |
| TR0685 | Thames River | 3 | 758 | 492330.36299 | 4793727.00102 |
| TR0694 | Thames River | 2 | 842 | 519872.84610 | 4789943.73762 |
| TR0696 | Thames River | 3 | 1299 | 516912.54169 | 4787954.03715 |
| TR0699 | Thames River | 2 | 627 | 495438.28772 | 4793243.28119 |
| TR0704 | Thames River | 3 | 693 | 486564.29495 | 4793773.40473 |
| TR0707 | Thames River | 2 | 727 | 474445.14018 | 4795350.62903 |
| TR0708 | Thames River | 2 | 723 | 474132.16077 | 4795530.17333 |
| TR0715 | Thames River | 3 | 978 | 508556.65139 | 4789139.96501 |
| TR0738 | Thames River | 3 | 1044 | 464741.98761 | 4742825.39704 |
| TR0747 | Thames River | 2 | 633 | 465456.64271 | 4739692.46291 |
| TR0748 | Thames River | 2 | 1414 | 453201.19648 | 4739250.50992 |
| TR0752 | Thames River | 2 | 2748 | 458484.35736 | 4734663.33680 |
| TR0762 | Thames River | 3 | 1870 | 460278.77704 | 4736561.46427 |
| TR0766 | Thames River | 2 | 902 | 501094.77662 | 4788278.01325 |
| TR0778 | Thames River | 3 | 1366 | 479400.50304 | 4790794.98263 |
| TR0787 | Thames River | 2 | 988 | 479391.15940 | 4790799.05841 |
| TR0796 | Thames River | 4 | 762 | 474982.69912 | 4791762.34492 |
| TR0818 | Thames River | 4 | 642 | 497692.03338 | 4788064.22782 |
| TR0827 | Thames River | 2 | 972 | 475397.15043 | 4788728.81906 |
| TR0828 | Thames River | 3 | 783 | 522366.16569 | 4781640.68741 |
| TR0841 | Thames River | 3 | 804 | 474702.21213 | 4789193.39531 |
| TR0856 | Thames River | 3 | 1276 | 512231.87957 | 4781982.24604 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|--------------|-------|-----------|--------------|---------------|
| TR0885 | Thames River | 3 | 1711 | 506873.82311 | 4779584.54606 |
| TR0893 | Thames River | 3 | 684 | 519822.40377 | 4777690.93972 |
| TR0910 | Thames River | 2 | 1198 | 485031.46060 | 4782554.33691 |
| TR0931 | Thames River | 2 | 1576 | 518024.59155 | 4777378.15022 |
| TR0944 | Thames River | 3 | 870 | 502801.11044 | 4778257.25694 |
| TR0956 | Thames River | 2 | 1673 | 488094.80532 | 4774499.70458 |
| TR0977 | Thames River | 2 | 1593 | 513492.49632 | 4770953.88636 |
| TR0980 | Thames River | 2 | 761 | 480993.96046 | 4775939.99720 |
| TR1015 | Thames River | 3 | 1160 | 513500.80998 | 4770870.00652 |
| TR1023 | Thames River | 3 | 1036 | 487731.75980 | 4770732.62619 |
| TR1024 | Thames River | 3 | 717 | 512734.72936 | 4769342.59194 |
| TR1033 | Thames River | 3 | 1169 | 500082.98816 | 4768387.66414 |
| TR1034 | Thames River | 3 | 712 | 471442.06467 | 4771799.02841 |
| TR1046 | Thames River | 3 | 655 | 482195.37786 | 4768386.37496 |
| TR1048 | Thames River | 3 | 1015 | 470650.26411 | 4767817.18264 |
| TR1055 | Thames River | 3 | 1281 | 503950.98561 | 4757687.16564 |
| TR1072 | Thames River | 3 | 2110 | 514380.44278 | 4756105.79972 |
| TR1079 | Thames River | 3 | 885 | 506259.92650 | 4763276.01547 |
| TR1090 | Thames River | 3 | 1228 | 514743.62240 | 4761858.51962 |
| TR1246 | Thames River | 3 | 687 | 496884.76027 | 4762919.39301 |
| TR1311 | Thames River | 3 | 1689 | 503532.41024 | 4759761.12372 |
| TR1338 | Thames River | 3 | 654 | 513932.79118 | 4757234.61812 |
| TR1349 | Thames River | 4 | 1560 | 491852.07285 | 4752626.62412 |
| TR1358 | Thames River | 3 | 1100 | 464017.09440 | 4751020.54297 |
| TR1375 | Thames River | 2 | 1968 | 445962.99237 | 4735737.90084 |
| TR1394 | Thames River | 3 | 1524 | 456294.39032 | 4732499.91351 |
| TR1395 | Thames River | 2 | 921 | 451972.31953 | 4730606.78005 |
| TR1409 | Thames River | 2 | 970 | 456771.12825 | 4730718.07211 |
| TR1413 | Thames River | 3 | 1015 | 456749.35281 | 4730611.32900 |
| TR1414 | Thames River | 2 | 657 | 456768.83398 | 4730609.53122 |
| TR1443 | Thames River | 3 | 1922 | 450926.41073 | 4729121.80341 |
| TR1449 | Thames River | 3 | 615 | 446590.04400 | 4727902.22744 |
| TR1463 | Thames River | 2 | 628 | 440778.11817 | 4728445.80368 |
| TR1478 | Thames River | 3 | 629 | 454129.98097 | 4725067.00134 |
| TR1487 | Thames River | 3 | 963 | 448525.30388 | 4725819.39240 |
| TR1490 | Thames River | 4 | 947 | 444257.43512 | 4725822.15449 |
| TR1539 | Thames River | 4 | 2096 | 446204.60494 | 4721869.94241 |
| TR1543 | Thames River | 2 | 608 | 437005.18187 | 4721710.69277 |
| TR1556 | Thames River | 4 | 1654 | 442458.63212 | 4721142.09540 |
| TR1561 | Thames River | 3 | 720 | 442459.62563 | 4720957.36439 |
| TR1569 | Thames River | 3 | 1193 | 435972.30288 | 4720412.00619 |
| TR1587 | Thames River | 2 | 890 | 439455.95928 | 4717987.04576 |
| TR1591 | Thames River | 3 | 834 | 430883.76971 | 4717348.74377 |
| TR1614 | Thames River | 3 | 748 | 421135.58146 | 4715241.63360 |
| TR1615 | Thames River | 2 | 651 | 432142.97196 | 4716722.60252 |
| TR1616 | Thames River | 2 | 779 | 432188.49897 | 4716725.84520 |
| TR1618 | Thames River | 3 | 2542 | 439409.85866 | 4715538.90965 |
| TR1659 | Thames River | 3 | 779 | 421156.53228 | 4715236.36243 |
| TR1670 | Thames River | 2 | 1286 | 425651.15649 | 4712992.99934 |
| TR1696 | Thames River | 3 | 672 | 422115.79199 | 4712195.13894 |
| TR1704 | Thames River | 3 | 1991 | 422524.07172 | 4709267.31629 |
| TR1711 | Thames River | 4 | 1998 | 423152.89187 | 4708041.96667 |
| TR1730 | Thames River | 3 | 725 | 422521.38690 | 4709257.97235 |
| TR1737 | Thames River | 2 | 1182 | 423141.99388 | 4708024.11432 |
| TR1752 | Thames River | 3 | 697 | 422654.91627 | 4708296.98992 |

| Site Code | Watershed | Order | Area (ha) | Easting | Northing |
|-----------|--------------|-------|-----------|--------------|---------------|
| TR1754 | Thames River | 2 | 739 | 416553.79799 | 4707788.05611 |
| TR1756 | Thames River | 2 | 1320 | 416558.42048 | 4707776.23255 |
| TR1773 | Thames River | 2 | 2283 | 422833.76588 | 4702860.54863 |
| TR1775 | Thames River | 3 | 1955 | 411395.03083 | 4705318.99973 |
| TR1779 | Thames River | 3 | 637 | 419240.71579 | 4704490.47181 |
| TR1789 | Thames River | 2 | 1103 | 422840.97239 | 4702851.19106 |
| TR1792 | Thames River | 3 | 974 | 415578.24400 | 4702754.44838 |
| TR1809 | Thames River | 3 | 1208 | 411479.67840 | 4702852.93697 |
| TR1816 | Thames River | 2 | 819 | 412228.12531 | 4702593.55051 |
| TR1825 | Thames River | 2 | 974 | 418881.64461 | 4699506.16965 |
| TR1828 | Thames River | 3 | 673 | 408269.06935 | 4701793.87841 |
| TR1836 | Thames River | 2 | 765 | 418659.58841 | 4699162.44045 |
| TR1837 | Thames River | 2 | 706 | 420722.48204 | 4697287.41240 |
| TR1844 | Thames River | 2 | 980 | 420720.23962 | 4697274.05160 |
| TR1847 | Thames River | 3 | 753 | 404151.76547 | 4696970.24985 |
| TR1848 | Thames River | 2 | 1322 | 415373.95918 | 4696728.85417 |
| TR1849 | Thames River | 3 | 694 | 413121.10000 | 4695743.44533 |
| TR1852 | Thames River | 2 | 842 | 415268.99557 | 4696303.19112 |
| TR1855 | Thames River | 3 | 929 | 405182.43409 | 4695394.05352 |
| TR1857 | Thames River | 2 | 733 | 405182.47066 | 4695387.61963 |
| TR1862 | Thames River | 2 | 1023 | 412963.36246 | 4695238.00156 |
| TR1863 | Thames River | 2 | 756 | 412118.14581 | 4694245.85155 |
| TR1874 | Thames River | 2 | 833 | 408847.55792 | 4693630.77891 |
| TR1880 | Thames River | 2 | 1206 | 410659.12668 | 4693274.99909 |
| TR1886 | Thames River | 3 | 1232 | 410692.66052 | 4689630.85140 |
| TR1907 | Thames River | 3 | 787 | 403009.39382 | 4689762.65887 |
| TR1911 | Thames River | 2 | 659 | 410704.83714 | 4689638.61878 |
| TR1924 | Thames River | 2 | 1037 | 402314.92666 | 4689108.13868 |
| TR1931 | Thames River | 3 | 733 | 396872.17487 | 4688480.35844 |
| TR1955 | Thames River | 2 | 947 | 402641.14264 | 4687252.75785 |
| TR1967 | Thames River | 3 | 768 | 399434.12370 | 4687286.36236 |
| TR1972 | Thames River | 2 | 1573 | 406585.86882 | 4688964.86773 |
| TR1980 | Thames River | 3 | 1222 | 403347.14491 | 4686046.96063 |
| TR1985 | Thames River | 3 | 1008 | 392422.76724 | 4687124.09767 |
| TR1987 | Thames River | 3 | 1890 | 396482.97232 | 4685798.26120 |
| TR1992 | Thames River | 3 | 1695 | 400174.06259 | 4685818.86741 |
| TR1995 | Thames River | 3 | 704 | 385476.49181 | 4686277.16534 |
| TR2001 | Thames River | 2 | 794 | 401518.98119 | 4684854.30812 |
| TR2010 | Thames River | 3 | 1387 | 401408.60674 | 4684860.55399 |
| TR2011 | Thames River | 3 | 710 | 392651.96135 | 4684807.45677 |
| TR2019 | Thames River | 2 | 1232 | 392726.90736 | 4679201.59141 |
| TR2077 | Thames River | 3 | 1242 | 392784.73436 | 4679203.37201 |
| TR2079 | Thames River | 3 | 1702 | 387091.06104 | 4676377.49850 |
| TR2133 | Thames River | 3 | 766 | 387091.06104 | 4676377.49850 |
| TR2137 | Thames River | 2 | 1735 | 380267.53607 | 4676846.44536 |
| TR2144 | Thames River | 3 | 1052 | 414983.97779 | 4708713.95691 |

Appendix 2 - Watershed membership and position of basin outflows in Universal Transverse Mercator Zone 17N (North American Datum 1983) of 160 rural basins in southwestern Ontario sampled for fish and benthic macroinvertebrates.

| Site Code | Watershed | Easting | Northing |
|-----------|---------------|--------------|---------------|
| GR0503 | Grand River | 554624.44225 | 4881734.72526 |
| GR0603 | Grand River | 550029.25980 | 4878782.54344 |
| GR0645 | Grand River | 546445.52216 | 4870719.04345 |
| GR0696 | Grand River | 559636.52579 | 4868792.02943 |
| GR0757 | Grand River | 559449.53624 | 4862776.99836 |
| GR0793 | Grand River | 538499.30494 | 4862718.45341 |
| GR0832 | Grand River | 538543.73536 | 4861592.06906 |
| GR0861 | Grand River | 556498.68726 | 4853543.01435 |
| GR0876 | Grand River | 557724.80837 | 4853848.32347 |
| GR0916 | Grand River | 547647.50576 | 4849719.11565 |
| GR0930 | Grand River | 562153.41746 | 4845421.07150 |
| GR0931 | Grand River | 562165.45909 | 4845422.86594 |
| GR0932 | Grand River | 530466.19547 | 4851719.75959 |
| GR0955 | Grand River | 569498.61685 | 4841222.71447 |
| GR0981 | Grand River | 545649.21525 | 4843222.40425 |
| GR0997 | Grand River | 538302.91783 | 4838696.38838 |
| GR1000 | Grand River | 571155.25143 | 4837658.05681 |
| GR1012 | Grand River | 530160.13634 | 4844320.81700 |
| GR1044 | Grand River | 550929.31386 | 4834518.24447 |
| GR1057 | Grand River | 534846.32773 | 4833682.66369 |
| GR1068 | Grand River | 565418.85142 | 4830598.27437 |
| GR1069 | Grand River | 548049.42910 | 4828733.21986 |
| GR1075 | Grand River | 511786.36431 | 4835232.96334 |
| GR1083 | Grand River | 534835.85853 | 4833672.97940 |
| GR1091 | Grand River | 540279.11591 | 4826028.31636 |
| GR1096 | Grand River | 532603.83645 | 4829443.93202 |
| GR1137 | Grand River | 570754.57193 | 4823749.10786 |
| GR1160 | Grand River | 517395.82155 | 4829801.81634 |
| GR1194 | Grand River | 529550.84900 | 4823761.39994 |
| GR1211 | Grand River | 529432.97102 | 4823075.19158 |
| GR1213 | Grand River | 540628.49105 | 4821306.83188 |
| GR1221 | Grand River | 568622.17607 | 4813881.18987 |
| GR1248 | Grand River | 525393.36319 | 4821559.90095 |
| GR1259 | Grand River | 513005.19732 | 4820276.06540 |
| GR1264 | Grand River | 557503.19001 | 4814155.68520 |
| GR1288 | Grand River | 520860.58050 | 4815148.52504 |
| GR1302 | Grand River | 507432.58019 | 4817454.92581 |
| GR1309 | Grand River | 531654.27322 | 4813591.83567 |
| GR1310 | Spencer Creek | 572082.77504 | 4802674.50937 |
| GR1312 | Grand River | 531673.33826 | 4813592.58340 |
| GR1314 | Grand River | 525180.98723 | 4810363.82179 |
| GR1341 | Grand River | 518037.52041 | 4812423.92535 |
| GR1342 | Grand River | 514690.38819 | 4812732.20290 |
| GR1357 | Grand River | 563422.96726 | 4796743.62007 |
| GR1384 | Grand River | 534511.75250 | 4806496.79327 |
| GR1392 | Grand River | 523074.33717 | 4807514.05974 |
| GR1406 | Grand River | 565993.03890 | 4797144.09722 |
| GR1419 | Grand River | 571154.05600 | 4794975.72122 |

| Site Code | Watershed | Easting | Northing |
|-----------|--------------|--------------|---------------|
| GR1443 | Grand River | 546655.89937 | 4799177.04000 |
| GR1456 | Thames River | 510128.00160 | 4803687.86067 |
| GR1461 | Grand River | 542520.65922 | 4794158.21585 |
| GR1464 | Grand River | 555924.61352 | 4795333.84412 |
| GR1489 | Grand River | 532913.15494 | 4796575.44217 |
| GR1503 | Grand River | 521980.64179 | 4793571.78923 |
| GR1536 | Grand River | 521967.40303 | 4793561.22834 |
| GR1582 | Grand River | 562788.27452 | 4784088.57771 |
| GR1607 | Grand River | 540456.98372 | 4786597.81235 |
| GR1632 | Grand River | 541819.94335 | 4783067.05798 |
| GR1653 | Grand River | 576473.26857 | 4773811.46617 |
| GR1658 | Grand River | 580556.92537 | 4771627.69878 |
| GR1668 | Grand River | 547103.99300 | 4782398.10064 |
| GR1776 | Grand River | 548195.65437 | 4774107.15676 |
| GR1790 | Grand River | 529800.38207 | 4771715.15337 |
| GR1828 | Grand River | 561980.57568 | 4771569.40850 |
| GR1840 | Grand River | 541793.87514 | 4772322.47378 |
| GR1882 | Grand River | 536538.17668 | 4772003.17863 |
| GR1905 | Grand River | 538605.11331 | 4771524.00631 |
| GR1926 | Grand River | 529783.49587 | 4771694.08720 |
| GR1995 | Grand River | 587201.50944 | 4761030.91668 |
| GR2056 | Grand River | 589209.18334 | 4757490.81866 |
| GR2063 | Grand River | 615683.35528 | 4751265.74696 |
| GR2067 | Grand River | 574079.65311 | 4758641.94132 |
| GR2232 | Grand River | 609815.74811 | 4743355.48542 |
| LP0298 | Long Point | 535266.77756 | 4767408.72056 |
| LP0344 | Long Point | 545024.08714 | 4763313.66775 |
| LP0378 | Long Point | 538294.07647 | 4762117.44276 |
| LP0382 | Long Point | 539390.53928 | 4761157.88777 |
| LP0397 | Long Point | 527867.38929 | 4762143.79111 |
| LP0482 | Long Point | 543922.99850 | 4754083.41901 |
| LP0507 | Long Point | 533199.50935 | 4753710.66137 |
| LP0520 | Long Point | 533848.36307 | 4754170.86682 |
| LP0555 | Long Point | 518938.78402 | 4753566.44158 |
| LP0591 | Long Point | 530897.84068 | 4751430.76755 |
| LP0630 | Long Point | 536618.29405 | 4747062.14246 |
| LP0655 | Long Point | 537172.85431 | 4743770.92450 |
| LP0677 | Long Point | 530950.17376 | 4742388.98326 |
| LP0691 | Long Point | 530965.87918 | 4742379.15982 |
| LP0700 | Long Point | 541201.22370 | 4736510.05666 |
| LP0705 | Long Point | 511821.65351 | 4738298.28670 |
| LP0712 | Long Point | 540641.22340 | 4737343.28494 |
| LP0725 | Long Point | 524341.18979 | 4739376.68828 |
| LP0738 | Long Point | 530369.60482 | 4734915.84001 |
| LP0745 | Long Point | 530570.83924 | 4734614.97720 |
| LP0747 | Long Point | 511805.66409 | 4738289.49949 |
| LP0749 | Long Point | 530361.16612 | 4734915.88227 |
| LP0754 | Long Point | 543723.11560 | 4725173.73093 |
| LP0778 | Long Point | 524301.43667 | 4724520.89174 |
| LP0789 | Long Point | 521600.41804 | 4728524.86543 |
| LP0796 | Long Point | 530989.68683 | 4726792.82659 |
| LP0815 | Long Point | 517379.71691 | 4724516.62093 |
| LP0823 | Long Point | 525030.53454 | 4724742.15848 |
| LP0841 | Long Point | 525028.85846 | 4724732.87838 |
| LP0895 | Long Point | 518576.69682 | 4722498.29760 |

| Site Code | Watershed | Easting | Northing |
|------------------|------------------|----------------|-----------------|
| LP0925 | Long Point | 543927.59871 | 4716163.83267 |
| TR0327 | Thames River | 484140.70648 | 4824821.97427 |
| TR0337 | Thames River | 486247.70151 | 4822897.62296 |
| TR0415 | Thames River | 489821.33806 | 4818672.11480 |
| TR0435 | Thames River | 501425.34033 | 4809338.33696 |
| TR0461 | Thames River | 502563.87657 | 4812003.90634 |
| TR0520 | Thames River | 488910.87729 | 4810123.91678 |
| TR0606 | Thames River | 510428.98248 | 4794822.82694 |
| TR0608 | Thames River | 503415.66906 | 4795695.62149 |
| TR0615 | Thames River | 474231.13824 | 4801116.22859 |
| TR0633 | Thames River | 474221.33346 | 4801122.44493 |
| TR0643 | Thames River | 489344.14319 | 4797094.29673 |
| TR0644 | Thames River | 478826.15579 | 4799578.61057 |
| TR0660 | Thames River | 475434.56851 | 4798129.04937 |
| TR0664 | Thames River | 495932.65950 | 4795117.23872 |
| TR0671 | Thames River | 495943.84253 | 4795117.53829 |
| TR0675 | Thames River | 501974.42430 | 4790400.88821 |
| TR0694 | Thames River | 519872.84610 | 4789943.73753 |
| TR0704 | Thames River | 486564.29495 | 4793773.40464 |
| TR0748 | Thames River | 453201.19648 | 4739250.50987 |
| TR0766 | Thames River | 501094.77662 | 4788278.01316 |
| TR0787 | Thames River | 479391.15940 | 4790799.05832 |
| TR0827 | Thames River | 475397.15043 | 4788728.81897 |
| TR0856 | Thames River | 512231.87957 | 4781982.24596 |
| TR0885 | Thames River | 506873.82311 | 4779584.54598 |
| TR0893 | Thames River | 519822.40377 | 4777690.93965 |
| TR0931 | Thames River | 518024.59155 | 4777378.15014 |
| TR0944 | Thames River | 502801.11044 | 4778257.25686 |
| TR1023 | Thames River | 487731.75980 | 4770732.62612 |
| TR1024 | Thames River | 512734.72936 | 4769342.59187 |
| TR1033 | Thames River | 500082.98816 | 4768387.66407 |
| TR1311 | Thames River | 503532.41024 | 4759761.12365 |
| TR1338 | Thames River | 513932.79118 | 4757234.61806 |
| TR1358 | Thames River | 464017.09440 | 4751020.54291 |
| TR1443 | Thames River | 450926.41073 | 4729121.80337 |
| TR1587 | Thames River | 439455.95928 | 4717987.04572 |
| TR1616 | Thames River | 432188.49897 | 4716725.84517 |
| TR1696 | Thames River | 422115.79199 | 4712195.13891 |
| TR1704 | Thames River | 422524.07172 | 4709267.31626 |
| TR1754 | Thames River | 416553.79799 | 4707788.05608 |
| TR1773 | Thames River | 422833.76588 | 4702860.54861 |
| TR1789 | Thames River | 422840.97239 | 4702851.19103 |
| TR1825 | Thames River | 418881.64461 | 4699506.16963 |
| TR1828 | Thames River | 408269.06935 | 4701793.87839 |
| TR1836 | Thames River | 418659.58841 | 4699162.44043 |
| TR1849 | Thames River | 413121.10000 | 4695743.44531 |
| TR1855 | Thames River | 405182.43409 | 4695394.05350 |
| TR1880 | Thames River | 410659.12668 | 4693274.99908 |
| TR1886 | Thames River | 410692.66052 | 4689630.85139 |
| TR1911 | Thames River | 410704.83714 | 4689638.61877 |
| TR1955 | Thames River | 402641.14264 | 4687252.75784 |
| TR1980 | Thames River | 403347.14491 | 4686046.96062 |
| TR1985 | Thames River | 392422.76724 | 4687124.09765 |
| TR1987 | Thames River | 396482.97232 | 4685798.26119 |
| TR1992 | Thames River | 400174.06259 | 4685818.86740 |

| Site Code | Watershed | Easting | Northing |
|------------------|------------------|----------------|-----------------|
| TR2019 | Thames River | 392726.90736 | 4679201.59141 |
| TR2079 | Thames River | 387091.06104 | 4676377.49850 |

Appendix 3 - United States Environmental Protection Agency Rapid Habitat Assessment Protocol data sheets for low gradient streams. Numbers 6, 9, and 10 were used to calculate local stressor score.

HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS (FRONT)

| | | | |
|---------------------------------|--|------------------------------|-------------------------|
| STREAM NAME _____ | | LOCATION _____ | |
| STATION # _____ RIVERMILE _____ | | STREAM CLASS _____ | |
| LAT _____ LONG _____ | | RIVER BASIN _____ | |
| STORET # _____ | | AGENCY _____ | |
| INVESTIGATORS _____ | | | |
| FORM COMPLETED BY _____ | | DATE _____ AM _____ PM _____ | REASON FOR SURVEY _____ |

| Habitat Parameter | Condition Category | | | |
|--|---|---|---|--|
| | Optimal | Suboptimal | Marginal | Poor |
| 1. Epifaunal Substrate/ Available Cover | Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient). | 30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale). | 10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed. | Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| 2. Pool Substrate Characterization | Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common. | Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present. | All mud or clay or sand bottom; little or no root mat; no submerged vegetation. | Hard-pan clay or bedrock; no root mat or vegetation. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| 3. Pool Variability | Even mix of large-shallow, large-deep, small-shallow, small-deep pools present. | Majority of pools large-deep; very few shallow. | Shallow pools much more prevalent than deep pools. | Majority of pools small-shallow or pools absent. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| 4. Sediment Deposition | Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition. | Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools. | Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent. | Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| 5. Channel Flow Status | Water reaches base of both lower banks, and minimal amount of channel substrate is exposed. | Water fills >75% of the available channel; or <25% of channel substrate is exposed. | Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed. | Very little water in channel and mostly present as standing pools. |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |

Parameters to be evaluated in sampling reach

HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS (BACK)

| Habitat Parameter | Condition Category | | | |
|---|--|---|---|-------------|
| | Optimal | Suboptimal | Marginal | Poor |
| 6. Channel Alteration Channelization or dredging absent or minimal; stream with normal pattern. | Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present. | Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted. | Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely. | |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| 7. Channel Sinuosity The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.) | The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line. | The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line. | Channel straight; waterway has been channelized for a long distance. | |
| SCORE | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| 8. Bank Stability (score each bank) Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected. | Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion. | Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods. | Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars. | |
| SCORE __ (LB) | Left Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE __ (RB) | Right Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| 9. Vegetative Protection (score each bank) More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally. | 70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent, more than one-half of the potential plant stubble height remaining. | 50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining. | Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height. | |
| SCORE __ (LB) | Left Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE __ (RB) | Right Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| 10. Riparian Vegetative Zone Width (score each bank riparian zone) Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone. | Width of riparian zone 12-18 meters; human activities have impacted zone only minimally. | Width of riparian zone 6-12 meters; human activities have impacted zone a great deal. | Width of riparian zone <6 meters; little or no riparian vegetation due to human activities. | |
| SCORE __ (LB) | Left Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE __ (RB) | Right Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |

Parameters to be evaluated broader than sampling reach

Appendix 4 – Complete list of fish species collected and the number and proportion of the 160 sites at which each species was found.

| Species Name | Common Name | # of Sites Present | % of Sites Present |
|--|---------------------------|--------------------|--------------------|
| <i>Lampetra appendix</i> | American Brook Lamprey | 6 | 4 |
| <i>Dorosoma cepedianum</i> | Gizzard Shad | 5 | 3 |
| <i>Moxostoma Spp</i> | Redhorse spp. | 3 | 2 |
| <i>Hypentelium nigricans</i> | Northern Hogsucker | 4 | 2 |
| <i>Catostomus commersonii commersonii</i> | White Sucker | 106 | 66 |
| <i>Cyprinus carpio</i> | Common Carp | 13 | 8 |
| <i>Semotilus atromaculatus atromaculatus</i> | Northern Creek Chub | 122 | 76 |
| <i>Nocomis biguttatus</i> | Hornyhead Chub | 9 | 6 |
| <i>Margariscus nachtriebi</i> | Northern Pearl Dace | 7 | 4 |
| <i>Phoxinus neogaeus</i> | Finescale Dace | 1 | <1 |
| <i>Phoxinus eos</i> | Northern Redbelly Dace | 38 | 24 |
| <i>Rhinichthys obtusus</i> | Western Blacknose Dace | 98 | 61 |
| <i>Rhinichthys cataractae cataractae</i> | Great Lakes Longnose Dace | 9 | 6 |
| <i>Campostoma anomalum pullum</i> | Central Stoneroller | 44 | 28 |
| <i>Cyprinella spiloptera</i> | Spotfin Shiner | 15 | 9 |
| <i>Luxilus cornutus frontalis</i> | Northern Common Shiner | 42 | 26 |
| <i>Luxilus chrysocephalus</i> | Striped Shiner | 16 | 10 |
| <i>Notropis rubellus</i> | Rosyface Shiner | 11 | 7 |
| <i>Notropis volucellus</i> | Mimic Shiner | 20 | 13 |
| <i>Notropis heterolepis</i> | Blacknose Shiner | 6 | 4 |
| <i>Pimephales notatus</i> | Bluntnose Minnow | 73 | 16 |
| <i>Pimephales promelas promelas</i> | Fathead Minnow | 35 | 22 |
| <i>Hybognathus hankinsoni</i> | Brassy Minnow | 3 | 2 |
| <i>Ameiurus melas</i> | Black Bullhead | 2 | 1 |
| <i>Ameiurus nebulosus</i> | Brown Bullhead | 6 | 4 |
| <i>Ameiurus natalis</i> | Yellow Bullhead | 6 | 4 |
| <i>Noturus flavus</i> | Stonecat | 1 | <1 |
| <i>Salvelinus fontinalis</i> | Brook Trout | 14 | 9 |
| <i>Salmo trutta</i> | Brown Trout | 10 | 6 |
| <i>Orcorhynchus mykiss</i> | Rainbow Trout | 11 | 7 |
| <i>Esox lucius</i> | Northern Pike | 4 | 2 |
| <i>Umbra limi</i> | Central Mudminnow | 33 | 21 |
| <i>Labidesthes sicculus</i> | Northern Brook Silverside | 1 | <1 |
| <i>Perca flavescens</i> | Yellow Perch | 3 | 2 |
| <i>Percina maculata</i> | Blackside Darter | 14 | 9 |
| <i>Percina caprodes semifasciata</i> | Northern Logperch | 1 | <1 |
| <i>Etheostoma nigrum nigrum</i> | Central Johnny Darter | 91 | 57 |
| <i>Etheostoma blennioides blennioides</i> | Northern Greenside Darter | 8 | 5 |
| <i>Etheostoma exile</i> | Iowa Darter | 12 | 8 |
| <i>Etheostoma microperca</i> | Least Darter | 27 | 17 |
| <i>Etheostoma caeruleum</i> | Rainbow Darter | 15 | 9 |
| <i>Etheostoma flabellare</i> | Barred Fantail Darter | 12 | 8 |

| Species Name | Common Name | # of Sites Present | % of Sites Present |
|--|--------------------------|---------------------------|---------------------------|
| <i>Micropterus dolomieu</i> | Smallmouth Bass | 5 | 3 |
| <i>Micropterus salmoides</i> | Largemouth Bass | 21 | 13 |
| <i>Lepomis Cyanellus</i> | Green Sunfish | 20 | 13 |
| <i>Lepomis peltastes</i> | Northern Longear Sunfish | 1 | <1 |
| <i>Lepomis gibbosus</i> | Pumpkinseed | 29 | 18 |
| <i>Lepomis macrochirus</i> <i>macrochirus</i> | Bluegill | 8 | 5 |
| <i>Ambloplites rupestris</i> | Northern Rockbass | 21 | 13 |
| <i>Cottus Spp.</i> | Sculpin Spp. | 25 | 16 |
| <i>Neogobius melanostomus</i> | Round Goby | 2 | 1 |
| <i>Culaea inconstans</i> | Brook Stickleback | 89 | 56 |

Appendix 5 – Complete list of benthic macroinvertebrate taxa collected and the number and proportion of the 160 sites at which each species was found.

| Taxa Name | # of Sites Present | % of Sites Present |
|-------------------------------|---------------------------|---------------------------|
| Amphipoda Gammaridae | 36 | 23 |
| Amphipoda Hyalellidae | 62 | 39 |
| Bivalvia Pisidiidae | 135 | 84 |
| Coleoptera Curculionidae | 7 | 4 |
| Coleoptera Dytiscidae | 31 | 19 |
| Coleoptera Elmidae | 146 | 91 |
| Coleoptera Gyrinidae | 2 | 1 |
| Coleoptera Haliplidae | 38 | 24 |
| Coleoptera Hydrophilidae | 18 | 11 |
| Coleoptera Psephenidae | 14 | 9 |
| Coleoptera Scirtidae | 1 | <1 |
| Collembola Isotomatidae | 8 | 5 |
| Decapoda | 16 | 10 |
| Diptera Athericidae | 1 | <1 |
| Diptera Ceratopogonidae | 126 | 79 |
| Diptera Chaorboridae | 1 | <1 |
| Diptera Chironomidae | 158 | 99 |
| Diptera Chironiminae | 137 | 86 |
| Diptera Diamesinae | 12 | 8 |
| Diptera Orthocladinae | 124 | 78 |
| Diptera Prodiamesinae | 10 | 6 |
| Diptera Tanypodinae | 136 | 85 |
| Diptera Tanytarsini | 105 | 66 |
| Diptera Dixidae | 5 | 3 |
| Diptera Empididae | 62 | 39 |
| Diptera Ephrydidae | 6 | 4 |
| Diptera Muscidae | 1 | <1 |
| Diptera Psychododae | 18 | 11 |
| Diptera Ptychopteridae | 2 | 1 |
| Diptera Simuliidae | 44 | 28 |
| Diptera Stratiomyiidae | 6 | 4 |
| Diptera Tabanidae | 44 | 28 |
| Diptera Tipulidae | 75 | 47 |
| Ephemeroptera Ephemeridae | 1 | <1 |
| Ephemeroptera Baetidae | 74 | 46 |
| Ephemeroptera Caenidae | 53 | 33 |
| Ephemeroptera Ephemerellidae | 18 | 11 |
| Ephemeroptera Heptageniidae | 49 | 31 |
| Ephemeroptera Leptohyphidae | 29 | 18 |
| Ephemeroptera Leptophlebiidae | 33 | 21 |
| Gastropoda Ancyliidae | 17 | 11 |
| Gastropoda Hydrobyiidae | 7 | 4 |
| Gastropoda Lymnaeidae | 33 | 21 |
| Gastropoda Physidae | 82 | 51 |
| Gastropoda Planorbidae | 42 | 26 |
| Gastropoda Valvatidae | 11 | 7 |
| Hemiptera Belostomatidae | 1 | <1 |
| Hemiptera Corixidae | 63 | 39 |
| Hemiptera Pleidae | 1 | <1 |

| Taxa Name | # of Sites Present | % of Sites Present |
|------------------------------|--------------------|--------------------|
| Hemiptera Veliidae | 5 | 3 |
| Hirudinea | 48 | 30 |
| Isopoda Assellidae | 75 | 47 |
| Lepidoptera | 7 | 4 |
| Megaloptera Corydalidae | 8 | 5 |
| Megaloptera Sialidae | 30 | 19 |
| Odanata Aeshnidae | 19 | 12 |
| Odanata Calopterygidae | 40 | 25 |
| Odanata Coenagrionidae | 33 | 21 |
| Odanata Cordulegasridae | 5 | 3 |
| Odanata Gomphidae | 2 | 1 |
| Odanata Libellulidae | 6 | 4 |
| Oligochaeta | 129 | 81 |
| Plecoptera Capniidae | 22 | 14 |
| Plecoptera Leutridae | 1 | <1 |
| Plecoptera Nemouridae | 6 | 4 |
| Plecoptera Perlidae | 3 | 2 |
| Plecoptera Perlodidae | 6 | 4 |
| Plecoptera Taeniopterygidae | 16 | 10 |
| Prostigmata Arrenuridae | 5 | 3 |
| Prostigmata Hydromidae | 1 | <1 |
| Prostigmata Hygrobatidae | 75 | 47 |
| Prostigmata Hygrophantidae | 5 | 3 |
| Prostigmata Lebertiidae | 91 | 57 |
| Prostigmata Limnesiidae | 1 | <1 |
| Prostigmata Mideopsidae | 6 | 4 |
| Prostigmata Oribatidae | 6 | 4 |
| Prostigmata Pionidae | 7 | 4 |
| Prostigmata Sperchonidae | 42 | 26 |
| Prostigmata Torrenticolidae | 8 | 5 |
| Prostigmata Unionicolidae | 15 | 9 |
| Trichoptera Brachycentridae | 3 | 2 |
| Trichoptera Dipseudopsidae | 1 | <1 |
| Trichoptera Glossosmatidae | 4 | 3 |
| Trichoptera Helicopsychidae | 12 | 8 |
| Trichoptera Hydropsychidae | 76 | 48 |
| Trichoptera Hydroptilidae | 6 | 4 |
| Trichoptera Lepidostomatidae | 2 | 1 |
| Trichoptera Leptoceridae | 47 | 29 |
| Trichoptera Limnephilidae | 60 | 38 |
| Trichoptera Molannidae | 4 | 3 |
| Trichoptera Philopotamidae | 16 | 10 |
| Trichoptera Phyganiidae | 28 | 18 |
| Trichoptera Polycentropidae | 11 | 7 |
| Trichoptera Psycomyiidae | 10 | 6 |
| Trichoptera Rhyacophilidae | 6 | 4 |
| Turbellaria | 39 | 24 |