

**Zoobenthic Community Composition and Chironomidae (Diptera) Mouthpart  
Deformities as Indicators of Sediment Contamination in the Lake Huron-Lake  
Erie Corridor of the Laurentian Great Lakes**

By

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## ABSTRACT

Zoobenthos are widely used indicators of ecological quality, integrating changes in habitat condition over time. This thesis investigated community composition and incidence of larval chironomid mouthpart deformities to assess benthic condition in the Lake Huron-Lake Erie Corridor.

To test the “Reference-Degraded Continuum” multivariate approach of zoobenthic community assessment, a series of analyses were used to identify two unique groupings of least-contaminated reference sites, each with characteristic relative abundances of zoobenthic genera and associated habitat features. Statistically significant negative relationships between biological condition and sediment contamination were found for each group. Indicator taxa were identified.

Six of 43 Chironomidae genera were assessed for mouthpart deformities. Overall incidence of deformities varied from 0.57% to 5.88% among zones. Only *Chironomus* exhibited significant among-zone variation, reflecting gross levels of sediment contamination.

The combined use of community and individual indicators was more diagnostic of benthic habitat quality than use of either approach alone.

**To my parents and parents-in-law,  
husband and son  
for their love and constant support**

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## **Chapter 1 General Introduction**

This research assesses the composition of the benthic invertebrate fauna and the condition of the sediments in which they dwell in the Lake Huron-Lake Erie Corridor of the Laurentian Great Lakes. The corridor consists of the St. Clair River, Lake St. Clair and the Detroit River. It contains two Areas of Concern (AOCs) as designated by the International Joint Commission (IJC). The St. Clair River AOC includes the main river, its delta channels and coastal watersheds in both the U. S. and Canada. The Detroit River AOC includes the Detroit River and its watersheds (Government Canada (GC) 2003). Since the corridor is a crucial part of the Great Lakes, its environmental (water, sediment and biota) quality is especially important. Knowledge of the benthic fauna and their response to the toxic chemical contaminants in the sediments is consequently of great value (Great Lakes Institute (GLI), University of Windsor 1982). The long-term value of this study is in linking the two Remedial Action Plan (RAP) programs within the Lake Huron-Lake Erie Corridor, providing an integrated framework by which to identify the spatial scale and specific locations at which degradation occurs. This represents a key element needed to plan remediation strategies that will ultimately permit delisting of sediment contamination and zoobenthic beneficial use impairments.

### **Areas of Concern (AOCs), Remedial Action Plans (RAPs) and Beneficial Use Impairments (BUIs) of the Great Lakes**

The Laurentian Great Lakes of North America and their connecting channels are a unique natural resource, containing about 84 percent of North America's surface freshwater and about 21 percent of the world's supply. More than 30 million people live

in the Great Lakes Basin currently (URL <http://epa.gov/grtlakes/basicinfo.html>). The Great Lakes basin has been home to indigenous peoples for thousands of years (Cornell 2003), and has been threatened by toxic inputs from human activities along its shores for hundreds of years (Hartig 2003). To protect this valuable resource, the U.S. and Canadian governments interacted through an agency known as the International Joint Commission (IJC) and signed the Great Lakes Water Quality Agreement (GLWQA) in 1972 and renewed it in 1978. This document coined the term “area of concern (AOC)” to describe any Great Lakes location whose environmental condition was deemed to unacceptable to the populace. An AOC is “a geographic area in the Great Lakes that fails to meet the General and Specific Objectives of the Agreement where such failure has caused or is likely to cause impairment of beneficial use or of the area’s ability to support aquatic life”. There are currently 41 AOCs (GC 2003). A Beneficial Use Impairment (BUI) was defined as a change in the environment sufficient to cause measurable negative impacts to one or more of 14 environmental and economic attributes listed by IJC (Table 1.1). Creation of a “Remedial Action Plan (RAP)” was recommended for each AOC by the IJC in 1987 to serve as an important step toward virtual elimination of persistent toxic substances and toward restoring and protecting the impaired beneficial uses.

One of the most widespread BUIs is “degradation of benthos”, which occurs when “benthic community composition exhibits attributes that would characterize a degraded community“. Attributes of a degraded community include:

- a) An indicator species characteristic of degraded environmental conditions is dominant;
- b) A keystone species expected in a specific habitat is absent or has been replaced by an invading species;

- c) Taxa designated as ecosystem objectives for a specific zone have not attained the recommended density, biomass, or productivity;
- d) The composite (multimetric) biotic score determined for the area does not fall within a range previously designated as indicative of unimpaired quality;
- e) A suite of species (multivariate assemblage) collected from the area is very different (statistically significant different,  $p < 0.01$ ) from the assemblage of species expected to be found in reference areas with the same physical environmental characteristics;
- f) The taxa richness per unit of benthic density is below that expected of a particular environment (Detroit River Canadian Cleanup (DRCC) 2006).

#### **Study Area and Contaminant Inputs**

The Lake Huron-Lake Erie Corridor is a 143 km long connecting waterway that links lakes Huron and Erie. Water takes 7 -9 days to flow from Lake Huron to Lake Erie in the main channel (Hudson et al. 1986). It is an important transportation route - millions of tons of commercial shipping transit the corridor annually (Muth et al. 1986); it supports a rich and diverse community ranging from sediment-dwelling zoobenthos to valuable sport fish species, and it is also a spawning and nursery ground for fish populations in lakes Huron and Erie (Muth et al. 1986). The Lake Huron-Lake Erie Corridor is a freshwater resource, a source of food for aboriginal Canadians and water for industries and human consumption (Upper Great Lake Connecting Channels Study (UGLCCS) 1988a). The sediment and water quality of the corridor greatly affect the ecosystem of downstream Lake Erie, since contributes 93% of Lake Erie's source water (Panek et al. 2003, Oliver and Bourbonniere 1985).

The corridor is greatly affected by anthropogenic stresses. Major contaminant inputs to the corridor are petrochemicals and diverse industrial chemicals, sewage and pesticides (GLI, University of Windsor 1982; Hudson et al. 1986; Hudson and Ciborowski 1996a). Long-term activities of large petrochemical complexes adjacent to the Upper St. Clair River near Sarnia, Ontario have contributed diverse organic pollutants, including octachlorostyrene (OCS), perchloroethylene (perc), hexachloroethane (HCE), hexachlorobutadiene (HCBd), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), pentachlorobenzene (PCB) and polycyclic aromatic hydrocarbons (PAHs), etc. (Environment Canada (EC) and Ontario Ministry of the Environment (OMOE) 1986). Mercury and lead have been the metals of most concern in St. Clair River (UGLCCS 1988a).

The Walpole Delta First Nation Reserve is a part of a large freshwater delta complex known as St. Clair Flat, located at the northeastern portion of Lake St. Clair (Cumming 1995). The Walpole Delta is part of the St. Clair River AOC (GC 2003). Since it is downstream of Sarnia, and about 47% of the St. Clair River water enters Lake St. Clair by the channels around it (Leach 1991), there is considerable evidence that water flowing through the Walpole delta plays an important role in transporting contaminants in the corridor. However, there has been limited research in this area.

Lake St. Clair is shallow and productive (Leach 1991). It serves as a sediment "filtration" system. Coarse sediment is deposited in the St. Clair delta, whereas most fine-grained materials are transported directly to the Detroit River and Lake Erie (UGLCCS 1988b). Although it is not designated as an AOC, Lake St. Clair is potentially affected by the St. Clair River (Oliver and Bourbonniere 1985; Leach 1991), because about 98% of the lake's water is contributed by the St. Clair River (Leach 1991). The highest

contaminant concentrations are found near the centre of the lake in the area of greatest water depth and fine-grained sediments (UGLCCS 1988b). Sediment-associated organic contaminants such as HCB, OCS, HCBd and QCB originated mainly from industrial activities in Sarnia (Oliver and Bourbonniere 1985; Leach 1991). Several trace metals exceed the Ministry of Environment Ontario (MOE) and U.S. Environmental Protection Agent (US EPA) dredging guidelines in the area near the Cut-off channel on the U. S. side of the lake. Among these trace metals, cadmium concentrations were the highest observed in Lake St. Clair (UGLCCS 1988b). Overall condition of Lake St. Clair appears good. However, with a large industry complex upstream and a growing population on the shoreline, the lake is subject to continuing anthropogenic stresses (Leach 1991).

The vicinity of Detroit - Windsor is one of the most industrialized areas in the world (Hartig and Stafford 2003). Trace elements in the Detroit River, such as mercury, arsenic, cadmium, chromium, copper, lead, manganese and zinc, and organic pollutants such as PCBs and solvent extractables (oil and grease) all exceed the dredging guideline for open water disposal (Thornley and Hamdy 1984; Hudson et al. 1986; Szalinska et al. 2006) in at least some locations. The lower section of the Detroit River on the U.S. shore (i.e., Trenton Channel) is the most severely polluted area in the whole corridor because of its habitat characteristics (Hudson et al. 1986). Sediment-associated contaminants include trace metals, organochlorine pesticides, PCBs and PAHs (Hudson et al. 1986; Besser et al. 1996; Drouillard et al. 2006; Szalinska et al. 2006). The persistent and bioaccumulative nature of mercury and PCBs make them toxic chemicals of especial concern. They were among the first contaminants to be reported in the Detroit River, and are good examples of the problems associated with the unmonitored release of toxic chemicals into ecosystems (Read et al. 2003).

### **Using Zoobenthos Distribution to Assess Local Conditions**

An indicator is *“a piece of evidence or signal that tells us something about the conditions around us. It is a tool that gives a clue about the “bigger picture” by looking at a small piece of the puzzle, or at several pieces together* (EC and US EPA 1999)”. In ecology, “bioindicators” become important tools for the assessment and monitoring of the effects of anthropogenic stresses to the ecosystem (Danz et al. 2005). Sediment toxicity is best evaluated by assessing the responses of biota differing in sensitivity to contaminants (Thornley 1985). Taxa that have been used to develop bioindicators of stress include zooplankton (Barbiero 2001; Sampaio 2002), aquatic plants (Hudson et al. 1986), fishes (Baghat 2005; Danz et al. 2005) and zoobenthos (Krieger 1984; Ciborowski et al. 1995; Kilgour et al. 2000).

Zoobenthos (bottom-dwelling invertebrates) are especially suitable biomonitors because they are relatively immobile, tend to spend most of their lives within a limited area, and are easy to capture (Ciborowski 2003). They therefore can better reflect sediment conditions where they were collected making them easier to monitor than most other organisms (Ciborowski and Corkum 1988; Reynoldson and Zarull 1989; Reynoldson et al. 1989; Covich et al. 1999; Zimmer et al. 2000). Their direct association with contaminants in sediments has made them especially popular as biological indicators of local sediment quality (Thornley and Hamdy 1984; Oliver 1984; Hudson et al. 1986; Ciborowski and Corkum 1988; Farara and Burt 1993; Canfield 1998; Frondorf 2001; Carter et al. 2006). The degradation of zoobenthos is recognized as one of the “BUI” by IJC in the corridor system.

If environmentally sensitive zoobenthos are absent or occur only in low densities, or the community is dominated by certain pollution-tolerant species, this area is possibly

contaminated by toxic chemicals (DRCC 1999). In areas of Lake Erie with good water quality and sediment conditions, one expects to find 100 per m<sup>2</sup> or more *Hexagenia* mayfly larvae in depositional zones (slow-flowing areas with soft substrates) (Ciborowski 2003). However, *Hexagenia* density of '20 per m<sup>2</sup> in depositional regions implies that anoxic or toxic conditions may sporadically occur due to organic pollution (Ciborowski 2003). *Hexagenia* larvae are acutely sensitive to anoxia and will die when the dissolved oxygen is less than 1 mg/L for more than 24 h (Winter et al. 1996). Depositional communities characterized by very high densities of oligochaete worms 3,000 per m<sup>2</sup>) and Chironomidae midge larvae, and a low diversity of zoobenthos should be considered degraded due to organic enrichment. Very low densities of worms and all other benthic genera in severely polluted site may indicate that metals and chemicals are sources of toxicity in the sediments (Ciborowski 2003). The shift from a community dominated by Chironomidae midge larvae to oligochaete worms is one of the first signs of eutrophication (Saether 1979). Davis et al. (1991) and Thornley (1985) advocated using caddisfly larvae (Trichoptera) as clean-water bioindicators because their abundance often declines in areas of poor water and sediment quality. Davis et al. (1991) suggested that communities associated with high flow and coarse substrates (erosional areas) may be less vulnerable to oil pollution while in slower-flow depositional areas, the oil was mixed into sediment and eliminated the caddisflies.

Benthic surveys of the corridor have been undertaken every 5-10 y since the mid 1950s, documenting the extent and degree of degradation of bottom sediments (Hiltunen and Manny 1982; Thornley and Hamdy 1984; Hudson et al. 1986; EC and OMOE 1979, 1986; Farara and Burt 1993 and Wood 2004). The condition of the zoobenthic communities in the St. Clair River was assessed in 1968, 1977 (EC and OMOE 1979) and

1985 (EC and OMOE 1986). The 1968 survey indicated that the benthic community was impaired on the Ontario side of the river, downstream from the petrochemical complex. The results of the 1977 and 1985 surveys showed that the condition of the benthic community had significantly improved, but was still impaired in the immediate area of the petrochemical industry.

The benthic community of Lake St. Clair was assessed in 1977 (Hiltunen and Manny 1982), 1983 (Hudson et al. 1986) and 1991 (Leach 1991). The high diversity of macrozoobenthos, together with a moderate abundance of oligochaetes, indicated that quality of the benthic environment was high throughout Lake St. Clair.

In 1968, the bottom fauna over large tracts of the Detroit River suggested that sediments and water quality were degraded. Mayflies were found in only about 25 percent of the locations sampled and in low numbers (10-20/m<sup>2</sup>; Thornley and Hamdy 1984); Immediately downstream of the confluence of the Rouge and Detroit Rivers, pollution tolerant worms numbered over 1000,000 per m<sup>2</sup> in both 1968 and 1980 surveys, indicating long-term, severe, organic enrichment in the Detroit River (Thornley and Hamdy 1984). Few changes in either the distribution or abundance of mayfly nymphs were seen between the 1980 survey, the 1983 survey (Hudson et al. 1986) and a study done in 1991 (Farara and Burt 1993).

Since degraded benthos is one of the BUIs listed in the corridor AOCs, improvement in the benthic community can be used to assess the progress of RAPs and the future delisting assessments. The analysis of biological communities is a necessary part of the total evaluation of a freshwater system (Saether 1979; Canfield 1998; Carter et al. 2006).



### **Zoobenthos and contaminants**

Toxic effects of anthropogenic compounds may influence survival and produce detectable changes in community composition or eradication of the benthic community as described above (IJC 1987; Ciborowski 2003). However, effects may be sublethal, reducing the fitness of individuals and/or eliciting teratogenic or mutagenic effects (Hudson 1994). Zoobenthos that live in or on moderately contaminated sediments can bioaccumulate the compounds. Some species (e.g., Chironomidae (Diptera)) can break down and metabolize organic chemicals, and exhibit significantly elevated incidences of deformities (IJC 1987; Ciborowski et al. 1995; Ciborowski 2003). The expression of morphological deformities of chironomids is believed to be an important bioindicator for detecting and assessing the nature, extent, and significance of toxic chemicals in aquatic ecosystem (Saether 1979; Warwick and Tisdale 1988; Warwick 1988, 1989, 1990a; Hudson and Ciborowski 1996a, b; Burt et al. 2003).

### **Thesis Objectives**

My thesis comprised two topics related to zoobenthic status in the Huron-Erie corridor. Firstly, I used a multivariate statistical analytical approach to describe the zoobenthic community attributes most characteristic of corridor locations in which sediment and water quality have been least affected (“reference”) and most affected (“degraded”) by trace metals, pesticides, and organic chemicals. I then derived biological indicator scales that permit one to assess the full range of conditions of the Lake Huron-Lake Erie Corridor aquatic ecosystem. Secondly, I documented the distribution of genera of Chironomidae (Diptera) and used the incidence of mouthpart deformities to assess the degree of environmental degradation (heterogeneity in the incidence of deformities

among sites). This study comprised part of a larger project undertaken in collaboration with Dr. G. Douglas Haffner and Dr. Ken G. Drouillard (Great Lakes Institute for Environmental Research, University of Windsor), funded by Ontario Ministry of the Environment, Environment Canada and the Great Lakes Sustainability Fund. The overall project objective was to investigate environmental changes to the Lake Huron-Lake Erie Corridor ecosystem as a result of the anthropogenic stresses (discharge of persistent organic contaminants and trace metals into waters).

In Chapter 2, zoobenthic samples collected during 3 studies from a total of 311 sites in the Detroit River in 1991 (Farara and Burt 1993) and 1999 (Wood 2004) and throughout the Lake Huron-Lake Erie Corridor in 2004/5 were amalgamated into one dataset to document changes in the benthic condition of the Lake Huron-Lake Erie corridor, including the Walpole delta. By using principal component analysis (PCA) of contaminant concentrations in sediments to identify a suite of stressor variables, each site within the dataset was assigned a score based on a “Sum of relative maximum (SumRel)” stress by which the “reference” sites and the “degraded” sites were identified. Zoobenthic assemblage data and a suite of environmental variables were then used to assess the quality of these sites along the contaminant gradient and to develop zoobenthic community indicators. This entailed using cluster analysis, discriminant function analysis (DFA) and ordination analysis. My expectation from the cluster analysis was that different groups of reference sites could be clearly separated based on their biological assemblages; I expected the DFA to show that key environmental variables controlling zoobenthic communities in rivers such as near-bottom water velocity (Rae 1985; Ciborowski 2003), substrate type or grain size (McLachlan and Cantrell 1976; Reynoldson and Zarull 1989; Kilgour et al. 2000), water depth (Kilgour et al. 2000) etc.

could separate groups of sites and consequently, zoobenthic communities. By performing Bray-Curtis ordination of zoobenthic composition (Gauch 1982) using the 'best' (sites with lowest SumRel) and 'most degraded' (sites with highest SumRel) sites as end points, I expected to define the bioindicator communities of reference and degraded sites respectively. Position of any other site along the gradient (based on zoobenthic composition) would define their relative environmental quality.

In Chapter 3, the distribution of Chironomidae genera was observed in 12 zones within the corridor in 2004/5. Genera that were widespread enough to assess for mentum deformities were selected. The incidence of mentum deformities of these selected genera was compared with the baseline levels from previous studies by using the replicated G-statistic Goodness of Fit test (Hudson and Ciborowski 1996a; Burt et al. 2003). My expectation was that significant spatial and taxonomic variation would be identified in the incidence of mentum deformities in this study. The zones with significant elevated incidence of deformities could be considered degraded by anthropogenic stresses.

The final chapter summarizes the results of the studies described above, identified problems associated with the use of these bioindicators, and recommended changes in methodology. Finally, possible directions for future research were generally discussed.

Since the corridor is a crucial part of the Great Lakes containing two AOCs, its environmental (water, sediment and biota) quality is especially important. Knowledge of the zoobenthos at the organism level and community level, which by many are considered to be especially good indicators of water and sediment quality, and their response to the toxic chemical contaminants in the sediments is consequently of great value (GLI, University of Windsor 1982).

Table 1.1. Beneficial Use Impairment (BUI) outlined by the International Joint Commission and status in the Lake Huron-Lake Erie Corridor in January, 2003

BUI	Description	Status in the corridor	
		St. Clair River AOC	Detroit River AOC
1	Restrictions on fish and wildlife consumption	Y	Y
2	Tainting of fish and wildlife flavor		Y
3	Degradation of fish and wildlife populations	?	?
4	Fish tumors or other deformities		Y
5	Bird or animal deformities or reproductive problems		?
6	Degradation of benthos	Y	Y
7	Restrictions on dredging activities	Y	Y
8	Restrictions on undesirable algae		
9	Restrictions on drinking water consumption, or taste and odor problems		Y
10	Beach closing	Y	Y
11	Degradation of aesthetics	Y	Y
12	Added costs to agriculture or industry		
13	Degradation of phytoplankton and zooplankton populations		
14	Loss of fish and wildlife habitat	Y	Y

Y: Impaired

?: Require further assessment

Blank: Not impaired

Adapted from Canada's PAP progress report 2003 (Government of Canada)

## **Chapter 2**

**A multivariate approach to develop zoobenthic community indicators of  
sediment contamination and assess environmental degradation in  
the Lake Huron - Lake Erie Corridor**

## 2.1 Summary

Zoobenthic community composition has been widely used as an indicator of sediment contamination in aquatic systems. Zoobenthic data collected from 311 Lake Huron-Lake Erie Corridor sites in 1991, 1999 or 2004/5 were analyzed by using a “Reference-Degraded Continuum (RDC)” multivariate approach. Principal component analysis (PCA) of the sites’ sediment chemical attributes (16 variables representing trace elements, PCBs, hydrophobic pesticides and other organochlorine compounds) identified 4 independent groups of contaminants. Each of the 4 principal components was converted to a 0.0-1.0 scale, and the scores for each site were summed to provide a “SumRel” measure of sediment quality. The 62 least-disturbed (lowest degree of sediment contamination) sites were designated “reference” and the 62 most-disturbed sites (highest concentrations of sediment-associated contaminants) were designated “degraded”.

Cluster analysis identified two groups of reference sites based on relative abundances of 15 zoobenthic taxa. One cluster was dominated by biota with adaptations typical of soft-substrate depositional conditions (Chironomidae, Ephemeroptera (*Hexagenia*, *Caenis*), Nematoda, and Acari). The other cluster contained taxa more typical of hard-substrate or erosional environmental conditions (Amphipoda, *Dreissena*, net-spinning Trichoptera, Chironomidae, and Hydrozoa). A discriminant function analysis (DFA) model distinguished between the sites at which these 2 biologically distinct cluster groups occurred on the basis of sediment median particle size, water depth, and dissolved oxygen concentration. The DFA function was applied to data from each of the 311 sites to predict the type of zoobenthic community expected, given the local environmental conditions at the time of collection. Two

hundred and fifty-five of the sites were predicted to have 'soft substrate group' taxa whereas 56 of the sites were expected to have 'hard substrate group' taxa.

Bray-Curtis ordination with subjective end-point selection was used to assess variation in zoobenthic community composition with respect to the sediment contamination scores. For each cluster two end points, representing the extremes of sediment contamination were defined. The endpoint benthic assemblage of taxa representing the least contaminated end and most contaminated end of the gradient were created by determining the centroid (mean relative abundance of each taxon) of the 4-5 sites with the lowest and highest SumRel scores, respectively. The relative position of a site along this gradient defined its biological quality, identified by a 'zoobenthic condition index (ZCI)' score ranging from 0.0 (the "most contaminated" endpoint) to 1.0 (the theoretical "best achievable" score).

Quantile regression was then used to determine the relationship between the median, 10<sup>th</sup> and 90<sup>th</sup> quantiles of ZCI score and sediment contamination score (SumRel score) for each of the two cluster groups. Statistically significant negative relationships between the zoobenthic community composition and sediment contamination scale for both clusters were found. Oligochaeta dominated the fauna of both depositional and erosional degraded sites. However, the ZCI score for sites in depositional cluster was only weakly correlated with the sediment contamination gradient.

A "Detroit River case study" was performed to test (and confirm) that the inclusion of near-bottom water velocity in the DFA model could give better classification by defining three zoobenthic assemblages communities, especially in (depositional-erosional) mixing zones.

By including assessment of the degraded condition in addition to reference condition sites, the RDC multivariate approach used in this study improves upon existing multivariate techniques and provides an alternative way to assess aquatic environmental condition by using zoobenthic community composition as indicators.



## 2.2 Introduction

Sediments play a dominant role in aquatic ecosystems by providing habitats for benthic invertebrate organisms. They trap and hold nutrients and detritus that drive food web (Crane et al. 2000). However, they also harbour hydrophobic contaminants, which become bound to organic material and fine mineral particles and persist long after point sources of pollution have been reduced or disappeared (Oliver and Bourbonniere 1985; Reynoldson and Zarull 1989; DRCCC 1999; Crane et al. 2000). Sediments also act as a contaminant “source”, because contaminants in sediments are continually changing in response to abiotic and biotic conditions and sometimes can be released back into the water and move through the food web (Malins and Ostrander 1991; DRCCC 1999). Contaminated sediments have been found in almost all water bodies in the world, including the Laurentian Great Lakes in North America. Sediment quality is a major concern in the Great Lakes, since it has long been adversely affected by anthropogenic sources such as industry, agriculture, urbanization, and other human activities (Krieger 1984; Oliver 1985; Reynoldson et al. 1989; EC and EPA 1999; Hartig 2003; GC 2003; Bhagat 2005).

The chemical approach used to assess sediment quality by many environmental scientists in early years has been criticized because some toxic chemicals could not be readily detected with existing analytical techniques (Chapman and Long 1983), and determination the concentrations of various chemicals present in the sediments *per se*, although sensitive and accurate, provide limited evidence of the biological effects of the anthropogenic pollutants, or do not reflect the actual ecological state (Long and Chapman 1985; Reynoldson and Zarull 1989; Warwick 1991; Reynoldson et al. 1995; Adams 2002; Adams et al. 2002; Simboursa and Zenetos 2002). In some aquatic environmental studies, only biological factors and habitat variables were analyzed to

determine whether habitat characteristics control patterns of community composition (Green and Vascotto 1978; Kilgour 2000). However, simply plotting distributions of taxa and environmental variables in a large data matrix and looking for patterns may not effectively predict environmental condition overall (Green and Vascotto 1978).

Many approaches have been developed to assess aquatic conditions relative to anthropogenic disturbances. Combined analysis of physical, chemical and biological data is necessary to link cause (habitat characteristics, sediment contaminants) and their effects (condition of biological communities), and to provide an accurate and integrated ecological assessment of aquatic ecosystem conditions (Diggins and Stewart 1998; Turak, et al. 1999; Adriaenssens et al. 2007). As sediment dwelling organisms, zoobenthos were widely investigated as one type of the biological factor, and they can integrate changes in environmental conditions over time (Adriaenssens et al. 2007). The statistical analytical methods that assess benthic invertebrate distribution and abundance as an indicator of habitat degradation have been a continuing focus of research (Thornley and Hamdy 1984; Hudson et al. 1986; Warwick 1991; Farara and Burt 1993; Death 1995; Kilgour 2000; Carter et al. 2006).

Besser et al. (1996) used the “Sediment Quality Triad” (SQT) approach of Chapman and Long (1983) to assess sediment contamination in the Trenton Channel of the Detroit River. This approach uses a combination of sediment chemistry (contamination), toxicity of environmental samples (laboratory bioassays) and zoobenthic species composition and densities of the resident biota to define and bound the extent of sediment contamination. The approach demonstrated a linkage between levels of contaminants and community response, and provided an understandable method for the assessment of polluted areas in water ecosystems (Reynoldson and Zarull 1989). However, this method did not take into account natural habitat variation,

which is considered to be the major factor to which the biota respond (Covich et al. 1999).

Multivariate analysis is an important statistical tool in community ecology since many ecological problems involve numerous variables and numerous samples, and the purpose of multivariate analysis is to integrate these data, summarizing the variables, removing redundancy in correlated variables, and revealing the underlying structures (Gauch 1982). In recent decades, multivariate approaches to developing bioindicators of anthropogenic stress and assessing the degree of disturbance at test locations have been widely used by many researchers (Reynoldson et al. 1997). Although the term 'multivariate analysis' refers to a host of techniques used to interpret many variables simultaneously (Gauch 1982), in the literature of pollution ecology, it is used to distinguish analyses that employ formal multivariate statistical methods from procedures collectively referred to as a "multimetric approach" (Reynoldson et al. 1997). The multimetric approach involves defining a series of measures thought to represent 'biotic integrity' (each measure termed a 'metric'), and adding the scores of each metric to form a composite (multimetric) index.

The fundamental feature of this approach is to use sites representing the 'reference condition' as a "control" against which test-site conditions are compared (Reynoldson et al. 1997). The reference condition is represented by a group of least disturbed sites organized by selected physical, chemical and biological characteristics (Reynoldson et al. 1997). Sites that are evaluated for similarity with the reference condition were defined as test sites. The reference sites are evaluated to determine whether they are biologically homogeneous or whether they can be grouped into distinct assemblages. When distinct assemblages occur, the characteristic biological communities at reference sites are each related to a set of habitat attributes that

typically determine community composition and are known to be little affected by most human activities at the sampling sites (e.g., longitude, latitude, water depth, bottom flow velocity and substrate type (Norris 1995)). By using multivariate classification techniques, the reference sites are classified into groups based on uniformity of these habitat attributes. New sites whose conditions are to be evaluated (test sites) are then each matched with the reference sites with which they share the most similar habitat attributes. The taxa that should occur at an individual site are predicted from the biological community previously found to be characteristic of the corresponding reference sites. By knowing what should be the original biological community at a river site, one can assess the degree to which human activities have altered that community based on presence and absence of these indicator taxa (Norris 1995).

Multivariate approaches are being increasingly used to empirically determine the associations among biological community composition, the habitat attributes to support particular community and various anthropogenic stresses (Green and Vascotto 1978; Reynoldson et al. 1995; Besser et al. 1996; Reynoldson et al. 1997; Bhagat 2005). Turak et al. (1999) using multivariate analyses determined that the use of environmental attributes to predict zoobenthic assemblages has potential as a method for detecting natural and anthropogenic disturbances to the ecological condition of rivers, even over a large spatial scale. Reynoldson et al. (1995) introduced a multivariate application of the reference condition method called the BEAST (Benthic Assessment of Sediment) to analyze benthic data in the Laurentian Great Lakes. They used the model to assess the zoobenthic assemblages of Collingwood Harbour, an Area of Concern designated by IJC, relative to reference sites. This study provided a relevant and realistic method for determining environmental impact and defining

ecological targets (Reynoldson et al. 1995). The multivariate approach is thought likely to be “the best technique for determining the impact of stress on compositional variability within a community” (Adams 2002). However, this approach still has opponents. The drawback to this approach is that it is said to be more complex than other methods, and is difficult to convey to managers and the general public (Barbour et al. 1996). With the development of new statistical software, the complexities of initial model construction may be hidden (Reynoldson et al. 1997).

To date, the multivariate methods used to assess zoobenthic condition in aquatic systems define the reference condition only. Some models classify communities by the presence/absence of species (Norris 1995; Carlisle and Meador 2007; Hargett et al. 2007), whereas others use densities of each taxon (Reynoldson et al. 1995). The reference condition methods have several limitations (Ciborowski et al. 2003; Bhagat 2005). First, the classification of test sites is limited to a binary designation – either ‘equivalent to reference’ or ‘different than reference’. Secondly, there is no comparative basis for assessing the relative condition of a test site that falls outside the range of reference conditions (i.e., “is a ‘different than reference’ site slightly degraded or severely degraded?”). Thirdly, these methods do not define different gradients of stress within a study area. Furthermore, the bimodal nature of presence/absence data has the potential problem to consider the “accidental occurrence” as “presence”, possibly making it hard to show clear effects of different types of stressors on the community composition as a whole. The absolute abundance (density) data may be of limited value in assessing zoobenthic community response to habitat disturbance when there are very large differences in overall abundance among samples that may be due to factors extraneous to the gradient of interest (e.g., weather

conditions on the day of sampling; time elapsed since a flood; variable efficiency of a sampler).

To address these limitations, Ciborowski et al. (2003) recommended a modified multivariate assessment approach called “Reference-Degraded Continuum” (RDC). In addition to defining the reference condition, this method also defined the complementary extreme, termed the “degraded condition” (agreed by consensus or other means to represent the most degraded or undesirable sites in a system). By using ordination techniques, all sites with similar habitat characteristics and zoobenthic community were bounded by a “best environmental conditions endpoint” at one end of an environmental condition scale and by a “most degraded endpoint” at the other end of the scale. The relative biological condition of all sites along the reference-degraded gradient can be evaluated by this method (Ciborowski et al. 2003; Bhagat 2005). This method emphasized the attributes of biological variables characteristic of the reference and degraded conditions rather than just reference conditions, which can be used in a practical manner to assist in management decisions. Bhagat (2005) used relative abundance of fishes rather than density (catch per unit effort) to identify characteristic communities, and identified species assemblages that reflected natural habitat attributes among reference and degraded conditions at Great Lakes coastal margins. In this paper, we use the “Reference-Degraded Continuum” multivariate approach to develop zoobenthic community indicators and assess the environmental quality of a Great Lakes connecting channel, the Lake Huron-Lake Erie Corridor aquatic ecosystem.

The goals of this study were to

- 1) Use the distribution of 3 classes of sediment contaminants (trace elements, hydrophobic pesticides and other organochlorine chemicals) to classify

sites along a gradient ranging from least contaminated (reference condition) to most contaminated (putatively degraded condition);

- 2) use zoobenthic community composition observed at reference sites to guide the grouping of other sites;
- 3) determine the habitat attributes along which distinct zoobenthic assemblages of reference conditions are segregated;
- 4) establish zoobenthic community composition criteria for assessing the quality of sites (degree of sediment contamination) within a group;
- 5) Identify zoobenthic assemblages that best serve as “indicators” of the reference end and degraded end of the anthropogenic contamination gradient within the Lake Huron-Lake Erie Corridor.

## **2.3 Methods**

### **Study Area and Site Selection**

The Lake Huron-Lake Erie Corridor was partitioned into three zones: St. Clair River, Lake St. Clair (include St. Clair Delta) and Detroit River (Figure 2.1). Sampling site locations were assigned prior to the survey implementation using a stratified random design (Szalinska et al. 2006). Collections were made at 100 sites throughout the corridor (except the Walpole Delta) during July-August, 2004; an additional 13 sites were sampled in the Walpole Delta in August 2005 (Figure 2.2) based on the same design to create an integrated database of the corridor. Twenty locations were sampled from the St. Clair River zone, which consisted of the upper and middle portion of the river. Thirty sites in open waters of Lake St. Clair, and 43 St. Clair Delta sites (30 sites in 2004 and 13 sites in 2005) were sampled. The Delta sites included locations in the downstream portion of the St. Clair River, the Chenal Ecarte,

Chematogan Channel, the South Channel, the North Channel and the Middle Channel. The Detroit River zone (20 sampling sites) encompassed the entire Detroit River, from its mixing zone with Lake St. Clair downstream to the Detroit River/Western Lake Erie mixing zone (GLSF 2005).

To provide an estimate of temporal variability, we also compiled and incorporated data from two previous studies (Farara and Burt 1993; Wood 2004). Both of these Detroit River surveys used field protocols identical to the 2004 Lake Huron-Lake Erie Corridor survey. Information from three separate benthic surveys was combined both to provide a larger sample size and to provide sufficient information for the classification and interpretation of the biological conditions in the corridor.

#### **Field Sampling Procedure**

All sites were sampled from an anchored boat; sampling sites were located by differential Global Positioning System (GPS) to ensure consistency with pre-determined coordinates.

#### **Habitat Attributes**

At each sampling site, a suite of habitat attributes was recorded. The location of a site (longitude and latitude) was recorded based on the GPS reading. Water temperature ( $^{\circ}\text{C}$ ), conductivity ( $\mu\text{S}/\text{cm}$ ), dissolved oxygen saturation (%) and dissolved oxygen concentration ( $\text{mg}/\text{L}$ ) were measured by using a Hydrolab multimeter (0.5 m from sediment/water interface); the pH of water at the surface was measured using a portable electronic pH meter. Water velocity 0.5 meter below the surface was measured with an Ott C-3 portable current meter. Water depth, precise to the nearest 0.1 m, was also measured in the field from the length of the Ponar rope.



Sediment characteristics, including sediment type and odor were recorded when each Ponar grab sample was collected, as was sediment pH. A visual description of land use on the adjacent shoreline was made.

All field notes for all sites were archived. All field data can be assessed via the Lake Huron-Lake Erie Corridor Survey Database, which was specifically designed to contain the corridor data from 2004/5 survey (A. Kirkpatrick, University of Windsor, unpubl.; data are available on request from either J.J.H. Ciborowski or J. Zhang, University of Windsor)).

#### Zoobenthic Samples

Both zoobenthos and sediment samples were collected with a Petite Ponar grab sampler (Wildco Co., 15 x 15 cm<sup>2</sup> surface area). Grab sample fullness was recorded; a grab had to be at least 50% full of sediment to be acceptable for a zoobenthic sample. Three zoobenthic samples were collected per site. Samples were sieved in the field with a 250-µm mesh sieve bucket to remove fine materials. The contents remaining in the sieve bucket were emptied into a labeled plastic bag and were preserved in buffered formal-ethanol solution (5:2 v/v 95% ethanol: phosphate-buffered 100% formalin, diluted 1:1 with water in the field [note: 37% formaldehyde solution = 100% formalin solution]).

#### Sediment Sampling

Multiple grab samples were retrieved at a given site until a total volume of 2 L sediments was collected. The effort per sample (i.e., the number of grabs required to collect 2 L of sediments) was recorded for each sampling site. Sediment samples for organic analysis were preserved in hexane-rinsed glass containers. Sediment samples

for metals analysis were placed in clean, acid-rinsed plastic bags. All sediment samples were stored frozen.

### **Laboratory Procedures**

#### **Zoobenthic Sample Processing**

One replicate of zoobenthic samples from each sampling site was randomly selected and processed; the remaining two replicates were archived. Sample processing and sorting/identification methodology followed the “St. Clair-Detroit River and Lake Erie Projects sorting protocol (J.J.H. Ciborowski, University of Windsor, unpubl.)” and Ciborowski (1991). Zoobenthic samples were poured off into a stacked series of sieves (4 mm, 1 mm, 0.50 mm and 0.25 mm). Each size fraction of the sample was elutriated to separate the less dense detritus and animals from the inorganic sediments. Each portion was then transferred to a Petri plate. Zoobenthos were sorted from the debris of each size fraction under a dissecting microscope, identified to the lowest taxonomic rank possible using available keys (Wiggins 1996 (Trichoptera); Merritt and Cummins 2000 (other insects); Peckarsky et al. 1999 (noninsect zoobenthos)). As required (Chironomidae), slide mounts were made when identification required examination under a compound microscope (see chapter 3). Zoobenthos were then stored in 70% ethanol in labeled glass vials and archived at the University of Windsor.

Subsampling was used when large numbers of organisms or large quantities of detritus occurred in particular sieve-size fractions of a sample (Ciborowski 1991).

## Quality Control and Assurance

Ten samples were randomly selected for resorting to ensure sorting quality. This was completed immediately after the initial sorting. Sorting efficiency (proportion of total number of animals recovered during initial sorting) was 91% for one sample and 96% or greater for the remaining samples (Table 2.1).

## Sediment sample Processing

In the laboratory, sediment samples were thoroughly mixed to ensure homogeneity, and then split into portions for median particle size analysis, total organic carbon (TOC) content, organic contaminant analysis and metal analysis. Sediment designated for TOC, organic contaminants and metals was passed through a brass sieve to ensure a grain size of less than 2 mm, and then frozen until submitted for analysis. Chemical analyses and quality assurance were performed by collaborators in the Great Lakes Institute for Environmental Research (GLIER), University of Windsor.

The particle size distribution analysis was performed using a standard sieving method that involves passing the dried sediment through a graded series of sieves (4.00, 2.00, 1.00, 0.50, 0.25, 0.15, and 0.075 mm) and sieved in an automatic sieve shaker (CSC Scientific, USA) for 3-5 min. Each fraction was weighed, and particle size were described using phi units ( $\phi$ ), where

$$\phi = -\log_2 d$$

(d is particle size in mm. Note that a negative value is coarser than a positive value).

Sediment TOC content was determined using loss on ignition (LOI). The LOI procedure involved combusting pre-weighed dried sediment samples at 450 °C for 24 h. The organic carbon was subsequently determined gravimetrically by subtraction.

Organic contaminant analysis was based on Standard Operating Procedures-GLIER Lab (SOP No. 02-002). Concentrations of particular contaminants were detected using a Hewlett-Packard (Avondale, PA, USA) 5890 chromatograph equipped with a  $^{63}\text{Ni}$  electron capture detector (GC-ECD), a Hewlett-Packard 7673A autosampler and DB-5 column (J&W Scientific, CA, USA).

Metals analysis was based on Standard Operating Procedures-GLIER Inorganic Lab (SOP No. 01-003). Strong extraction (total metals concentrations) was performed using 3.0-g wet sediment samples placed in 50-mL glass beakers with 5 mL of 1:3 (nitric: hydrochloric acid). This mixture was heated to 100 °C for 5 h, and filtered with Whatman #4 filter paper. The supernatant was transferred to pre-weighed 125 mL LDPE bottles (Nalgene via Fisher Sci., Toronto, ON, Canada) and brought up to 100 g with purified water. Metal concentrations (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were analyzed using an Inductively Coupled Plasma Optical Emission Spectrophotometer (IRIS #701776, Thermo Jarrell Ash Corporation). Total Hg was measured using an atomic absorption spectrophotometer (AAS-300, Varian) equipped with a single element hollow cathode lamp and a vapor generation accessory unit (VGA-76, Varian). Liquid samples were introduced into the instrument via a Meinhard concentric glass nebulizer (TK-30-K2, JE Meinhard Associates Inc., California, USA) combined with a cyclonic spray chamber.

All methods used are accredited under Canadian Association for Environmental Analytical Laboratories (CAEAL), and the inter-laboratory testing is performed semiannual under their procedures (Szalinska et al. 2006; Drouillard et al. 2006).

## **2.4 Statistical Analysis Methods**

Statistical analyses involved generation and interpretation of three forms of site-specific data, each initially summarized by a site (rows) x variable (columns) matrix – a stressor variable matrix, a zoobenthic assemblage matrix, and an environmental data matrix.

The stressor matrix contained variables representing sediment contamination. The data from this matrix were summarized using principal component analysis (PCA), each component of which was then used to designate putative reference sites and degraded sites.

The zoobenthic matrix contained relative abundances of the taxa common to the Lake Huron-Lake Erie corridor, expressed as octaves ( $\text{Log}_2$  (relative abundance in a sample)). The information in this matrix was used to identify distinct zoobenthic assemblages at reference sites, and to document the differences in benthic invertebrate relative abundance relative to increasing environmental stress.

The environmental data matrix contained information relating to the physical conditions of the microhabitat from which zoobenthic and sediment samples were collected. Variables in this matrix were used to classify the distinct assemblages of zoobenthos identified by cluster analysis of the zoobenthic data.

### **Reference and degraded site designation**

The term ‘reference condition’ has been used to define the condition equivalent to pristine (sometimes, historical condition), or the condition in the absence of human disturbance (minimally-disturbed condition) (Stoddard et al. 2006); it is also used to describe the best remaining condition (or least-disturbed condition) in a region heavily modified by human activities (Stoddard et al. 2006), like the Lake Huron-Lake Erie

Corridor system. In this project, we defined the reference condition as the condition that exists in ecosystems that are least-disturbed by anthropogenic stressors (Host et al. 2005).

Since the Lake Huron-Lake Erie Corridor has been disturbed by many human activities, dozens of elements that are potentially toxic as well as many different PCB congeners, many types of PAHs, and all sorts of other hydrophobic organic contaminants are found in the sediments here. However, because these contaminants come from specific classes of pollution, the concentrations of many compounds in the sediments tend to be correlated. A principal component analysis (PCA) was used as a means of reducing the large numbers of contaminants into a smaller number of statistically independent suites of chemicals, each of which may exert its own effects on the biota.

The underlying objective of the reference and degraded site designation in this project was to use PCA of physico-chemical attributes to identify sites with sediment quality relatively least and most affected by metals, hydrophobic organochlorine pesticides and other hydrophobic organic compounds, respectively.

### Sampling Sites

Sampling sites used for developing initial multivariate model were chosen from combined data from the 2004/5 Huron-Erie Corridor survey (105 sites), the 1991 (Farara and Burt 1993) and 1999 (Wood 2004) Detroit River surveys (77 sites and 129 sites, respectively). A total of 311 sampling sites were put in the analysis (locations summarized in Appendix I).

### Summarizing Contaminant Concentrations

The 2004/5 corridor survey analyzed nineteen elements (18 metals and arsenic), whereas in the 1991 and 1999 Detroit River surveys, thirteen (12 metals and arsenic) and nineteen (18 metals and arsenic) elements were reported, respectively. Consequently, thirteen elements (12 metals plus arsenic) were common enough to be included in the analysis. All 3 surveys reported concentrations of various PCB congeners. However, the methodology and detection limits for reporting the congeners improved greatly between 1990 and 2004, making a congener-by-congener analysis unreliable. Instead, the value of  $\Sigma$ PCBs reported in each survey was used in the analysis. Reports of pesticides, organic hydrocarbons (e.g., polycyclic aromatic hydrocarbons) and petrochemical byproducts were also variable among surveys. Consequently, a representative insecticide degradation product (p,p'-DDE) and a petrochemical byproduct (octachlorostyrene) were used as single-variable surrogates for the accumulation of agricultural pesticides and petrochemical contaminants, respectively. A total of 16 chemical variables were compiled for each site in the survey data matrix. The concentrations of each were transcribed from the 3 data sources into a single site (rows) x contaminant (columns) matrix.

Many contaminants were listed as occurring below the limits of detection. As PCA requires numeric information for each cell of the data matrix, we used the method of Szalinska et al. (2006) to generate surrogate values when contaminants were reported as non-detectable. Each non-detectable value was replaced with a randomly generated value of between 0.01 and 0.5X the detection limit of each chemical. All data were log-transformed prior to further analysis.

Principal component analysis was conducted on a correlation matrix of the selected 16 chemical variables, followed by varimax raw factor rotation. Five

principal component factor loadings of all the variables were extracted, explaining 84% of total variance (Table 2. 2).

The first principal component (PC1), with which aluminum, manganese, cobalt, nickel, iron, copper, and chromium were associated, was defined as “trace and minor metals”; the variables correlated with the second category (PC2) were lead, cadmium, zinc, mercury and Sum PCBs. Consequently, PC-2 was said to represent “trace metals and Sum PCBs”; the component (PC3 – “other organochlorine compounds”) grouped DDE and OCS together. Only arsenic was correlated with the fourth principal component. Each of the first four categories was considered to be an independent “stressor”. The only variable correlating with the fifth PC, calcium, represented the mineral content of the sediment (hardness), and was not considered to be a “stressor”. Accordingly, PC5 was excluded from the following analysis.

The principal component scores for each “stressor” at a site were scaled to a proportion of the maximum observed value, which is:

$$\text{Relative Scale (Rel)} = \frac{\text{Observation} - \text{Minimum}}{\text{Maximum} - \text{Minimum}}$$

Each site was assigned a “Sum of Relative (SumRel) contamination score”, representing the sum of the four PC-associated “Relative Scales”, based on the assumption that the zoobenthic community is affected equally by each of the stressors and that their effects are additive.

A site was classified as “reference site (REF)” if its “SumRel” placed it within the lowest quintile (lowest 20 percent) of the frequency distribution of all sites (Host et al. 2005). A site was classified as “degraded site (DEG)” if its “SumRel” placed it within the highest quintile (highest 20 percent) of the gradient of all sites. All other sites were classified as “test sites”.



### **Zoobenthic assemblages identification at reference sites**

#### **Summarizing Zoobenthic Density and Relative Abundance**

A total of 100 zoobenthic taxa was identified to the lowest taxonomic rank possible in the 2004/5 samples (data are available on request from either J.J.H. Ciborowski or J. Zhang, University of Windsor). For the purpose of statistical analysis, zoobenthic taxa rarely found (fewer than 5 percent of samples) were eliminated from subsequent analysis to avoid unduly weighting rare taxa (Thornley and Hamdy 1984). To produce consistency with 1991 and 1999 Detroit River datasets, individuals found in the 0.25 mm size fraction of the 2004/5 samples were excluded from further calculations. Furthermore, some genera were combined to produce family-level totals. The data set of Wood (2004) (1999 Detroit River survey) was the coarsest, consisting of 16 taxa designated as 'dominant'. Consequently, that taxonomic grouping was used in the multivariate statistical analyses (Appendix II). Wood (2004) reported the dominant taxa that live in depositional substrate are Oligochaeta (particularly Tubificidae), Chironomidae, burrowing mayflies (Ephemerae), Nematoda, and Gastropoda, whereas animals characteristic of erosional substrates include *Dreissena*, Amphipoda (*Gammarus* and *Echinogammarus* spp), Hydrozoa (*Hydra* and *Cordylophora*), Trichoptera (primarily net-spinning families Hydropsychidae, Psychomyiidae and Polycentropodidae) and Oligochaeta (particularly Tubificidae).

Zoobenthic relative abundance was expressed on an octave scale ( $\log_2 [100 \times (\text{proportion} + 0.01)]$ ) (Gauch 1982). Transformed data were used to reduce the weighting of dominant taxa (White and Irvine 2003).

## Identifying Zoobenthic Assemblages at Reference Sites

To identify groups of reference sites (hereafter referred to as 'REF' sites) with similar zoobenthic community composition, we used Ward's method of cluster analysis with the City-block (Manhattan) distance measure. Once clusters of REF sites had been identified, the zoobenthic taxa most important in distinguishing hierarchical clusters of sites were determined by calculating ANOVA-like F-ratios where  $F = (\text{Between cluster mean square})/(\text{Error mean square})$  for each taxon (Green and Voscatto 1978). Taxa with the highest F-ratios contributed most to the distinctiveness of pairs of clusters.

### Site classification

#### Summarizing Environmental Variable Data

The environmental data matrix was used to summarize natural physicochemical attributes of each sample site that are most important in determining differences in zoobenthic community composition in the absence of human-related stress. In running water systems, hydrodynamic properties (velocity, depth, Froude number, etc.) and substrate characteristics (particle size characteristics, organic content, etc.) typically dictate community composition (Norris 1995; Hargett et al. 2007). The Lake Huron-Lake Erie Corridor is up to 10 m deep, preventing us from collecting direct measurements of near-bottom flow characteristics at the point where each sample was collected. Subsurface water velocity readings were collected where possible, but these are often poorly correlated with near-bed flows. The following variables were available in the 1991 and 1999 survey reports and were compiled in the environmental data matrix: total organic carbon (LOI (%)), water depth (m), water temperature (°C),

dissolved oxygen concentration (mg/L), sediment median particle size ( $\phi$ ) and the location of a site (lake or river; longitude and latitude) (Appendix I).

All habitat attributes were  $\text{Log}(Y+1)$  transformed to improve homogeneity of variances and normality of the data, except for latitude, longitude, median particle size ( $\phi$  units), and the variable based on a categorical scale (lake or river).

#### Classification of Test and Degraded Sites

Once groups of compositionally similar REF sites had been determined through cluster analysis, a forward step-wise discriminant function analysis (DFA) was performed to identify the habitat attributes that would best separate individual clusters of REF sites. The DFA model was then used to determine to which REF cluster a particular “test site” or “degraded site (hereafter referred to as ‘DEG’ sites)” should belong, based on the diagnostic habitat attributes observed at each test site. Appendix III demonstrated the process by which the sites were assigned to different groups.

#### **Ordination of sampling sites based on zoobenthic relative abundance**

Once each site had been assigned to a particular REF clusters, Bray-Curtis ordination with subjective endpoint selection (McCune and Grace 2002) was used to identify which zoobenthic taxa were most strongly associated with the extremes of sediment quality as summarized by the SumRel scores for each of the cluster groups. Rather than using the single extreme endpoints of the cumulative frequency distributions, whose zoobenthic composition may or may not be typical of sediment quality at these locations, I selected the 4-5 sites (up to 10% of the most extreme SumRel values) with the lowest SumRel scores. I calculated the mean octave score of each taxon averaged over these 4-5 sites (i.e., the centroid of the group of sites in species relative abundance space), and used these means to represent a hypothetical

assemblage expected to be representative of the 'best' end of the SumRel gradient. This hypothetical 'best' site was included in the site x species matrix for Cluster C1 and identified as one endpoint of the Bray-Curtis ordination. The relative abundances (octaves) of taxa from the 4-5 sites with the highest SumRel scores were similarly averaged to create a hypothetical 'most degraded' site, which was also included in the ordination matrix, and identified as the other subjective endpoint of the Bray-Curtis ordination. The 'Best' and 'most degraded' sites thus represented the reference endpoint and degraded endpoint for each of the clusters.

The ordination procedure assigned a 'Zoobenthic Condition Index' score to each site in the analysis based upon its percent similarity to the two endpoints. A scatterplot of Zoobenthic Condition Index score (Y-axis) vs. Sediment quality (SumRel - X axis) was then used to identify the relative position of each site member of a cluster along the contaminant gradient.

Quantile regression analysis was used to relate trends in Benthic Condition Index (Bray-Curtis ordination score) to sediment quality (SumRel) using the SAS QUANTREG procedure (SAS Institute 2004). Regression coefficients representing the relationship between the median, 0.10, and 0.90 quantile linear regression lines and sediment quality (SumRel) were generated. The ordination scores were expected to be a negative function of decreasing sediment quality (increasing SumRel score). One-tailed tests were applied to evaluate the null hypothesis that the quantile regression coefficients were equal to zero.

The 0.10 quantile is the value exceeded by 90% of the Zoobenthic Condition Index scores for a particular sediment quality (SumRel) value. In particular, 90% of the sites with a SumRel value at the 'good' end of the sediment quality gradient will have Zoobenthic Condition Index scores larger than or equal to the 0.10 quantile

value. Consequently, I operationally defined this value as the ZCI score below which a site should be considered to have biological quality 'poorer than equivalent to reference'. I represented this value by a horizontal line drawn on the 'ZCI vs. SumRel' scatterplot for a cluster.

Correlations between plots of zoobenthic relative abundance at each site and the ZCI (Bray-Curtis ordination score) for that site were inspected for each taxon. Forward stepwise multiple regression analysis will identify taxa whose relative abundances (octaves) contributed significantly to ZCI score for sites in particular clusters.

Bray-Curtis ordinations were performed using PC-ORD<sup>®</sup>, version 4 (McCune and Mefford (MjM Software Design) 1999). Quantile regression analyses were performed using SAS, version 9.1.3 (SAS Institute 2004). All other statistical analyses were performed using Statistica<sup>®</sup> software package, version 6.0 (StatSoft Inc. 2001).

## **2.5 Results**

### **REF and DEG sites**

A total of 62 REF sites (20 percent of 311 sites) within the whole corridor were designated as being least contaminated by trace metals, pesticides and organic chemicals (lowest SumRel stressor scores). Another 62 sites that exhibited the highest SumRel stressor scores were designated as "DEG" sites. The mean ( $\pm$  1SE) concentration of 16 chemical variables (log (Y+1) transformed values) and four scaled PC factor scores in REF, test and DEG sites are summarized in Table 2.3. Most of the mean concentrations of chemicals in DEG sites were higher than those in REF sites, especially the trace metals, arsenic and the organic compounds, which were considered "toxic" to benthic fauna. Figures 2.3, 2.4 and 2.5 demonstrate the

distribution of REF and DEG sites in St. Clair River (2004), Lake St. Clair (2004/5) and Detroit River (1991, 1999 and 2004), respectively. Fourteen REF sites and no DEG sites were found in the St. Clair River; 37 REF sites and no DEG sites were located in Lake St. Clair; the Detroit River is the most degraded part of the Lake Huron-Lake Erie Corridor, all 62 DEG sites and 11 REF sites were located in the Detroit River.

### **Zoobenthic communities and Habitat Influences**

Based on the cluster analysis of 15 zoobenthic taxa relative abundance (octave scale), we identified 3 groups of REF sites (Figure 2.6, A, B1, B2). However, in the subsequent analyses, we found that the DFA model could not separate the 3 groups on the basis of the habitat variables available to us. For this reason, and based on the similarity of zoobenthic community composition, 2 clusters of REF sites in the cluster analysis were chosen (Figure 2.6). Cluster C1 was the largest group, consisting of 55 sites that were dominated by Chironomidae, Nematoda, *Caenis* (Ephemeroptera) and *Hexagenia* (Ephemeroptera), which are taxa characteristic of soft substrate or depositional zones of rivers; Cluster C2 consisted of 7 sites. It was characterized by a dominance of *Dreissena*, Amphipoda, Hydrozoa, Sphaeriidae, Turbellaria, Hydrosychidae (Trichoptera), and other net-spinning Trichoptera, which were characteristic of hard substrates or erosional river habitats (Table 2.4). Samples from both cluster sites had a preponderance of Oligochaeta (averaging 9-35% of the total; Figure 2.7).

The discriminant function analysis classified 59 of 62 REF sites correctly (Table 2.5). Four variables were accepted by the DFA model, three of them (water depth, sediment median particle size and dissolved oxygen concentration) were identified as

important in separating the clusters of REF sites (Table 2.6). The sites forming the cluster C1 zoobenthic assemblage (depositional) tended to be characterized by shallow water with fine substrate and high dissolved oxygen concentration, whereas cluster C2 zoobenthic assemblage sites (erosional) tended to have deep water, coarser substrate and lower dissolved oxygen concentration.

The DFA model thus generated was used to assign the nonreference sites to one of the two clusters based on those three variables (Appendix III).

The DFA classified 255 sites as belonging to Cluster C1 (putatively dominated by taxa characteristic of depositional zones), and assigned 56 sites to Cluster C2 (samples with taxa typical of erosional habitats). The sites assigned to Cluster C1 consisted of the original 55 REF sites, 47 DEG sites, and 153 test sites. The sites making up the cluster C2 group consisted of the original 7 REF sites, 15 DEG sites and 34 test sites. The cumulative frequency distributions of stressor scores for sites classified as belonging to clusters C1 and C2 are shown in Figures 2.8 and 2.9, respectively. Both frequency distributions were normally distributed because the scores are composites of principal component scores (of sediment contaminant concentrations), which by definition are normally distributed. The centroid-determined 'best' and 'most degraded' sites used 5 sites at the reference extreme and 4 sites at the degraded extreme of Cluster C1, respectively, and 4 sites at each end of the cluster C2 group.

### **Stressor Influences**

Bray-Curtis ordinations were performed on each of the 2 clusters of sites using subjectively defined endpoints ('best' and 'most degraded'). A matrix of sites (rows) x zoobenthic taxa (columns) was used to identify which types of zoobenthic taxa were

associated with particular types of sites. The relative position of each site member of the cluster between the two end-points indicated the relative environmental condition of these sites along the contaminant gradient.

#### Zoobenthic Condition Index (ZCI) vs. Sediment Contamination Score (SumRel)

Although there was great variation in the relationship between the Zoobenthic Condition Index (ZCI; site ordination scores) and sediment condition (SumRel) in cluster C1 sites, the relationship was negative and highly significant ( $r = -0.37$ ,  $p < 0.001$ ) (Figure 2.10). The slopes of the 10<sup>th</sup> and 90<sup>th</sup> percent quantiles were all significantly less than zero (Table 2.7), indicating that despite broad variation, both the highest and lowest ZCI scores observed tended to decrease with increasing sediment contamination (Figure 2.10).

The relationship between variation in Zoobenthic Condition Index score and sediment contamination was stronger for sites classified as supporting erosional taxa (C2, Figure 2.11). There was a negative and highly significant correlation between the ZCI scores and the SumRel contamination scores ( $r = -0.66$ ,  $p < 0.001$ ) (Figure 2.11). The slope of the 90<sup>th</sup> percent quantile was significantly less than zero (Table 2.7), indicating that the highest ZCI scores observed tended to decrease with increasing sediment contamination (Figure 2.11). Although the slope of the 10<sup>th</sup> percent quantile was not significantly less than zero, it is much more clearly a threshold response (Table 2.7).

#### REF sites vs. DEG sites

Ninety percent of sites have ZCI scores greater than the 10<sup>th</sup> percentile value for any given degree of sediment contamination value. In other multivariate models, such



as the BEAST (Reynoldson and Day 1995), a 90% confidence ellipse is used to define the boundaries of the reference condition. Sites that fall outside that ellipse are said to be “nonreference”. By the same logic, the 90% lower confidence limit for the ZCI score in reference conditions is the predicted 10<sup>th</sup> percentile value for the least degraded end of the sediment condition gradient (represented by a horizontal dashed line in Figure 2.10 and 2.11). For sites characterized by depositional taxa (Cluster C1), ZCI was variable at the low end of the stressor scale, but no site had a ZCI value of less than 0.1 when the relative sediment contamination (SumRel) score was less than about 1.0. From a biological perspective, any site with a SumRel score <1.0 is equivalent to reference, and the variability among sites must be entirely due to environmental factors other than sediment contamination. By the same token no cluster C1 site has a ZCI value of more than 0.1 when the relative SumRel score is greater than 2.4. At this level of SumRel, the influence of contamination overrides any other sources of environmental variability, and such locations should be considered to be biologically degraded.

For sites characterised by erosional taxa (Cluster C2), no site had a ZCI value of less than 0.27 when the relative sediment contamination (SumRel) score was less than about 1.55. From a biological perspective, any site with a SumRel score <1.55 is equivalent to reference, and the variability among sites must be entirely due to environmental factors other than sediment contamination. No cluster C2 site has a ZCI value of more than 0.27 when the relative SumRel score is greater than 2.0. At this level of SumRel, the influence of contamination overrides any other sources of environmental variability, and such locations should be considered to be biologically degraded.

Sediment contamination may or may not exert a significant effect on zoobenthic community composition at intermediate levels of sediment contamination on both clusters, but the 10<sup>th</sup> percentile regression line delineates the boundary above which 90% of ZCI scores are expected to occur for any particular sediment contamination (Sumrel) score.

#### Multiple regression analysis relating relative abundance of taxa to ZCI scores

Forward stepwise multiple regression analysis identified 10 taxa whose relative abundances (octaves) contributed significantly to ZCI score for sites in Cluster C1 (Table 2.8;  $R^2 = 0.91$ .  $n=255$ ). However, inspection of scatterplots of relative abundance of individual taxa vs. ZCI score in cluster C1 sites indicated that only Oligochaeta and Chironomidae occurred frequently enough in samples to show real pattern (for any ZCI score  $<0.10$  (degraded), the relative abundance of Oligochaeta was  $>40\%$  (Figure 2.12), Chironomidae constituted  $<8\%$  (Figure 2.13) of the sample, and most other major taxa (*Hexagenia*, *Caenis*, Ceratopogonidae, Trichoptera, Turbellaria, Gastropoda, *Dreissena*) were absent). A revised forward stepwise multiple regression analysis was performed including only these two taxa to generate a “ZCI predictive equation”. Oligochaeta contributed negatively to the ZCI score whereas Chironomidae contributed positively to the ZCI score (Table 2.9).

Forward stepwise multiple regression analysis identified 9 taxa whose relative abundances (octaves) contributed significantly to ZCI score for sites in cluster C2 (Table 2.10;  $R^2 = 0.97$ .  $n=56$ ). However, inspection of scatterplots of relative abundance of individual taxa vs. ZCI score in cluster C2 sites indicated that only Oligochaeta, Hydropsychidae, Chironomidae and *Dreissena* occurred frequently enough to show meaningful patterns (for any ZCI score  $<0.27$  (degraded), the relative

abundance of Oligochaeta was >55% (Figure 2.14), Chironomidae constituted <3% (Figure 2.15), Hydropsychidae and *Dreissena* constituted <2% of the sample (Figures 2.16, 2.17), respectively). A revised forward stepwise multiple regression analysis was performed with only these four taxa to produce a “ZCI predictive equation”. Oligochaeta contributed negatively to the ZCI score whereas the other three taxa contributed positively to the ZCI score (Table 2.11).

## **2.6 Discussion and Conclusions**

### **REF and DEG site designation - SumRel**

Reference sites are expected to be locations at which biota are exposed to the minimal degree of anthropogenic disturbance in the system. However, in large river systems like the Lake Huron-Lake Erie Corridor, such sites typically do not exist due to the effects of widespread, long-term human activities (Whittier et al. 2007). We identified the least-disturbed group of sites the Lake Huron-Lake Erie corridor to be considered “reference” (Stoddard et al. 2006), recognizing that they may not be in very good condition as compared with natural conditions. The lowest SumRel sediment contamination score in the system was 0.71 (site S38), which is much greater than the theoretical minimum that could occur (sum of the four lowest scaled PC factor values, <0.01). This implies that for the Lake Huron-Lake Erie Corridor, the “apex” of the stressor pyramid (Ciborowski et al. 2003) representing the true reference condition (complete absence of disturbance) no longer exists, our REF-designated sites are unlikely to be “minimally disturbed” even though they represent the “least-disturbed” sites in the system.

To assess overall sediment contamination, in the multivariate analysis, I performed a principal components analysis (PCA) of 16 chemical variables (metals,

pesticides and organic compounds), and 5 principal components summarized those original variables. Several different methods of REF site designation have been proposed, depending on the PC factor loadings. Bhagat (2005) chose the boundaries for REF and DEG sites based on the assumption that the biological community is limited by the single greatest stressor (Relative Maximum stressor value, RelMax). RelMax is thought to be the best measure when there are truly undisturbed sites (minimally disturbed) within a study area (Host et al. 2005). My results showed that 7 chemical variables (Al, Mn, Co, Ni, Fe, Cr and Cu) were highly correlated with the first principal component. The loadings of those variables ranged from 0.65 to 0.91, accounting for 32% of the total variance. Among these metals, Al, and Fe are common minor metals that are normally bound in the sediment, should not be considered “toxic” to the benthic fauna under normal water quality conditions. Overall, the elements associated with PC-1 were more related to sediment characteristics (clay content) than to contaminant stress. However, because some of the metals (Co, Ni, Cr and Cu) are often suspected to be toxic at high concentrations, we considered PC1 to be one independent “stressor”. The second component described variation of 5 variables (Hg, Pb, Zn, Cd and SumPCBs). These had loadings ranging from 0.58 to 0.81 and accounted for 26% of the variance. The third and fourth PCs accounted for 9% and 9% of the variation, respectively, and loadings ranged from 0.66 to 0.96 (Table 2.2).

Each of the principal components provided important descriptions of some aspect of overall sediment contamination, although most of the potential contaminant toxicity is likely associated with the variables summarized by PC2. Based on these results, and because as described above, the corridor system has been disturbed by human activities for a long period of time, I judged that the sum of the 4 relative

contaminant scores from the 4 PC factors (SumRel) was the most reasonable method to identify “least-disturbed sites” as REF sites and “most-disturbed sites” as DEG sites in the Lake Huron-Lake Erie Corridor.

To assess and confirm whether PC2 might dominate the toxicity stress gradient, I also reanalyzed the data, designating reference and degraded conditions based solely on the scores for PC2 (Appendix IV). My results indicated that using PC2 alone indeed improve the correlations, consistent with the idea that PC2- associated compounds account for much of the stress-response relationship between ZCI and sediment contamination score. However, the same taxa serve as indicator taxa indicated that using SumRel to identify reference and degraded sites and eventually develop zoobenthic indicators is still a reasonable method.

Some potentially important classes of compounds such as PAHs, and compounds such as pentachlorobenzene (QCB) and hexachlorobenzene (HCB) had to be left out of the analysis due to incomplete data. This limitation has potential to influence the accuracy of our REF and DEG site designation if their concentrations vary independently of the other suites of compounds.

Most of the REF sites in the Lake Huron-Lake Erie Corridor were located in the St. Clair River and Lake St. Clair, and few REF sites but all the DEG sites were located in the Detroit River, indicating that the Detroit River sediments are the most polluted in the corridor system, especially the areas around Belle Isle on the US side, Zug Island (downstream of the Rouge River), Mud Island (downstream of the Ecorse River), Trenton Channel and the downstream of Fighting Island along the main channel. These results are consistent with earlier findings of a number of Detroit River surveys that demonstrated elevated concentrations of trace metals, PCBs, OCs and PAHs at point locations downstream of Belle Isle, near the Rouge River outflow,

along Trenton Channel and downstream of Trenton Channel (UGLCCS 1988a; Drouillard et al. 2006; Szalinska et al. 2006). This suggested that the relative environmental quality in the St. Clair River and Lake St. Clair is better than that in the Detroit River, although the St. Clair River near the petroleum complex around Sarnia, Ontario and the Walpole Delta within Lake St. Clair have been reported to be disturbed by human activities for a long period of time, and both were included in the St. Clair River AOC by IJC (GC 2003).

The analysis designated 11 locations within the Detroit River as REF sites. Six of them were located in the river mouth area, around Peche Island and upstream of Belle Isle (Figure 2.5), indicating that the head of the Detroit River had relatively good sediment quality compared with other parts of the river.

### **Zoobenthic Assemblages**

The cluster analysis of REF sites revealed unique assemblages of zoobenthic taxa among groups of sites. Cluster C1 tended to be dominated by Oligochaeta, Nematoda, Ephemeroptera (*Hexagenia* and *Caenis*) and Chironomidae. All of these taxa are common types of zoobenthos living in soft substrates, and the community of cluster C1 was considered to be representative of the biota expected to be found in a “depositional” river zone. Sites making up cluster C2 contained high relative abundances of *Dreissena*, Amphipoda (*Gammarus* and *Echinogammarus*), Hydrozoa (*Hydra* and *Cordylophora*) and Trichoptera (mainly *Cheumatopsyche* and *Hydropsyche* net-spinning caddisflies), which are taxa that typically colonize hard surfaces, or build shelters beneath or between the rocks or hard substrates (Manny et al. 1986; Ciborowski 2003); the community of cluster C2 was considered typical of “erosional” areas.

However, some cluster C1 sites had different zoobenthic assemblage composition compared with others. For instance, the composition of cluster C1 sites 109C, A53, S55 and S69 was similar to one another: in addition to having a high relative abundance of Oligochaeta, Nematoda and Chironomidae, these sites also supported high relative abundances of *Dreissena* and Amphipoda, which are typically considered to be erosional taxa. These sites were likely best defined as “depositional-erosional mixed” sites. This might be due to the merging of two different zoobenthic cluster groups during the initial REF site classification stage. Designating three groups of zoobenthic assemblages might better separate sites based on similar zoobenthic community composition. However, none of the environmental variables available could be used to uniquely distinguish this third group from the other two.

### **Habitat Influences**

Since the DFA model distinguished the two zoobenthic assemblages largely on the basis of substrate type, and because substrate has been considered by others to be an important habitat variable influencing the benthic fauna (Wood 2004; Strayer et al. 2006), we had expected median particle size to be important in separating the clusters of sites. The discriminant function analysis indeed revealed that median particle size was perhaps the most important variable in the model ( $p < 0.001$ ). Water depth and dissolved oxygen concentration were also significantly different between two clusters of sites. The depositional cluster sites tended to have fine substrate, occurred in shallow water, and had high dissolved oxygen concentration, while sites found to have erosional-type zoobenthos had coarse substrate, low dissolved oxygen concentration and were in deep water. All three of these variables (median particle size, water depth and dissolved oxygen concentration) strongly correspond to near-bottom water

velocity, which is considered to be the key habitat attribute controlling zoobenthic communities in rivers (Rae 1985; Ciborowski 2003). However, near-bottom water velocity was unavailable for this analysis. This might explain why the classification model could not classify all sites to three clusters properly.

Site location (latitude and longitude) has also been reported as a primary explanatory factor (Turak et al. 1999). Since the Lake Huron-Lake Erie Corridor was composed of two rivers and a lake, each with different habitat characteristics (i.e., water velocity), we had expected “lake or river” and correspondingly, the location of a site to also be important variables separating groups of sites and zoobenthic assemblages. However, none of the geographically-based variables proved to be important diagnostic variables relative to the others identified by the discriminant function model. This suggests that none of the water bodies supports a zoobenthic fauna that isn’t found elsewhere in the corridor. It also suggests that sites in one river could be used as reference condition sites against which to compare conditions of sites in the other river or lake. The inability to identify suitable reference sites against which to compare the condition of the Detroit River has been often cited as a limitation in assessing the condition of the Detroit River zoobenthic community (Thornley and Hamdy 1985, Ferara and Burt 1992, Wood 2004).

### **Sediment Contamination Influences**

We used Bray-Curtis ordination to develop criteria for assessing the quality of sites (based on zoobenthic community composition) along the sediment contamination gradient previously defined by the REF and DEG site designation. My results showed clear distinctions and a strong relationship between the ZCI (Bray-Curtis ordination scores) and the SumRel (sediment contamination scores) only for sites classified as



belonging in cluster C2, the erosional cluster. Although a statistically significant correlation was found between the ZCI for depositional sites (cluster C1) and SumRel, the pattern was relatively “noisy”, and the overall proportion of variation accounted for was very low ( $R^2 = 0.11$ ). This likely reflects environmentally-unexplained heterogeneity in zoobenthic composition within this large group. Ultimately, the three zoobenthic assemblages identified by the cluster analysis should be classified to better illustrate the correlation pattern of the zoobenthic community composition and Sediment Contamination Score.

### **Synopsis**

Two groups of sites, each with distinct zoobenthic community composition were identified by cluster analysis, and the discriminant function analysis revealed that median particle size, water depth and dissolved oxygen concentration were important variables distinguishing between these two groups of sites. Statistically significant but relatively weak correlations between the zoobenthic community composition and sediment contamination score for both clusters were found, indicating that zoobenthic community composition can be used as a valid indicator of sediment quality in the Lake Huron-Lake Erie Corridor. However, only the erosional cluster exhibited a strong and clear correlation. The weak associations observed for the depositional sites were likely due to the lack of data regarding the key habitat factor, the near-bottom water velocity in this analysis. Inclusion of this factor might permit the DFA model to identify the habitat characteristics distinguishing three clusters of REF zoobenthic groups; better correlations between the zoobenthic community composition and sediment contamination score were expected.

Some of the lack of correlation could be due to inaccuracy of the measure of sediment contamination. For example, the dominant metals associated with PC-1 included Al and Fe, which are a normal component of most sediments. If PC-1 is in fact not an important stressor, its inclusion could result in the misordering of sites along the SumRel sediment contamination scale. Evidently, this is partially true in that ordination of zoobenthic assemblages to produce a ZCI with respect to a contamination scale based on PC-2 only produced stronger correlations than those derived using the SumRel scale. Nevertheless, both analyses found the same taxa to be most indicative of the reference and degraded conditions of both fine sediment and coarse sediment locations.

Although I could not directly measure near-bottom water velocity, estimates of the Detroit River velocities can be derived from simulation runs of a 3-dimensional hydrological model developed by Dr. S. Reistma (formerly of the University of Windsor). Since the near-bottom water velocity data were available only for the Detroit River sites (Reitsma et al. 2003) calculated by a 3-dimensional Detroit River Flow model, another analysis which included the near-bottom water velocity data in the DFA model was performed using the 1991, 1999 and 2004 Detroit River sites only ( $n = 213$ ). The multivariate analysis procedure was the same as that used for the whole-corridor analysis; results and discussion are summarized in the "Detroit River Case Study" below.

## 2.7 The Detroit River Case Study

### Results

Using varimax factor rotation, 5 principal component factor loadings of all 16 chemical variables (metals, pesticides and organic chemicals) were extracted, explaining 82% of total variance (Table 2.12); scores for each of the first four categories were highly correlated with concentrations of two or more metals and/or organic compounds and were considered to be an independent “stressor”. Scores of the last category (PC5) were correlated with concentrations of calcium and manganese only, elements that are not necessarily of anthropogenic origin. Consequently PC5 was not considered to be a “stressor” and was excluded from the analysis. The mean ( $\pm$  1SE) concentration of 16 chemical variables (log (Y+1) transformed values) and the SumRel scores in REF, test and DEG sites were summarized in Table 2.13. From this table, most of the mean concentrations of chemicals in DEG sites were higher than those in REF sites, especially the trace metals, arsenic and SumPCBs, which were considered “toxic” to benthic fauna. A total of 43 REF sites were selected as least-disturbed sites within the Detroit River (lowest SumRel contamination scores). Another 43 sites, which had the highest SumRel contamination scores (most-contaminated sediments) were defined as “DEG” sites. Figure 2.18 shows the distribution of REF and DEG sites in the Detroit River (1991, 1999 and 2004).

Based on the similarity of zoobenthic community composition of 16 zoobenthic taxa, 3 clusters of 43 REF Detroit River sites in the cluster analysis were chosen (Figure 2.19); Cluster DR1 consisted of 16 sites that were dominated by Chironomidae, Nematoda and *Hexagenia* (Ephemeroptera), taxa that are characteristic of depositional environmental conditions; Cluster DR2 consisted of 9

sites, dominated by a mixture of depositional taxa (such as Oligochaeta, Chironomidae, Nematoda), and erosional taxa (Amphipoda, Sphaeriidae and Hydrozoa). Consequently, cluster DR2 was considered to be a “mixed group”. Cluster DR3 was the largest group (18 sites), and the zoobenthos were dominated by Dreissena, Amphipoda, Hydrozoa, Turbellaria and Hydrosychidae (Trichoptera), taxa that are characteristic of erosional conditions in rivers (Figure 2.20) (Table 2.14).

The discriminant function analyses classified 33 of 43 REF sites correctly (Table 2.15); Six habitat variables were incorporated into the DFA model, five of them, near-bottom water velocity, median particle size, water temperature and two site location variables (latitude, longitude) were identified as important in separating the clusters of REF sites (Table 2.16). The DFA model thus generated was used to classify the test sites and DEG sites into corresponding clusters based on those five variables (Appendix IV). The distribution of 3 cluster sites within the Detroit River is summarized in Figure 2.21.

The cumulative frequency distributions of sediment contamination scores for sites classified as belonging to clusters DR1, DR2 and DR3 are shown in Figure 2.22, 2.23 and 2.24, respectively. Using the endpoint selection methods (objective) of Bray-Curtis ordination, the centroid-determined ‘best’ and ‘most degraded’ sites used 5 sites at the reference extreme and 4 sites at the degraded extreme of Cluster DR1, respectively; 5 sites at the reference extreme and 3 sites at the degraded extreme of Cluster DR2, respectively, and 4 sites at reference and degraded ends of the cluster DR3 group, respectively. All the ‘best’ and ‘most degraded’ endpoints were included in the ordination matrices. The relative position of each site member of the cluster between the two end-points indicated the relative environmental condition of these sites along the contaminant gradient.

Cluster DR1 (depositional group - slow-flowing water with fine substrate) consisted of 69 sites (13 REF sites, 18 DEG sites and 38 test sites). There was a negative and highly significant correlation between variation in ZCI score and sediment contamination ( $r = -0.37$ ,  $p < 0.01$ ) (Figure 2.25). The slope of the 90<sup>th</sup> percent quantile was significantly less than zero (Table 2.17), indicating that the highest ZCI scores observed tended to decrease with increasing sediment contamination. Although the slope of the 10<sup>th</sup> percent quantile was not significantly less than zero, it is much more clearly a threshold response (Table 2.17).

Forward stepwise multiple regression analysis identified 6 taxa whose relative abundances (octaves) contributed significantly to ZCI score for sites in Cluster DR1 (Table 2.18;  $R^2 = 0.90$ ,  $n=69$ ). However, inspection of scatterplots of relative abundance of individual taxa vs. ZCI score in cluster DR1 sites indicated that only Nematoda and Oligochaeta occurred frequently enough to show real pattern (for any ZCI score  $< 0.15$  (degraded), the relative abundance of Nematoda was  $< 21\%$  (Figure 2.26) and Oligochaeta was  $> 23\%$  (Figure 2.27) of the sample. A revised multiple regression analysis was performed including only these two taxa, to generate a “ZCI predictive equation”. Oligochaeta contributed negatively to the ZCI score whereas Nematoda contributed positively to the ZCI score (Table 2.19).

Cluster DR2 (mixed group) consisted of 72 sites (8 REF sites, 19 DEG sites and 45 test sites). There was a negative and highly significant correlation between variation in ZCI score and sediment contamination ( $r = -0.60$ ,  $p < 0.001$ ) (Figure 2.28). The slope of the 90<sup>th</sup> percent quantile was significantly less than zero (Table 2.17), indicating that the highest ZCI scores observed tended to decrease with increasing sediment contamination. Although the slope of the 10<sup>th</sup> percent quantile was not significantly less than zero, it is much more clearly a threshold response (Table 2.17).

Forward stepwise multiple regression analysis identified 4 taxa whose relative abundances (octaves) contributed significantly to ZCI score for sites in Cluster DR2 (Table 2.20;  $R^2 = 0.92$ .  $n=72$ ). These 4 taxa were included to generate a “ZCI predictive equation”, Oligochaeta and Gastropoda contributed negatively to the ZCI score whereas Chironomidae and Nematoda contributed positively to the ZCI score (Table 2.20). For any ZCI score  $<0.10$  (degraded), the relative abundance of Chironomidae was  $<4\%$  (Figure 2.29), Nematoda was  $<2\%$  (Figure 2.30) and Oligochaeta was  $>64\%$  of the sample.

Cluster DR3 (erosional group - fast-flowing water with coarse substrate) consisted of 72 sites (22 REF sites, 6 DEG sites and 44 test sites). There was a negative and highly significant correlation between variation in Zoobenthic Condition Index score and sediment contamination ( $r = -0.34$ ,  $p<0.01$ ) (Figure 2.31). Although the slopes of both the 90<sup>th</sup> and 10<sup>th</sup> percent quantiles were not significantly less than zero, there are clearly significant changes in the 'boundaries' as SumRel changes. This means that the ordination scores can be used as indicator scores even if the 'least squares' and median regression slopes aren't significantly different from zero (Table 2.17).

Forward stepwise multiple regression analysis identified 7 taxa whose relative abundances (octaves) contributed significantly to ZCI score for sites in Cluster DR3 (Table 2.21;  $R^2 = 0.98$ .  $n=72$ ). However, inspection of scatterplots of relative abundance of individual taxa vs. ZCI score in cluster DR3 sites indicated that only *Dreissena* and Oligochaeta were abundant enough to show real pattern (for any ZCI score  $<0.10$  (degraded), the relative abundance of *Dreissena* was  $<3\%$  (Figure 2.32) and Oligochaeta was  $>13\%$  of the sample (Figure 2.33). A revised multiple regression analysis was performed including only these two taxa, to generate a “ZCI predictive

equation". *Oligochaeta* contributed negatively to the ZCI score whereas *Dreissena* contributed positively to the ZCI score (Table 2.22).

There is evidence that the overall sediment quality of the Detroit River between 1991 and 2004 has changed (Figure 2.34). In the depositional cluster (cluster DR1), the mean SumRel contamination score in 2004 was marginally significantly lower than that in 1991 and 1999 ( $p < 0.05$ ), indicating that the sediment quality has improved in cluster DR1 sites in 2004; in the mixed cluster (cluster DR2), the mean SumRel in 2004 is highly significantly lower than that in 1991 ( $p < 0.001$ ), and significantly lower than that in 1999 ( $p < 0.01$ ), indicating that in 2004, the sediment quality in cluster DR2 sites is much better than that in early years; while in the erosional cluster (cluster DR3), although there is no statistically significant difference among the three years, there was a trend suggesting that the mean SumRel in 2004 is lower than that in 1991 and 1999, which means the sediment quality of erosional areas in 2004 is relatively better than that in previous years. However, there are 8 locations that were sampled in all three years (Table 2.23). A 'repeated measures ANOVA' was performed to compare the mean ordination score among 3 years at these 8 blocks of sites, there was no statistically significant difference among 3 years ( $p > 0.05$ ) (Figure 2.35), indicating that zoobenthic community condition in 2004 had not changed appreciably at these locations .

## **Discussion**

The distribution pattern of REF and DEG sites in the Detroit River case study is similar to that of the whole corridor study. Most of the REF sites in the Detroit River were located near the mouth, indicating that the sediment quality here is relatively better than in other parts of this river, especially the areas downstream of Belle Isle on

the US side, Zug Island (downstream of the Rouge River), Mud Island (downstream of the Ecorse River), Trenton Channel and the downstream of Fighting Island along the main channel and in the Canadian side. These areas were also reported to be the heavy metal “hot spots” by Szalinska et al. (2006).

Three clusters of sites, each with distinct zoobenthic assemblages were identified by the cluster analysis; they were a depositional group (cluster DR1), a mixed group (cluster DR2) and an erosional group (cluster DR3). The DFA model correctly classified most of the original REF sites. These three clusters were more precise in identifying the groups of zoobenthic communities within rivers. As we had predicted, the near-bottom water velocity as the key habitat factor significantly influenced the zoobenthic community composition. Also as we had originally expected, sediment median particle size and site location (longitude and latitude) were also significantly different among three clusters. It was surprising that water depth was not more diagnostic in the DFA model. However, a positive association ( $r = 0.61$ ,  $p < 0.001$ ) between water depth and near-bottom water velocity was found (Figure 2.36), and a negative association ( $r = -0.32$ ,  $p < 0.001$ ) between water depth and median particle size was observed (Figure 2.37), indicating that although the water depth was not accepted into the DFA model, it was weakly related to near-bottom water velocity and median particle size (the most two important habitat variables separate three groups of sites).

Since hydrophobic pollutants tend to settle in slow-flowing, depositional areas (oils and trace metals adhere to the organic matter in the soft substrates), we found more DEG sites in cluster DR1 and DR2 sites (18 and 19 sites, respectively). In erosional areas, the sediments and sediment-associated contaminants were likely washed away by fast-flowing water. These areas are likely less negatively affected by



human activities. Only 6 DEG sites were found in cluster DR3 sites, while the most REF sites (22 sites) were located in this cluster of sites. The sites near the Detroit River mouth area (around Peche Island and the upstream of Belle Isle) contained most of cluster DR3 sites (Figure 2.13); this might be the reason why the sediment quality here is relatively better than other parts of the river.

When performing the Bray-Curtis ordination techniques, better correlations between the biological condition (ZCI score) and the sediment contamination score (SumRel) were found for all three clusters compared with the whole corridor analysis (2 clusters), especially the cluster DR2 (mixed group) sites. They were isolated from the depositional group in this analysis, and showed strong and clear correlations between these two factors ( $r = -0.60$ ). However, the correlations between these two factors for the depositional cluster and erosional cluster were still not very strong. One possible reason for this result is attributed to the fact that the biological factor of a particular site (zoobenthos) was collected by ponar grab sampler, which is based on a fine spatial scale, while the near-bottom water velocity data was calculated by computer software using very coarse spatial scales.

Overall, the inclusion of near-bottom water velocity effectively improved the correlation between the benthic condition and the sediment contamination scales, indicating that it is a preferable way to assess environmental condition of rivers by using zoobenthic community composition as indicators.

The RDC approach has several relative merits compared with the established techniques. First, it gave a “contaminant gradient” bounded by two end-points, which can give the relative biological condition within a given area (i.e., the Lake Huron-Lake Erie Corridor). Secondly, the established techniques do not address the problem of “how degraded one site is” (there is no comparative basis for assessing the relative

condition of a test site that falls outside the range of reference conditions), whereas this method solved this problem by giving a contaminant gradient; lastly, Wood (1999) tried to use the BEAST multivariate method as a tool to investigate sediment quality assessment using zoobenthic community composition, but failed to find any correlation between anthropogenic stress caused by sediment contamination and the zoobenthic community composition. My method did find correlations between these two factors in different habitat characteristics, especially in the locations with relatively coarse substrates. By including assessment of the degraded condition in addition to reference condition sites, the RDC multivariate approach used in this study improves upon existing multivariate techniques and provides an alternative way to assess aquatic environmental condition by using zoobenthic community composition as indicators.

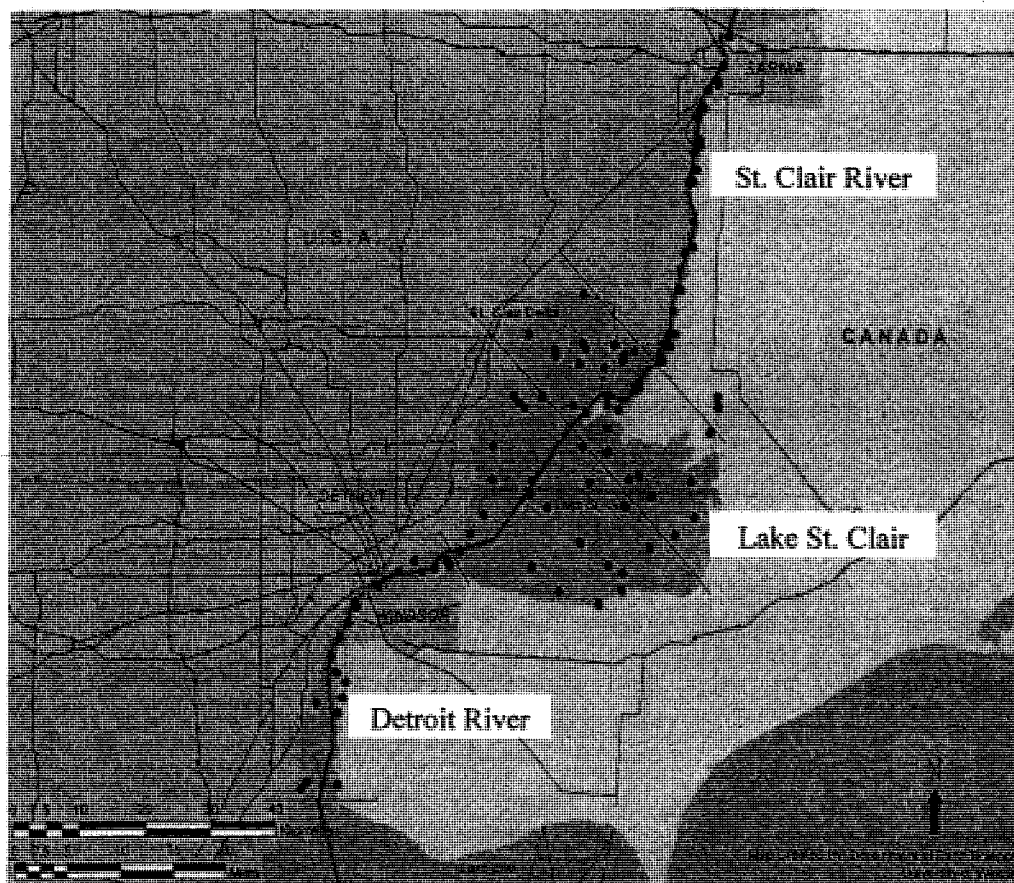


Figure 2.1. Location of sampling sites in the Lake Huron-Lake Erie Corridor, July-August, 2004 (three zones: St. Clair River, Lake St. Clair (include St. Clair Delta) and Detroit River). Map was made by Alice Grgicak-Mannion in Univeristy of Windsor

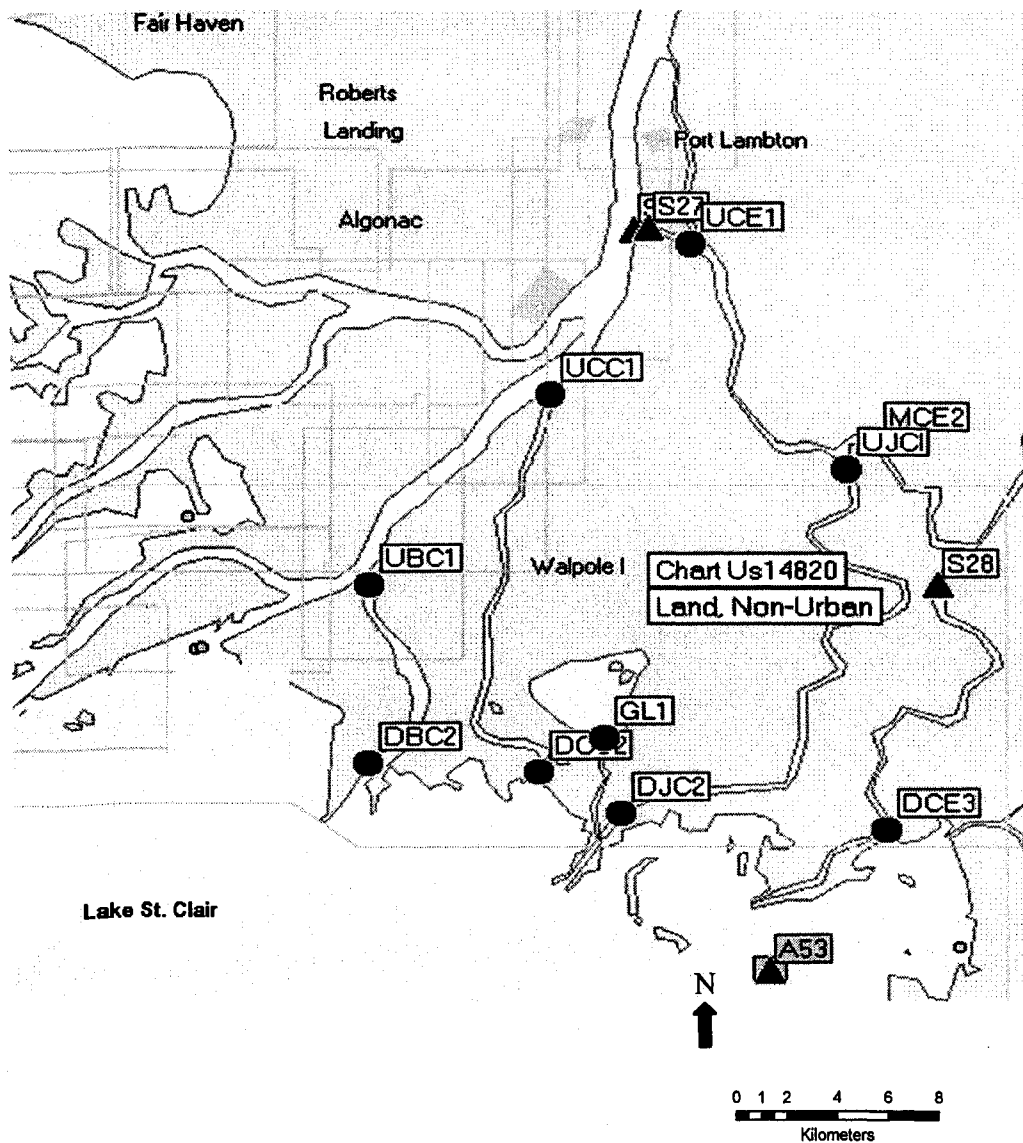


Figure 2.2. Location of sampling sites in the Walpole Delta (within Lake St. Clair), August 2005. Site locations corresponding to site labels are summarized in Appendix I)

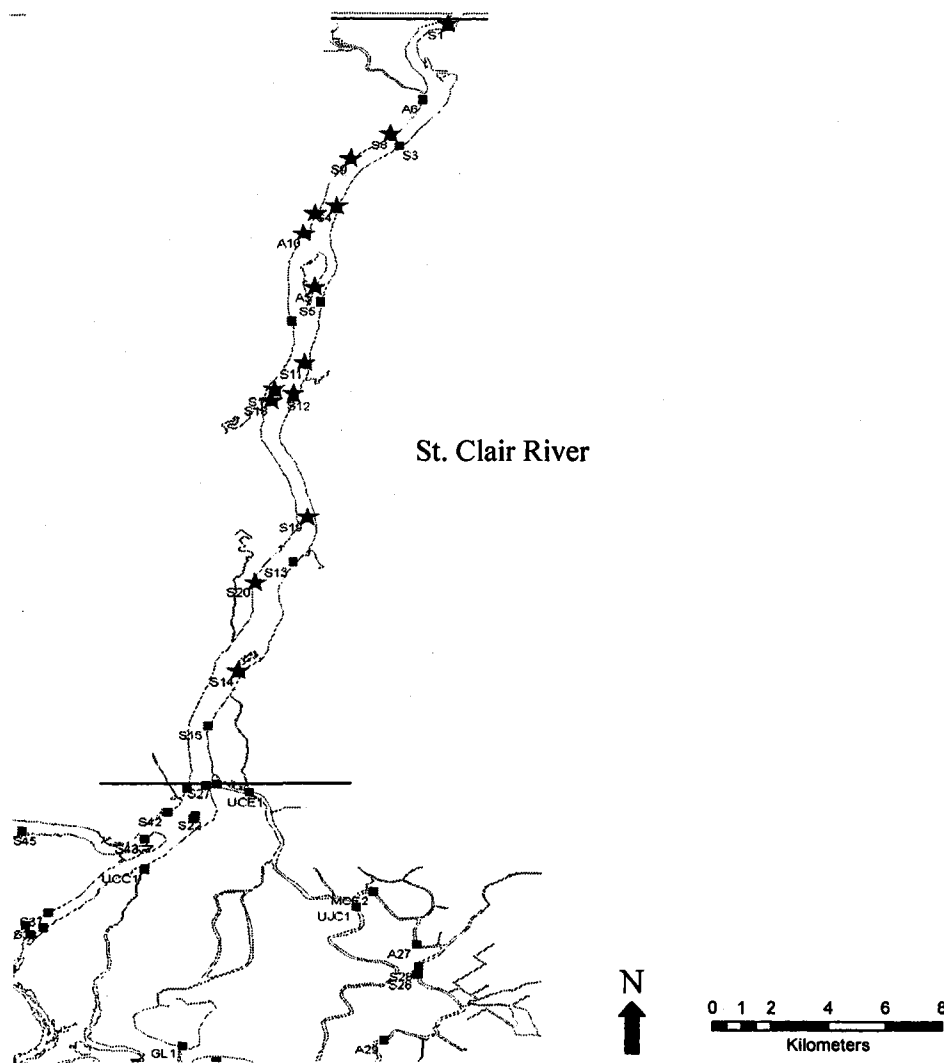


Figure 2.3. Distribution of the St. Clair River REF and DEG sites in the Lake Huron-Lake Erie Corridor analysis using 1991, 1999 and 2004 datasets (The site numbers showed up in the map are 2004 sampling sites). 5-point stars indicated “REF” sites; there are no “DEG” sites in the St. Clair River

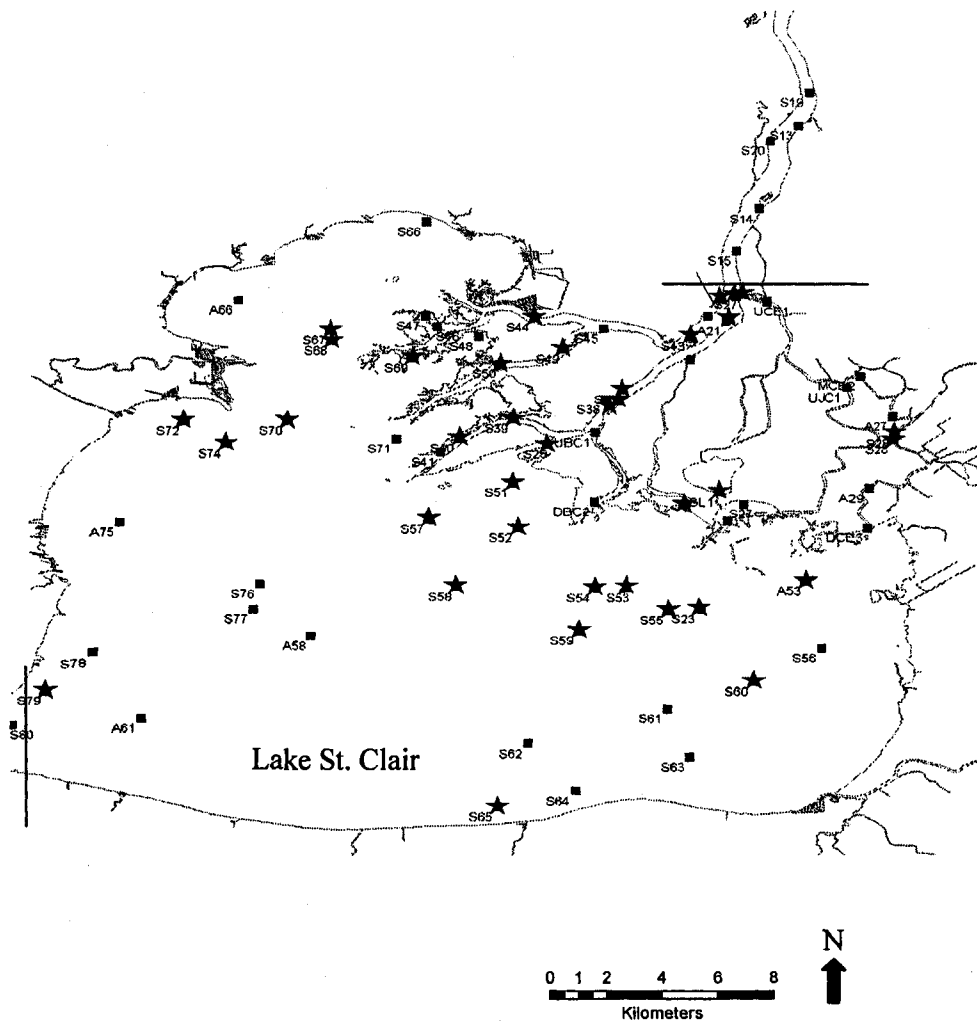


Figure 2.4. Distribution of the Lake St. Clair REF and DEG sites in the Lake Huron-Lake Erie Corridor analysis using 1991, 1999 and 2004 datasets (The site numbers showed up in the map are 2004/5 sampling sites). 5-point stars indicated “REF” sites; there are no “DEG” sites in Lake St. Clair

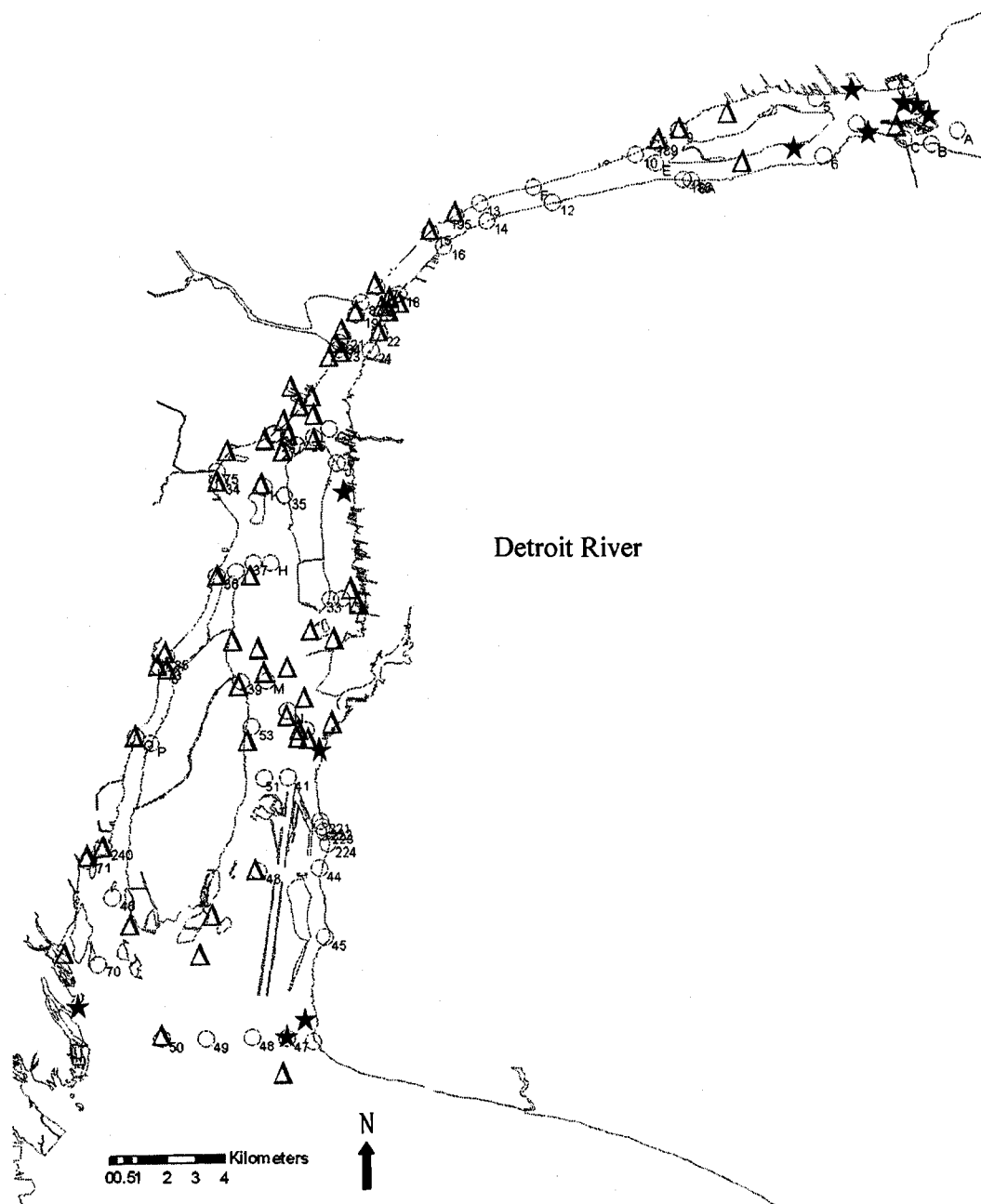


Figure 2.5. Distribution of the Detroit River REF and DEG sites in the Lake Huron-Lake Erie Corridor analysis using 1991, 1999 and 2004 datasets (The site numbers showed up in the map are 1991 sampling sites). 5-point stars indicated “REF” sites; triangles indicated “DEG” sites

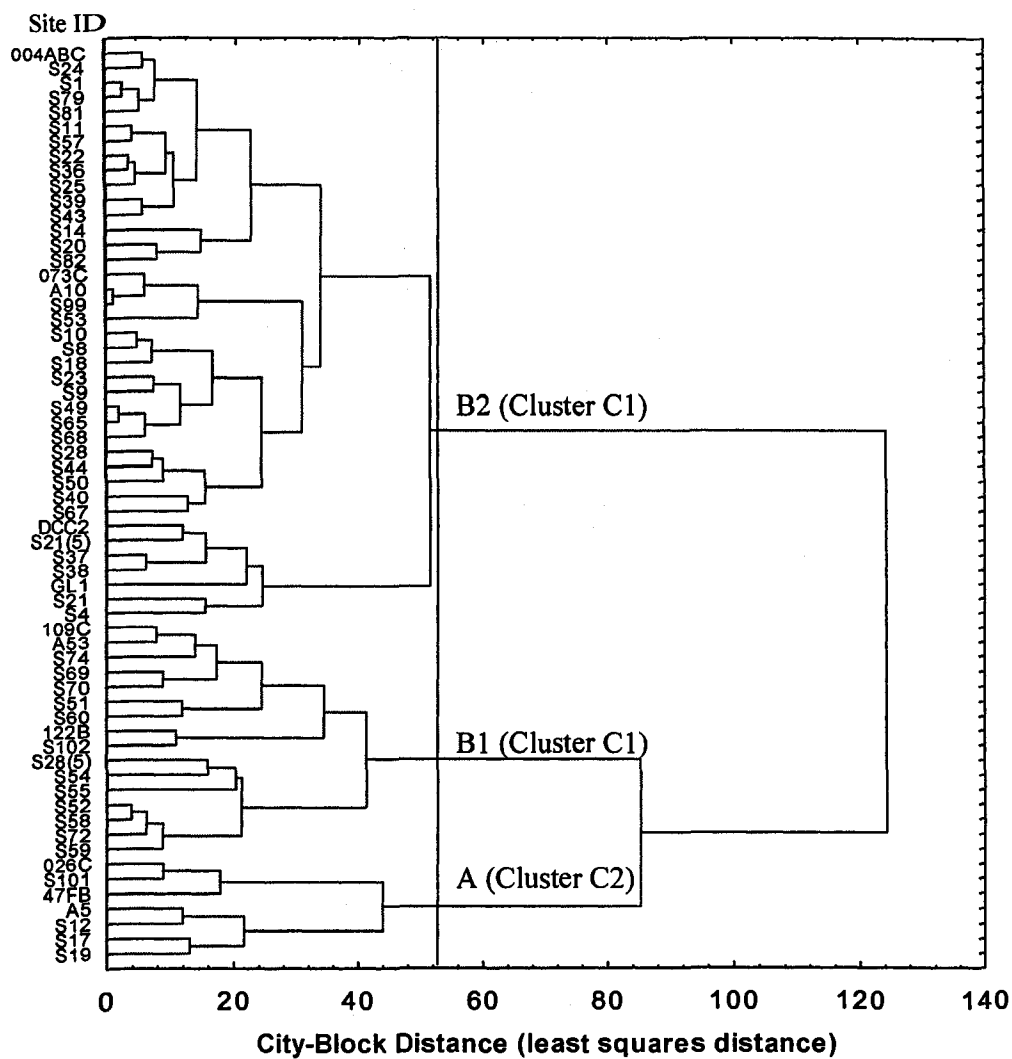


Figure 2.6 Dendrogram of REF sites (n = 62) grouped according to similar zoobenthic community composition in the 1991, 1999 and 2004/5 Lake Huron-Lake Erie Corridor analysis (Ward's method clustering city-block distances of octave-transformed relative abundances of zoobenthic taxa). Site locations corresponding to site labels are summarized in Appendix I



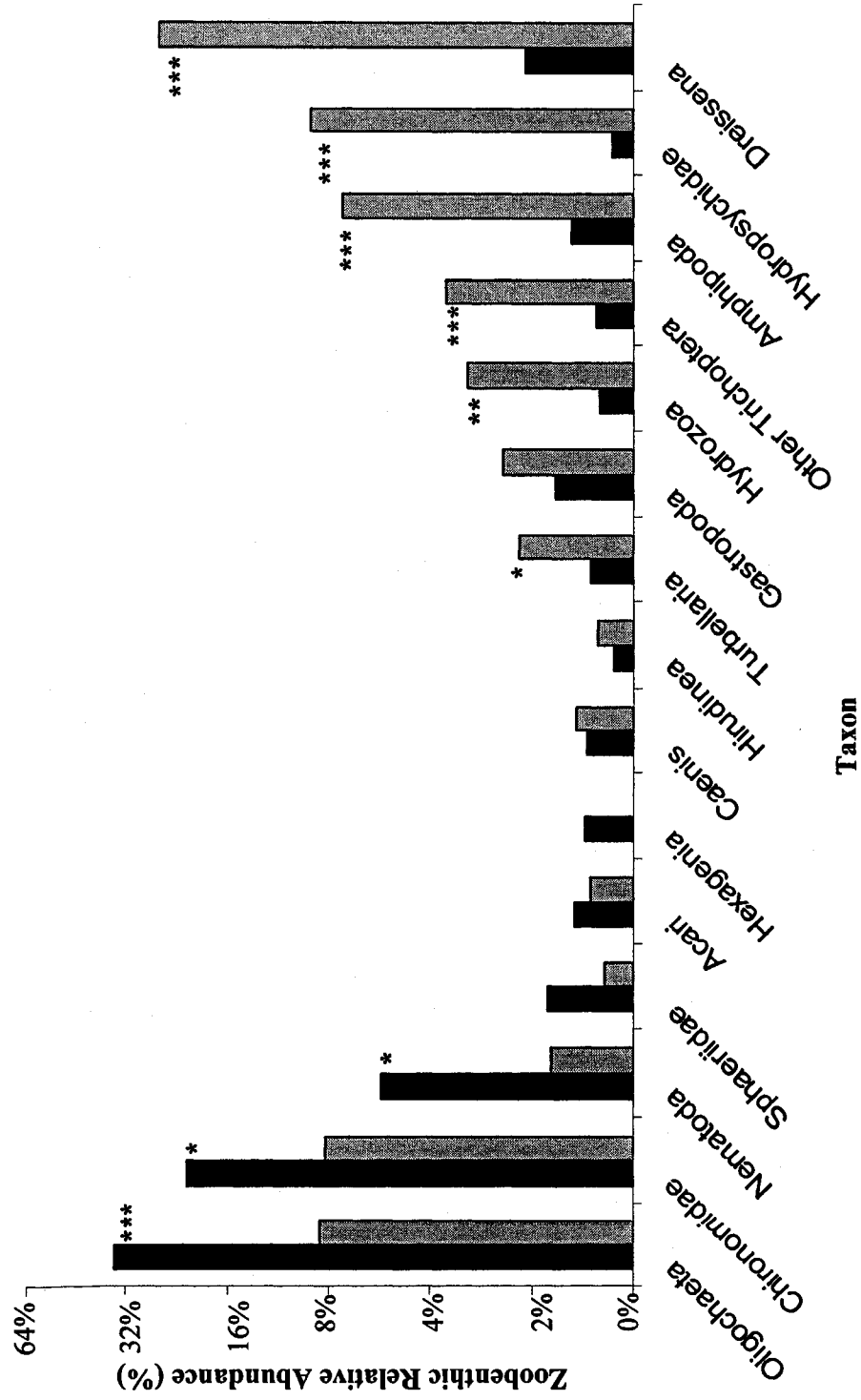


Figure 2.7. Mean value of taxa relative abundance (%) for two clusters of zoobenthic communities found in the Lake Huron-Lake Erie Corridor. Black bars indicate cluster C1 (depositional group), grey bars indicate cluster C2 (erosional group). Asterisk (\*) indicates significance level: \*\*\* highly different; \*\* moderately different; \* marginally different

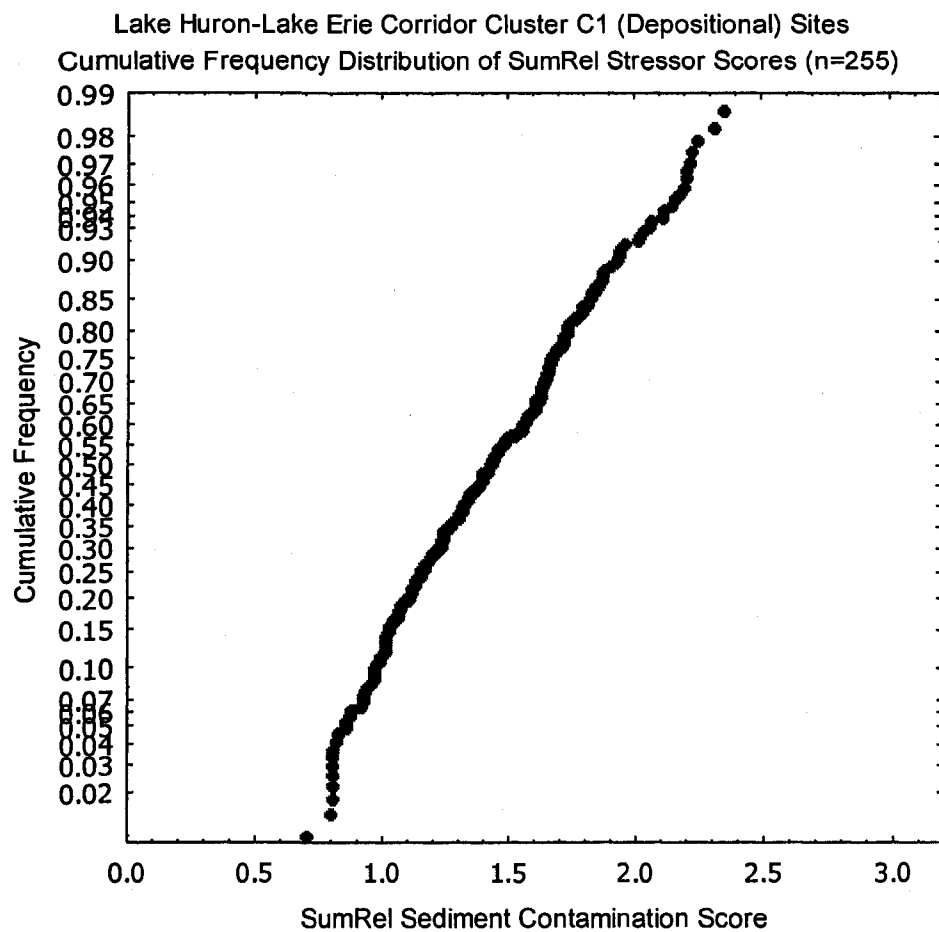


Figure 2.8. Cumulative Frequency Distribution of the sediment contamination scores (SumRel) for cluster C1 sites (n=255) in the Lake Huron-Lake Erie Corridor analysis

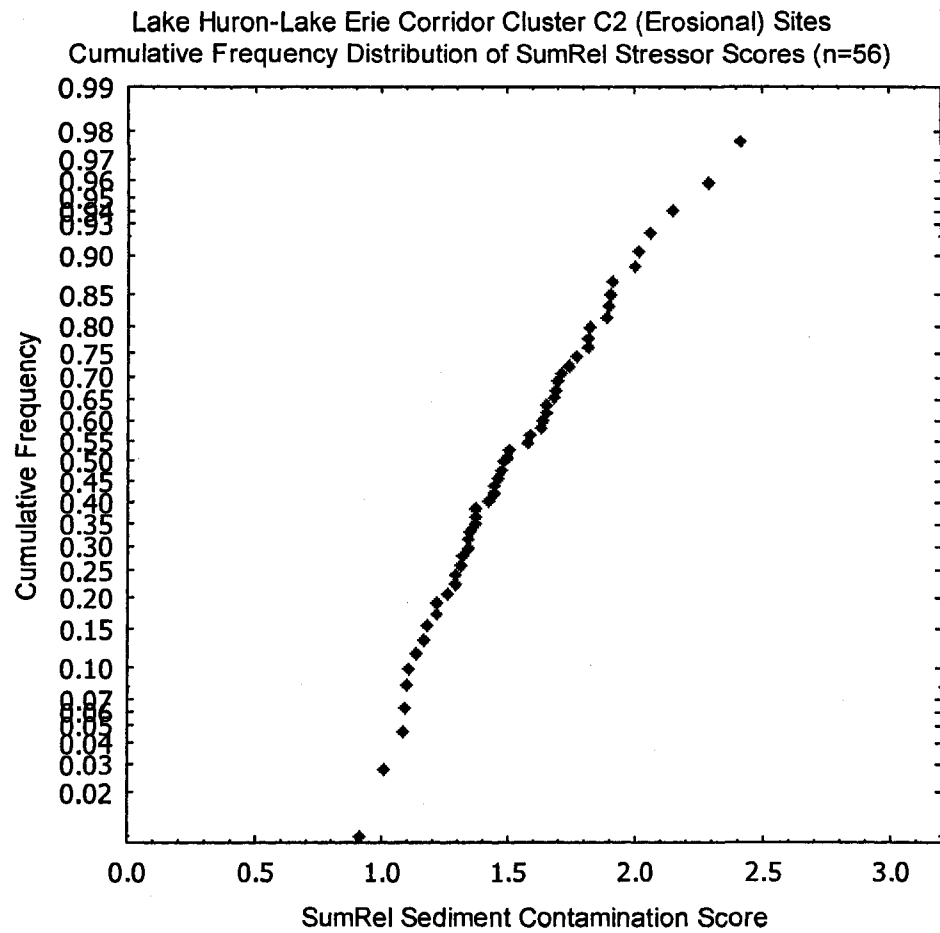


Figure 2.9. Cumulative Frequency Distribution of the sediment contamination scores (SumRel) for cluster C2 sites (n=56) in the Lake Huron-Lake Erie Corridor

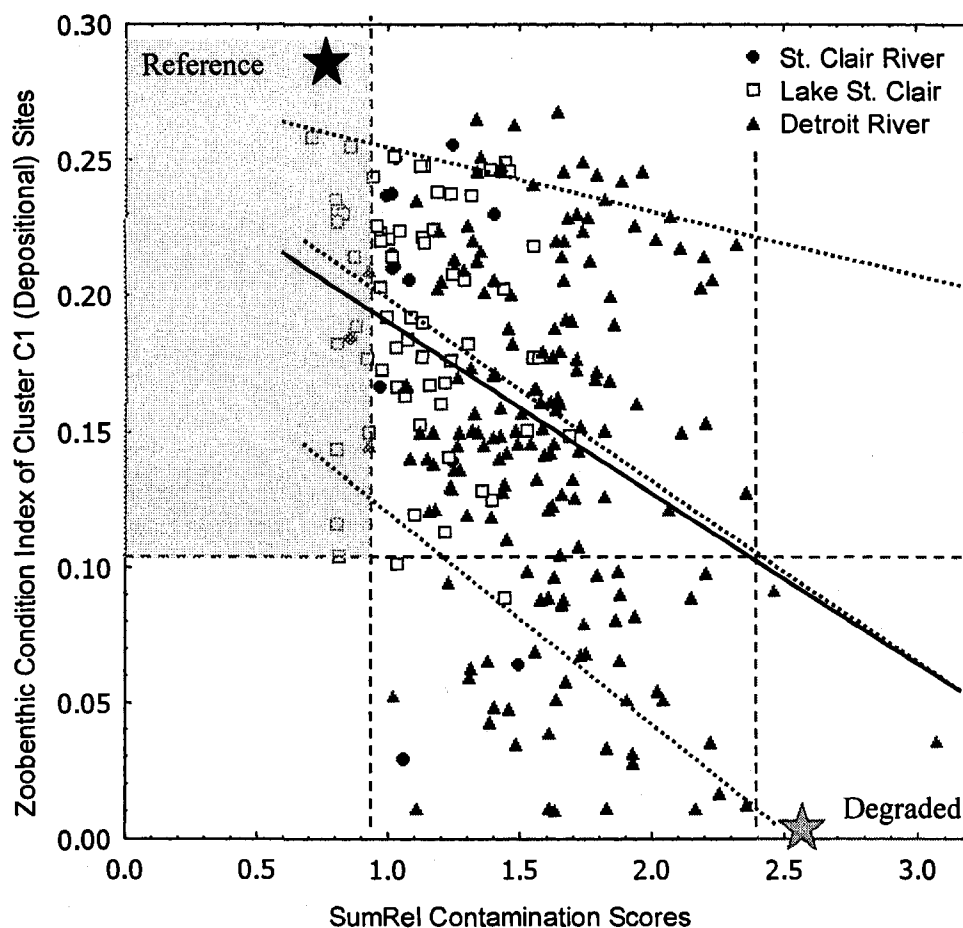


Figure 2.10. Relationship between Zoobenthic Condition Index (ZCI; Bray-Curtis zoobenthic relative abundance ordination scores) and the sediment contamination score (SumRel) for sites in cluster C1.  $n = 255$  sites. The site with black star indicates the REF endpoint (high ordination score together with low SumRel); the site with grey star indicates the DEG endpoint (low ordination score together with high SumRel). Solid line indicates the least square fit line; dashed lines indicate 0.9, median and 0.1 quantile linear regression lines, respectively. The horizontal and vertical lines separate the samples into sectors as would be identified by piecewise quantile regression. All sites with SumRel scores  $\leq 1.0$  have a ZCI score of 0.10 or greater. All sites with SumRel scores  $\geq 2.4$  have a ZCI score of  $< 0.10$ . Accordingly, depositional (C1) sites with ZCI scores  $> 0.10$  cannot be said to be degraded

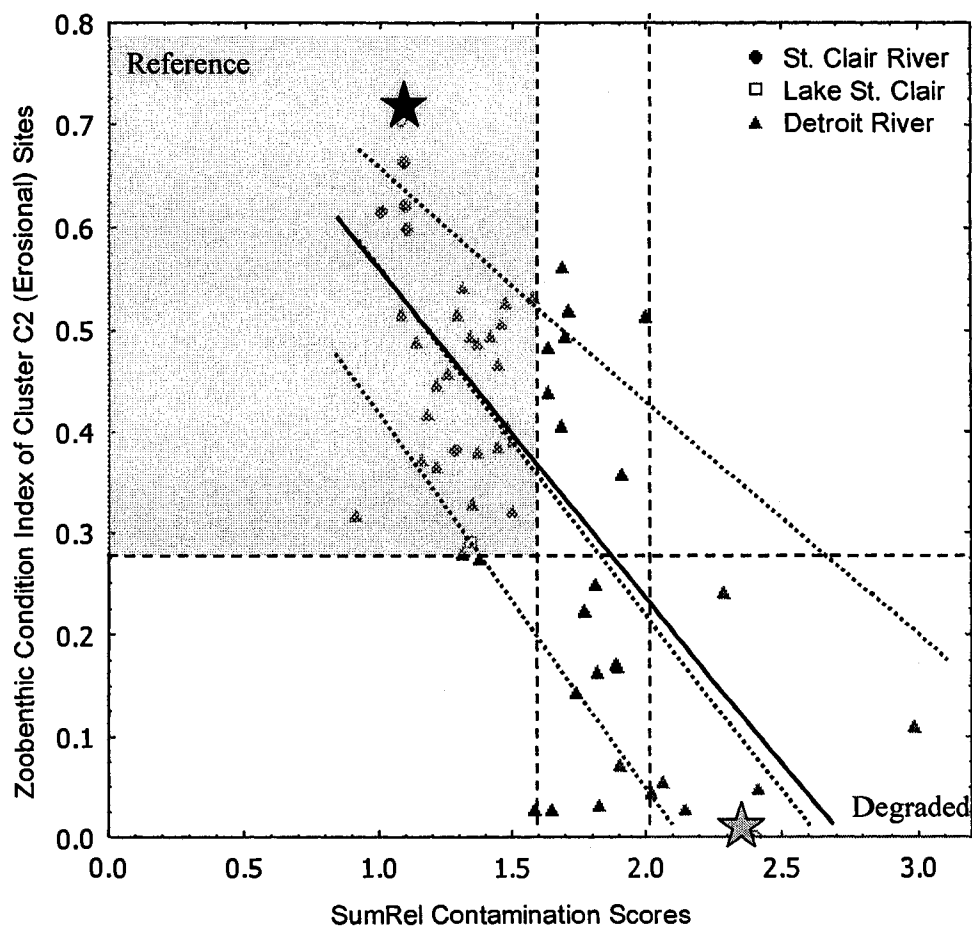


Figure 2.11. Relationship between Zoobenthic Condition Index (ZCI; Bray-Curtis zoobenthic relative abundance ordination scores) and the sediment contamination score (SumRel) for sites in cluster C2.  $n = 56$  sites. The site with black star indicates the REF endpoint (high ordination score together with low SumRel); the site with grey star indicates the DEG endpoint (low ordination score together with high SumRel). Solid line indicates the least square fit line; dashed lines indicate 0.9, median and 0.1 quantile linear regression lines, respectively. The horizontal and vertical lines separate the samples into sectors as would be identified by piecewise quantile regression. All sites with SumRel scores  $\leq 1.55$  have a ZCI score of 0.27 or greater. All sites with SumRel scores  $\geq 2.0$  have a ZCI score of  $< 0.27$ . Accordingly, erosional (C2) sites with ZCI scores  $> 0.27$  cannot be said to be degraded

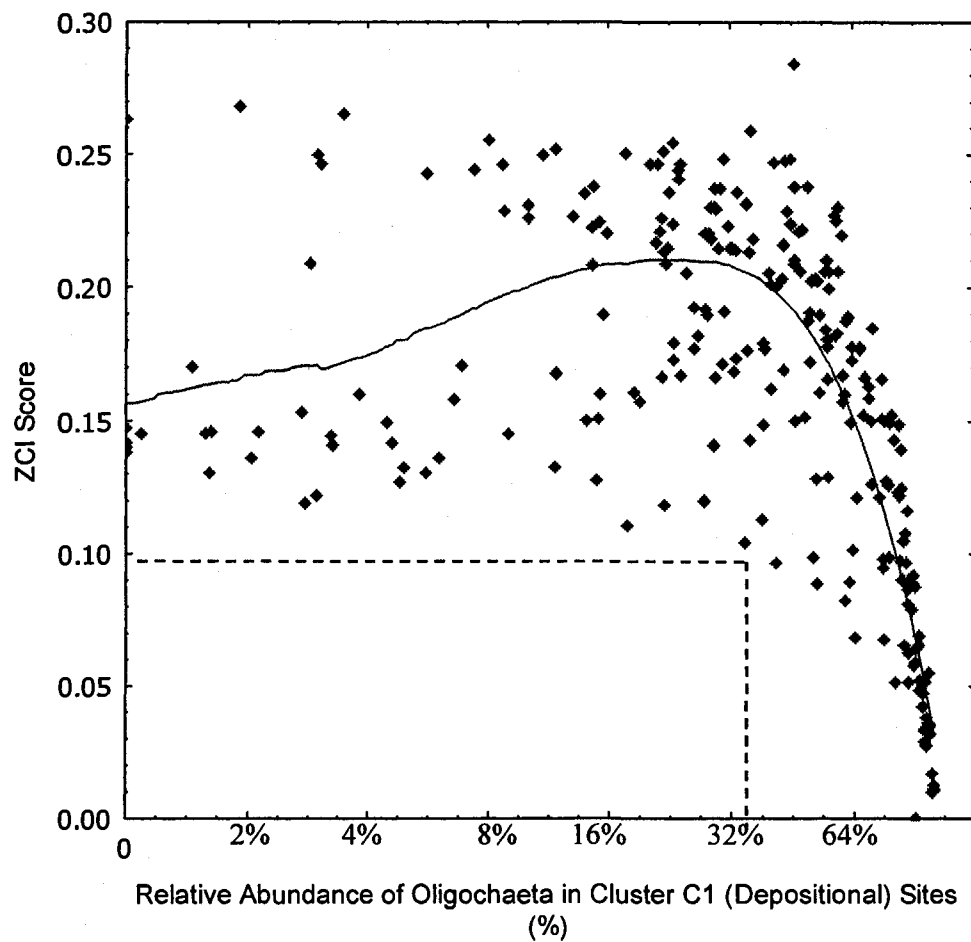


Figure 2.12. Relative abundance of Oligochaeta (%) in cluster C1 (Depositional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.10. Below a ZCI value of 0.10, the maximum relative abundance of Oligochaeta observed was more than 40% (vertical dashed line)

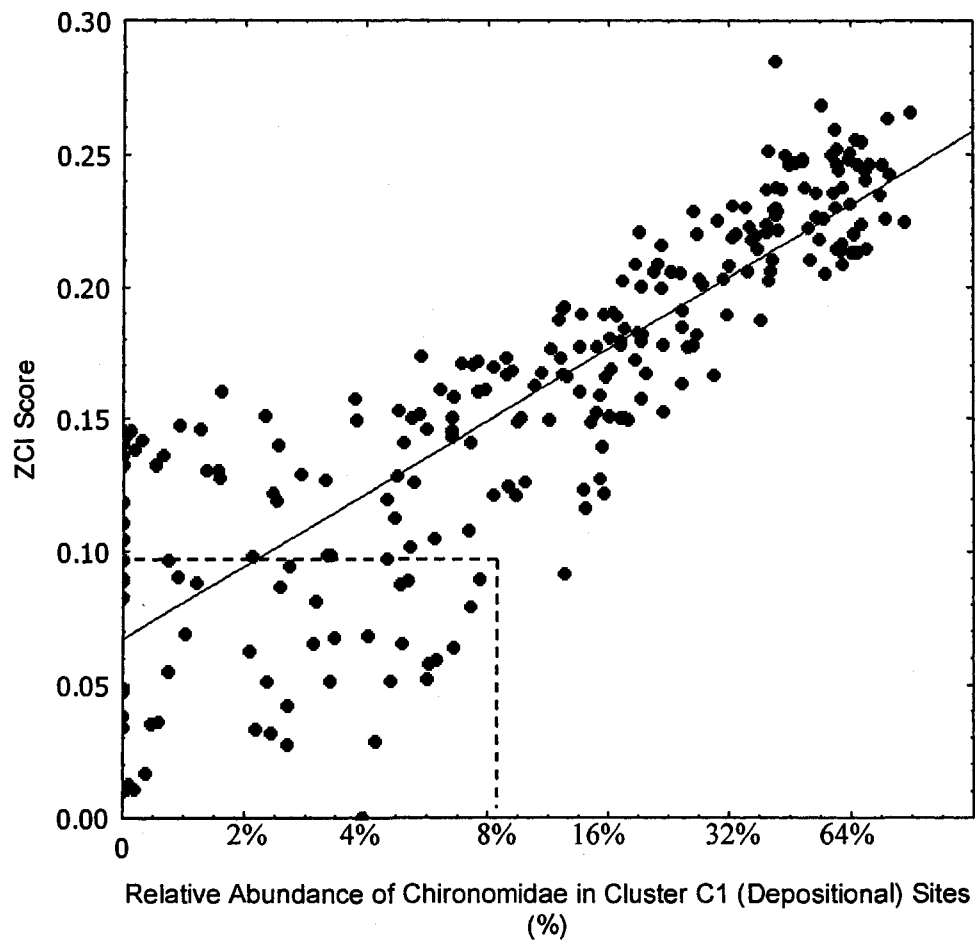


Figure 2.13. Relative abundance of Chironomidae (%) in cluster C1 (Depositional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.10. Below a ZCI value of 0.10, the maximum relative abundance of Chironomidae observed was less than 8% (vertical dashed line)

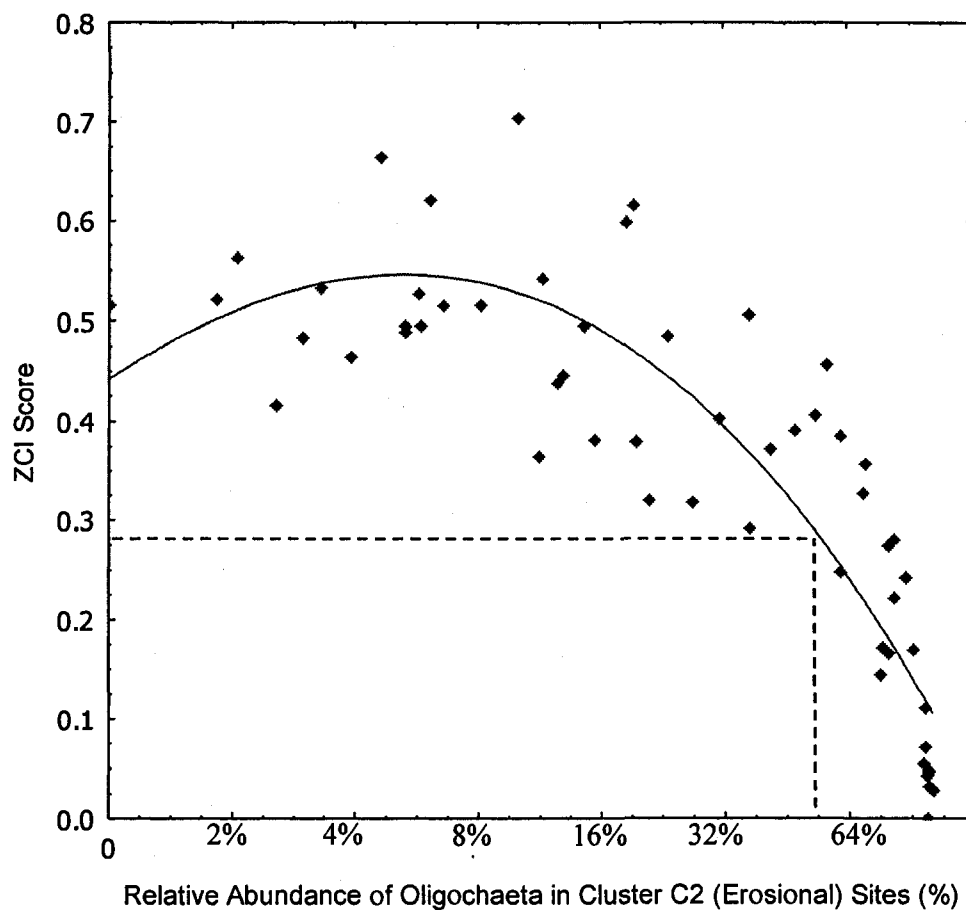


Figure 2.14. Relative abundance of Oligochaeta (%) in cluster C2 (Erosional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.27. Below a ZCI value of 0.27, the maximum relative abundance of Oligochaeta observed was more than 55% (vertical dashed line)



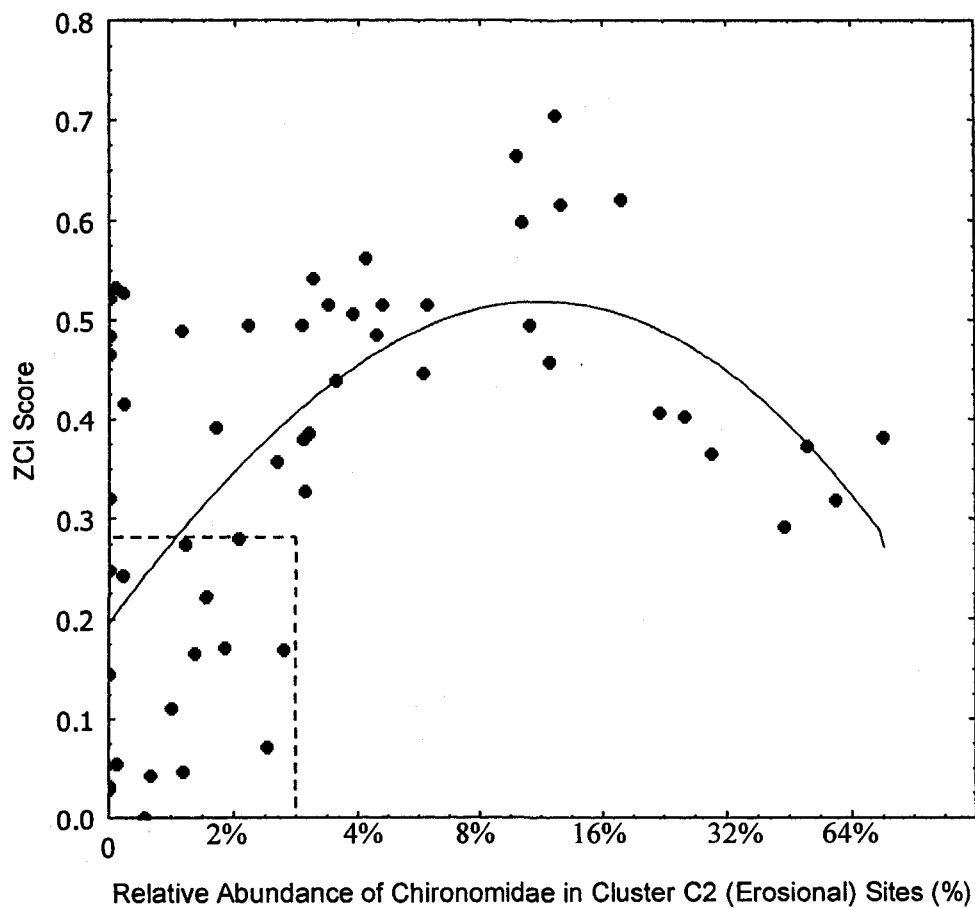


Figure 2.15. Relative abundance of Chironomidae (%) in cluster C2 (Erosional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.27. Below a ZCI value of 0.27, the maximum relative abundance of Chironomidae observed was less than 3% (vertical dashed line)

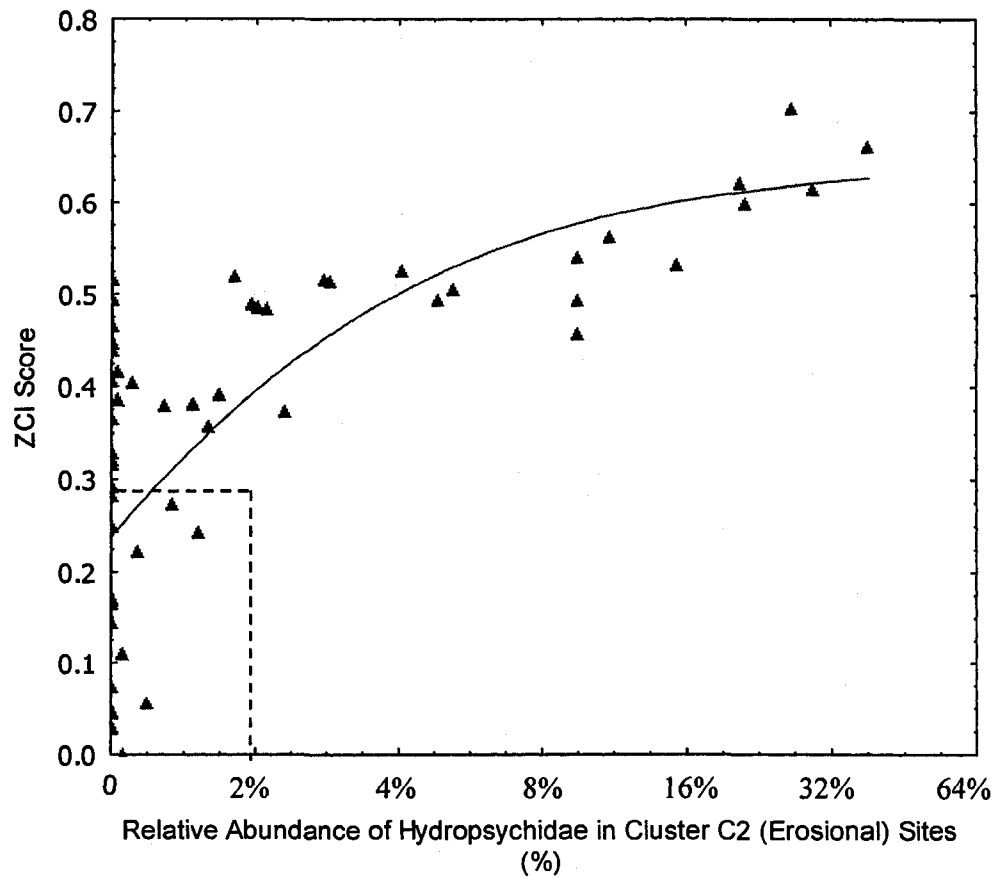


Figure 2.16. Relative abundance of Hydropsychidae (%) in cluster C2 (Erosional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.27. Below a ZCI value of 0.27, the maximum relative abundance of Hydropsychidae observed was less than 2% (vertical dashed line)

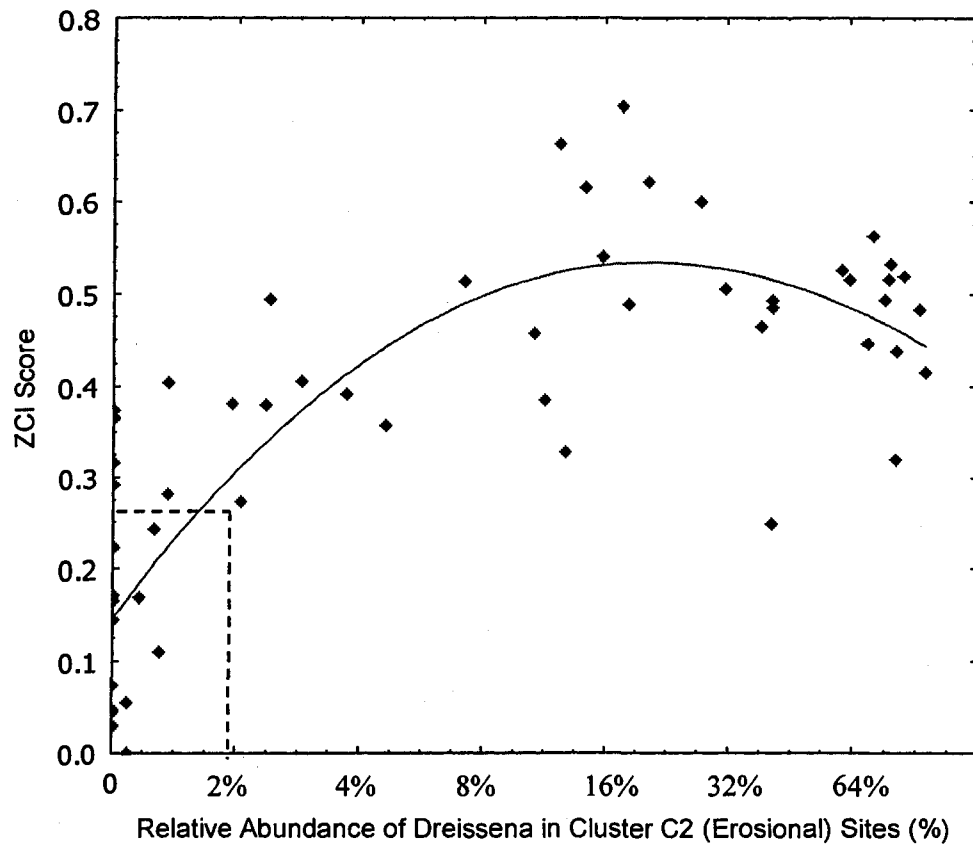


Figure 2.17. Relative abundance of *Dreissena* (%) in cluster C2 (Erosional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.27. Below a ZCI value of 0.27, the maximum relative abundance of *Dreissena* observed was less than 2% (vertical dashed line)

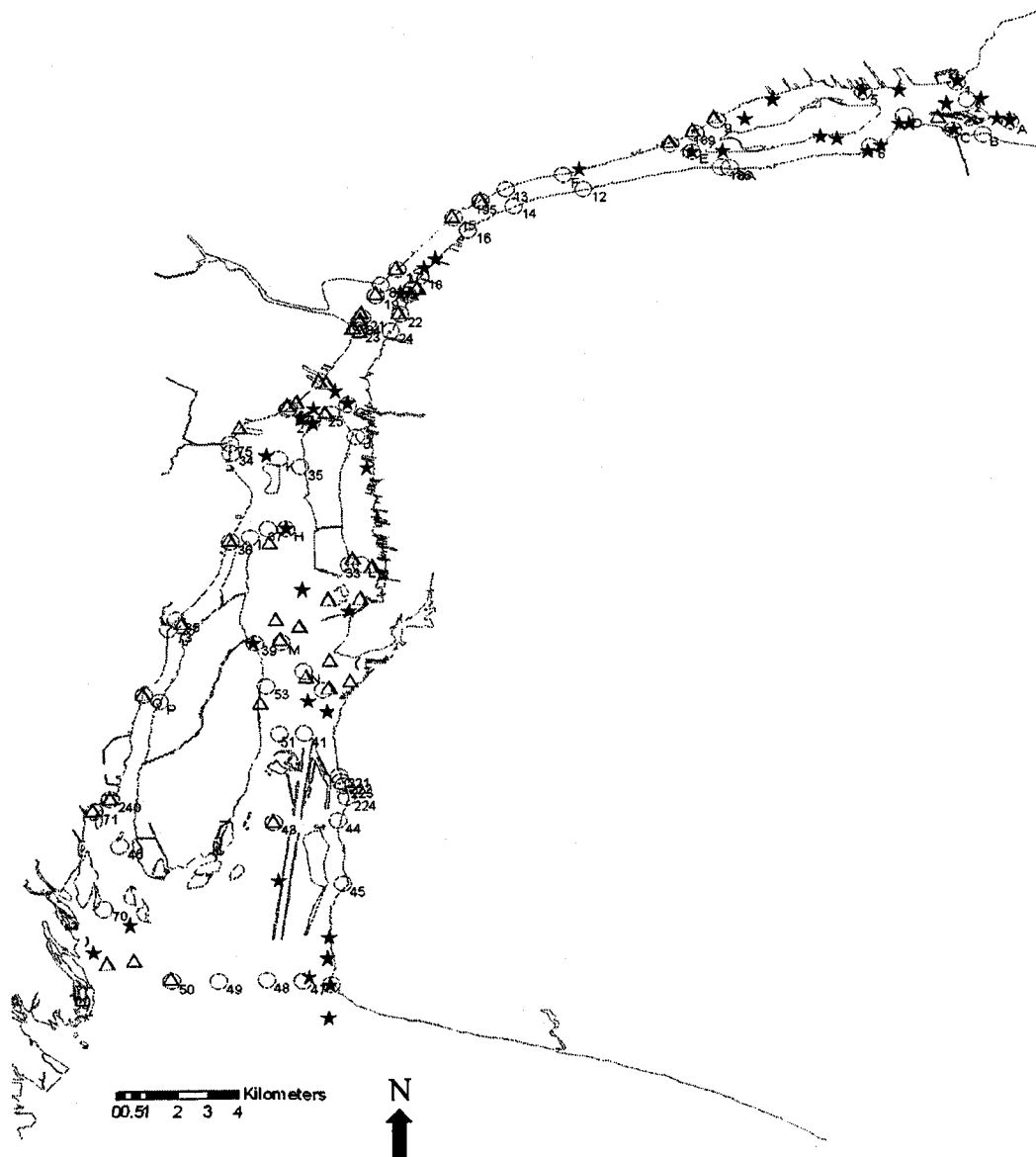


Figure 2.18. Distribution of REF and DEG sites in 1991, 1999 and 2004 Detroit River case study. (The site numbers shown in the map are 1991 sampling sites). 5-point stars indicate "REF" sites; triangles indicate "DEG" sites

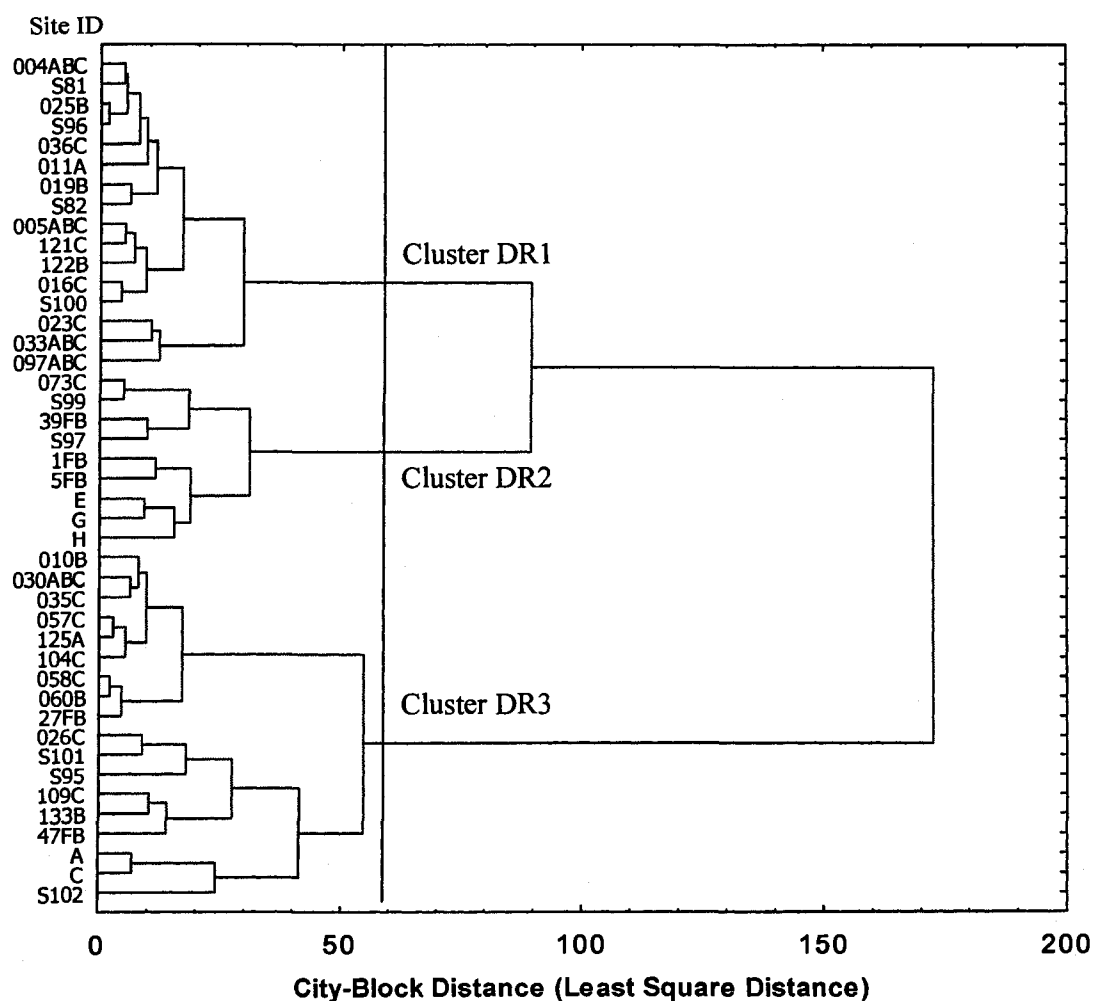


Figure 2.19. Dendrogram of REF Detroit River sites (n = 43) grouped according to similar zoobenthic community composition (Ward's method clustering city-block distances of octave-transformed relative abundances of zoobenthic taxa). Site locations corresponding to site labels are summarized in Appendix I)

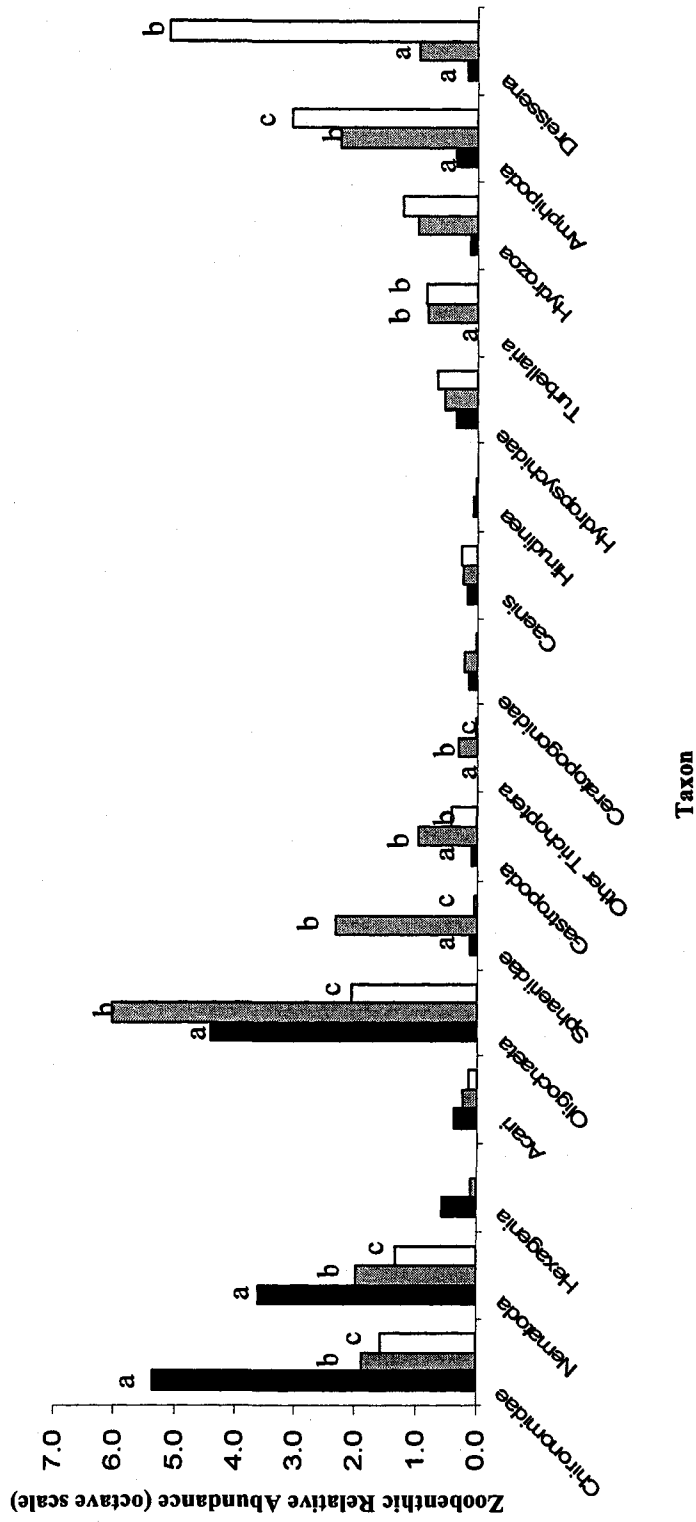


Figure 2.20. Mean value of taxa relative abundance (octave scale) for three clusters of REF zoobenthic communities found in the Detroit River. Black bars indicate cluster DR1 (depositional group), grey bars indicate cluster DR2 (mixed group) and white bars indicate cluster DR3 (erosional group). Members of a group with the same letter have means that are not significantly different from one another ( $p > 0.05$ )



Figure 2.21. Distribution of sampling sites belonging to particular clusters (the site numbers showed up in the map are 1991 sampling sites). 5-point stars indicate cluster DR1, black crosses indicate cluster DR2, triangles indicate cluster DR3

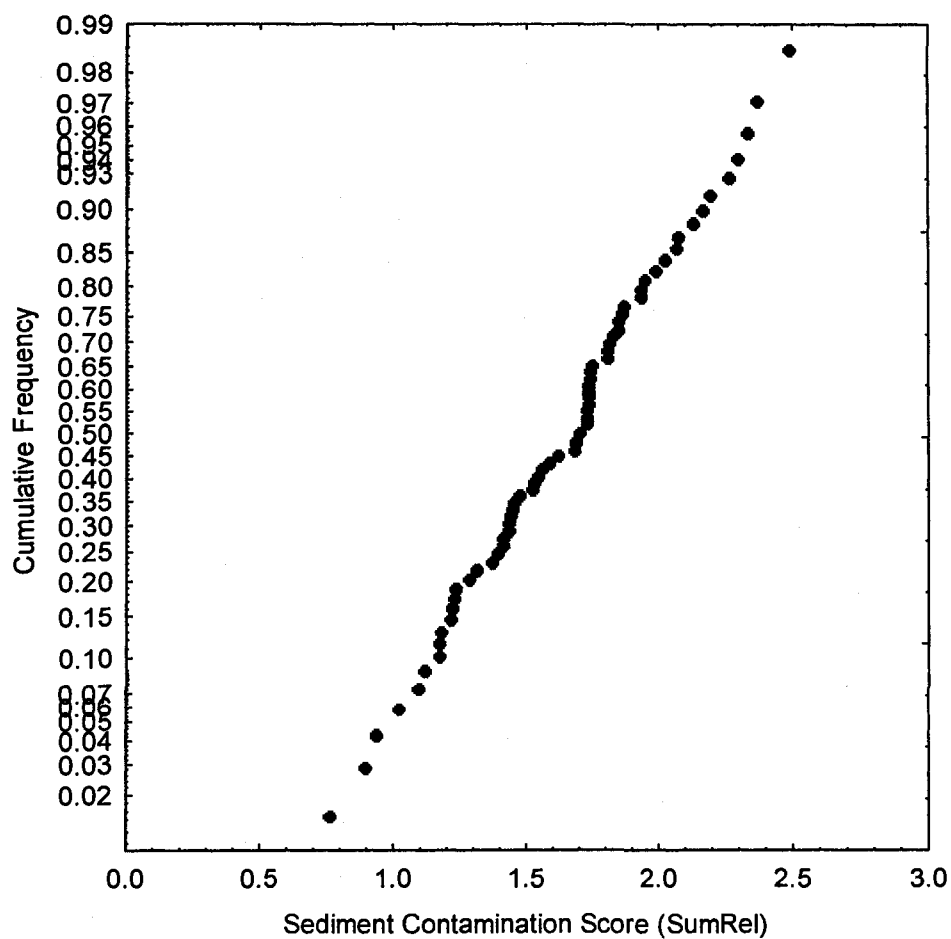


Figure 2.22. Cumulative Frequency Distribution of the sediment contamination scores (SumRel) for cluster DR1 sites (n=69) in the Detroit River case study



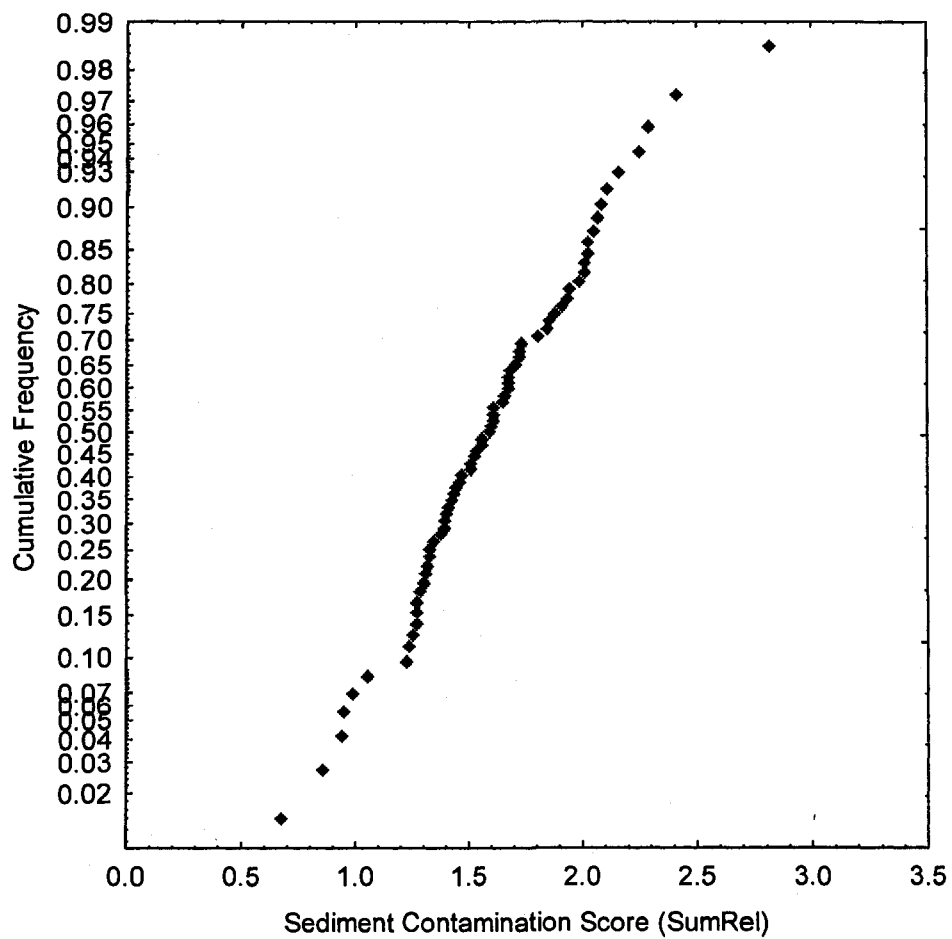


Figure 2.23. Cumulative Frequency Distribution of the sediment contamination scores (SumRel) for cluster DR2 sites (n=72) in the Detroit River case study

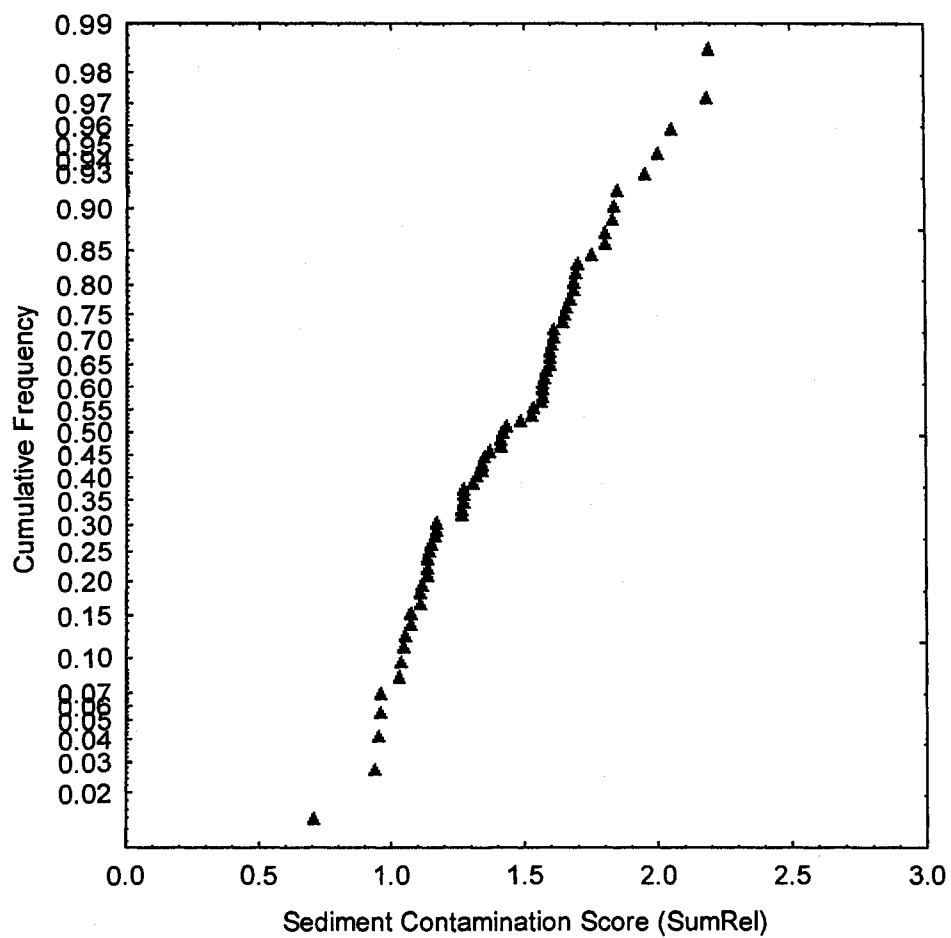


Figure 2.24. Cumulative Frequency Distribution of the sediment contamination scores (SumRel) for cluster DR3 sites (n=72) in the Detroit River case study

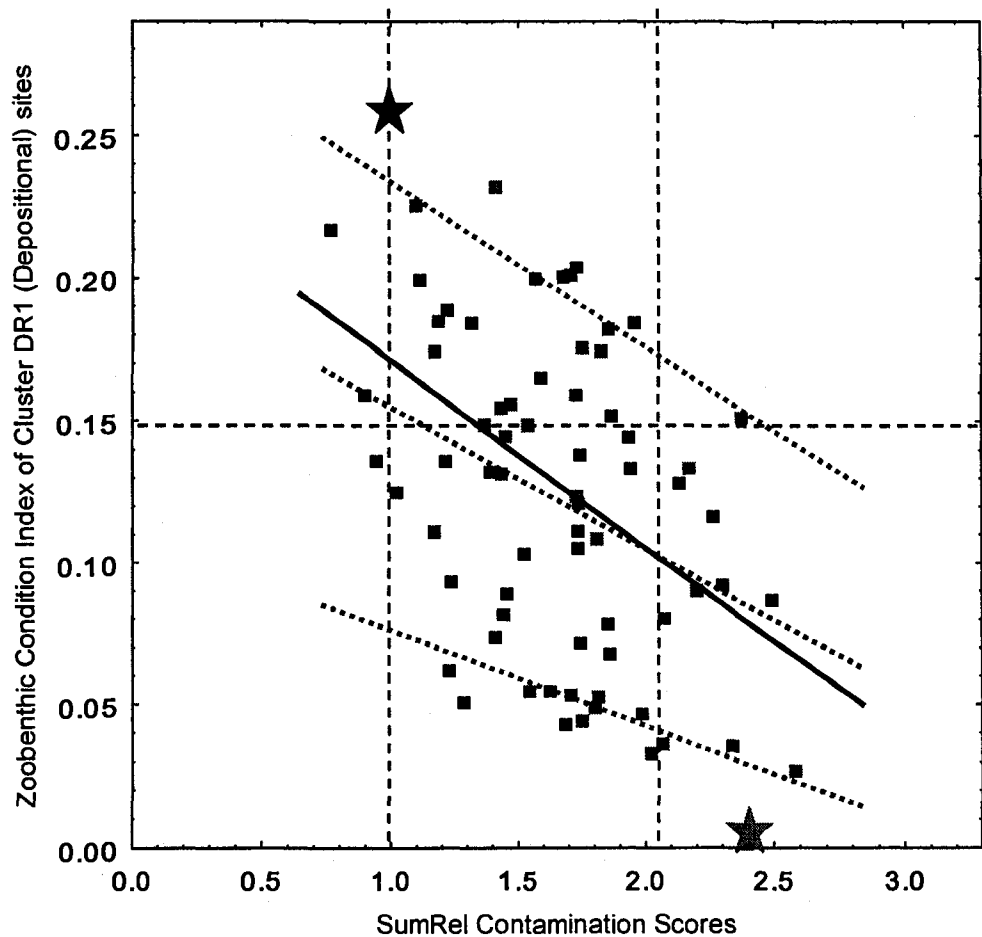


Figure 2.25. Relationship between Zoobenthic Condition Index (Bray-Curtis zoobenthic relative abundance ordination scores) and the sediment contamination score (SumRel) for sites in cluster DR1.  $n = 69$  sites. The site with black star indicates the REF endpoint (high ordination score together with low SumRel); the site with grey star indicates the DEG endpoint (low ordination score together with high SumRel). Solid line indicates the least square fit line; dotted lines indicate 0.9, median and 0.1 quantile lines, respectively. The horizontal and vertical lines separate the samples into sectors as would be identified by piecewise quantile regression. All sites with SumRel scores  $\leq 1.0$  have a ZCI score of 0.15 or greater. All sites with SumRel scores  $\geq 2.0$  have a ZCI score of  $< 0.15$ . Accordingly, depositional (DR1) sites with ZCI scores  $> 0.15$  cannot be said to be degraded

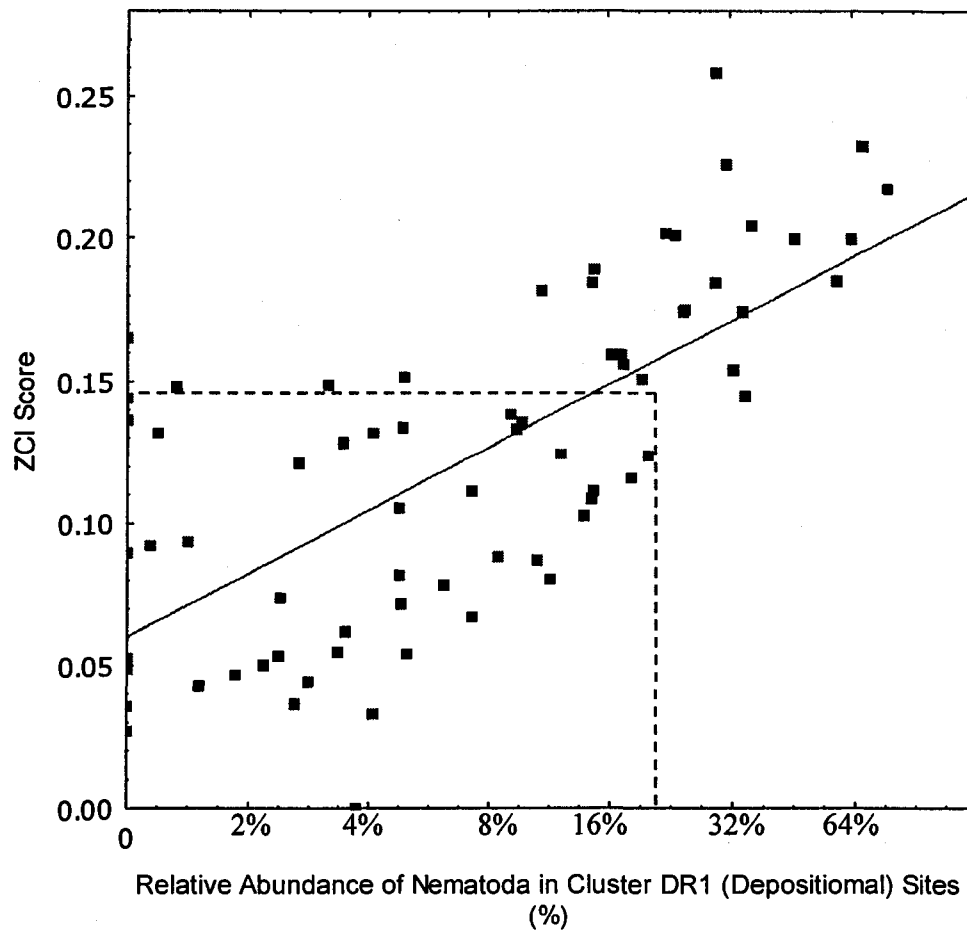


Figure 2.26. Relative abundance of Nematoda (%) in cluster DR1 (Depositional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.15. Below a ZCI value of 0.15, the maximum relative abundance of Nematoda observed was less than 20% (vertical dashed line)

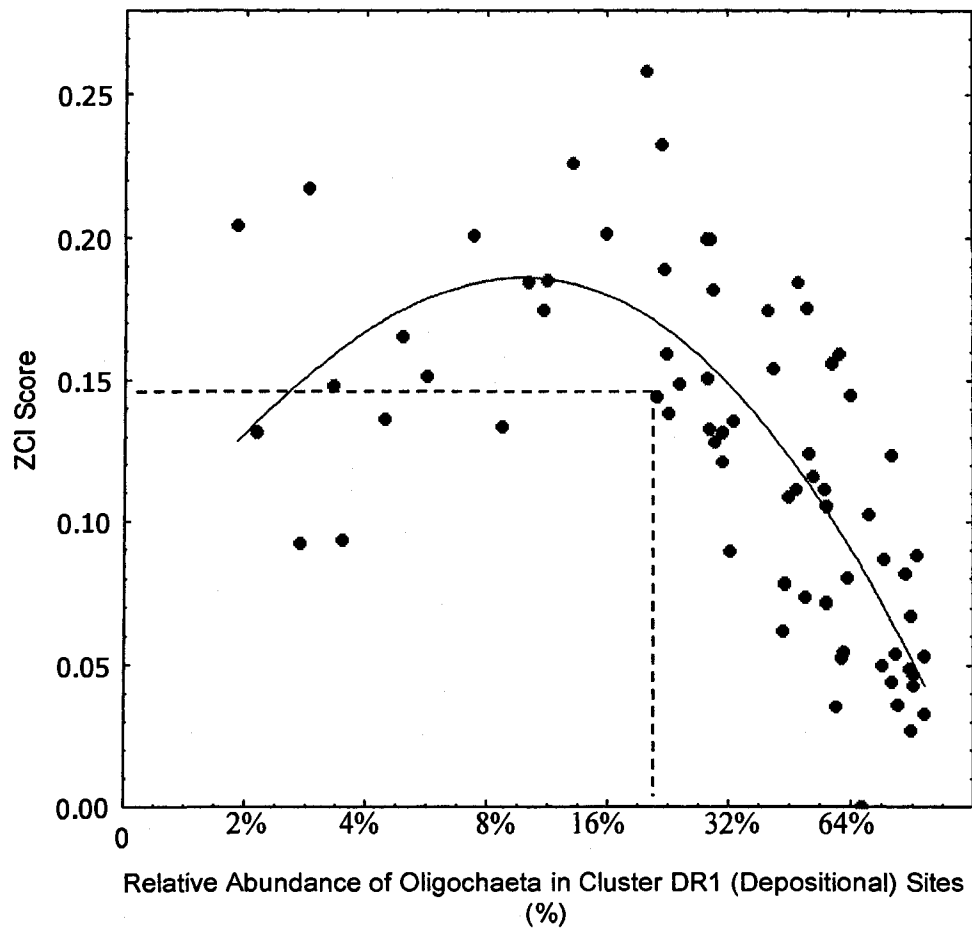


Figure 2.27. Relative abundance of Oligochaeta (%) in cluster DR1 (Depositional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.15. Below a ZCI value of 0.15, the relative abundance of Oligochaeta observed was more than 21% in most of the cluster DR1 sites (vertical dashed line)

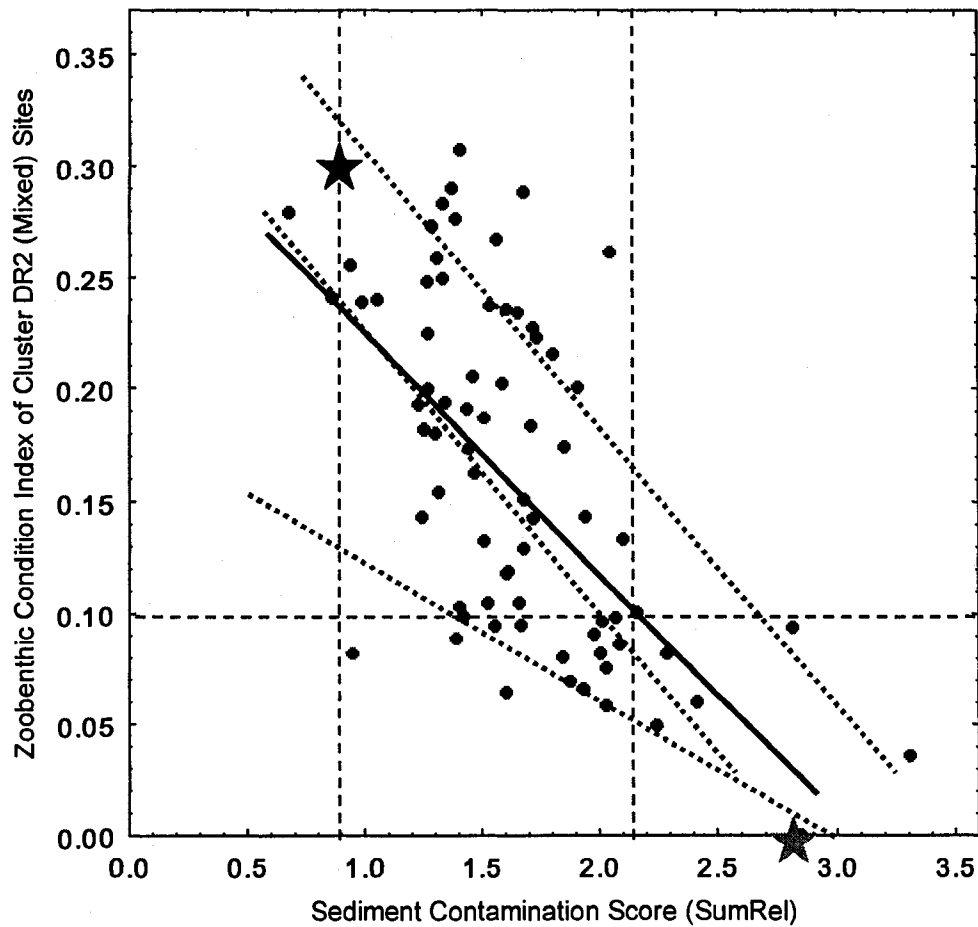


Figure 2.28. Relationship between Zoobenthic Condition Index (Bray-Curtis zoobenthic relative abundance ordination scores) and the sediment contamination scores (SumRel) for sites in cluster DR2.  $n = 72$  sites. The site with black star indicates the REF endpoint (high ordination score together with low SumRel); the site with grey star indicates the DEG endpoint (low ordination score together with high SumRel). Solid line indicates the least square fit line; dotted lines indicate 0.9, median and 0.1 quantile lines, respectively. The horizontal and vertical lines separate the samples into sectors as would be identified by piecewise quantile regression. All sites with SumRel scores  $\leq 0.90$  have a ZCI score of 0.10 or greater. All sites with SumRel scores  $\geq 2.1$  have a ZCI score of  $< 0.10$ . Accordingly, mixed (DR2) sites with ZCI scores  $> 0.10$  cannot be said to be degraded

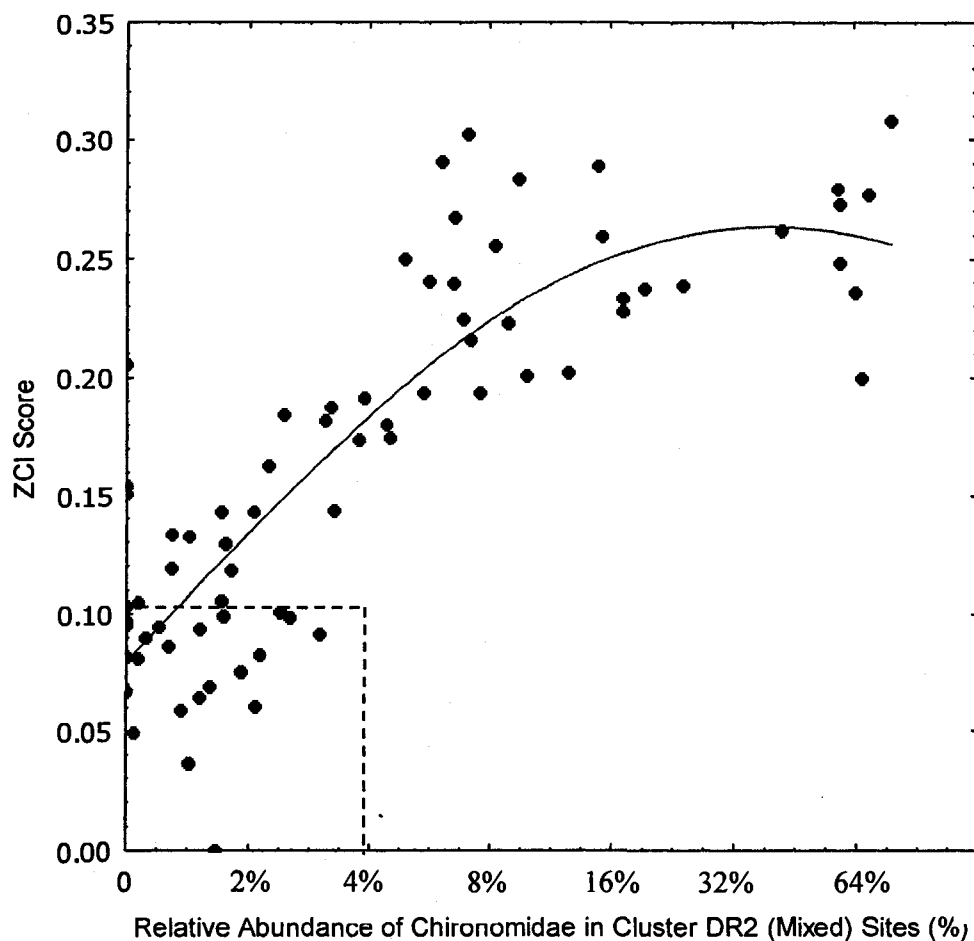


Figure 2.29. Relative abundance of Chironomidae (%) in cluster DR2 (Mixed) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.10. Below a ZCI value of 0.10, the maximum relative abundance of Chironomidae observed was less than 3.8% (vertical dashed line)

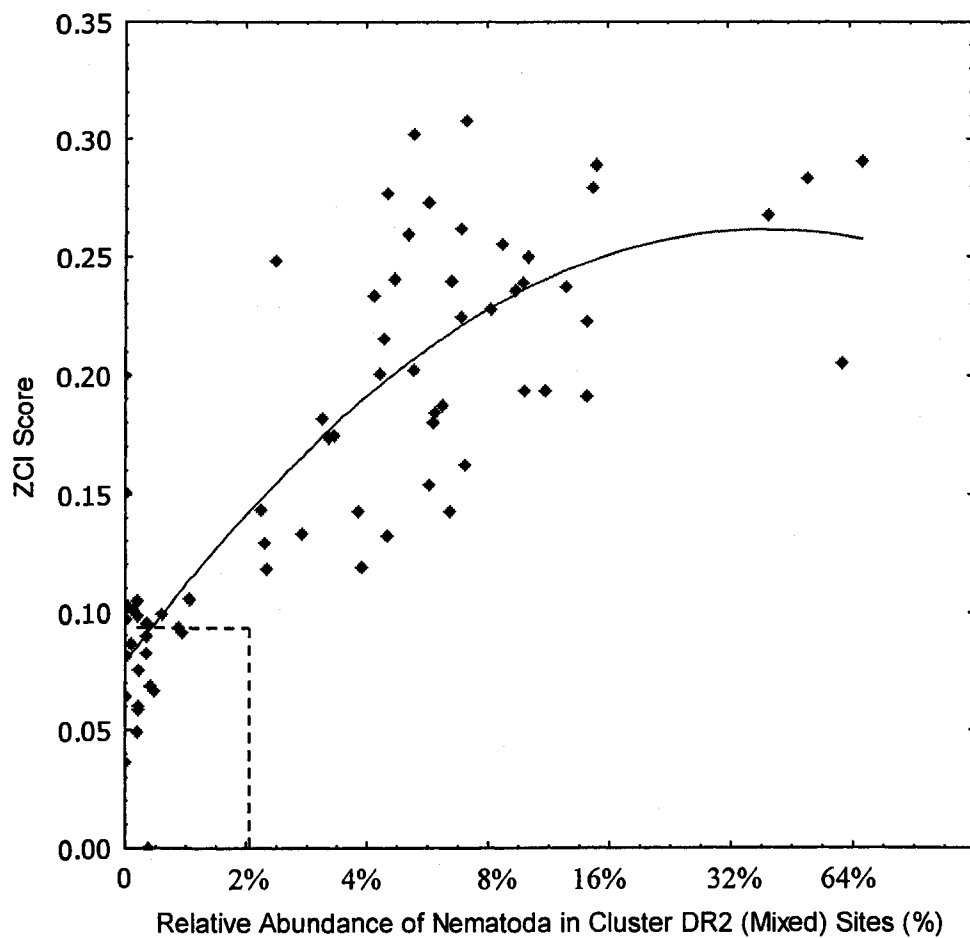


Figure 2.30. Relative abundance of Nematoda (%) in cluster DR2 (Mixed) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.10. Below a ZCI value of 0.10, the maximum relative abundance of Nematoda observed was less than 2% (vertical dashed line)



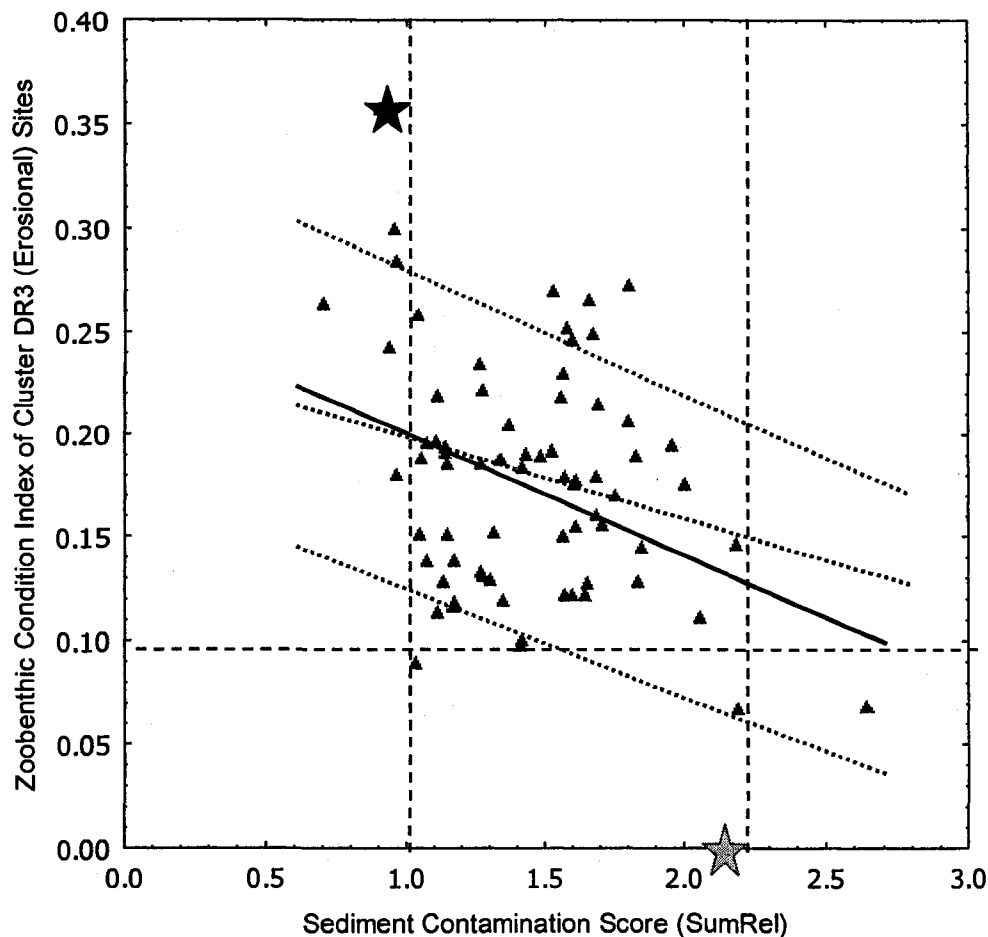


Figure 2.31. Relationship between Zoobenthic Condition Index (Bray-Curtis zoobenthic relative abundance ordination scores) and the sediment contamination scores (SumRel) for sites in cluster DR3.  $n = 72$  sites. The site with black star indicates the REF endpoint (high ordination score together with low SumRel); the site with grey star indicates the DEG endpoint (low ordination score together with high SumRel). Solid line indicates the least square fit line; dotted lines indicate 0.9, median and 0.1 quantile lines, respectively. The horizontal and vertical lines separate the samples into sectors as would be identified by piecewise quantile regression. All sites with SumRel scores  $\leq 0.95$  have a ZCI score of 0.10 or greater. All sites with SumRel scores  $> 2.2$  have a ZCI score of  $< 0.10$ . Accordingly, depositional (DR3) sites with ZCI scores  $> 0.10$  cannot be said to be degraded

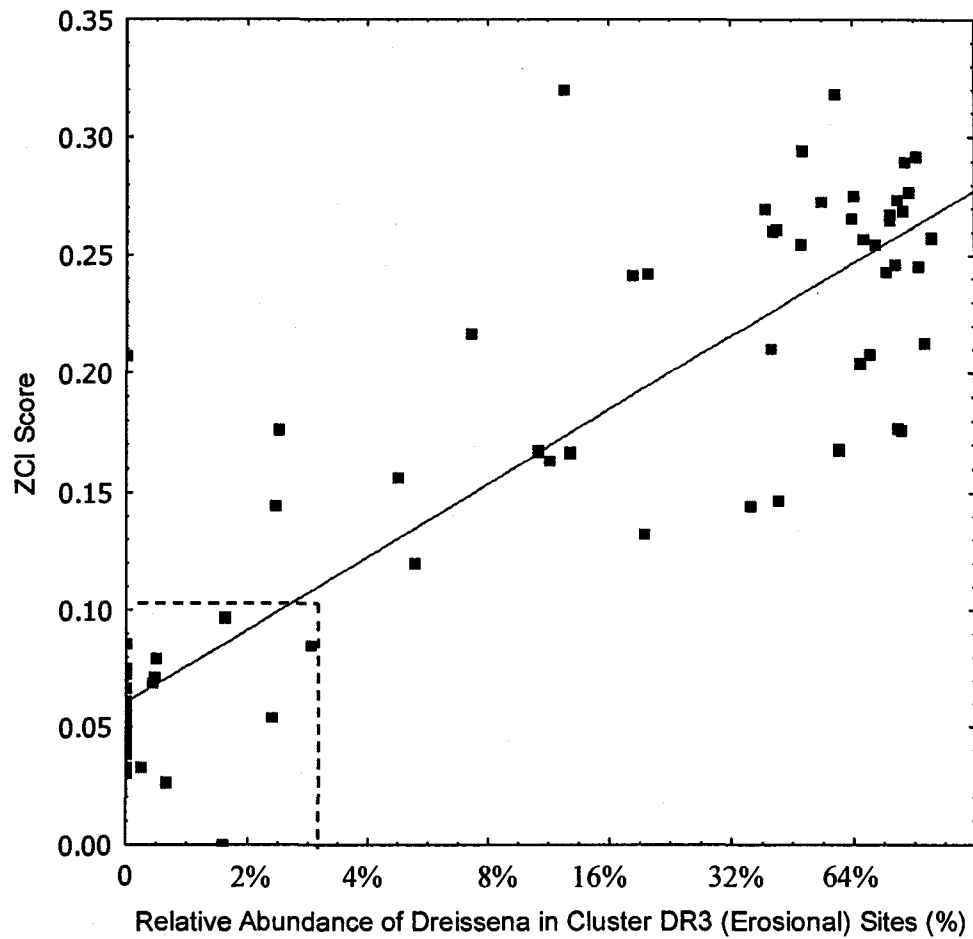


Figure 2.32. Relative abundance of *Dreissena* (%) in cluster DR3 (Erosional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites based on ZCI boundary score of 0.10. Below a ZCI value of 0.10, the maximum relative abundance of *Dreissena* observed was less than 3% (vertical dashed line)

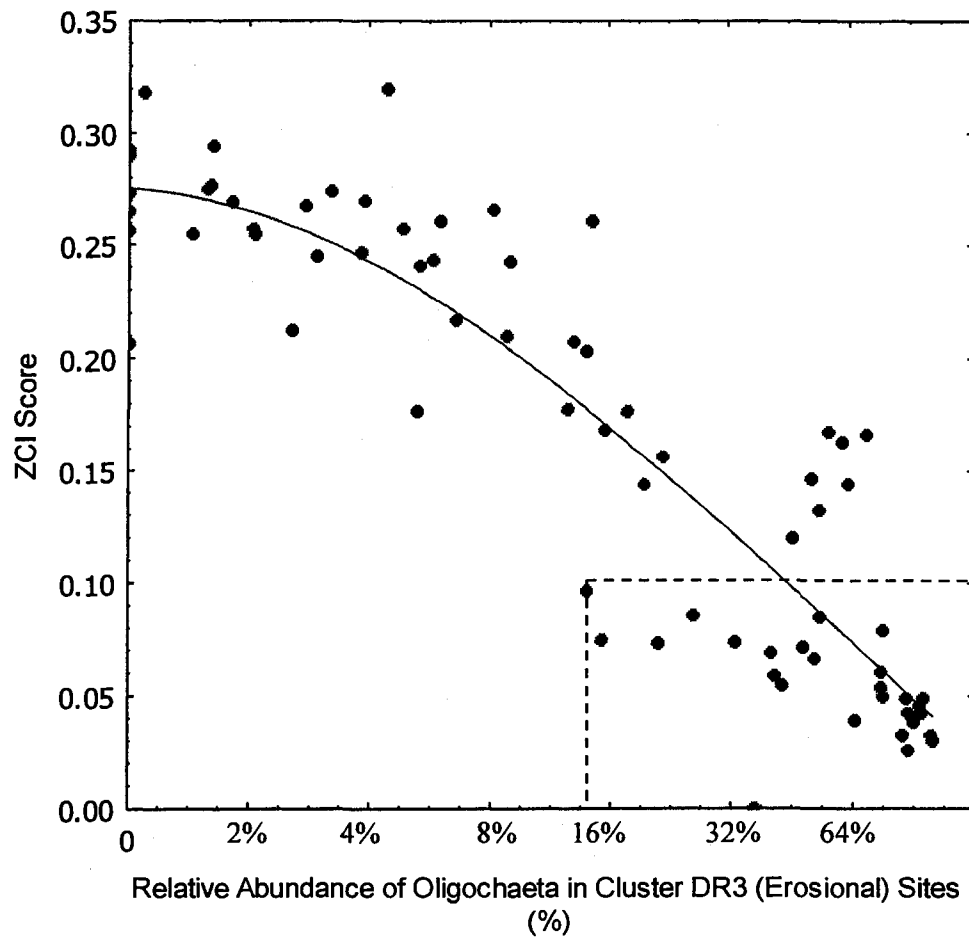
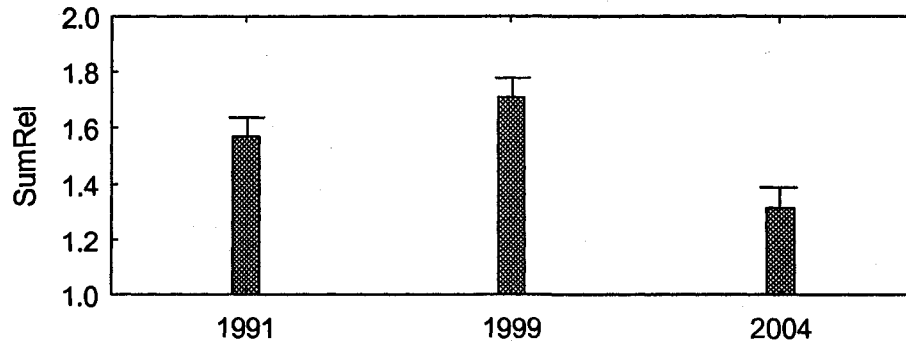


Figure 2.33. Relative abundance of Oligochaeta (%) in cluster DR3 (Erosional) sites along the ZCI gradient. Solid line is a distance-weighted least square fit through the data points. Horizontal dashed line represents the putative boundary between 'degraded' and less contaminated sites base on ZCI boundary score of 0.10. Below a ZCI value of 0.10, the minimum relative abundance of Oligochaeta observed was 16% (vertical dashed line)

Cluster DR1 (Depositional)

Current effect:  $F(2, 66) = 3.5491, p = .03436$

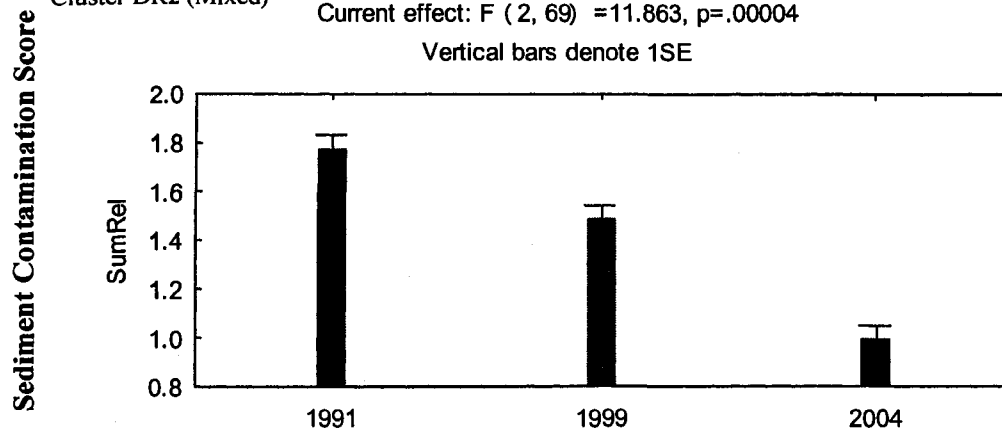
Vertical bars denote 1SE



Cluster DR2 (Mixed)

Current effect:  $F(2, 69) = 11.863, p = .00004$

Vertical bars denote 1SE



Cluster DR3 (Erosional)

Current effect:  $F(2, 69) = 1.8208, p = .16959$

Vertical bars denote 1SE

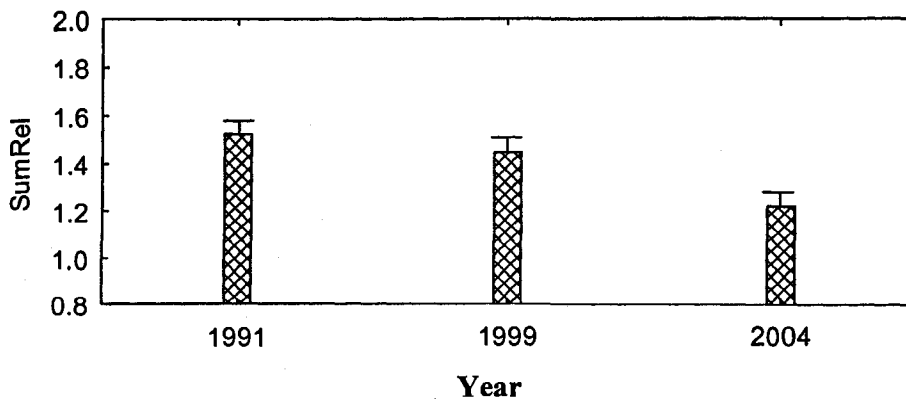


Figure 2.34. Mean SumRel sediment contamination scores of 3 cluster sites among years 1991, 1999 and 2004 in the Detroit River (Detroit River case study). Vertical bars denote 1 Standard Error

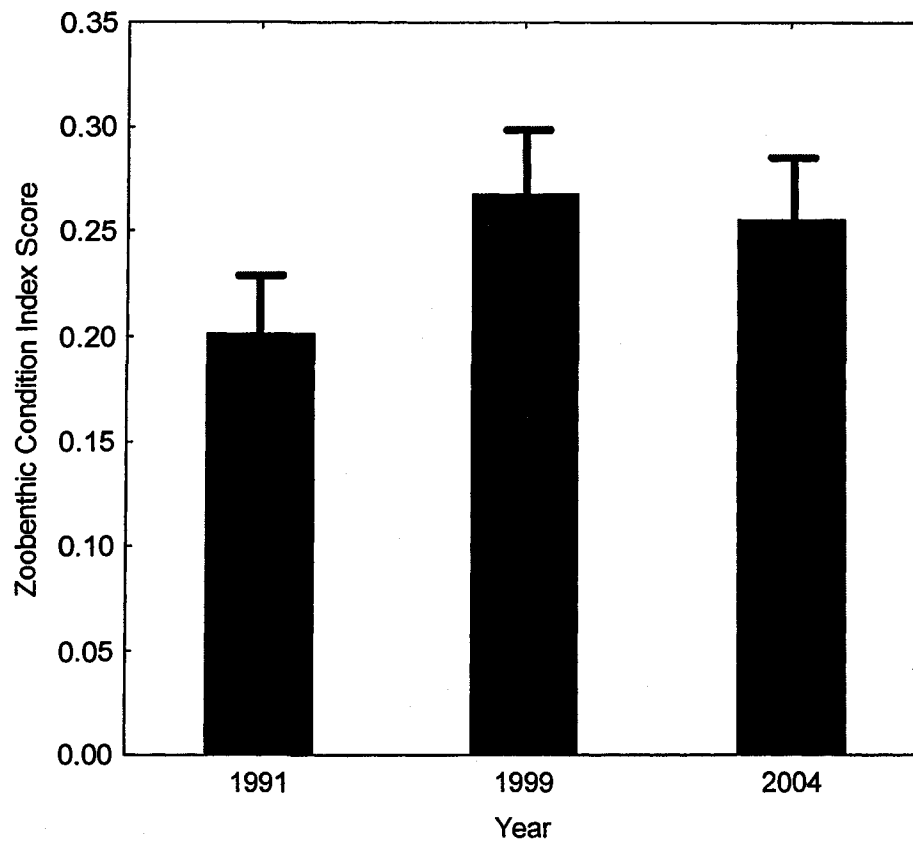


Figure 2.35. Comparison of mean Zoobenthic Condition Index (ordination scores) at 8 corresponding sites in the Detroit River among 3 years (1991, 1999 and 2004). Repeated measures ANOVA  $F_{[2,14]} = 3.15$ ,  $p = 0.074$ . Vertical bars denote 1 SE

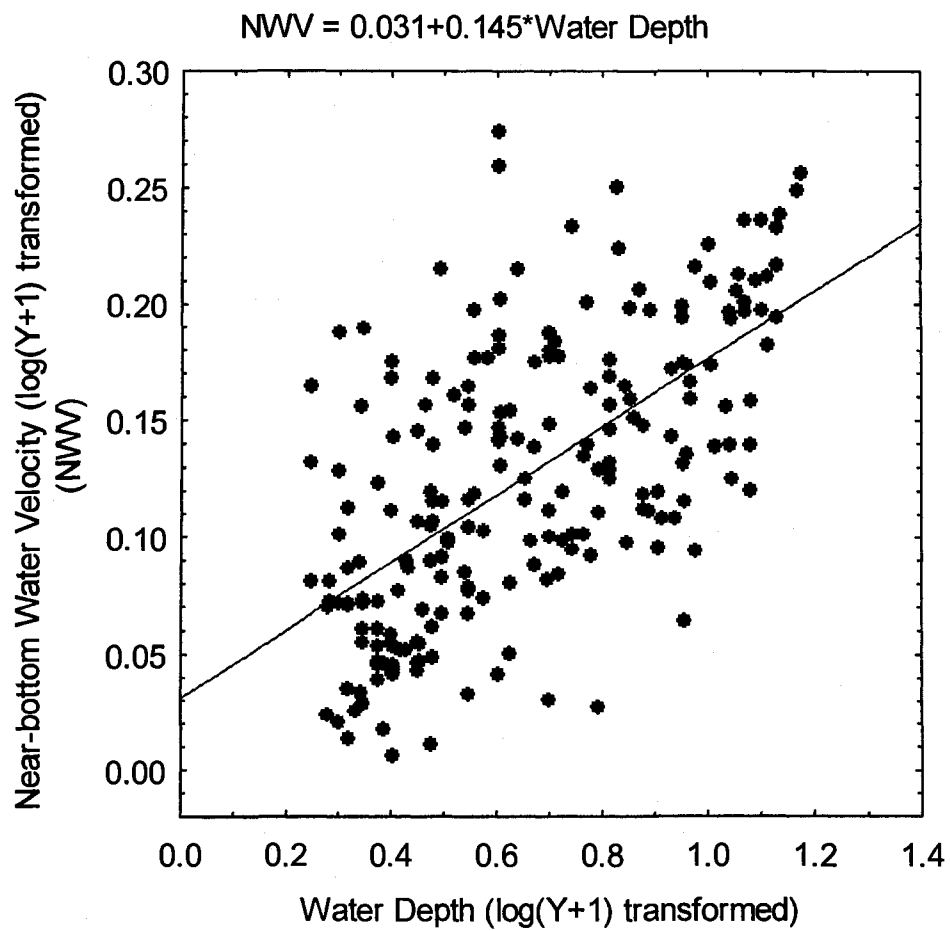


Figure 2.36. Correlation between near-bottom water velocity and water depth of sites sampled in the Detroit River 1991, 1999 and 2004 (n = 213)

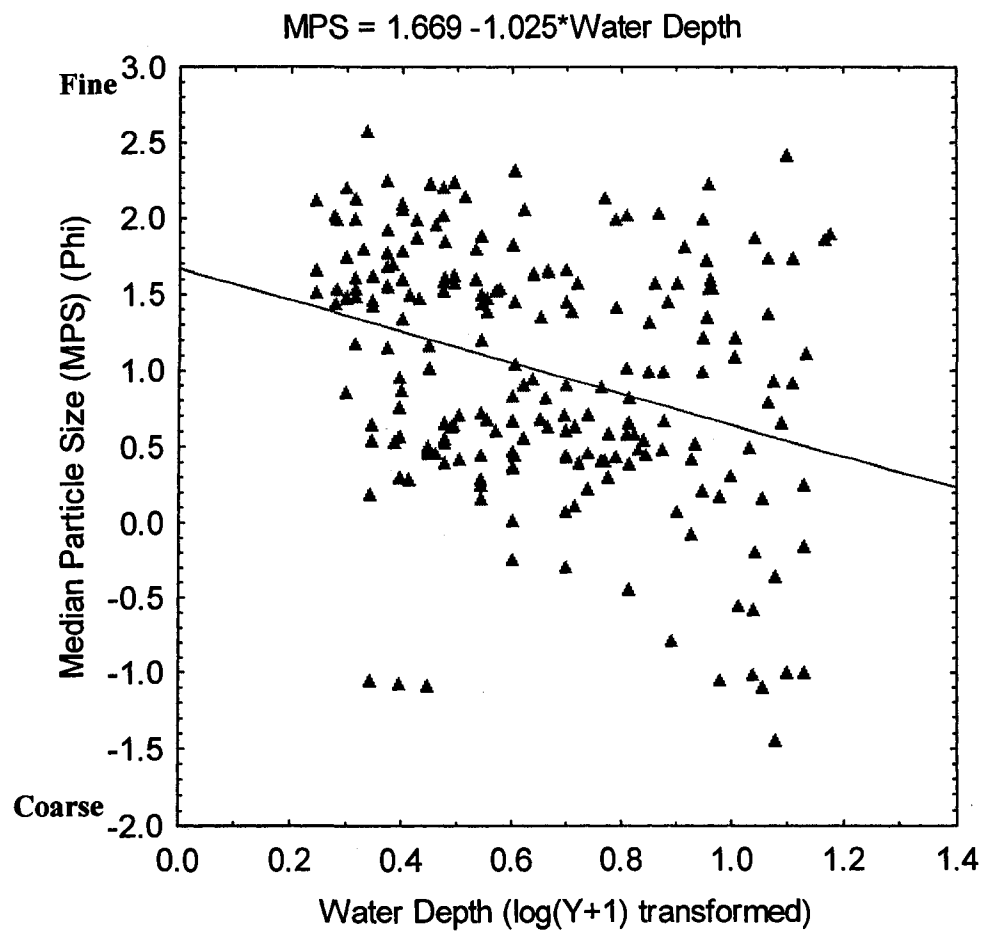


Figure 2.37. Correlation between median particle size and water depth of sites sampled in the Detroit River 1991, 1999 and 2004 ( $n = 213$ )

Table 2.1. Numbers of zoobenthos sorted and quality controlled by research assistants for the 2004/5 Lake Huron-Lake Erie Corridor survey

Site ID	Replicate	Number Missed	Total Number in the sample	Percent Efficiency (%)
S81	2	4	125	97
S24	2	7	328	98
S15	3	2	240	99
S52	3	3	113	97
S13	3	3	348	99
S96	1	7	75	91
S80	2	5	155	97
S68	1	8	256	97
S27	2	0	152	100
S59	3	15	389	96



Table 2.2. Correlation (factor loading) between values of 16 chemical variables measured at 311 Lake Huron-Lake Erie Corridor sites and 5 principal component factors. Variables combined in 5 factors are shown in bold face

Stressor variables	PC1	PC2	PC3	PC4	PC5
Co	<b>0.91</b>	0.18	0.16	0.12	0.04
Al	<b>0.90</b>	-0.04	0.02	0.08	0.19
Ni	<b>0.82</b>	0.50	0.13	0.14	0.06
Mn	<b>0.74</b>	0.38	0.16	0.15	0.36
Fe	<b>0.72</b>	0.41	0.10	0.15	0.11
Cr	<b>0.71</b>	0.62	0.10	0.13	0.03
Cu	<b>0.65</b>	0.64	0.03	0.14	0.02
Hg	-0.03	<b>0.81</b>	0.08	-0.07	0.17
Pb	0.42	<b>0.80</b>	0.07	0.17	0.02
Zn	0.55	<b>0.71</b>	0.07	-0.02	0.06
SumPCBs	0.27	<b>0.65</b>	0.44	0.18	-0.21
Cd	0.34	<b>0.58</b>	0.18	0.55	0.04
OCS	0.05	0.02	<b>0.86</b>	0.00	0.30
p,p'-DDE	0.31	0.34	<b>0.66</b>	0.07	-0.35
As	0.13	0.05	0.00	<b>0.96</b>	0.03
Ca	0.34	0.09	0.12	0.06	<b>0.84</b>
Explained Variance	5.18	4.10	1.52	1.42	1.18
Proportion of total variance	0.32	0.26	0.09	0.09	0.07
Cum. Proportion	0.32	0.58	0.67	0.76	<b>0.84</b>

Table 2.3. Mean ( $\pm$  1SE) concentration of 16 sediment chemicals (log (Y+1)) and PC factor scores among REF, TEST and DEG sites in 1991, 1999 and 2004/5 Lake Huron-Lake Erie Corridor Surveys

Sediment Chemicals	Mean $\pm$ 1SE		
	Reference Sites	Test Sites	Degraded Sites
Al (mg/g)	3.48 $\pm$ 0.02	3.85 $\pm$ 0.02	4.00 $\pm$ 0.03
As (ug/g)	0.33 $\pm$ 0.02	0.63 $\pm$ 0.02	1.17 $\pm$ 0.04
Ca (mg/g)	4.40 $\pm$ 0.03	4.52 $\pm$ 0.02	4.65 $\pm$ 0.02
Cd (ug/g)	0.11 $\pm$ 0.01	0.25 $\pm$ 0.01	0.58 $\pm$ 0.03
Co (ug/g)	0.61 $\pm$ 0.01	0.83 $\pm$ 0.01	0.96 $\pm$ 0.01
Cr (ug/g)	0.89 $\pm$ 0.02	1.33 $\pm$ 0.02	1.65 $\pm$ 0.04
Cu (ug/g)	0.86 $\pm$ 0.03	1.41 $\pm$ 0.02	1.72 $\pm$ 0.05
Fe (mg/g)	3.82 $\pm$ 0.02	4.20 $\pm$ 0.01	4.42 $\pm$ 0.05
Hg (ug/g)	0.07 $\pm$ 0.01	0.12 $\pm$ 0.01	0.14 $\pm$ 0.02
Mn (ug/g)	2.11 $\pm$ 0.02	2.41 $\pm$ 0.01	2.65 $\pm$ 0.03
Ni (ug/g)	0.88 $\pm$ 0.02	1.30 $\pm$ 0.01	1.52 $\pm$ 0.03
Pb (ug/g)	0.60 $\pm$ 0.02	1.20 $\pm$ 0.04	1.64 $\pm$ 0.07
Zn (ug/g)	1.41 $\pm$ 0.02	1.79 $\pm$ 0.03	2.15 $\pm$ 0.07
p,p'-DDE (ng/g)	0.12 $\pm$ 0.01	0.31 $\pm$ 0.02	0.60 $\pm$ 0.07
OCS (ng/g)	0.12 $\pm$ 0.01	0.18 $\pm$ 0.02	0.32 $\pm$ 0.05
SumPCBs (ng/g)	0.39 $\pm$ 0.04	1.17 $\pm$ 0.05	1.86 $\pm$ 0.11
PC1	0.34 $\pm$ 0.02	0.57 $\pm$ 0.01	0.67 $\pm$ 0.02
PC2	0.19 $\pm$ 0.01	0.30 $\pm$ 0.01	0.39 $\pm$ 0.02
PC3	0.23 $\pm$ 0.01	0.24 $\pm$ 0.01	0.31 $\pm$ 0.02
PC4	0.23 $\pm$ 0.01	0.34 $\pm$ 0.02	0.66 $\pm$ 0.03
SumRel	0.98 $\pm$ 0.01	1.45 $\pm$ 0.01	2.03 $\pm$ 0.03

Table 2.4. Analysis of Variance (one-way ANOVA) results of two clusters of zoobenthos in 62 REF sites in the Lake Huron-Lake Erie Corridor. The zoobenthic taxa most important in distinguishing hierarchical clusters of sites has highest F value. Asterisk (\*) indicates significance level: \*\*\* highly different; \*\* moderately different; \* marginally different

Taxon	Cluster C1 vs. Cluster C2				Mean ( $\pm$ 1SE) Relative Abundance of Zoobenthos		
	SS Between	df	SS Within	df	F	p	
Hydropsychidae	55.70	1	46.73	60	71.53	p<0.001***	Cluster C1 0.21 $\pm$ 0.09 Cluster C2 3.20 $\pm$ 0.76
Dreissena	82.37	1	168.91	60	29.26	p<0.001***	1.09 $\pm$ 0.23 4.73 $\pm$ 0.38
Amphipoda	32.46	1	90.50	60	21.52	p<0.001***	0.61 $\pm$ 0.17 2.90 $\pm$ 0.58
Oligochaeta	25.52	1	74.90	60	20.44	p<0.001***	5.12 $\pm$ 0.15 3.09 $\pm$ 0.33
Other Trichoptera	13.82	1	68.74	60	12.06	p<0.001***	0.38 $\pm$ 0.14 1.87 $\pm$ 0.81
Hydrozoa	10.52	1	59.30	60	10.65	p<0.01**	0.34 $\pm$ 0.11 1.64 $\pm$ 0.70
Nematoda	17.56	1	163.67	60	6.44	p<0.05*	2.49 $\pm$ 0.22 0.81 $\pm$ 0.41
Chironomidae	11.56	1	136.37	60	5.08	p<0.05*	4.40 $\pm$ 0.20 3.04 $\pm$ 0.30
Turbellaria	3.15	1	45.28	60	4.17	p<0.05*	0.43 $\pm$ 0.10 1.14 $\pm$ 0.60
Hexagenia	1.38	1	49.32	60	1.68	p>0.05	0.47 $\pm$ 0.12 0.00 $\pm$ 0.34
Sphaeriidae	2.01	1	76.18	60	1.59	p>0.05	0.86 $\pm$ 0.15 0.29 $\pm$ 0.22
Gastropoda	1.67	1	106.72	60	0.94	p>0.05	0.78 $\pm$ 0.18 1.29 $\pm$ 0.50
Hirudinea	0.17	1	24.46	60	0.42	p>0.05	0.19 $\pm$ 0.09 0.36 $\pm$ 0.36
Acari	0.17	1	51.93	60	0.19	p>0.05	0.58 $\pm$ 0.13 0.42 $\pm$ 0.29
Caenis	0.07	1	52.71	60	0.08	p>0.05	0.46 $\pm$ 0.12 0.56 $\pm$ 0.37

Table 2.5. Summary of observed number of Lake Huron-Lake Erie Corridor sites in each cluster (columns) identified by zoobenthic taxa relative abundances and membership predicted (rows) by discriminant function classification (Appendix III) on the basis of habitat characteristics measured at those sites

Group	% Correct	Observed	
		Cluster C1	Cluster C2
Cluster C1	98	54	1
Cluster C2	71	2	5
Total	95	56	6

Table 2.6. Habitat variables accepted into the DFA model describing discriminant functions and their mean ( $\pm$  1SE) in the 62 REF sites. Variables with bold face were determined by DFA model as significant in classifying REF site cluster membership. Asterisk (\*) indicates significance level: \*\*\* highly different; \*\* moderately different; \* marginally different

Habitat variables	Significance level	Mean $\pm$ 1SE	
		Cluster C1	Cluster C2
		Shallow water area with fine substrate and high DOC	Deep water area with coarse substrate and low DOC
<b>Median Particle Size (Phi)</b>	<b>p &lt; 0.001***</b>	1.41 $\pm$ 0.10	-0.39 $\pm$ 0.28
<b>Disssolved Oxygen Concentration (DOC)(mg/L)</b>	<b>p &lt; 0.01**</b>	9.50 $\pm$ 0.01	8.92 $\pm$ 0.03
<b>Water Depth (m)</b>	<b>p &lt; 0.05*</b>	2.77 $\pm$ 0.07	4.59 $\pm$ 0.22
Total Organic Carbon (Loss On Ignition %)	p > 0.05	1.36 $\pm$ 0.04	1.41 $\pm$ 0.12
Water Temperature (°C)	p > 0.05	20.03 $\pm$ 0.02	20.17 $\pm$ 0.05
Lake or River	p > 0.05		
Latitude	p > 0.05		
Longitude	p > 0.05		

Talbe 2.7. The parameter estimates and quantile regression equations of 90%, median and 10% quntile for 2 clusters in 1991, 1999 and 2004/5 Lake Huron-Lake Erie Corridor surveys. Asterisk (\*) indicates significance level: \*\*\* highly different; \*\* moderately different; \* marginally different

Clusters	Quantil	n	Intercept ( $\pm$ 1SE)	Reggression Coefficient ( $\pm$ 1SE)	t	p	Qauntile Equations
Cluster C1 (Depositional)	90%	255	$0.2758 \pm 0.015$	$-0.0245 \pm 0.010$	-2.47	<0.01**	$Y_{.90}=0.2758-0.0245*(SumRel)$
	median	255	$0.262 \pm 0.022$	$-0.0691 \pm 0.015$	-4.56	<0.001***	$Y_{.50}=0.2620-0.0691*(SumRel)$
	10%	255	$0.1876 \pm 0.033$	$-0.0757 \pm 0.018$	-4.15	<0.001***	$Y_{.10}=0.1876-0.0757*(SumRel)$
Cluster C2 (Erosional)	90%	56	$0.8536 \pm 0.119$	$-0.2117 \pm 0.079$	-2.67	<0.01**	$Y_{.90}=0.8536-0.2117*(SumRel)$
	median	56	$0.889 \pm 0.159$	$-0.3475 \pm 0.109$	-3.2	<0.01**	$Y_{.50}=0.8879-0.3475*(SumRel)$
	10%	56	$0.747 \pm 0.335$	$-0.3541 \pm 0.218$	-1.63	>0.05	$Y_{.10}=0.7470-0.3541*(SumRel)$

Table 2.8. Forward stepwise multiple regression of relative abundances of 16 taxa vs. ZCI scores for cluster C1 sites.  $F_{[10,244]} = 242.77$   $p < 0.0001$   $R^2 = 0.91$

	<b>B <math>\pm</math> 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.096 $\pm$ 0.007	14.327	0.000	
<b>Chironomidae</b>	0.027 $\pm$ 0.001	36.641	0.000	0.717
<b>Oligochaeta</b>	-0.010 $\pm$ 0.001	-10.484	0.000	0.137
<b>Gastropoda</b>	0.004 $\pm$ 0.001	3.407	0.001	0.016
<b>Dreissena</b>	0.004 $\pm$ 0.001	5.199	0.000	0.010
<b>Hexagenia</b>	0.006 $\pm$ 0.001	4.424	0.000	0.007
<b>Hydrozoa</b>	0.007 $\pm$ 0.002	3.907	0.000	0.006
<b>Acari</b>	0.006 $\pm$ 0.002	3.227	0.001	0.007
<b>Caenis</b>	0.004 $\pm$ 0.001	2.706	0.007	0.004
<b>Sphaeriidae</b>	0.004 $\pm$ 0.001	3.270	0.001	0.003
<b>Ceratopogonidae</b>	0.005 $\pm$ 0.002	2.419	0.016	0.003

Table 2.9. Revised forward stepwise multiple regression of relative abundances of 2 taxa vs. ZCI scores for cluster C1 sites.  $F_{[2,252]}=735.87$   $p<0.0001$   $R^2= 0.85$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.136 ± 0.005	25.409	0.000	
<b>Chironomidae</b>	0.027 ± 0.001	35.410	0.000	0.717
<b>Oligochaeta</b>	-0.014 ± 0.001	-15.348	0.000	0.137

$$ZCI = 0.136 + 0.027*Chironomidae - 0.014*Oligochaeta$$



Table 2.10. Forward stepwise multiple regression of relative abundances of 16 taxa vs. ZCI scores for cluster C2 sites.  $F_{[9,46]}=187.53$   $p<0.0001$   $R^2=0.97$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.216 ± 0.027	7.867	0.000	
<b>Oligochaeta</b>	-0.023 ± 0.004	-5.852	0.000	0.625
<b>Hydropsychidae</b>	0.022 ± 0.004	4.949	0.000	0.138
<b>Chironomidae</b>	0.028 ± 0.004	7.452	0.000	0.054
<b>Dreissena</b>	0.030 ± 0.003	10.414	0.000	0.071
<b>Hydrozoa</b>	0.017 ± 0.003	6.124	0.000	0.051
<b>Gastropoda</b>	0.018 ± 0.005	3.842	0.000	0.019
<b>Amphipoda</b>	0.015 ± 0.004	4.011	0.000	0.008
<b>Nematoda</b>	0.013 ± 0.004	2.928	0.005	0.003
<b>Other Trichoptera</b>	0.020 ± 0.007	2.882	0.006	0.004

Table 2.11. Revised forward stepwise multiple regression of relative abundances of 4 taxa vs. ZCI scores for cluster C2 sites.  $F_{[4,51]}=101.43$   $p<0.0001$   $R^2= 0.89$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.295 ± 0.050	5.908	0.000	
<b>Oligochaeta</b>	-0.028 ± 0.007	-3.793	0.000	0.625
<b>Hydropsychidae</b>	0.039 ± 0.007	5.916	0.000	0.138
<b>Chironomidae</b>	0.034 ± 0.005	6.442	0.000	0.054
<b>Dreissena</b>	0.031 ± 0.005	5.714	0.000	0.071

$$\text{ZCI} = 0.295 - 0.028 \cdot \text{Oligochaeta} + 0.039 \cdot \text{Hydropsychidae} + 0.034 \cdot \text{Chironomidae} + 0.031 \cdot \text{Dreissena}$$

Table 2.12. Correlation (factor loading) between values of 16 chemical variables measured at 213 Detroit River sites and 5 principal component factors. Variable combined in 5 factors are shown in bold face

Stressor variables	PC1	PC2	PC3	PC4	PC5
<b>Pb</b>	<b>0.89</b>	0.00	0.11	0.07	0.11
<b>Cu</b>	<b>0.86</b>	0.30	0.07	0.01	0.11
<b>Zn</b>	<b>0.84</b>	0.28	0.10	-0.07	0.15
<b>Cr</b>	<b>0.82</b>	0.41	0.11	0.01	0.15
<b>Hg</b>	<b>0.77</b>	-0.08	0.07	0.03	-0.11
<b>Ni</b>	<b>0.71</b>	0.62	0.15	0.02	0.16
<b>Cd</b>	<b>0.64</b>	0.18	0.08	0.62	0.06
<b>SumPCBs</b>	<b>0.61</b>	-0.16	0.57	0.05	-0.08
<b>Fe</b>	<b>0.54</b>	0.45	0.00	0.04	0.28
<b>Al</b>	0.03	<b>0.91</b>	-0.04	0.04	0.19
<b>Co</b>	0.32	<b>0.88</b>	0.18	0.06	0.07
<b>DDE</b>	0.21	0.08	<b>0.85</b>	-0.05	-0.13
<b>OCS</b>	-0.03	0.19	<b>0.79</b>	0.07	0.28
<b>As</b>	-0.06	0.02	-0.01	<b>0.96</b>	0.03
<b>Ca</b>	0.07	0.13	0.00	0.05	<b>0.94</b>
<b>Mn</b>	0.48	0.44	0.12	0.02	<b>0.64</b>
Explained Variance	5.47	2.85	1.80	1.34	1.62
Proportion of total variance	0.34	0.18	0.11	0.08	0.10
Cum. Proportion	0.34	0.52	0.63	0.72	<b>0.82</b>

Table 2.13. Mean ( $\pm$  1SE) concentration of 16 sediment chemicals (log (Y+1)) and PC factor scores among REF, TEST and DEG sites in the Detroit River Case Study (1991, 1999 and 2004)

Sediment Chemicals	Mean $\pm$ 1SE		
	Reference Sites	Test Sites	Degraded Sites
Al (mg/g)	3.67 $\pm$ 0.04	3.90 $\pm$ 0.02	<b>4.00 <math>\pm</math> 0.04</b>
As (ug/g)	0.44 $\pm$ 0.06	0.76 $\pm$ 0.03	<b>1.17 <math>\pm</math> 0.06</b>
Ca (mg/g)	4.46 $\pm$ 0.03	4.53 $\pm$ 0.02	<b>4.67 <math>\pm</math> 0.03</b>
Cd (ug/g)	0.11 $\pm$ 0.02	0.31 $\pm$ 0.01	<b>0.62 <math>\pm</math> 0.02</b>
Co (ug/g)	0.70 $\pm$ 0.02	0.86 $\pm$ 0.01	<b>0.97 <math>\pm</math> 0.02</b>
Cr (ug/g)	1.14 $\pm$ 0.04	1.41 $\pm$ 0.02	<b>1.71 <math>\pm</math> 0.04</b>
Cu (ug/g)	1.18 $\pm$ 0.04	1.48 $\pm$ 0.03	<b>1.81 <math>\pm</math> 0.04</b>
Fe (mg/g)	4.04 $\pm$ 0.04	4.26 $\pm$ 0.02	<b>4.45 <math>\pm</math> 0.04</b>
Hg (ug/g)	0.03 $\pm$ 0.02	0.12 $\pm$ 0.01	<b>0.17 <math>\pm</math> 0.02</b>
Mn (ug/g)	2.28 $\pm$ 0.03	2.45 $\pm$ 0.02	<b>2.70 <math>\pm</math> 0.03</b>
Ni (ug/g)	1.11 $\pm$ 0.03	1.37 $\pm$ 0.02	<b>1.57 <math>\pm</math> 0.03</b>
Pb (ug/g)	0.89 $\pm$ 0.07	1.31 $\pm$ 0.04	<b>1.78 <math>\pm</math> 0.07</b>
Zn (ug/g)	1.41 $\pm$ 0.07	1.86 $\pm$ 0.04	<b>2.31 <math>\pm</math> 0.07</b>
p,p'-DDE (ng/g)	0.19 $\pm$ 0.05	0.40 $\pm$ 0.03	<b>0.52 <math>\pm</math> 0.05</b>
OCS (ng/g)	0.10 $\pm$ 0.03	0.15 $\pm$ 0.02	<b>0.31 <math>\pm</math> 0.03</b>
SumPCBs (ng/g)	0.83 $\pm$ 0.11	1.37 $\pm$ 0.06	<b>1.88 <math>\pm</math> 0.11</b>
<b>SumRel</b>	<b>1.06 <math>\pm</math> 0.03</b>	<b>1.55 <math>\pm</math> 0.02</b>	<b>2.16 <math>\pm</math> 0.03</b>

Table 2.14. Analysis of Variance (one-way ANOVA) results of three clusters of zoobenthos in 43 REF sites in the Detroit River Case Study. The zoobenthic taxon most important in distinguishing hierarchical clusters of sites has highest F value. Asterisk (\*) indicates significance level: \*\*\* highly different; \*\* moderately different; \* marginally different

Taxon	Cluster (DR1, DR2) vs. Cluster DR3						Cluster DR1 vs Cluster DR2						Mean ( $\pm$ ISE) Relative Abundance of zoobenthos			
	SS			SS			SS			SS			Cluster DR1		Cluster DR2	
	Between	df	Within	Between	df	Within	Between	df	Within	Between	df	Within	Cluster DR1	Cluster DR2	Cluster DR3	Cluster DR3
Dreissena	212.47	1	72.29	40	117.56	p<0.001 ***	3.75	1	72.29	40	2.07	p>0.05	0.17 $\pm$ 0.34	0.98 $\pm$ 0.45	<b>5.16 <math>\pm</math> 0.32</b>	
Chironomidae	41.93	1	56.37	40	29.76	p<0.001 ***	69.07	1	56.37	40	49.01	p<0.001 ***	<b>5.34 <math>\pm</math> 0.30</b>	1.88 $\pm$ 0.40	1.57 $\pm$ 0.28	
Sphaeriidae	14.29	1	18.29	40	31.24	p<0.001 ***	29.04	1	18.29	40	63.50	p<0.001 ***	0.12 $\pm$ 0.17	<b>2.36 <math>\pm</math> 0.23</b>	0.05 $\pm$ 0.16	
Oligochaeta	100.61	1	81.69	40	49.26	p<0.001 ***	15.01	1	81.69	40	7.35	p<0.001 ***	4.44 $\pm$ 0.36	<b>6.06 <math>\pm</math> 0.48</b>	2.09 $\pm$ 0.34	
Amphipoda	31.58	1	89.75	40	14.07	p<0.001 ***	21.86	1	89.75	40	9.74	p<0.001 ***	0.36 $\pm$ 0.37	2.31 $\pm$ 0.50	<b>3.10 <math>\pm</math> 0.35</b>	
Other Trichoptera	0.16	1	1.89	40	3.33	p<0.05 *	0.57	1	1.89	40	12.07	p<0.001 ***	0.00	<b>0.31 <math>\pm</math> 0.07</b>	0.03 $\pm$ 0.05	
Nematoda	21.55	1	133.47	40	6.46	p<0.05 *	14.88	1	133.47	40	4.46	p<0.05 *	<b>3.60 <math>\pm</math> 0.46</b>	1.99 $\pm$ 0.61	1.34 $\pm$ 0.43	
Turbellaria	1.95	1	28.92	40	2.69	p>0.05	3.88	1	28.92	40	5.37	p<0.05 *	0.00	0.82 $\pm$ 0.28	<b>0.85 <math>\pm</math> 0.20</b>	
Gastropoda	0.09	1	19.05	40	0.19	p>0.05	4.21	1	19.05	40	8.84	p<0.001 ***	0.11 $\pm$ 0.17	<b>0.96 <math>\pm</math> 0.23</b>	0.44 $\pm$ 0.16	
Hexagenia	1.13	1	14.10	40	3.20	p>0.05	1.29	1	14.10	40	3.66	p>0.05	0.57 $\pm$ 0.16	0.10 $\pm$ 0.20	0.00 $\pm$ 0.14	
Hydrozoa	4.55	1	85.92	40	2.12	p>0.05	4.23	1	85.92	40	1.97	p>0.05	0.13 $\pm$ 0.37	0.99 $\pm$ 0.49	1.23 $\pm$ 0.35	
Hirudinea	0.02	1	0.69	40	1.18	p>0.05	0.02	1	0.69	40	1.24	p>0.05	0.08 $\pm$ 0.03	0.02 $\pm$ 0.04	0.00 $\pm$ 0.03	
Acari	0.31	1	10.30	40	1.20	p>0.05	0.11	1	10.30	40	0.44	p>0.05	0.38 $\pm$ 0.13	0.24 $\pm$ 0.17	0.14 $\pm$ 0.12	
Ceratopogonidae	0.28	1	7.37	40	1.54	p>0.05	0.02	1	7.37	40	0.14	p>0.05	0.16 $\pm$ 0.11	0.22 $\pm$ 0.14	0.02 $\pm$ 0.10	
Hydropsychidae	0.49	1	35.10	40	0.56	p>0.05	0.18	1	35.10	40	0.20	p>0.05	0.37 $\pm$ 0.23	0.55 $\pm$ 0.31	0.68 $\pm$ 0.22	
Caenis	0.04	1	12.96	40	0.13	p>0.05	0.03	1	12.96	40	0.08	p>0.05	0.17 $\pm$ 0.14	0.23 $\pm$ 0.19	0.27 $\pm$ 0.13	

Table 2.15. Summary of observed number of the Detroit River sites in each cluster (columns) identified by zoobenthic taxa relative abundances and membership predicted (rows) by discriminant function classification (Appendix IV) on the basis of habitat characteristics measured at those sites

Group	% Correct	Observed		
		Cluster DR1	Cluster DR2	Cluster DR3
Cluster DR1	69	11	1	4
Cluster DR2	67	1	6	2
Cluster DR3	89	1	1	16
Total	77	13	8	22

Table 2.16. Habitat variables put into the DFA model describing discriminant functions and their mean ( $\pm 1$ SE) in the 43 Detroit River REF sites. Variables with bold face were determined by DFA model as significant in classifying Detroit River REF site cluster membership. Asterisk (\*) indicates significance level: \*\* moderately different; \* marginally different

Habitat variables	Significance level	Mean $\pm 1$ SE		
		Cluster DR1	Cluster DR2	Cluster DR3
		Slow-flowing water Fine substrate low water temperature	Coarse substrate High water temperature	Fast-flowing water Coarse substrate Low water temperature
Near-bottom Water Velocity (m/s)	<b><math>p &lt; 0.01^{**}</math></b>	$0.23 \pm 0.03$	$0.30 \pm 0.04$	<b><math>0.45 \pm 0.03</math></b>
Median Particle Size (Phi)	<b><math>p &lt; 0.01^{**}</math></b>	<b><math>1.13 \pm 0.19</math></b>	$0.42 \pm 0.26$	$0.42 \pm 0.18$
Water Temperature ( $^{\circ}$ C)	<b><math>p &lt; 0.05^{*}</math></b>	$18.52 \pm 0.02$	<b><math>20.11 \pm 0.03</math></b>	$18.58 \pm 0.02$
Longitude	<b><math>p &lt; 0.05^{*}</math></b>			
Latitude	<b><math>p &lt; 0.05^{*}</math></b>			
Dissolved Oxygen Concentration (mg/L)	$p > 0.05$	$8.52 \pm 0.05$	$8.88 \pm 0.06$	$8.48 \pm 0.04$
Water Depth (m)	$p > 0.05$	$2.68 \pm 0.15$	$2.69 \pm 0.20$	$5.34 \pm 0.14$
Total Organic Carbon (Loss On Ignition %)	$p > 0.05$	$1.48 \pm 0.09$	$1.50 \pm 0.12$	$1.50 \pm 0.08$

Talbe 2.17. The parameter estimates and quantile regression equations of 90%, median and 10% quntile for 3 clusters in the Detroit River Case Study. Asterisk (\*) indicates significance level: \*\*\* highly different; \*\* moderately different; \* marginally different

Clusters	Quantil	n	Intercept ( $\pm$ 1SE)	Regression Coefficient ( $\pm$ 1SE)	t	p	Qauntile Equations
<b>Cluster DR1 (Depositional)</b>	90%	69	0.2896 $\pm$ 0.0477	-0.0582 $\pm$ 0.0283	-2.06	<0.05*	$Y_{.90}=0.2896-0.0582*(SumRel)$
	median	69	0.2020 $\pm$ 0.0411	-0.0478 $\pm$ 0.0235	-2.03	<0.05*	$Y_{.50}=0.2020-0.0478*(SumRel)$
	10%	69	0.1090 $\pm$ 0.0437	-0.0353 $\pm$ 0.0268	-1.32	>0.05	$Y_{.10}=0.1090-0.0353*(SumRel)$
<b>Cluster DR2 (Mixed)</b>	90%	72	0.4497 $\pm$ 0.0780	-0.1251 $\pm$ 0.0532	-2.35	<0.05*	$Y_{.90}=0.4497-0.1251*(SumRel)$
	median	72	0.3617 $\pm$ 0.0283	-0.1249 $\pm$ 0.0179	-6.97	<0.001***	$Y_{.50}=0.3617-0.1249*(SumRel)$
	10%	72	0.1855 $\pm$ 0.0646	-0.0606 $\pm$ 0.0411	-1.48	>0.05	$Y_{.10}=0.1855-0.0606*(SumRel)$
<b>Cluster DR3 (Erosional)</b>	90%	72	0.3430 $\pm$ 0.0735	-0.0604 $\pm$ 0.0473	-1.28	>0.05	$Y_{.90}=0.3430-0.0604*(SumRel)$
	median	72	0.2419 $\pm$ 0.0372	-0.0433 $\pm$ 0.0249	-1.74	<0.05*	$Y_{.50}=0.2419-0.0433*(SumRel)$
	10%	72	0.1766 $\pm$ 0.0464	-0.0493 $\pm$ 0.0364	-1.35	>0.05	$Y_{.10}=0.1766-0.0493*(SumRel)$



Table 2.18. Forward stepwise multiple regression of relative abundances of 13 taxa vs. ZCI scores for cluster DR1 sites.  $F_{[6,62]}=91.521$   $p<0.0001$   $R^2= 0.90$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.133 ± 0.011	12.015	0.000	
Nematoda	0.020 ± 0.001	15.223	0.000	0.487
Oligochaeta	-0.017 ± 0.002	-11.305	0.000	0.300
Acari	0.016 ± 0.003	5.833	0.000	0.067
Hydropsychidae	0.011 ± 0.003	3.743	0.000	0.020
Hexagenia	-0.008 ± 0.002	-3.563	0.001	0.014
Chironomidae	0.003 ± 0.001	2.525	0.014	0.010

Table 2.19. Revised forward stepwise multiple regression of relative abundances of 2 taxa vs. ZCI scores for cluster DR1 sites.  $F_{[2,66]} = 121.80$   $p < 0.0001$   $R^2 = 0.79$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.161 ± 0.012	13.909	0.000	
Nematoda	0.020 ± 0.002	11.938	0.000	0.487
Oligochaeta	-0.020 ± 0.002	-9.635	0.000	0.300
ZCI = 0.161 + 0.020*Nematoda - 0.020*Oligochaeta				

Table 2.20. Forward stepwise multiple regression of relative abundances of 14 taxa vs. ZCI scores for cluster DR2 sites.  $F_{[4,67]}=190.94$   $p<0.0001$   $R^2= 0.92$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.110 ± 0.010	10.508	0.000	
<b>Chironomidae</b>	0.020 ± 0.002	11.650	0.000	0.678
<b>Nematoda</b>	0.026 ± 0.002	13.891	0.000	0.222
<b>Gastropoda</b>	-0.008 ± 0.003	-2.821	0.006	0.010
<b>Oligochaeta</b>	-0.004 ± 0.002	-2.804	0.006	0.009

$$\text{ZCI} = 0.110 + 0.020 \cdot \text{Chironomidae} + 0.026 \cdot \text{Nematoda} - 0.008 \cdot \text{Gastropoda} - 0.004 \cdot \text{Oligochaeta}$$

Table 2.21. Forward stepwise multiple regression of relative abundances of 15 taxa vs. ZCI scores for cluster DR3 sites.  $F_{[7,64]}=523.63$   $p<0.0001$   $R^2= 0.98$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.141 ± 0.008	17.063	0.000	
<b>Dreissena</b>	0.017 ± 0.001	17.397	0.000	0.818
<b>Oligocheata</b>	-0.016 ± 0.001	-13.956	0.000	0.115
<b>Turbellaria</b>	0.010 ± 0.002	4.693	0.000	0.029
<b>Amphipoda</b>	0.007 ± 0.001	6.423	0.000	0.007
<b>Hydrozoa</b>	0.007 ± 0.001	5.856	0.000	0.005
<b>Sphaeriidae</b>	-0.009 ± 0.002	-4.867	0.000	0.007
<b>Nematoda</b>	0.003 ± 0.001	2.601	0.012	0.002

Table 2.22. Revised forward stepwise multiple regression of relative abundances of 2 taxa vs. ZCI scores for cluster DR3 sites.  $F_{[2,69]}=480.62$   $p<0.0001$   $R^2= 0.93$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.141 ± 0.008	15.235	0.000	
<b>Dreissena</b>	0.018 ± 0.002	11.181	0.000	0.818
<b>Oligochaeta</b>	-0.021 ± 0.002	-10.901	0.000	0.115

$$\text{ZCI} = 0.141 + 0.018 * \text{Dreissena} - 0.021 * \text{Oligochaeta}$$

Table 2.23. Location of eight blocks of Detroit River sites that were sampled in 1991, 1999 and 2004

Block	Site ID	Sampling year	Latitude	Longitude
1	<b>5FB</b>	1991	42.354	-82.959
	<b>003ABC</b>	1999	42.354	-82.944
	<b>S101</b>	2004	42.354	-82.948
2	<b>2FB</b>	1991	42.351	-82.928
	<b>008A</b>	1999	42.351	-82.923
	<b>S82</b>	2004	42.351	-82.923
3	<b>E</b>	1991	42.333	-83.009
	<b>015C</b>	1999	42.337	-83.011
	<b>S85</b>	2004	42.337	-83.012
4	<b>H</b>	1991	42.206	-83.131
	<b>065C</b>	1999	42.211	-83.125
	<b>S93</b>	2004	42.211	-83.125
5	<b>L</b>	1991	42.194	-83.108
	<b>070B</b>	1999	42.202	-83.105
	<b>S89</b>	2004	42.201	-83.107
6	<b>35FB</b>	1991	42.227	-83.127
	<b>078B</b>	1999	42.230	-83.136
	<b>S97</b>	2004	42.230	-83.136
7	<b>73FB</b>	1991	42.172	-83.165
	<b>101C</b>	1999	42.172	-83.161
	<b>S98</b>	2004	42.172	-83.160
8	<b>70FB</b>	1991	42.079	-83.184
	<b>145B</b>	1999	42.073	-83.176
	<b>S100</b>	2004	42.073	-83.175

## **Chapter 3**

### **Use of Chironomidae (Diptera) Mouthpart Deformities to Assess Environmental Degradation in the Lake Huron-Lake Erie Corridor**

### 3.1 Summary

The spatial distribution and mentum deformities of Chironomidae (Diptera) were examined in 12 zones within the Lake Huron-Lake Erie Corridor. Five thousand and seven larvae belonging to 43 genera were collected in summer 2004 and 2005. The dominant tribe Chironomini contained 73% of all the chironomids examined. Total numbers of 3117 larvae of six genera (*Chironomus*, *Phaenopsectra/Tribelo*, *Dicrotendipes*, *Polypedilum*, *Procladius* and *Tanytarsus*) were found to be widespread and sensitive enough to test mentum deformities. Both spatial and taxonomic variations were identified in the incidence of mentum deformities in this study (G-statistic Goodness of Fit test). Overall incidence of mentum deformities of *Chironomus* is 5.43% (SE=1.15%, n=387), displayed high variation compared with 2.65% baseline level. All other genera show homogenous among sites (0.32% to 2.64 %). The environmentally degraded zones have significantly elevated mentum deformities (1CDR: overall  $4.43 \pm 1.31\%$ , n=248; *Chironomus*  $16.00 \pm 7.33\%$ , n=25. 3LSC: overall  $3.06 \pm 0.62\%$ , n=752; *Chironomus*  $12.24 \pm 3.31\%$ , n=98. 1ADR: overall  $5.88 \pm 2.16\%$ , n=119; *Dicrotendipes*  $25.00 \pm 21.65\%$ , n=4 and *Procladius*  $25.00 \pm 15.31\%$ , n=8). While the relatively unpolluted zones have low incidence of deformities overall (0.57% to 0.72%), elevate incidences detected elsewhere indicated that the mentum deformity bioindicator can reflect the degree of chemical pollution. However, zones in downstream portions of the Detroit River have very low density of chironomids, and the few individuals collected were not deformed, possibly because high concentration of diverse chemicals killed all but the most tolerant chironomids and the sample sizes are too limited to perform this test.



### **3.2 Introduction**

Due to the rapid growth in agriculture and industry over the last six decades, the quality of aquatic ecosystems in the world has been seriously threatened by persistent chemicals, including substances such as trace metals, pesticide residues and other pollutants (Warwick 1990a, 1990b). To monitor environmental quality and support remedial actions, scientists need a sensitive technique to determine biological responses to contaminant stresses (Warwick 1990a; Clarke 1993). Increasing attention has focused on the responses of affected communities or organisms as general indicators of environmental degradation (Krieger 1984; Thornley 1985; Warwick 1988, 1990a; Dermott 1991; Diggins and Stewart 1993; Vermeulen 1995; Burt et al. 2003; Bhagat 2005).

Not all environmental changes can be detected by alterations in biological communities. Individual organisms tend to respond to the stressors before population and community changes can be detected, and are thought likely to be more sensitive indicators of degradation (Warwick 1990a). Aquatic larval midges (Diptera: Chironomidae) are reported to be one such group of zoobenthos (Pinder 1986; Warwick 1988, 1990a; Dickman 1992; Hudson and Ciborowski 1996a). Chironomidae are among the most widely distributed and abundant freshwater zoobenthic families in the world. They can be collected in all types of habitat and levels of contamination (Pinder 1986). The larval stage is the longest and most sensitive stage of the chironomid life cycle. These factors make them important in ecosystem function. Because they are benthic, larvae are directly exposed to sediment-associated contaminants (Warwick 1990a). When toxicity of sediment-associated contaminants is significantly higher than reference areas, chironomid larvae may exhibit significantly elevated incidences of deformities, including

mouthpart and antennal malformations, and thickened exoskeletons and head capsules (IJC 1987; Warwick 1988; Hudson and Ciborowski 1996a, 1996b; Burt et al. 2003). Deformities are assumed to be associated with anthropogenic stress, but not natural stress (Diggins and Stewart 1993; Burt et al. 2003). Warwick (1988) proposed that although morphological deformities in chironomid larvae occasionally occur in unpolluted areas, the incidence of deformities became elevated significantly in environmentally degraded locations. Thus, morphological deformities of chironomids have considerable potential to be “a biological screening tool for detecting and assessing the nature, extent, and significance of toxic chemicals in freshwater ecosystems (Warwick 1988)”.

Antennal deformities were investigated by many researchers because, as a receptor organ, it was expected to be more sensitive to contaminants than other body parts (Warwick 1985, 1988, 1990a; Warwick and Tisdale 1988; Janssens de Bisthoven et al. 1998). However, Warwick (1988, 1990a) suggested that beyond a certain contaminant concentration, the antennal response might be overwhelmed, and more discernible responses may be found in other less sensitive morphological structures including harder mouthparts such as the mentum and mandibles. Because chironomid mouthparts have consistently imparted the most information in contaminant-affected locations, they have become increasingly used to document the presence of anthropogenic stress on organisms (Warwick 1988, 1990b; Hudson 1994; Hudson and Ciborowski 1996a, 1996b; Groenendijk et al. 1998; Burt 1998; Burt et al. 2003).

Several researchers have found that some chironomid genera appear to be more susceptible to morphological deformities than others (Hare and Carter 1976; Wiederholm 1984; Warwick 1988, 1989, 1990a). Hudson and Ciborowski (1996a) assessed the incidence of deformed mouthparts (menta) in chironomids collected in the St. Clair and

Detroit rivers. The incidence of deformities in *Chironomus* and *Phaenopsectra/Tribelos* varied significantly from <2-3% at relatively uncontaminated sites to 6-20% at more contaminated locations. Both genera were broadly distributed and sensitive enough for use in deformity studies. However, within the same tribe (Chironomini), *Cryptochironomus*, *Polypedilum*, and *Stictochironomus* showed uniformly low incidences of deformities at all sampling sites across a contaminant gradient. Diggins and Stewart (1993) had similar conclusions when they surveyed the Buffalo River, NY and assessed the correlation between incidence of deformities in larval midges and the degree of sediment pollution by trace metals.

Diggins and Stewart (1993) also agreed with the contention of Warwick (1989) and Dermott (1999) that *Chironomus* is more sensitive to contaminants than *Procladius*, but *Procladius* might be more tolerant to contaminants. They found that the incidence of *Procladius* ligula deformities was elevated in areas containing high industrial contaminant levels in the areas where *Chironomus* was greatly affected, or had been eliminated. This suggested that *Procladius* might be a bioindicator in the most degraded environments, where no other taxa could survive.

Although chironomid mouthpart deformities have been increasingly used as indicators of environmental stresses, many studies have been based on small sample sizes, which result in large standard errors. Burt and Ciborowski (1999) performed a meta-data analysis on the results of 28 reports utilizing chironomid deformity as an indicator of contamination. Four of the studies failed to find significantly elevated incidences of deformities in the contaminated sites. This might be the result of using small sample sizes. Hudson and Ciborowski (1996a) determined that for a doubling in the incidence of deformities over 3% background levels to be judged significant ( $p < 0.05$ ) with a power of

80%, at least 125 individuals from each sample must be examined to provide the necessary statistical power. Burt et al. (2003) reported baseline incidence of mouthpart deformities of five widespread chironomid genera (*Chironomus*, 2.65%; *Procladius*, 2.73%; *polypedilum*, 4.31%; *Tanytarsus*, 1.98% and *Heterotrissocladius*, 1.84%). Only when the lower boundary of the incidence of deformities (proportion deformed – 1 standard error) exceeds these baseline levels, can one conclude that there is a significant elevation in the incidence of mentum deformities, implying that contamination is having a negative impact on the microhabitat where the chironomids live.

This study represents a 1-year evaluation of the distribution of Chironomidae collected within the Lake Huron-Lake Erie Corridor, and on morphological abnormalities (mentum or ligula) of the common genera. The objectives of this paper are to:

- 1) Document the distribution of larval Chironomidae along the contaminant gradient in Lake Huron-Lake Erie Corridor in 2004/5; and
- 2) Evaluate variability in the incidence of mentum (ligula) deformities among the common taxa to determine which genera are sensitive enough to be used as bioindicators;
- 3) Use the incidence of mouthpart deformity of indicator genera to assess the environmental degradation in the Lake Huron-Lake Erie Corridor (test the heterogeneity in the incidence of deformities among zones).

To be considered “common”, I used the criterion that 40 or more individuals of one ‘susceptible’ genus had to occur in more than one zone. A “susceptible genus” is one previously reported to have exhibited morphological abnormalities in relation to anthropogenic stresses.

### **3.3 Methods**

#### **Study Sites**

The Lake Huron-Lake Erie Corridor connects southern Lake Huron to the western basin of Lake Erie (Hudson et al. 1986). It contains many industrial and agriculturally stressed areas, including the large petrochemical complex around Sarnia, Ontario, Walpole Island, and most parts of the Detroit River. It is also the major source of contaminant input to Lake Erie (Panet et al. 2003; Oliver and Bourbonniere 1985).

A total of 113 sites had been sampled in the Lake Huron-Lake Erie Corridor survey in 2004 and 2005 (see chapter 2). Adjacent sites were pooled to form 12 zones (Figure 3.1). Four zones (1ASR, 2CSR, 3ASR and 4CSR) were grouped in the St. Clair River; they are upstream and downstream in U.S. and Canadian sides, respectively. The St. Clair delta was divided into three parts. The first group represents Anchor Bay (1LSC), which has historically been assumed to be a relatively unpolluted area; the second group included the North Channel, Middle Channel and Dickenson Island of the St. Clair delta (2LSC); and the last group included samples from the South Channel, Chenal Ecarte and Walpole Island (3LSC). Zone 4LSC represented pooled sample data from the open water area of Lake St. Clair. Four zones were grouped in the Detroit River; zone 1ADR is on the U.S. side of Belle Isle, 2CDR is around Peche Island and the Canadian side of Belle Isle. The next two were located in the downstream in U. S. side and in Canadian side, respectively (3ADR and 4CDR).

#### **Chironomid Sample Processing**

Chironomid larvae were sorted from the benthic samples (4 mm, 1 mm and 0.5 mm fractions) and preserved in 70% ethanol solution as summarized in Chapter 2. The

heads of individual larvae were removed and placed on a microscope slide in a drop of CMC-9AF<sup>®</sup> aqueous mounting medium (Master's Chemical Company, Des Plaines, Illinois) ventral side up. The corresponding body was placed beside the head. A cover slip was placed on the slide and gentle pressure was applied to the slip to separate the mouthparts and properly orient the head capsule. The slide was set aside and allowed to clear for 24-48 h and then sealed with nail polish for long term preservation.

Chironomids were identified to genus as possible under a compound microscope using keys of Oliver and Russell (1983) and Wiederholm (1983). Individuals that were poorly mounted or damaged were excluded from the analysis. Deformities in the structure of the mentum (or ligula of Tanypodinae) were examined at the same time as larvae were identified. Deformities are defined as any morphological feature that departs from normal configuration (Warwick 1988), which is restricted to developmental abnormalities and does not include wear or damage to the structure that is incurred during the life cycle and the natural variability in morphology (Warwick 1996). In this paper, missing or extra teeth on the mentum (or ligula of Tanypodinae) and medial köhn gap of chironomid larvae were defined as deformities. No other morphological features were examined for deformities (Hudson and Ciborowski 1996a).

### **Statistical Analysis**

Incidence of deformities was expressed as "proportion  $\pm$  1 standard error (SE)" of deformed larvae at each zone for each genus. Standard error was determined from the binomial theorem as  $SE = \text{SQRT} [(pq)/n]$ , where  $p$  is the proportion of deformed specimens,  $q$  is  $(1-p)$ , the proportion of undeformed specimens, and  $n$  is the sample size.

To test the degree of heterogeneity in the incidence of deformities among the common genera ( $H_0$ : incidence of deformities is equal among all common genera), a G-statistic Goodness of Fit test (Sokal and Rohlf 1981) was used.

To determine whether the incidence of deformities at a location was significantly elevated, one-tailed G-statistic Goodness of Fit tests (Sokal and Rohlf 1981) were used. Baseline incidences against which the null hypotheses were tested ( $H_0$ : incidence of deformities < baseline) were based on values reported in the literature. The baseline levels of *Chironomus* (2.65%), *Procladius* (2.73%), *polypedilm* (4.31%) and *Tanytarsus* (1.98%) were reported by Burt et al. (2003), the baseline level of *Phaenopsectra/Tribelos* (2.90%) was based on Hudson and Ciborowski (1996a). Since the incidence of mentum deformity in all genera pooled from the Great Lakes reference sites is  $2.1 \pm 0.2\%$  (Burt et al. 2003), the baseline level of *Dicrotendipes* was considered as 2.30% in this study.

### 3.4 Results

#### Distribution of chironomid genera

A total of 5,007 Chironomidae larvae representing 43 taxa was collected from 12 sampling zones within Lake Huron-Lake Erie Corridor. The greatest proportion of these belonged to the tribe Chironomini, comprising 73% of all chironomids collected (Table 3.1). Within this group, *Polypedilum*, *Dicrotendipes*, *Chironomus*, *Cryptochironomus* and *Phaenopsectra/Tribelos* formed the most important components of the fauna. The second largest component of the Chironomidae community was Tanytarsini. *Tanytarsus* was the most abundant taxon in this group. Following this was the Tanypodinae (32% of this group were *Procladius*), Orthocladiinae and others. Appendix VI summarizes the distribution of all chironomid taxa in the corridor.

Since *Chironomus*, *Dicrotendipes*, *Phaenopsectra/Tribelos*, *Polypedilum* and *Tanytarsus* were abundant enough to be considered common, and all of them were previously reported to exhibit morphological responses to anthropogenic stresses (Hare and Carter 1976; Wiederholm 1984; Warwick 1988, 1990a; Burt et al. 2003), these five taxa were chosen for statistical analysis. Although *Procladius* was not abundant enough to be considered as common, it has been reported to exhibit elevated incidence of deformities when the habitat is severely polluted (Dermott 1991; Burt et al. 2003) and *Procladius* was retained for mentum deformity analysis as well.

#### **Incidence of mentum deformities**

A total of 3,117 individuals belonging to the six major taxa (*Chironomus*, *Dicrotendipes*, *Phaenopsectra/Tribelos*, *Polypedilum*, *Procladius*, and *Tanytarsus*) were examined for mentum (or ligula) deformities. Table 3.2 described the normal arrangement of teeth in the mentum / ligula of these taxa. The most common type of deformity of *Chironomus*, *Dicrotendipes*, *Phaenopsectra/Tribelos*, *Polypedilum* and *Tanytarsus* observed in this study was a missing lateral tooth, which comprised 57% of all deformities. Three deformed *Procladius* specimens were found, all having one extra tooth in their ligula. A detailed description and number of mentum deformities in the six taxa were summarized in Appendix VII. There was significant heterogeneity in the overall incidence of mentum deformities among these six taxa (G-statistic Goodness of Fit test,  $G = 17.46$ ,  $df = 5$ ,  $p < 0.01$ ). *Dicrotendipes*, *Phaenopsectra/Tribelos*, *Polypedilum*, *Procladius* and *Tanytarsus* exhibited relatively low overall incidence of deformities, ranging from 0.32% to 2.64%. Only *Chironomus* exhibited higher incidence of deformities of 5.43% (Table 3.3).



Zone 1ADR (at the head of the Detroit River around Belle Isle on the US side) had the highest overall incidence of deformities ( $5.88 \pm 2.16\%$ ,  $n = 119$ ); the second highest overall incidence of deformity within the corridor zones was 2CSR, the upstream end of the St. Clair River on the Canadian side ( $4.44 \pm 1.31\%$ ,  $n = 248$ ). No deformed individuals were found in zones 3ADR (downstream on the US side of the Detroit River;  $n = 20$ ) and 4CDR (the Canadian side of the most downstream part of the Detroit River;  $n = 45$ ) (Table 3.4).

*Chironomus* exhibited significant among-zone variation in the incidence of mentum deformities (One-tailed G-statistic Goodness of Fit test,  $G = 24.24$ ,  $df = 11$ ;  $p < 0.05$ ). Zones 2CSR (the upstream end of the St. Clair River on the Canadian side) and 3LSC (Walpole Island region) had incidences of deformity that were significantly higher than the baseline value of 2.65% (G-statistic Goodness of Fit test,  $G = 8.19$ ,  $P < 0.01$  and  $G = 18.886$ ,  $p < 0.001$ , respectively). They are  $16.00 \pm 7.33\%$  ( $n = 25$ ) in zone 2CSR and  $12.24 \pm 3.31\%$  ( $n = 98$ ) in zone 3LSC.

All other genera displayed homogeneity in mentum deformities among the corridor regions (One-tailed G-statistic Goodness of Fit test; *Dicrotendipes*:  $G = 14.26$ ,  $df = 11$ ,  $p > 0.05$ ; *Phaenopsectra/Tribelos*:  $G = 10.53$ ,  $df = 11$ ,  $p > 0.05$ ; *Polypedilum*:  $G = 9.17$ ,  $df = 10$ ,  $p > 0.05$ ; *Procladius*:  $G = 10.87$ ,  $df = 10$ ,  $p > 0.05$  and *Tanytassus*:  $G = 4.46$ ,  $df = 11$ ,  $p > 0.05$ ). However, compared with the baseline levels, *Dicrotendipes* in zone 2CSR ( $8.89 \pm 4.24\%$ ,  $n = 45$ ) and zone 1ADR ( $25.00 \pm 21.65\%$ ,  $n = 4$ ) had elevated incidence of deformities; *Procladius* in zone 1ADR ( $25.00 \pm 15.31\%$ ,  $n = 8$ ) had elevated incidence of deformities (summarized in Table 3.5).

### 3.5 Discussion and Conclusions

#### Variation in Deformities among Taxa

The incidence of mouthpart deformities of Chironomidae has been investigated by many scientists since the 1980s, most of whom have reported an association between deformities of some Chironomidae genera and anthropogenic contamination (Wiederholm 1984; Warwick 1985, 1988, 1990a, 1990b; Dickman et al. 1992; Diggins and Stewart 1993; Hudson and Ciborowski 1996a; Martinez et al. 2002; Burt et al. 2003). In the current study, 6 of 43 taxa identified were examined for and were found to be widespread and sensitive enough to evaluate for the incidence of mentum deformities. All of the taxa have been recognized previously as contaminant tolerant and show elevated incidence of deformities in contaminated areas. Burt et al. (2003) indicated that as the degree of contamination increases, the genera of chironomids responding will shift from sensitive taxa like *Heterotrissocladius* and *Tanytarsus* to *Polypedilum* and to more tolerant genera *Chironomus* and *Procladius*. Wiederholm (1984) and Burt et al. (2003) reported that *Tanytarsus* have low incidences of mentum deformities in unpolluted sites whereas a relatively high proportion exhibit deformed menta in strongly polluted sites. Hudson and Ciborowski (1996a) found only *Phaenopsectra/Tribelos* and *Cryptochironomus* at the heavily contaminated Trenton Channel sites, but *Phaenopsectra/Tribelos* and *Chironomus* had elevated incidences of mentum deformities in the environmentally degraded locations in the Lake Huron-Lake Erie Corridor system. Dickman et al. (1992) found that *Chironomus*, *Dicrotendipes* and *Polypedilum* were pollution tolerant chironomids common in the study sites where other genera cannot survive. *Procladius* was widely accepted to be more tolerant of industrial contamination than *Chironomus*, although they are not as susceptible to deformities as *Chironomus*

(Warwick 1988, 1990b; Diggins and Stewart 1993; Dermott 1999). My findings are consistent with these reports.

In addition to the major six genera analyzed, *Cricotopus* was abundant in three zones of this study (1ASR, 2CSR and 3LSC) and could be considered common. I found two deformed individuals of this genus (in zone 2CSR; both missing a lateral tooth). *Cryptochironomus* was also abundant in three zones (1ASR, 3ASR and 4CSR). Each zone had one deformed individual (missing a lateral tooth). *Pseudochironomus* was abundant in zones 1LSC and 2LSC. I found one case of mentum deformity (extra lateral teeth) in zone 2LSC. *Paratanytarsus* abundant only at zone 3LSC and had two deformed individuals (both missing a lateral tooth). *Ablabesmyia* was found in all of the zones but it was abundant only in one zone. There was one deformed individual in zone 3ASR and one in zone 3LSC (both had an extra ligula tooth). *Stictochironomus* was abundant at zone 4LSC with one deformed individual (missing a lateral tooth) only.

Other genera in which mentum deformities were found (*Cricotopus*, *Cryptochironomus*, *Pseudochironomus*, *Paratanytarsus*, *Stictochironomus* and *Ablabesmyia*) have been occasionally reported in the literature. Martinez et al. (2002) found deformed *Cricotopus* (9.75%) in the Coeur d' Alene River system, Idaho, USA. Tennesen and Gottfried (1983) reported deformed ligula in *Ablabesmyia* (4.0%) in artificial lakes and coal stripmine ponds in Alabama. Hudson and Ciborowski (1996a) reported that *Stictochironomus* had low incidence of deformity in Anchor Bay ( $1.1 \pm 0.8\%$ ,  $n = 174$ ) and elevated incidence of deformity in Walpole Island ( $4.8 \pm 1.9\%$ ,  $n = 126$ ) in the Lake Huron-Lake Erie Corridor system. Warwick (1990a, 1990b) reported finding deformities in many other genera for the first time. Since we do not have enough data to determine expected baseline incidences, these genera were not included in the

current analysis. Further research is required to test mentum deformities in a broader suite of genera. Inclusion of these rarer taxa will give scientists new perspectives with which to analyze the responses of chironomid communities to contaminants (Warwick 1990b).

### **Types of Deformities**

Most of the deformed menta in this analysis consisted of extra or missing lateral teeth. No between-zone differences in the type of deformities were observed. However, some researchers reported that the medial köhn gaps of *Chironomus* were more common deformities associated with higher contamination level (Warwick and Tisdale 1988; Hudson and Ciborowski 1996b; Burt et al. 2003). Medial köhn gaps were a type of deformity characterized by a large gap in the mentum. The presence of the gap may or may not involve the loss of one or more of the tripartite median teeth (Warwick and Tisdale 1988). These gaps were found in the heavily polluted Teltowkanal in Berlin, Germany (Köhn and Frank 1980, cited by Warwick and Tisdale 1988). Hudson and Ciborowski (1996b) conducted a lab-based experiment that exposed *Chironomus salinarius* group Kieffer larvae to mixtures of contaminated Trenton Channel sediments and uncontaminated, formulated sediment in different ratios. They also reported that the medial köhn gaps occurred only in the most heavily contaminated treatments (1:0 and 1:1 dilutions). Medial köhn gaps of *Chironomus* were found only in zone 3LSC (Walpole Island region) and zone 2CDR (downstream of Peche Island, the Canadian side of the mouth of Detroit River). Both of these two zones were found to be degraded by anthropogenic stresses in current study. However, since the medial köhn gaps accounted for only 1.29% (5 out of 387 individuals, see Appendix 3.2) of the incidence of

deformities of *Chironomus* in this study, they are likely too rare to be of great diagnostic value.

### **Associations between Deformities and Classes of Chemicals**

Although in this study, the incidence of deformed individuals in contaminated zones such as upstream sections of the St. Clair River and Walpole Island vicinity was significantly higher than in relatively unpolluted areas such as the Anchor Bay and the open water area of Lake St. Clair, we could not determine which types of contaminants led to this pattern. Concentrations of the organochlorine compounds, such as 1245-TCB, 1234-TCB, QCB, HCB, OCS and trace metals such as cobalt, nickel, copper, and chromium are very high in the upstream end of the St. Clair River around Sarnia (zone 2CSR). The concentration of pesticide residues in Walpole Island vicinity is much higher than other areas within the corridor, and the concentrations of mercury, lead, zinc, cadmium, DDE and Sum PCB are very high in zone 1ADR, the U.S. side of Belle Isle (see Chapter 2). However, we could not find any single contaminant or class of contaminants to which induction of deformities could be directly attributed. Mentum deformities believed to be the result of industrial or agricultural (pesticide-related) contaminants rather than domestic wastes (Pinder 1986; Diggins and Stewart 1993). Warwick (1990b) found that most severely deformed larvae in Lac St. Louis were from an area seriously contaminated by PCBs and heavy metals. Janssens de Bisthoven et al. (1998) studied Belgian lowland rivers and concluded that mentum deformities appeared to be potential predictors of lead levels in the sediments and larvae. Martinez et al. (1996) assessed the potential association between mentum deformities and trace elements in Chironomidae in the Coeur d' Alene River system, Idaho, USA, which is contaminated

with trace metals. They found significant correlation between all metal concentrations except Ni and deformity rates. Vermeulen (1995) believed that heavy metals and several organic xenobiotics such as pesticides, PAHs and PCBs are referred to as causal compounds based on some field studies; however, there was no relationship between the organic loading and deformities. Since the Lake Huron-Lake Erie Corridor is a complex waterway polluted by diverse industrial, agricultural, recreational and municipal contaminants, further research is necessary to elucidate the responses of mentum deformities to specific chemicals.

#### **Spatial Distribution of Deformed Larvae**

Zone 2CSR is located at the upstream end of the St. Clair River on the Canadian side. This is where clean water from Lake Huron enters the corridor system. However, it is also the place where petrochemical byproducts entered the St. Clair River since there is a large petrochemical complex around Sarnia, Ontario (MOE 1986; EC and EPA 1988). Elevated incidences of deformities were found in this area in this study.

Zone 1LSC is located at Anchor Bay area, which is considered to be a relatively unpolluted reference area in the corridor system. Relatively few chironomids were collected in the Anchor Bay reference area. Although one *Polypedilum* individual of 11 larvae collected was deformed ( $9.09 \pm 8.67\%$ ,  $n = 11$ ), the overall sample size was too small to determine a precise estimate of the incidence of deformities.

Zone 3LSC is located at the junction of downstream of the St. Clair River and Walpole Delta, also include the South Channel and Chenal Ecarte. About half of water from the St. Clair River flushes from here to the centre of Lake St. Clair, and then through Peche Island, the mouth of the Detroit River to Lake Erie (Leach 1991;

UGLCCS 1988). Walpole Island is a First Nation reserve. The major land uses in Walpole Island vicinity are agriculture, so pesticides are a major type of pollutant input to the corridor system via Walpole Island. Pollutants carried by the St. Clair River water from the main river channels also tend to settle down here since the flow velocity here is much lower than that in the St. Clair River (UGLCCS 1988b). Elevated incidences of deformed mentum of *Chironomus* ( $12.24 \pm 3.31\%$ ,  $n = 98$ ) were also found in this location. This result is similar to the findings of Hudson and Ciborowski (1996a) that Walpole Island organisms were most prone to deformities.

Zone 4LSC is within the open water area of Lake St. Clair. Low incidences of mentum deformities were found in this zone ( $0.72 \pm 0.51\%$ ,  $n = 278$ ), indicating that environment condition here is generally good. Thornley (1985) and Leach (1991) also reported that Lake St. Clair supports organisms mainly associated with relatively unpolluted waters, primarily due to the large inflow of clean Lake Huron water.

A high density of industries is located on both the U.S. and Canadian shorelines of the Detroit River. Diverse industrial chemicals and pesticides are discharged into the Detroit River, with municipal wastewater entering in the vicinity of Detroit and Windsor. Hudson and Ciborowski (1996a) reported that Peche Island had a high proportion of deformed *Chironomus* ( $16.7 \pm 2.1\%$ ,  $n = 305$ ). Their conclusion was confirmed by the current analysis. Zone 1ADR, the downstream of Peche Island in the U.S. side (beside Belle Isle), which has the highest overall incidence of mentum deformities ( $5.88 \pm 2.16\%$ ,  $n = 119$ ) could be considered as ecological degraded area. The incidence of mentum deformities in *Dicrotendipes* in this area was also elevated ( $25 \pm 21.65\%$ ,  $n = 4$ ), and it is also the only zone in this analysis to have elevated deformed ligula in *Procladius* ( $25 \pm 15.31\%$ ,  $n = 8$ ). However, the sample sizes for both deformed genera in this zone were

too low to be able to draw any definitive conclusion. It is necessary to collect more individuals from this zone to create a robust analysis.

The lower portion of the Detroit River (zones 3ASR and 4CSR), which contained the most degraded sites in the corridor system (see Chapter 2) is the most severely polluted area in the whole corridor because of the industrial pollutants and its habitat characteristics (Hudson et al. 1986; Szalinska 2006). These zones had few individuals of chironomids or any other type of zoobenthos except for oligochaetes. No deformities were found in larvae from these zones. This might fit the hypothesis given by Warwick (1990b) that when the toxicity of contaminants elevated to a certain level, the chironomids might be eradicated, and were therefore not collected. In these areas, it is difficult or impossible to collect enough chironomids (more than 125 larvae from each population; Hudson and Ciborowski 1996a) to perform a suitably powerful analysis of deformities. Since the sample size is so small ( $n = 20$  in zone 3ADR and  $n = 45$  in zone 4CDR) of all six taxa examined, and the community composition of these zones have been altered by anthropogenic contaminants, the incidence of mentum deformities in Chironomidae might not be a good way to evaluate the environmental conditions in areas as polluted as these. A laboratory-based toxicity test might be a better way to evaluate the degree of contaminant in the downstream of the Detroit River (Ciborowski et al. 1995; Hudson and Ciborowski 1996b).

### **Synopsis**

Chironomini was the dominant tribe in this study, comprising 73% of all the chironomids collected in the corridor system in 2004/5. Six genera were widespread enough to assess for mentum deformities. Significant spatial and taxonomic variation



was identified in the incidence of mentum deformities in this study. However, only *Chironomus* display high variation in incidence of mentum deformities overall. Zones around the Canadian shoreline of upstream end of the St. Clair River (Sarnia region) and Walpole Island had significantly elevated *Chironomus* mentum deformities. The Canadian shoreline of upstream St. Clair River also had elevated *Dicrotendipes* mentum deformities. Elevated deformities of *Dicrotendipes* mentum and *Procladius* ligula were found in the U. S. side of Belle Isle in the Detroit River, indicating that these locations were degraded by anthropogenic stresses. Further study is required to specify the point sources of chemicals in these areas. Compared with previous studies, this study has larger sample size; however, it has still not achieved the sample sizes recommended by Hudson and Ciborowski (1996a) to provide suitable power to assess individual sites (at least 125 larvae from each population). The most heavily polluted zones such as downstream portions of the Detroit River had very low densities of Chironomidae and other zoobenthic taxa except for oligochaetes, so that no statistical trends were evident. Since the community composition has been so obviously altered in this area, the incidence of deformities in Chironomidae is not a suitable or even necessary way to evaluate the environmental conditions of such areas.

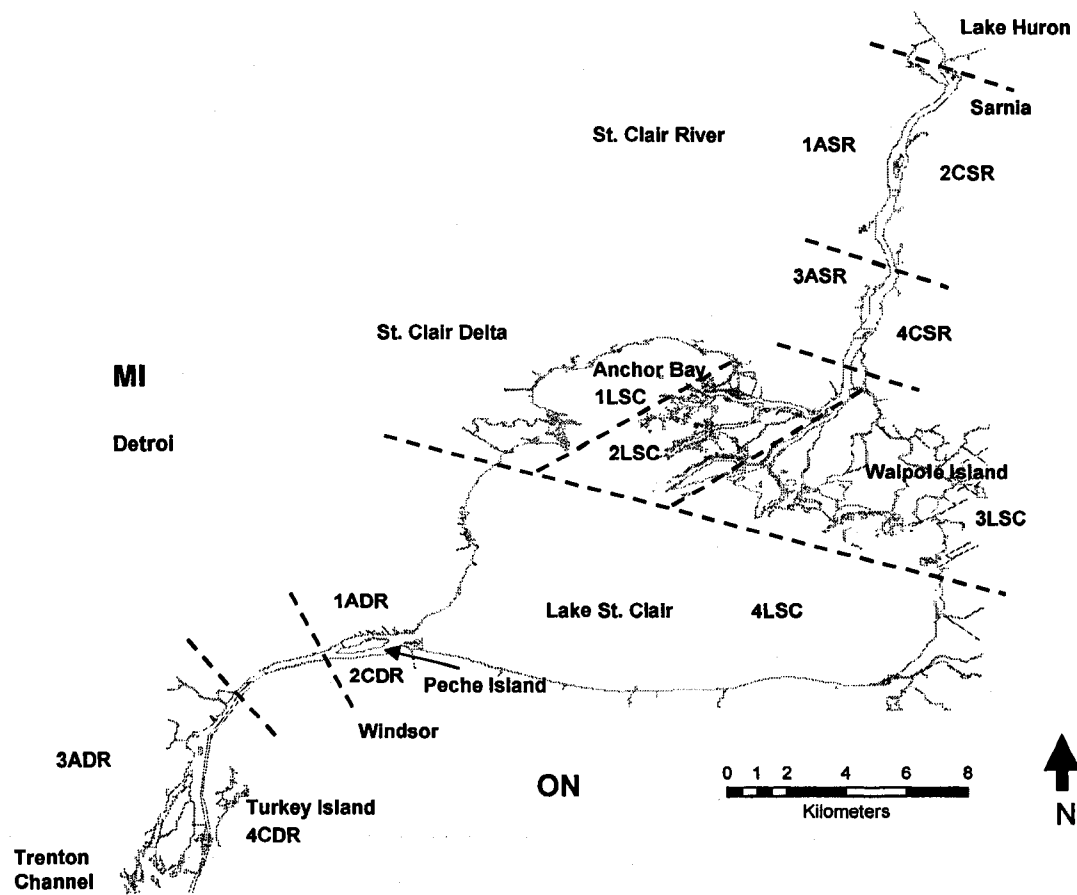


Figure 3.1. Location of 12 zones in the Lake Huron-Lake Erie Corridor for Chironomidae mouthpart deformity study in 2004/5

Table 3.1. Summary of numbers of chironomid larvae collected from 12 Lake Huron-Lake Erie Corridor zones, 2004/5

Site ID	Chironominae			Tanypodinae	Orthocladiinae	Prodiamesinae	Diamesinae
	chironomini	Tanytarsini					
St. Clair River	1ASR	480	20	54	101	7	0
	2CSR	324	8	18	55	0	2
	3ASR	60	2	10	3	0	1
	4CSR	261	25	9	11	0	1
Lake St. Clair	1LSC	174	90	37	8	0	1
	2LSC	751	46	53	25	19	8
	3LSC	883	170	121	91	3	4
	4LSC	324	104	81	15	0	4
Detroit River	1ADR	162	6	33	8	0	0
	2CDR	168	38	11	2	1	1
	3ADR	26	7	4	2	0	0
	4CDR	47	6	3	0	0	0
Total		3660	522	434	321	30	22

Table 3.2. Arrangement of teeth in the mentum / ligula of *Chironomus*, *Dicrotendipes*, *Phaenopsectra* / *Tribelos*, *Polypedilum*, *Procladius* and *Tanytarsus*

Genus	No. of Median Teeth (Mentum / Ligula)	No. of Lateral Teeth (pairs) (Mentum)
<i>Chironomus</i>	1 trifid	6
<i>Dicrotendipes</i>	1	6
<i>Phaenopsectra</i> / <i>Tribelos</i>	2	7
<i>Polypedilum</i>	2	7
<i>Procladius</i>	5	N/A
<i>Tanytarsus</i>	1 simple, bifid or trifid	5

Table 3.3. Incidence of mentum deformity ( $\% \pm \text{SE}$ ) of six genera collected from the Lake Huron-Lake Erie Corridor, 2004/5

Genus	Deformed Mentum ( $\% \pm 1\text{SE}$ )	Sample size (n)
<i>Chironomus</i>	$5.43 \pm 1.15$	387
<i>Dicrotendipes</i>	$2.64 \pm 0.67$	569
<i>Phaenopsectra/Tribelos</i>	$1.90 \pm 0.77$	316
<i>Polypedilum</i>	$2.08 \pm 0.39$	1395
<i>Procladius</i>	$2.16 \pm 1.23$	139
<i>Tanytarsus</i>	$0.32 \pm 0.32$	311

Table 3.4. Overall incidence of deformities (proportion  $\pm$  1SE) of six taxa at 12 zones in the Lake Huron-Lake Erie Corridor, 2004/5

Site ID	Deformed Mentum/Ligula (% $\pm$ SE)	Sample size (n)
1ASR	2.49 $\pm$ 0.74	441
2CSR	4.43 $\pm$ 1.31	248
3ASR	2.63 $\pm$ 2.60	38
4CSR	1.65 $\pm$ 0.82	243
1LSC	0.57 $\pm$ 0.57	176
2LSC	1.65 $\pm$ 0.52	606
3LSC	3.06 $\pm$ 0.62	752
4LSC	0.72 $\pm$ 0.51	278
1ADR	5.88 $\pm$ 2.16	119
2CDR	2.65 $\pm$ 1.31	151
3ADR	0	20
4CDR	0	45

Table 3.5. Incidence of mentum deformities (%  $\pm$  SE) among the Lake Huron-Lake Erie corridor sampling zones for each of genera examined. Numbers in parenthesis represent sample size; incidence of mentum deformities (%  $\pm$  1SE) higher than the baseline levels are shown in bold face

Site ID	<i>Chironomus</i>	<i>Dicrotendipes</i>	<i>Phaenopsectra</i> / <i>Tribelos</i>	<i>Polypedium</i>	<i>Procladius</i>	<i>Tanytarsus</i>
1ASR	2.90 $\pm$ 2.02 (69)	0 (25)	3.64 $\pm$ 1.79 (110)	2.03 $\pm$ 1.01 (197)	2.86 $\pm$ 2.82 (35)	0 (5)
2CSR	16.00 $\pm$ 7.33 (25)	8.89 $\pm$ 4.24 (45)	0 (8)	2.53 $\pm$ 1.25 (158)	0 (11)	0 (1)
3ASR	0 (3)	0 (9)	0 (1)	5.00 $\pm$ 4.87 (20)	0 (4)	0 (1)
4CSR	0 (2)	2.99 $\pm$ 1.47 (134)	0 (17)	0 (75)	0 (5)	0 (10)
1LSC	0 (17)	0 (9)	0 (37)	9.09 $\pm$ 8.67 (11)	0 (13)	0 (89)
2LSC	0 (30)	0 (69)	0 (32)	2.12 $\pm$ 0.70 (425)	0 (16)	2.94 $\pm$ 2.90 (34)

Table 3.5. Continued

Site ID	<i>Chironomus</i>	<i>Dicrotendipes</i>	<i>Phaenopsectra</i> / <i>Tribelos</i>	<i>Polypedilum</i>	<i>Procladius</i>	<i>Tanytarsus</i>
3LSC	12.24 ± 3.31 (98)	2.31 ± 1.36 (216)	6.90 ± 4.71 (29)	1.23 ± 0.61 (325)	0 (19)	0 (65)
4LSC	0 (22)	2.44 ± 2.41 (41)	0 (53)	1.96 ± 1.94 (51)	0 (23)	0 (88)
1ADR	0 (5)	25.00 ± 21.65 (4)	0 (16)	4.71 ± 2.30 (85)	25.00 ± 15.31 (8)	0 (1)
2CDR	3.49 ± 1.98 (86)	0 (13)	0 (4)	2.44 ± 2.41 (41)	N.A. (0)	0 (7)
3ADR	0.00 (5)	0 (2)	0 (4)	N.A. (0)	0 (3)	0 (6)
4CDR	0 (25)	0 (2)	0 (5)	0 (7)	0 (2)	0 (4)



## **Chapter 4 General Discussion and Conclusions**

### **4.1 General Discussion**

Numerous studies using zoobenthos as indicators have been conducted to assess habitat quality in freshwater ecosystems. These studies have often proposed that zoobenthos serve as good indicators of anthropogenic stresses either at the community level or at the level of the individual (Thornley and Hamdy 1984; Ciborowski and Corkum 1988; Warwick 1988, 1989, 1990a, Burt et al. 2003). The purpose of my research has been to assess the habitat quality of the Lake Huron-Lake Erie Corridor aquatic ecosystem by using the zoobenthic community composition and the incidence of chironomid mouthpart deformity as indicators. Although it is important, few studies have directly compared the efficacy of zoobenthic indicators at community and individual levels in assessing water and sediment quality to evaluate how anthropogenic contaminants affect the overall ecosystem health. For instance, in chironomid mouthpart deformity studies, if the incidence of deformity at a site was not elevated above the baseline level, one could not draw an absolute conclusion. Two alternative explanations could be indicated: either the anthropogenic stresses in this site were not sufficient to produce deformities, or the stresses were at such high levels that most of the organisms have been killed, and/or the surviving organisms have developed a resistance to the stresses (Burt 1999). In such situation, a community level assessment is necessary to give a complementary explanation. In contrast, in some cases, habitat changes cannot be detected by community indicators, whereas individual organisms are likely to be more sensitive indicators of degradation. For instance, in my study, the Canadian side of the upper end of the St. Clair River (near Sarnia) and the Walpole Delta in Lake St. Clair did not contain any sites contaminated

enough to be classified as 'degraded', and their community composition was not distinctly altered. However, the chironomid mouthpart deformity study (Chapter 3) revealed elevated incidences of deformities around these areas, indicating that these areas are environmentally degraded at certain levels. The combination of using community and individual indicators to assess habitat quality is more powerful than using either of them individually.

The long-term assessment of ecosystem condition is important to improve our understanding of natural variability. In Chapter 2, I compiled data from two previous Detroit River studies (Farara and Burt 1993; Wood 2004) and the current corridor study (2004/5), both to document historical changes in the biological condition of the Lake Huron-Lake Erie Corridor and to provide a large enough database to permit delineation of putative reference and degraded conditions, based on sediment contamination.

The "Reference-Degraded Continuum" (RDC) multivariate approach was used to integrate physical, chemical and biological variables in this chapter. This is the first application of this technique to zoobenthos. When environment quality is uniformly good (equivalent to reference), the zoobenthic community is believed to be unique in areas with different benthic habitat characteristics (Manny et al. 1986; Ciborowski 2003). The results of Chapter 2 confirmed this and showed that near-bottom water velocity, water depth and temperature, substrate type (median particle size), dissolved oxygen concentration and the geographic location of sites within the corridor are possible factors by which distinct associations of zoobenthic taxa exist in reference areas. Although the Bray-Curtis ordination analysis indicated that the relationship between biological conditions (relative abundances of zoobenthic taxa) and the sediment contamination scores (Sumrel) was strongest in hard-substrate locations,

correlations were found between these two factors for all types of sites, especially when near-bottom water velocity was included as a classification variable in the DFA model in the Detroit River case study.

The first investigation (Chapter 2) suggests that in a system like the Lake Huron-Lake Erie Corridor, the RDC approach was an effective way to assess substrate quality by using zoobenthic community composition as an indicator overall. However, some REF sites, which were expected to have low SumRel scores and high ZCI scores, were distributed across the entire zoobenthic ordination gradient (Y-axis). In contrast, some DEG sites had relatively high ZCI scores (indicating good biological condition), especially those in depositional sites. This suggests that either some of reference sites might not be representative of good environmental quality, or that other biological factors influence the zoobenthic assemblages at those locations. Possible reasons were:

- 1) Although the “least-disturbed” sites were designated as reference sites in the analysis, the ‘true’ reference condition (minimally disturbed – i.e., truly uncontaminated sites) no longer exists; lack of appropriate “reference sites” together with no clear “contaminant gradient” might limit the use of this approach to assess the habitat quality;
- 2) Sixteen chemicals (metals, pesticides and other organochlorine compounds) were selected to perform the initial reference and degraded site designation. However, some potentially important classes of compounds such as PAHs, and compounds such as pentachlorobenzene (QCB) and hexachlorobenzene (HCB) were left out of the analysis due to incomplete data. The reference sites with poor biological conditions might have high concentrations of classes of toxic chemicals that were not included in current analysis. Alternatively, the designation of relatively benign materials as stressors (e.g., inclusion of PC-1

scores in the SumRel total) may reduce the strength of correlation. Ordination of zoobenthic community composition with respect to PC-2 alone (Appendix IV) provided evidence for this possibility. However, that analysis identified the same taxa as being the key bioindicators of reference and degraded conditions;

3) Factors other than anthropogenic stresses can influence zoobenthic community composition in similar habitat situations - food quantity, food type and aquatic plant cover, etc. (Covich et al. 1999; Doisy and Rabeni 2001). Human activities unrelated to pollutants may also negatively influence the zoobenthic community composition, such as the alteration of shorelines and loss of wetlands (Leach 1991);

4) the near-bottom water velocity data were estimated from a hydrodynamic model based on a coarse spatial scale, but the zoobenthic data were collected based on a fine spatial scale (one ponar sample per site), so the near-bottom water velocity might not be sensitive enough to document the subtle differences in velocity among sites.

Chironomid mouthpart deformities have been used extensively as an indicator of water and sediment quality (Warwick and Tisdale 1988; Warwick 1988, 1989, 1990a; Hudson and Ciborowski 1996a, b). However, sample size is an important factor influencing the suitability of this indicator. At least 125 larvae from each population should be used to provide suitable power (recommended by Hudson and Ciborowski (1996a)). If sample sizes at individual sites were too small to perform the statistical analysis, the adjacent sites were pooled to form larger zones. In Chapter 3, I pooled the 2004/5 corridor sampling sites to 12 zones to create larger sample sizes, and then looked at the spatial and taxonomic variation in incidence of chironomid

mentum deformities along the corridor. However, sample sizes of larvae collected at individual sample sites in the corridor system in 2004/5 survey were so small that statistical analysis is not viable, which limited the power of my research.

The results derived from Chapter 3 showed that both spatial and taxonomic variation was identified in the incidence of chironomid mentum deformities (G-statistic Goodness of Fit test). Genera differed in their sensitivity to contaminants; *Chironomus* had the greatest incidence of deformities. With the increasing of the contaminant concentration, the incidence of mentum deformity generally increased. Significant spatial differences were found in the incidence of mentum deformities of *Chironomus*, indicating that deformities are a potentially effective indicator of water and sediment quality.

## 4.2 General Conclusion

The RDC approach provided a method to identify differences in zoobenthic community composition associated with environmental variability in the Lake Huron-Lake Erie Corridor, and to develop a zoobenthic condition index that permits one to assess the effects differences in sediment contamination. In the chironomid mouthpart deformity study, both spatial and taxonomic variation was identified across the corridor. The results of these two studies provided complementary information and together gave an overall assessment of the corridor biological condition. In severely degraded areas (i.e., the lower portion of the Detroit River, which contained most of the degraded sites in my first study), the zoobenthic community composition has been so obviously altered (dominated by oligochaetes and had low densities of all other taxa) that the incidence of mouthpart deformities in Chironomidae is unsuitable for evaluating environmental conditions. In areas not designated 'degraded' in the first study, but still disturbed to a certain extent (i.e., the upstream end of the St. Clair River on the Canadian side, Walpole Island region and the head of the Detroit River around Belle Isle on the US side), elevated incidences of mentum deformities of chironomids were found in the second study. All other areas not designated either 'reference' or 'degraded' in the first study and lacking evidence of elevated incidences of mentum deformities in the second study had relative better biological condition. Sites designated 'reference' in the first study are likely the 'best available' sites, and support benthic assemblages representing the best biological condition compared with other areas in the Lake Huron-Lake Erie ecosystem. Overall, both community level and individual level assessments of biological condition are useful approaches to determine the effects of sediment contamination.

There is evidence that the overall sediment quality of the Detroit River has improved between 1991 and 2004. However, a comparison of the mean ZCI scores among 3 years at 8 blocks of sites, failed to show statistically significant differences among 3 years, indicating that the condition of zoobenthic communities in 2004 has not markedly improved.

### **4.3 Future Research**

Sediments in the Lake Huron-Lake Erie Corridor have long been contaminated by industrial, agricultural and municipal inputs, especially by persistent chemicals, such as PCBs, PAHs, organochlorine pesticides and trace elements. The reference and degraded site designation used in this study relied on the compilation of the 16 chemicals for each site in the survey data. However, contaminants excluded from analysis due to incomplete data also have the potential to influence zoobenthic community composition (i.e., PAHs, HCB, QCB, etc.). Additional analyses of the sediments are needed to provide more complete stressor information in future studies. This would permit better maps of the contaminant gradient and associated biological communities in the river systems to be drawn.

In multivariate analysis, an important step is to use habitat attributes to classify groups of sites with similar zoobenthic community composition. However, the key habitat attribute, near-bottom water velocity, was not available for the St. Clair River and Lake St. Clair sampling sites, and the Detroit River sites used coarse-grained data from a hydrodynamic model. Although it is reassuring to know that even such a coarse level of resolution can greatly improve the ability to classify benthic habitat, this limitation limited the discriminatory ability of the DFA model and the ordination technique. In future studies, a special effort should be made to collect near-bottom water velocity data in the field studies if possible, so that this factor can be taken into account to improve site classification at the whole corridor scale.

Although significant spatial and taxonomic variation was identified in the incidence of mentum deformities in this study, small sample size is still a problem that limits the power of such investigations, especially in zones with high proportions of mouthpart deformity but small sample sizes, such as zone 2CSR (the upstream end of



the St. Clair River on the Canadian side) and 1ADR (downstream of Peche Island in the U.S. side, beside Belle Isle). Future research requires that a field sampling method that permits one to collect more individual specimens from such areas, to reduce the standard error of these zones.

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Appendix I. Locations and habitat attributes of 311 sampling sites in the Lake Huron-Lake Erie Corridor in 1991, 1999 and 2004/5 surveys. Lake or River type are: Detroit River (DR), Lake St. Clair (LSR), and St. Clair River (SCR)

Site ID	Lake or River	Latitude	Longitude	Total Organic Carbon (LOI %)	Water Depth (m)	Water Temperature (°C)	Dissolved Oxygen Concentration (mg/L)	Median Particle Size (Phi)
003ABC	DR	42.35	-82.94	1.14	5.94	15.00	9.80	0.56
004ABC	DR	42.35	-82.93	0.78	3.20	15.50	10.60	0.57
005ABC	DR	42.34	-82.95	0.53	1.22	17.00	9.00	0.64
007ABC	DR	42.34	-82.94	4.17	8.08	16.00	9.30	2.24
008A	DR	42.35	-82.92	0.82	2.13	16.00	12.50	0.65
009B	DR	42.36	-82.92	0.96	3.20	16.00	10.00	0.90
010B	DR	42.35	-82.98	1.43	3.35	22.02	8.68	0.95
011A	DR	42.34	-82.99	1.57	6.25	21.96	8.55	1.58
012A	DR	42.34	-82.99	1.13	1.52	22.52	10.08	1.60
013A	DR	42.35	-82.99	1.99	1.22	22.36	9.30	1.62
014B	DR	42.33	-83.02	1.47	8.23	21.87	8.64	1.54
015C	DR	42.34	-83.01	1.27	6.55	22.00	8.24	1.00
016C	DR	42.33	-82.96	0.87	1.52	19.66	7.54	0.87
017B	DR	42.34	-83.00	1.69	3.35	21.96	8.77	1.64
018A	DR	42.33	-83.01	2.55	12.65	19.37	7.12	1.12
019B	DR	42.33	-83.00	2.19	3.66	20.34	8.11	1.66
021B	DR	42.33	-82.99	0.97	12.50	19.68	7.16	0.25
022B	DR	42.33	-82.98	1.67	11.89	19.26	7.09	1.74
023C	DR	42.34	-82.95	0.97	1.52	19.47	7.13	1.34
024C	DR	42.35	-82.97	4.89	7.92	21.98	8.65	2.00
025B	DR	42.34	-82.97	2.76	1.37	20.15	6.66	2.25
026C	DR	42.34	-82.97	1.80	8.99	19.68	6.88	0.31

027B	DR	42.33	-83.03	1.70	11.58	12.15	9.20	2.42
029C	DR	42.31	-83.08	1.39	9.14	14.40	10.50	1.22
030ABC	DR	42.32	-83.06	1.81	11.89	16.66	9.18	0.92
031A	DR	42.32	-83.05	6.67	12.50	12.04	9.36	-0.16
033ABC	DR	42.29	-83.09	1.45	9.14	19.13	6.55	1.10
034C	DR	42.30	-83.09	19.15	11.58	19.07	6.85	-1.00
035C	DR	42.29	-83.09	3.99	12.50	18.99	7.39	-1.00
036C	DR	42.30	-83.09	2.18	10.67	19.03	7.46	1.74
037B	DR	42.30	-83.08	3.91	13.72	20.00	8.50	1.86
042C	DR	42.29	-83.10	16.28	5.18	19.29	7.29	2.00
043ABC	DR	42.29	-83.10	5.31	14.02	18.81	7.17	1.90
044A	DR	42.25	-83.11	1.67	1.58	16.00	9.20	1.50
045B	DR	42.25	-83.11	5.72	1.52	16.00	9.40	2.06
047ABC	DR	42.27	-83.11	1.23	11.28	15.20	6.92	0.67
048C	DR	42.26	-83.11	0.65	10.67	16.39	9.59	1.38
049A	DR	42.27	-83.11	6.02	13.00	12.90	9.20	0.91
050B	DR	42.26	-83.12	5.48	5.79	17.31	6.16	1.35
052B	DR	42.26	-83.12	10.06	6.55	16.74	9.56	0.67
054B	DR	42.26	-83.12	2.00	7.00	17.22	6.14	1.58
055C	DR	42.25	-83.12	0.87	10.36	15.91	9.80	0.17
057C	DR	42.24	-83.13	1.82	10.67	16.42	8.40	0.80
058C	DR	42.25	-83.12	0.94	8.08	16.27	9.54	1.55
059ABC	DR	42.24	-83.12	3.08	1.17	16.41	9.07	2.58
060B	DR	42.25	-83.12	0.88	7.92	16.00	9.00	0.21
064B	DR	42.21	-83.13	7.11	1.52	21.97	10.31	1.79
065C	DR	42.21	-83.12	2.06	1.68	21.75	10.33	1.88
066A	DR	42.20	-83.12	3.61	1.37	22.46	10.76	1.77
067B	DR	42.20	-83.11	5.54	1.52	20.85	8.30	2.06
068B	DR	42.20	-83.11	2.13	6.71	20.80	9.36	1.46

069A	DR	42.21	-83.10	6.25	0.99	21.72	10.45	2.21
070B	DR	42.20	-83.10	5.65	1.07	21.02	8.57	2.14
071B	DR	42.23	-83.13	1.25	10.06	21.05	9.57	-0.19
072A	DR	42.23	-83.13	2.44	1.22	21.70	10.83	1.47
073C	DR	42.23	-83.11	1.12	7.62	20.68	9.61	0.52
074B	DR	42.22	-83.11	6.88	1.68	21.13	10.61	1.70
075A	DR	42.24	-83.13	2.81	6.10	21.23	9.04	1.31
076ABC	DR	42.25	-83.13	8.22	3.05	21.57	10.50	2.00
077B	DR	42.23	-83.14	4.48	8.00	20.76	7.66	1.36
078B	DR	42.23	-83.14	2.35	1.07	21.86	10.61	1.49
079C	DR	42.24	-83.14	7.43	1.07	21.89	6.58	2.00
080C	DR	42.24	-83.14	2.43	0.76	21.49	5.18	1.52
081B	DR	42.19	-83.10	6.35	1.52	14.79	14.10	2.09
082A	DR	42.15	-83.12	5.93	1.83	15.00	8.70	2.23
083B	DR	42.15	-83.12	1.97	9.75	15.00	8.90	0.50
084A	DR	42.16	-83.12	4.07	1.42	15.80	8.60	1.71
085C	DR	42.15	-83.12	2.74	2.13	15.00	9.10	1.63
086A	DR	42.15	-83.12	2.73	2.13	15.00	9.20	1.58
088C	DR	42.20	-83.11	6.81	10.06	14.00	14.00	1.88
089A	DR	42.15	-83.11	6.08	1.14	15.00	9.05	1.80
090B	DR	42.18	-83.11	2.09	10.97	14.10	14.00	0.93
091C	DR	42.17	-83.13	9.25	1.37	16.50	10.50	1.55
092ABC	DR	42.17	-83.13	2.62	1.98	16.50	9.00	1.53
093C	DR	42.18	-83.12	3.16	4.27	18.30	14.00	1.58
094C	DR	42.18	-83.12	1.53	5.49	14.20	14.00	0.59
095A	DR	42.18	-83.14	1.12	1.07	17.00	8.80	1.55
096A	DR	42.18	-83.13	3.56	1.68	16.00	8.80	2.00
097ABC	DR	42.19	-83.12	6.67	1.37	14.40	14.00	1.92
098C	DR	42.20	-83.15	3.21	6.40	16.50	8.70	2.03

099C	DR	42.15	-83.14	5.42	1.37	16.00	9.80	1.56
100C	DR	42.20	-83.14	2.44	1.98	16.00	8.80	1.58
101C	DR	42.17	-83.16	8.03	0.76	17.00	10.40	2.12
102B	DR	42.18	-83.14	1.18	1.22	17.50	10.20	0.56
103A	DR	42.17	-83.14	1.44	1.22	16.20	9.20	1.43
104C	DR	42.15	-83.12	2.01	6.10	19.53	8.20	1.00
105C	DR	42.09	-83.12	4.88	2.29	19.41	7.56	2.14
106B	DR	42.09	-83.15	2.51	3.05	20.18	8.77	2.32
107C	DR	42.09	-83.14	5.17	1.98	19.96	8.08	2.21
108B	DR	42.12	-83.12	6.91	1.98	20.82	8.77	2.02
109C	DR	42.14	-83.12	1.10	3.05	19.50	8.12	1.05
10FB	DR	42.34	-83.02	2.10	5.50	20.10	7.63	0.40
111C	DR	42.13	-83.14	1.91	1.37	20.03	7.22	1.69
113B	DR	42.14	-83.17	1.32	5.00	19.83	6.67	0.58
114B	DR	42.14	-83.17	2.27	5.49	19.71	6.88	1.02
115ABC	DR	42.10	-83.15	3.20	0.76	20.40	9.77	1.66
116B	DR	42.11	-83.18	1.13	3.05	19.79	7.49	0.45
117ABC	DR	42.11	-83.18	2.14	4.88	19.56	7.64	0.41
118A	DR	42.11	-83.18	3.76	5.79	19.58	7.55	0.49
119B	DR	42.10	-83.18	2.34	2.44	19.33	7.38	1.80
121C	DR	42.04	-83.12	2.47	3.96	17.11	5.75	0.71
122B	DR	42.06	-83.12	0.56	1.43	17.02	9.52	0.54
123A	DR	42.05	-83.13	7.14	5.49	16.60	8.50	2.02
124A	DR	42.06	-83.13	6.30	4.88	16.42	9.55	2.14
125A	DR	42.06	-83.13	1.16	7.92	16.43	9.77	1.00
126A	DR	42.06	-83.14	3.20	2.13	17.80	10.48	2.24
127B	DR	42.06	-83.14	3.03	3.66	17.87	8.07	1.65
128B	DR	42.08	-83.15	1.79	1.00	20.00	9.20	1.49
129A	DR	42.08	-83.13	1.05	2.74	19.14	9.98	0.61

12FB	DR	42.32	-83.04	2.80	9.30	19.23	8.40	-0.56
130A	DR	42.08	-83.13	4.99	1.89	19.33	10.58	1.96
131B	DR	42.08	-83.12	5.77	3.20	18.99	10.61	2.06
132B	DR	42.07	-83.12	0.60	0.99	16.77	9.92	0.53
133B	DR	42.09	-83.13	1.55	1.83	20.62	11.76	1.02
134C	DR	42.09	-83.17	0.57	1.22	20.50	8.80	0.55
135A	DR	42.09	-83.17	3.89	0.91	18.80	10.36	1.54
136B	DR	42.06	-83.19	2.80	1.07	22.29	11.60	1.18
137A	DR	42.06	-83.19	0.56	1.00	24.70	11.80	1.48
138B	DR	42.06	-83.18	2.45	2.44	21.19	9.05	1.60
139B	DR	42.08	-83.17	1.82	1.37	20.50	9.70	1.16
13FB	DR	42.32	-83.07	1.90	7.50	20.77	7.90	-0.07
140C	DR	42.06	-83.18	1.29	2.74	19.95	9.29	1.53
141B	DR	42.08	-83.16	4.14	3.05	20.50	8.80	1.46
142C	DR	42.09	-83.17	3.14	1.07	19.71	9.90	1.61
143B	DR	42.07	-83.18	1.69	2.59	19.27	9.49	1.39
144B	DR	42.07	-83.18	3.61	4.11	19.36	9.58	1.39
145B	DR	42.07	-83.18	2.65	2.59	19.47	9.66	0.69
146B	DR	42.07	-83.17	6.32	2.59	19.51	9.74	1.48
147A	DR	42.08	-83.19	12.71	0.76	23.14	8.62	2.32
148B	DR	42.09	-83.19	1.75	3.66	20.44	9.20	0.64
149C	DR	42.08	-83.18	2.59	0.91	21.02	9.36	2.00
14FB	DR	42.31	-83.06	1.30	4.50	19.57	7.07	0.23
150B	DR	42.09	-83.18	5.62	4.00	19.56	9.44	1.67
15FB	DR	42.31	-83.08	9.70	5.20	21.43	6.73	0.44
16FB	DR	42.31	-83.08	4.40	2.80	19.17	8.33	2.18
17FB	DR	42.29	-83.10	4.70	7.00	20.00	7.00	0.08
186FB	DR	42.33	-83.00	2.30	11.00	23.30	3.00	-0.35
189FB	DR	42.34	-83.01	7.00	3.00	19.90	7.63	-0.25



18FB	DR	42.29	-83.09	5.80	4.00	19.23	6.63	1.46
195FB	DR	42.32	-83.07	4.40	4.00	21.40	6.77	0.44
198FB	DR	42.29	-83.09	6.83	7.20	21.87	7.96	1.82
199FB	DR	42.29	-83.09	6.83	6.50	21.73	2.98	0.48
19FB	DR	42.28	-83.10	5.70	10.00	20.00	7.00	-0.57
1FB	DR	42.36	-82.93	1.50	6.00	20.00	9.97	0.45
200FB	DR	42.29	-83.09	6.83	8.20	22.17	8.47	1.60
21FB	DR	42.28	-83.11	6.10	3.50	19.30	9.10	0.69
221FB	DR	42.12	-83.11	6.83	3.00	22.00	7.59	0.68
222FB	DR	42.12	-83.11	6.83	5.70	24.37	7.10	0.58
223FB	DR	42.12	-83.11	6.83	3.00	22.73	7.70	0.38
224FB	DR	42.12	-83.11	2.90	1.00	24.47	7.26	0.86
22FB	DR	42.28	-83.10	4.90	3.00	22.47	8.67	1.82
23FB	DR	42.27	-83.11	6.50	4.20	20.20	8.03	0.64
240FB	DR	42.12	-83.18	3.00	2.00	22.90	6.90	0.41
24FB	DR	42.27	-83.10	2.60	4.00	19.05	8.75	0.08
25FB	DR	42.25	-83.12	2.20	2.20	19.27	6.72	0.43
26FB	DR	42.25	-83.13	6.83	6.70	23.10	11.90	1.05
27FB	DR	42.24	-83.12	1.80	1.20	19.17	6.73	0.19
28FB	DR	42.24	-83.11	6.20	3.00	19.60	6.71	0.83
2FB	DR	42.35	-82.93	1.60	3.00	18.57	9.73	0.47
33FB	DR	42.19	-83.11	2.30	4.80	23.93	5.93	0.89
34FB	DR	42.23	-83.15	1.80	4.20	20.40	9.30	0.12
35FB	DR	42.23	-83.13	6.83	1.00	20.23	8.90	1.75
36FB	DR	42.20	-83.15	2.40	8.50	20.77	7.40	0.18
37FB	DR	42.21	-83.14	1.50	3.00	20.40	8.13	0.37
39FB	DR	42.17	-83.14	1.60	1.50	21.37	9.37	0.30
40FB	DR	42.15	-83.12	2.20	2.50	21.17	8.00	0.72
41FB	DR	42.14	-83.13	1.10	4.00	23.70	8.60	-0.29

42FB	DR	42.05	-83.12	2.20	2.50	23.70	8.30	0.26
43FB	DR	42.11	-83.13	3.70	2.00	23.10	12.40	1.61
44FB	DR	42.11	-83.11	2.30	2.50	24.53	8.13	0.29
45FB	DR	42.09	-83.11	3.60	2.00	24.47	7.67	1.85
46FB	DR	42.10	-83.18	1.50	2.00	21.27	8.43	0.54
47FB	DR	42.06	-83.12	0.49	4.00	23.60	8.23	0.45
48FB	DR	42.06	-83.14	1.20	5.50	23.37	8.30	0.66
49FB	DR	42.05	-83.15	2.50	4.50	23.53	6.60	0.46
50FB	DR	42.05	-83.16	1.60	4.50	23.47	6.70	0.71
51FB	DR	42.14	-83.13	2.90	2.50	24.17	8.57	1.50
53FB	DR	42.15	-83.14	1.90	1.50	17.03	6.55	0.58
5FB	DR	42.35	-82.96	1.30	2.50	19.53	8.27	0.45
62FB	DR	42.25	-83.13	7.70	2.50	19.13	6.97	0.16
6FB	DR	42.34	-82.96	3.60	3.50	18.33	9.03	1.35
70FB	DR	42.08	-83.18	1.80	1.80	21.47	8.33	0.49
71FB	DR	42.11	-83.19	2.10	1.80	21.93	7.73	0.46
73FB	DR	42.17	-83.17	6.83	5.30	24.03	11.53	1.96
75FB	DR	42.23	-83.15	3.30	3.00	20.60	7.07	0.78
83FB	DR	42.29	-83.10	8.00	5.50	20.37	6.77	1.67
84FB	DR	42.28	-83.11	7.50	2.50	18.90	8.37	1.45
85FB	DR	42.18	-83.16	5.10	5.50	21.17	7.27	0.99
8A	DR	42.33	-83.00	6.83	4.80	23.27	8.10	0.42
9FB	DR	42.34	-83.00	2.50	6.80	19.80	9.07	-0.79
A	DR	42.34	-82.91	1.50	4.30	17.63	10.30	0.40
B	DR	42.34	-82.92	5.20	3.60	17.60	9.70	0.82
C	DR	42.34	-82.93	1.30	5.50	17.47	8.80	-0.44
D	DR	42.35	-82.95	2.90	5.50	18.37	10.23	0.82
E	DR	42.33	-83.01	1.20	4.00	19.23	7.73	0.61
F	DR	42.33	-83.05	2.00	7.50	20.80	6.97	0.43

G	DR	42.25	-83.11	1.40	1.80	19.63	6.55	0.51
H	DR	42.21	-83.13	4.40	1.90	20.80	9.16	0.46
I	DR	42.20	-83.14	1.00	2.00	20.60	8.52	0.57
J	DR	42.24	-83.11	2.20	1.50	20.07	10.50	0.96
K	DR	42.23	-83.13	2.00	3.00	20.17	8.03	0.02
L	DR	42.19	-83.11	4.60	2.50	24.23	8.43	1.88
M	DR	42.17	-83.13	2.20	2.00	21.07	9.23	0.67
N	DR	42.16	-83.13	3.70	2.20	21.00	7.52	0.71
O	DR	42.15	-83.17	6.83	5.00	20.63	7.60	0.30
P	DR	42.15	-83.17	2.20	1.50	21.03	9.04	0.75
S100	DR	42.07	-83.18	2.09	2.80	19.35	9.12	1.54
S101	DR	42.35	-82.95	1.78	7.00	18.54	9.64	1.22
S102	DR	42.35	-82.92	1.30	1.50	18.33	10.10	-1.07
S81	DR	42.34	-82.95	0.94	1.80	18.84	9.16	-1.08
S82	DR	42.35	-82.92	1.13	2.50	18.14	10.01	1.21
S83	DR	42.32	-83.05	1.95	11.00	18.48	9.60	-1.44
S84	DR	42.35	-82.99	6.24	2.10	18.43	9.90	0.63
S85	DR	42.34	-83.01	3.76	5.20	18.77	9.53	1.42
S87	DR	42.29	-83.09	3.03	10.40	18.29	9.45	-1.10
S89	DR	42.20	-83.11	4.02	8.00	19.17	9.37	1.72
S90	DR	42.30	-83.09	1.87	8.50	18.92	9.40	-1.05
S93	DR	42.21	-83.12	3.56	1.60	19.12	9.81	0.29
S94	DR	42.16	-83.12	5.03	1.80	19.45	9.66	1.17
S95	DR	42.18	-83.11	1.83	10.00	19.36	4.46	-1.02
S96	DR	42.07	-83.12	0.56	0.90	20.72	9.94	1.44
S97	DR	42.23	-83.14	1.25	1.70	18.93	9.52	1.48
S98	DR	42.17	-83.16	7.15	0.90	19.76	9.97	2.02
S99	DR	42.06	-83.19	0.86	1.20	20.96	10.37	-1.05
A23	LSC	42.57	-82.58	3.23	3.00	19.10	10.09	1.97

A27	LSC	42.56	-82.42	2.80	1.00	18.40	10.30	1.32
A28	LSC	42.55	-82.42	3.20	0.50	18.90	12.80	1.40
A29	LSC	42.51	-82.43	6.18	0.50	19.30	9.70	1.07
A53	LSC	42.45	-82.47	2.94	5.20	22.28	8.70	2.11
A58	LSC	42.42	-82.74	3.67	6.20	16.94	9.94	2.20
A66	LSC	42.63	-82.78	1.84	2.70	20.67	10.38	1.44
DBC2	LSC	42.51	-82.58	2.54	4.50	22.54	7.30	2.06
DCC2	LSC	42.50	-82.53	6.90	1.00	26.62	8.68	1.65
DCE3	LSC	42.49	-82.44	5.31	3.30	22.68	6.91	1.82
DJC2	LSC	42.49	-82.51	2.06	10.00	22.77	7.25	2.13
GL1	LSC	42.51	-82.52	1.07	0.40	26.89	8.04	1.61
MCE2	LSC	42.59	-82.44	4.23	3.00	22.40	7.37	1.87
S21	LSC	42.64	-82.51	1.71	6.00	18.02	10.00	1.16
S21(5)	LSC	42.64	-82.51	1.59	6.00	22.22	7.28	1.63
S22	LSC	42.62	-82.51	0.81	1.00	19.35	10.20	1.09
S23	LSC	42.44	-82.53	2.17	1.80	19.20	9.79	1.70
S24	LSC	42.57	-82.57	1.47	1.20	10.20	9.67	1.83
S25	LSC	42.54	-82.61	1.06	2.10	17.91	10.12	1.70
S27	LSC	42.50	-82.50	3.99	3.00	18.50	10.80	1.46
S27(5)	LSC	42.64	-82.50	2.76	1.70	22.25	7.40	1.57
S28	LSC	42.55	-82.42	1.84	0.50	15.80	9.70	1.54
S28(5)	LSC	42.55	-82.42	1.53	0.50	22.50	7.00	1.56
S36	LSC	42.64	-82.51	1.52	1.00	20.85	9.86	1.70
S37	LSC	42.58	-82.57	1.21	1.00	20.31	9.99	1.56
S38	LSC	42.57	-82.58	0.54	0.80	20.01	10.03	1.54
S39	LSC	42.56	-82.63	0.85	2.00	20.96	9.17	1.60
S40	LSC	42.55	-82.66	1.75	8.00	20.61	9.44	1.87
S41	LSC	42.54	-82.67	1.00	9.00	20.48	9.57	1.88
S42	LSC	42.62	-82.52	3.93	2.00	20.80	9.65	2.02

S43	LSC	42.61	-82.53	2.55	2.00	20.38	9.49	2.09
S44	LSC	42.62	-82.62	1.95	10.00	20.88	10.28	1.68
S45	LSC	42.62	-82.58	2.81	4.00	20.35	9.46	2.06
S46	LSC	42.62	-82.67	3.47	0.90	21.56	10.15	2.33
S47	LSC	42.62	-82.68	2.11	6.00	20.99	9.30	2.05
S48	LSC	42.61	-82.65	1.43	5.50	20.52	10.08	1.52
S49	LSC	42.60	-82.60	2.60	7.00	21.17	8.78	1.84
S50	LSC	42.59	-82.63	2.15	2.20	20.73	9.85	1.59
S51	LSC	42.52	-82.63	0.54	0.60	19.92	9.94	1.48
S52	LSC	42.49	-82.63	0.76	4.30	20.75	9.45	1.47
S53	LSC	42.45	-82.57	0.51	1.20	22.45	9.32	1.53
S54	LSC	42.45	-82.58	0.60	4.30	21.15	8.91	1.52
S55	LSC	42.44	-82.54	1.78	4.60	21.26	9.06	1.59
S56	LSC	42.41	-82.46	2.21	4.30	20.47	8.69	1.16
S57	LSC	42.49	-82.67	2.12	5.50	19.33	9.44	2.31
S58	LSC	42.45	-82.66	1.77	7.30	19.85	9.27	1.61
S59	LSC	42.42	-82.59	1.62	7.60	21.41	9.26	2.42
S60	LSC	42.39	-82.50	1.62	5.00	20.49	8.83	2.40
S61	LSC	42.37	-82.54	2.05	5.80	20.63	8.60	2.25
S62	LSC	42.35	-82.62	2.46	6.40	20.95	8.69	1.59
S63	LSC	42.34	-82.53	1.95	5.60	21.11	9.03	0.73
S64	LSC	42.32	-82.59	1.71	4.00	20.87	9.82	-1.20
S65	LSC	42.31	-82.64	0.67	1.20	20.15	9.68	1.51
S66	LSC	42.68	-82.68	2.41	2.70	21.52	10.82	1.83
S67	LSC	42.62	-82.73	1.88	3.70	20.46	9.53	2.20
S68	LSC	42.61	-82.73	1.44	3.00	21.53	9.06	1.80
S69	LSC	42.60	-82.68	1.84	1.20	22.89	10.10	1.86
S70	LSC	42.56	-82.75	0.72	4.60	20.60	10.25	1.31
S72	LSC	42.56	-82.81	1.07	3.40	21.84	10.98	1.30

S74	LSC	42.54	-82.78	1.51	4.90	20.65	9.19	1.65
S78	LSC	42.41	-82.86	2.61	5.00	18.90	9.42	2.06
S79	LSC	42.39	-82.88	1.17	4.00	19.23	9.33	1.11
S80	LSC	42.36	-82.90	2.37	6.00	18.22	9.53	1.30
UBC1	LSC	42.55	-82.58	2.89	0.50	23.42	8.75	2.02
UCC1	LSC	42.60	-82.53	3.12	0.60	23.18	7.97	1.81
UCE1	LSC	42.63	-82.49	2.21	4.50	22.17	7.48	1.22
UJC1	LSC	42.58	-82.45	3.44	1.00	22.48	7.22	2.30
A10	SCR	42.90	-82.47	3.44	1.80	19.17	10.21	1.73
A5	SCR	42.88	-82.46	1.10	5.00	19.78	9.03	-1.30
A6	SCR	42.97	-82.42	4.90	1.50	20.15	9.25	1.73
S1	SCR	43.01	-82.41	0.41	4.60	20.77	10.03	1.43
S3	SCR	42.95	-82.43	1.19	3.00	20.33	10.26	1.20
S4	SCR	42.92	-82.45	1.46	4.60	20.03	9.97	1.10
S5	SCR	42.87	-82.46	2.76	1.50	19.86	8.50	1.68
S8	SCR	42.95	-82.43	1.00	2.40	19.85	9.77	1.60
S9	SCR	42.94	-82.45	0.77	5.50	19.85	9.72	1.59
S10	SCR	42.91	-82.46	1.09	1.50	19.12	10.12	1.54
S11	SCR	42.84	-82.47	1.01	1.50	20.05	8.63	1.53
S12	SCR	42.83	-82.47	1.51	6.00	20.08	9.21	-0.99
S13	SCR	42.75	-82.47	1.08	1.00	20.61	10.00	-0.67
S14	SCR	42.69	-82.49	1.19	6.50	20.48	9.75	1.63
S15	SCR	42.67	-82.51	3.24	2.90	20.61	10.29	1.57
S16	SCR	42.86	-82.47	1.53	4.00	19.25	10.54	1.28
S17	SCR	42.83	-82.48	1.95	3.00	19.33	10.10	-1.09
S18	SCR	42.82	-82.48	1.50	4.00	19.57	10.40	1.63
S19	SCR	42.77	-82.47	1.64	2.40	20.56	9.78	-1.32
S20	SCR	42.74	-82.49	2.18	3.50	20.93	10.36	1.17

Appendix II. Densities (/m<sup>2</sup>) of 16 dominant taxa found at 311 sampling sites in the Lake Huron-Lake Erie Corridor 1991, 1999 and 2004/5 surveys

Site ID	Oligochaeta	Nematoda	Chironodidae	Ceratopogonidae	Hexagenia	Caenis	Hydropsychidae	Other Trichoptera	Amphipoda	Dreissena	Acarina	Hydra	Hirudinae	Turbellaria	Gastropoda	Sphaeriidae
003ABC	116	14	65	0	7	0	134	0	670	2025	0	0	0	0	0	0
004ABC	507	406	2913	0	0	0	0	0	0	29	29	0	0	0	14	0
005ABC	779	1283	732	0	22	0	0	0	0	4	29	0	0	0	11	0
007ABC	145	11	320	0	84	2	0	0	216	7370	0	212	14	0	0	0
008A	174	29	725	0	14	0	14	0	2754	11826	0	0	0	0	29	0
009B	609	0	2261	0	0	43	0	0	0	0	43	0	43	0	0	0
010B	11	0	152	0	0	0	22	0	1076	1120	11	11	0	0	0	0
011A	174	435	739	43	0	0	0	0	0	0	43	0	0	0	0	0
012A	188	29	565	14	0	29	0	0	0	0	0	0	0	0	0	0
013A	1304	522	3957	0	0	0	0	0	43	304	0	0	87	0	0	0
014B	333	174	275	0	0	0	0	0	275	449	0	0	0	0	0	0
015C	183	35	78	0	0	0	0	0	35	113	0	43	0	0	0	0
016C	674	565	457	0	0	0	0	0	0	0	0	22	0	0	0	0
017B	261	913	2783	0	87	0	0	0	0	0	43	0	0	0	0	0
018A	0	14	58	0	72	0	0	0	391	1899	0	14	0	0	0	0
019B	1000	652	2826	0	0	0	174	0	87	0	0	0	0	0	0	0
021B	43	0	87	0	0	0	0	0	87	696	0	0	0	0	0	0
022B	130	0	87	0	0	0	0	0	130	652	0	0	0	0	0	0
023C	739	43	826	0	43	43	0	0	0	43	0	0	0	0	0	0
024C	196	43	174	0	0	0	22	0	13413	14630	0	2478	0	0	174	0
025B	2565	696	4783	0	0	0	0	0	0	0	0	0	0	0	0	0
026C	87	22	43	0	0	0	22	0	130	761	0	87	0	65	0	0
027B	87	0	0	0	0	0	43	0	348	1696	43	0	0	0	0	0
029C	43	0	0	0	0	0	0	0	0	217	0	0	0	0	0	0

030ABC	58	27	46	0	0	0	72	0	507	2560	0	0	0	0	0	0
031A	9	0	26	0	0	0	83	0	117	587	0	0	0	0	0	0
033ABC	452	5	546	0	65	0	14	0	22	0	0	10	7	0	0	0
034C	87	0	0	0	0	0	0	0	1739	1130	0	43	0	0	0	0
035C	54	0	22	0	0	0	0	0	65	315	0	0	0	0	0	0
036C	22	0	130	0	0	0	0	0	0	0	0	0	0	0	0	0
037B	87	174	43	0	0	0	43	0	31043	51652	0	5522	0	2609	0	0
042C	2783	43	609	0	0	0	0	0	87	130	0	43	0	0	0	0
043ABC	0	0	20	0	0	0	67	0	2341	2797	0	110	0	0	0	0
044A	565	22	348	0	0	0	0	0	0	0	0	0	0	0	0	0
045B	609	0	43	0	0	0	0	0	0	0	0	0	0	0	43	0
047ABC	7	3	0	0	0	0	6	0	83	674	7	0	0	0	18	0
048C	22	33	11	0	11	0	0	0	0	0	0	0	11	0	0	0
049A	191	26	9	0	0	0	0	0	0	157	0	0	0	0	0	0
050B	40957	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
052B	1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
054B	130	0	0	0	0	0	0	0	0	87	0	0	0	0	0	0
055C	232	0	0	0	0	0	14	0	0	130	0	0	0	0	0	0
057C	0	0	0	0	0	0	0	0	84	861	0	0	0	0	0	0
058C	0	22	43	0	0	0	43	0	565	64130	0	0	0	0	0	0
059ABC	243	21	345	0	0	17	0	0	196	0	32	0	0	0	0	0
060B	22	0	0	0	0	0	0	0	0	2065	0	0	0	0	0	0
064B	130	87	3696	0	0	2000	130	0	130	0	130	0	43	0	0	0
065C	43	304	43	0	0	348	0	0	43	0	0	0	0	0	0	0
066A	0	217	2783	0	0	435	43	0	43	0	43	0	0	0	0	0
067B	2217	652	1870	0	87	0	0	0	0	0	0	0	0	0	0	0
068B	739	0	1391	0	0	0	0	0	0	0	0	0	0	0	0	0
069A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
070B	2522	174	1000	174	43	391	87	0	43	130	0	0	0	0	0	0
071B	43	0	0	0	0	0	0	0	0	174	0	0	0	0	0	0
072A	304	130	1609	130	43	565	0	0	0	0	0	0	0	0	0	0



073C	130	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
074B	1391	217	435	43	0	217	217	0	0	261	0	0	43	0	0	0
075A	522	0	0	0	0	0	0	0	0	348	0	0	0	0	0	0
076AB	4065	29	7	0	0	0	7	0	0	0	29	0	0	0	7	0
077B	957	1261	217	0	0	0	0	0	0	0	130	0	0	0	0	0
078B	942	101	43	14	0	0	0	0	14	0	0	0	0	0	0	0
079C	2696	87	43	0	0	0	0	0	0	0	0	0	0	0	0	0
080C	3043	43	0	0	0	0	87	0	0	0	0	0	0	0	0	0
081B	652	0	391	43	43	0	0	0	0	0	0	0	0	0	0	0
082A	348	130	826	43	130	43	43	0	43	0	0	0	0	0	0	0
083B	304	0	304	0	174	0	87	0	0	1217	0	0	0	0	0	0
084A	1043	348	609	0	43	0	0	0	0	0	0	0	0	0	0	0
085C	87	130	739	0	0	0	0	0	0	0	0	0	0	0	0	0
086A	435	0	957	0	0	0	0	0	0	0	0	0	0	0	0	0
088C	696	87	130	0	0	0	0	0	0	0	0	0	0	0	0	0
089A	261	174	304	43	87	0	0	0	0	43	0	0	0	0	43	0
090B	65	22	152	0	65	0	22	0	130	1870	0	0	0	0	0	0
091C	87	43	739	0	43	87	0	0	87	0	43	0	0	0	0	0
092AB	94	80	1601	7	87	0	7	0	116	0	29	0	0	0	14	0
093C	696	87	609	0	87	0	0	0	0	0	0	0	0	0	0	0
094C	0	0	0	0	0	0	0	0	130	739	0	0	0	0	0	0
095A	1261	87	478	217	0	43	0	0	0	0	43	0	43	43	87	0
096A	435	130	826	0	87	0	43	0	43	0	0	0	0	0	0	0
097AB	87	14	3087	14	58	29	0	0	188	0	0	0	14	0	0	0
098C	19	0	57	0	0	0	0	0	0	0	0	0	19	0	0	0
099C	783	130	130	0	43	0	0	0	0	0	0	0	174	0	0	0
100C	2217	43	391	0	0	0	0	0	0	0	0	0	43	0	0	0
101C	1348	0	174	0	0	0	0	0	0	0	0	0	0	0	0	0
102B	1043	4043	304	391	0	43	0	0	0	0	174	0	0	0	0	0
103A	1261	435	217	826	0	43	0	0	0	0	217	0	0	0	0	0
104C	87	22	0	0	0	0	0	0	5283	9609	0	217	0	0	0	0

105C	913	652	1565	43	43	0	43	0	696	0	0	0	304	0	0	0
106B	913	1391	435	0	0	0	0	0	87	0	0	0	0	0	43	0
107C	1000	304	870	43	43	0	0	0	0	0	0	0	0	0	0	0
108B	1870	652	2696	478	0	43	43	0	565	0	174	0	43	0	43	0
109C	478	826	261	0	0	0	0	0	609	87	43	0	0	0	0	0
10FB	3249	6	25	0	0	0	0	0	38	0	0	13	0	26	44	718
111C	2783	1696	1174	0	0	43	0	0	0	0	43	0	43	0	174	0
113B	65	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0
114B	957	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
115AB	1696	159	5130	43	0	43	29	0	87	58	43	0	29	0	14	0
116B	2935	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
117AB	1783	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
118A	326	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
119B	5935	261	457	0	0	0	0	0	65	0	174	0	22	0	0	0
121C	217	1217	609	0	87	0	0	0	0	0	0	0	0	0	0	0
122B	109	4500	1174	0	0	0	0	0	0	0	0	0	0	0	43	0
123A	543	65	522	22	87	0	0	0	0	22	0	0	0	0	0	0
124A	130	304	565	0	261	43	0	0	0	0	0	0	0	0	0	0
125A	22	43	22	0	0	0	0	0	391	3087	0	0	0	0	0	0
126A	391	22	304	0	0	0	0	0	239	304	43	0	0	0	0	0
127B	1739	1348	783	43	304	0	0	0	0	43	43	0	0	0	0	0
128B	1022	54	22	0	0	11	0	0	0	0	0	0	0	0	0	0
129A	543	1217	1761	130	22	174	43	0	348	2739	22	0	0	0	0	0
12FB	397	6	6	0	0	0	257	25	250	5064	0	1891	0	276	244	6
130A	304	435	1304	0	0	0	0	0	0	0	0	0	0	0	0	0
131B	87	3348	5087	739	43	87	0	0	43	0	0	0	0	0	130	0
132B	565	217	174	0	0	0	0	0	0	0	0	0	0	0	0	0
133B	2893	1054	2778	38	0	38	115	0	2490	2356	38	0	0	57	153	0
134C	77	115	0	0	0	0	0	0	0	0	0	0	0	0	0	0
135A	1935	935	696	43	0	0	22	0	109	0	174	0	0	22	0	0
136B	522	109	109	22	0	0	0	0	0	0	22	0	0	0	0	0

137A	3478	174	0	0	0	0	0	0	0	0	0	0	0	0	43	0
138B	1826	957	87	0	0	0	0	0	0	0	0	0	0	0	0	0
139B	739	43	152	0	0	87	0	0	43	0	0	0	0	0	0	0
13FB	2179	58	38	0	0	0	32	38	141	128	0	692	0	103	321	968
140C	1239	87	65	0	0	0	0	0	0	0	0	0	0	0	0	0
141B	5283	761	65	0	0	22	0	0	0	0	0	0	0	0	0	0
142C	783	1739	174	43	0	0	0	0	0	0	43	0	0	0	0	0
143B	1826	1696	0	0	0	0	0	0	87	0	0	0	0	0	0	0
144B	1565	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0
145B	1391	413	43	0	0	0	0	0	0	0	22	0	0	0	0	0
146B	5913	478	0	0	0	0	0	0	0	0	0	0	0	0	43	0
147A	3087	391	130	0	0	0	0	0	0	0	0	0	0	0	0	0
148B	783	0	43	0	0	0	43	0	0	0	0	0	0	0	0	0
149C	54	174	0	0	0	0	0	0	0	0	33	0	0	0	0	0
14FB	5286	45	550	6	45	0	84	96	15544	385	0	5352	26	417	129	558
150B	5261	217	87	0	0	0	0	0	0	0	43	0	0	0	0	0
15FB	26511	19	13	0	0	0	51	0	64	26	0	51	0	77	77	852
16FB	4769	1032	288	0	13	0	6	6	96	6	6	231	0	32	13	308
17FB	13531	13	301	0	0	0	115	83	1038	712	13	2352	0	423	230	795
186FB	366	51	6	0	0	0	2275	52	250	12557	0	0	0	199	148	0
189FB	6365	38	6	0	0	0	38	0	179	19	0	231	0	38	88	334
18FB	7878	45	339	0	26	0	0	6	26	13	0	109	0	6	6	346
195FB	35608	64	0	0	0	0	0	0	51	0	13	0	0	38	173	398
198FB	8867	109	211	0	0	0	0	6	58	13	0	13	0	6	6	71
199FB	3431	32	63	0	0	0	0	0	90	6	0	0	0	0	0	186
19FB	211	13	186	0	0	0	71	13	179	237	0	2404	0	192	83	212
1FB	12062	77	1114	0	77	0	1788	378	6839	1135	0	1026	0	359	300	1616
200FB	8288	231	351	0	0	0	13	6	410	564	0	103	0	6	19	64
21FB	14922	269	45	0	0	0	0	0	0	0	6	173	0	6	26	0
221FB	8782	654	1153	77	45	26	90	44	1859	3115	0	0	26	179	96	660
222FB	2776	115	609	12	18	0	0	96	558	19595	6	0	89	109	45	26

223FB	563	96	383	0	0	0	45	25	308	26294	0	6	6	519	430	6
224FB	3974	231	1180	19	0	6	0	19	198	1282	6	0	6	321	13	32
22FB	7141	32	165	0	12	0	6	0	90	679	0	13	0	13	64	83
23FB	11456	13	167	0	0	0	0	19	90	244	0	83	83	147	267	2846
240FB	11967	6	173	0	0	0	0	0	19	0	6	0	0	0	18	218
24FB	831	71	44	0	6	19	32	37	750	2974	13	391	0	340	166	205
25FB	2135	462	327	0	6	6	96	24	994	3852	13	846	13	64	353	327
26FB	34536	6	82	0	12	0	0	0	185	26	0	0	0	122	6	513
27FB	968	32	57	0	6	83	19	19	794	58805	0	199	6	83	379	359
28FB	12975	212	441	6	6	45	6	88	526	2244	45	3461	31	1128	82	308
2FB	1519	340	25	0	0	0	19	44	1942	55523	0	5474	0	1897	1282	301
33FB	504	32	0	6	0	0	282	82	673	23659	0	6	0	90	308	0
34FB	65556	0	44	0	0	0	0	0	6	0	6	0	6	13	12	77
35FB	4976	1038	620	13	19	359	6	51	147	19	19	0	6	38	51	551
36FB	18695	6	50	0	0	0	0	0	147	0	0	0	6	109	45	90
37FB	9044	308	31	0	13	0	26	13	403	32	13	0	13	71	397	327
39FB	8076	635	634	327	0	77	0	0	981	6	71	13	0	77	102	532
40FB	2027	244	306	6	0	64	32	44	929	6827	6	359	6	103	134	90
41FB	2115	776	170	0	6	6	250	31	128	1814	19	83	141	32	359	0
42FB	3023	38	58	0	0	6	353	140	1852	21185	6	90	12	1179	230	71
43FB	23152	1000	2667	13	0	0	32	39	449	6	0	13	0	45	538	2128
44FB	3818	64	204	0	6	0	116	6	2077	19768		6	31	769	500	205
45FB	5457	449	319	0	13	0	38	12	77	6		6	0	26	31	51
46FB	21223	1224	359	19	0	19	0	0	532	0	26	0	26	288	724	359
47FB	19	13	13	0	0	0	0	0	109	45			0	32	6	0
48FB	1423	737	344	0	0	0	0	6	840	6			0	0	24	256
49FB	4115	455	1660	0	0	0	0	186	346	147	0	13	6	83	442	378
50FB	18076	19	217	0	0	0	0	0	96	0	0	6	0	0	647	4756
51FB	14935	1167	3178	0	0	0	1012	366	1301	6	13	77	77	641	2526	2211
53FB	7570	776	6165	0	6	0	26	288	2628	90	122	13	13	583	4679	2263
5FB	2463	13	52	0	0	0	96	0	276	6	0	19	0	45	0	314

62FB	10263	0	45	0	0	0	6	6	128	32			13	0	103	32
6FB	2889	83	653	0	64	6	0	0	147	160	6	570	13	218	32	1115
70FB	10589	654	64	0	0	0	0	0	115	0	6	147	0	192	166	301
71FB	33678	45	396	0	0	0	19	0	71	0	6	6	13	724	371	45
73FB	17549	19	6	0	0	0	0	0	0	0	0	0	6	0	6	0
75FB	100444	58	25	0	0	0	6	0	51	0	0	0	147	19	32	0
83FB	26271	26	351	0	6	0	0	0	13	0	6	0	0	13	38	147
84FB	5879	6	26	0	0	0	6	0	154	51	0	77	0	0	859	45
85FB	12401	19	18	0	0	0	0	0	38	0	0	0	6	0	45	0
8A	1346	128	210	0	19	0	0	70	840	25986	6	0	0	115	185	423
9FB	433	0	116	0	0	0	853	44	378	141	6	6980	0	141	199	769
A	621	109	32	0	0	0	90	45	269	4897	0	5455	0	340	500	6
A10	59956	1467	2044	0	0	0	0	0	0	0	0	0	0	0	0	0
A23	15689	222	3733	0	578	0	0	0	44	0	0	0	0	0	0	0
A27	1156	44	44	0	0	0	0	0	0	0	0	0	0	0	0	0
A28	5689	578	1778	0	311	0	0	311	0	0	0	0	622	0	0	622
A29	267	178	267	0	44	0	0	0	0	0	0	0	0	0	0	0
A5	311	0	1022	0	0	0	1200	2356	44	1200	0	0	0	0	0	0
A53	5244	9333	2444	0	0	222	0	0	889	844	0	0	0	0	0	400
A58	4800	1422	356	0	0	0	0	0	0	1289	0	0	0	44	1244	267
A6	82311	4178	5111	0	0	0	0	0	0	0	0	0	0	0	0	0
A66	1467	1067	5289	0	0	0	0	44	311	2267	0	0	0	0	222	89
B	5089	115	351	6	173	13	0	6	391	64	0	38	0	45	52	45
C	1142	0	129	0	0	13	250	18	494	4570	0	19384	0	71	308	32
D	5129	391	479	0	32	0	0	45	1635	19	0	38	0	135	712	2211
DBC2	10978	1911	3511	178	2533	1733	0	0	0	0	311	0	0	311	0	0
DCC2	6489	533	7467	0	0	1244	0	133	1911	489	133	0	0	133	2756	0
DCE3	9422	11378	3467	0	756	0	0	0	0	0	0	0	0	0	0	0
DJC2	17289	311	800	0	489	0	0	0	0	0	0	0	0	222	0	444
E	1120	128	36	0	0	0	0	13	115	103	0	218	0	13	96	211
F	2833	0	19	0	0	0	12	0	64	38	0	308	0	32	58	225

<b>G</b>	7242	487	95	0	6	6	0	13	160	32	0	321	0	90	173	340
<b>GL1</b>	267	89	933	0	844	89	0	89	356	0	44	0	0	0	222	0
<b>H</b>	4178	1327	582	6	45	186	0	75	3372	981	13	6	13	71	666	1526
<b>I</b>	9433	359	43	0	0	0	0	6	147	19	6	0	0	13	122	109
<b>J</b>	1898	64	58	0	0	51	32	19	1186	47389	32	1288	6	103	551	96
<b>K</b>	11180	385	97	0	0	0	19	6	205	0	26	0	0	103	724	962
<b>L</b>	4760	192	346	0	13	0	6	19	57	0	0	0	0		12	224
<b>M</b>	2775	833	615	0	89	6	12	63	1763	2205	0	26	6	38	122	833
<b>MCE2</b>	13022	2178	4178	178	400	0	0	0	0	0	0	0	0	0	0	0
<b>N</b>	2039	872	179	0	0	38	25	63	1366	8468	6	269	6	109	756	321
<b>O</b>	7210	26	38	0	6	0	0	0	13	0	6	0	0		0	39
<b>P</b>	31039	2064	878	71	6	19	6	51	679	0	90	0	90	436	2262	2513
<b>S1</b>	400	44	444	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>S10</b>	9333	222	6844	0	0	0	0	178	0	0	267	44	0	267	0	44
<b>S100</b>	12933	5511	1689	0	0	0	0	0	0	0	0	0	0	0	0	89
<b>S101</b>	444	0	356	0	0	533	400	0	1511	9111	0	178	0	222	0	0
<b>S102</b>	0	5156	89	0	0	0	0	0	44	0	0	889	0	89	44	0
<b>S11</b>	14356	1200	7511	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>S12</b>	3778	0	2400	0	0	400	5867	3289	489	2756	0	0	978	0	578	89
<b>S13</b>	2711	533	14089	0	0	356	89	89	0	178	0	0	0	0	89	578
<b>S14</b>	1556	844	5822	0	178	44	267	44	0	0	0	311	0	0	267	0
<b>S15</b>	2044	1333	18667	0	178	800	5022	0	0	178	0	0	0	178	400	0
<b>S16</b>	7556	1067	15733	0	0	222	933	1644	0	0	0	0	0	133	0	0
<b>S17</b>	844	89	444	0	0	0	978	89	178	1289	133	222	0	0	489	89
<b>S18</b>	13511	133	18622	0	178	0	0	0	0	0	0	0	0	0	0	44
<b>S19</b>	311	0	756	0	0	0	3200	311	489	978	89	2222	0	0	222	0
<b>S20</b>	3867	1333	1911	0	89	89	356	1511	0	0	44	0	0	0	0	0
<b>S21</b>	5511	0	1422	0	0	44	0	44	89	311	0	222	0	0	756	578
<b>S21(5)</b>	2000	267	3733	0	44	2178	44	0	0	133	133	0	44	0	889	133
<b>S22</b>	24044	1244	44089	0	0	44	0	0	0	0	178	400	0	0	0	44
<b>S23</b>	10933	311	6444	0	0	89	489	0	0	0	89	222	0	0	89	89

S24	2933	1200	7511	400	0	533	0	0	0	222	44	0	0	0	0	0
S25	6089	133	4400	0	0	89	0	0	0	0	44	0	0	0	0	0
S27	12356	0	7511	0	0	133	0	0	0	0	0	0	0	0	0	844
S27(5)	78000	2267	12800	0	0	1911	0	0	0	0	0	0	0	0	0	356
S28	4311	178	1600	0	0	0	0	0	178	44	0	0	0	44	133	89
S28(5)	3333	622	2933	444	889	178	0	0	0	5733	133	0	0	178	1156	622
S3	10044	44	1733	0	0	0	0	44	0	0	89	44	0	0	89	0
S36	756	0	1689	0	0	0	0	0	0	0	0	0	0	0	0	0
S37	3333	356	10089	0	0	756	0	0	0	89	222	0	0	0	44	222
S38	6311	0	10356	0	0	844	0	0	0	44	0	133	0	0	133	178
S39	12089	133	2889	0	1067	0	0	0	0	489	0	0	0	0	0	222
S4	978	0	1289	0	0	44	0	533	133	89	133	89	0	0	0	44
S40	3556	89	978	0	133	0	0	44	0	0	0	0	0	400	44	1289
S41	667	89	267	0	0	0	0	0	0	0	0	0	0	0	0	44
S42	11378	711	23556	0	178	400	44	0	578	0	444	0	0	89	444	0
S43	6933	356	7422	0	667	44	0	0	0	0	0	44	0	0	178	0
S44	1200	178	711	0	0	0	0	0	44	0	0	0	0	44	0	133
S45	9867	489	10533	0	533	44	0	0	0	0	311	0	0	0	0	0
S46	3689	1778	5467	0	222	222	0	0	89	0	311	0	89	133	978	0
S47	1111	311	4889	0	489	0	0	44	0	0	89	0	0	0	0	1200
S48	1467	133	2044	0	0	0	0	0	133	0	0	0	0	0	0	0
S49	26711	0	7111	0	44	0	0	0	0	0	0	0	0	0	0	178
S5	47600	31600	18578	444	0	622	0	0	0	222	0	0	489	489	2622	2622
S50	2311	400	7022	0	0	0	0	0	0	0	0	0	0	89	489	178
S51	89	44	44	0	0	0	0	0	0	0	44	0	0	0	0	178
S52	800	356	578	0	0	0	0	0	0	1333	0	0	0	44	0	0
S53	44	0	0	0	0	0	0	0	0	0	0	0	0	0	89	0
S54	889	133	0	0	0	0	0	0	0	1111	0	44	0	222	44	133
S55	5911	4311	2667	0	44	44	0	0	533	4178	44	2844	1378	0	178	1378
S56	4844	1644	2089	0	0	0	0	0	1333	9600	0	0	0	0	667	800
S57	6756	311	1467	0	44	0	0	0	0	0	0	0	0	133	0	0

S58	3022	1511	400	0	0	0	0	0	0	6400	0	0	0	89	0	44
S59	10044	3378	667	0	0	0	0	0	0	1867	0	0	89	0	0	0
S60	4089	2222	267	0	222	0	0	0	0	622	0	0	44	0	0	44
S61	7022	9733	2178	0	178	0	0	0	0	844	0	0	311	0	356	0
S62	89	2711	400	0	44	0	0	0	44	1067	0	0	0	0	0	0
S63	1333	2000	133	0	0	0	0	0	0	0	89	0	0	44	0	0
S64	222	133	267	0	0	0	0	0	0	0	0	0	0	0	0	0
S65	2356	0	356	0	0	0	0	0	0	0	0	0	0	0	0	44
S66	2178	5778	1644	0	222	0	0	44	89	9422	0	0	311	0	311	44
S67	4800	400	1467	0	89	0	0	133	44	0	44	0	178	267	1378	133
S68	4978	44	1689	0	0	0	0	0	0	0	0	0	311	0	0	178
S69	6044	1333	3733	0	89	0	0	311	2089	222	0	0	400	178	0	89
S70	2311	1422	6044	0	267	0	0	0	578	267	44	0	89	133	0	44
S72	5022	2844	2133	0	267	0	0	0	0	8267	89	0	0	0	44	178
S74	4756	2800	5733	0	800	0	0	0	444	1600	1067	0	0	0	89	400
S78	889	2044	2044	0	0	0	0	0	89	1022	44	0	133	0	311	0
S79	400	178	800	0	0	0	0	0	0	0	0	0	0	0	0	0
S8	10178	267	3378	0	0	0	0	133	0	44	222	44	0	0	89	0
S80	2978	1244	622	0	44	0	0	0	0	0	44	0	0	44	0	0
S81	756	400	1689	0	0	0	0	0	0	0	44	0	0	0	0	44
S82	2800	756	889	0	0	0	222	0	0	0	44	0	0	0	0	0
S83	444	89	89	0	0	0	356	44	1911	622	0	622	0	44	0	0
S84	5156	267	1022	0	0	133	0	0	133	89	0	0	0	0	133	0
S85	22711	7778	7778	0	311	0	0	0	0	0	0	0	0	0	0	0
S87	311	0	44	0	0	0	89	0	311	933	0	267	0	356	0	0
S89	3644	178	578	0	0	0	0	0	44	0	0	0	0	0	0	44
S9	1689	0	1244	0	0	0	133	44	0	0	0	0	0	0	0	44
S90	4667	889	133	0	44	0	0	0	267	800	0	0	0	0	0	0
S93	44	44	44	0	0	356	0	0	44	0	0	0	0	0	44	178
S94	7867	2267	3556	0	0	0	0	0	0	0	222	0	0	0	0	0
S95	2044	89	400	0	0	89	311	0	133	356	0	0	0	267	0	0



<b>S96</b>	400	89	311	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>S97</b>	6267	667	622	0	0	0	0	0	44	0	133	0	0	222	0	667
<b>S98</b>	53689	12756	844	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>S99</b>	4400	178	222	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>UBC1</b>	311	622	8756	0	0	3111	0	89	0	44	0	0	933	0	1778	0
<b>UCC1</b>	11022	2356	12578	178	444	1067	0	0	0	0	0	0	0	0	0	0
<b>UCE1</b>	2044	0	4667	267	533	800	844	133	0	0	133	0	0	0	978	133
<b>UJC1</b>	14400	1556	2933	0	489	0	0	0	0	0	0	0	0	0	0	0

Appendix III. Classifications of the Lake Huron-Lake Erie Corridor REF and other sites based on discriminant function analysis (DFA) ('G' refers to group/cluster number). The 'Observed' column contains the grouping of the REF sites based on zoobenthic community composition. Column '1' contains the groupings of other sites based on the DFA analysis with 8 habitat variables. Sites with asterisk (\*) were misclassified by the DFA model. When the classification probability was near 0.5, a "best judgment" method was used to assign the sites to proper group based on the zoobenthic community composition

Site ID	Observed	1	2
004ABC	G_1:1	G_1:1	G_2:2
073C	G_1:1	G_1:1	G_2:2
109C	G_1:1	G_1:1	G_2:2
122B	G_1:1	G_1:1	G_2:2
A10	G_1:1	G_1:1	G_2:2
A53	G_1:1	G_1:1	G_2:2
DCC2	G_1:1	G_1:1	G_2:2
GL1	G_1:1	G_1:1	G_2:2
S1	G_1:1	G_1:1	G_2:2
S10	G_1:1	G_1:1	G_2:2
S102	G_1:1	G_1:1	G_2:2
S11	G_1:1	G_1:1	G_2:2
S14	G_1:1	G_1:1	G_2:2
S18	G_1:1	G_1:1	G_2:2
S20	G_1:1	G_1:1	G_2:2
S21	G_1:1	G_1:1	G_2:2
S21(5)	G_1:1	G_1:1	G_2:2
S22	G_1:1	G_1:1	G_2:2
S23	G_1:1	G_1:1	G_2:2
S24	G_1:1	G_1:1	G_2:2
S25	G_1:1	G_1:1	G_2:2
S28	G_1:1	G_1:1	G_2:2
S28(5)	G_1:1	G_1:1	G_2:2
S36	G_1:1	G_1:1	G_2:2
S37	G_1:1	G_1:1	G_2:2
S38	G_1:1	G_1:1	G_2:2
S39	G_1:1	G_1:1	G_2:2
S4	G_1:1	G_1:1	G_2:2
S40	G_1:1	G_1:1	G_2:2
S43	G_1:1	G_1:1	G_2:2
S44	G_1:1	G_1:1	G_2:2
S49	G_1:1	G_1:1	G_2:2
S50	G_1:1	G_1:1	G_2:2

Appendix III. Continued.

Site ID	Observed	1	2
S51	G_1:1	G_1:1	G_2:2
S52	G_1:1	G_1:1	G_2:2
S53	G_1:1	G_1:1	G_2:2
S54	G_1:1	G_1:1	G_2:2
S55	G_1:1	G_1:1	G_2:2
S57	G_1:1	G_1:1	G_2:2
S58	G_1:1	G_1:1	G_2:2
S59	G_1:1	G_1:1	G_2:2
S60	G_1:1	G_1:1	G_2:2
S65	G_1:1	G_1:1	G_2:2
S67	G_1:1	G_1:1	G_2:2
S68	G_1:1	G_1:1	G_2:2
S69	G_1:1	G_1:1	G_2:2
S70	G_1:1	G_1:1	G_2:2
S72	G_1:1	G_1:1	G_2:2
S74	G_1:1	G_1:1	G_2:2
S79	G_1:1	G_1:1	G_2:2
S8	G_1:1	G_1:1	G_2:2
*S81	G_1:1	G_2:2	G_1:1
S82	G_1:1	G_1:1	G_2:2
S9	G_1:1	G_1:1	G_2:2
S99	G_1:1	G_1:1	G_2:2
026C	G_2:2	G_2:2	G_1:1
*47FB	G_2:2	G_1:1	G_2:2
A5	G_2:2	G_2:2	G_1:1
*S101	G_2:2	G_1:1	G_2:2
S12	G_2:2	G_2:2	G_1:1
S17	G_2:2	G_2:2	G_1:1
S19	G_2:2	G_2:2	G_1:1
003ABC	---	G_1:1	G_2:2
005ABC	---	G_1:1	G_2:2
007ABC	---	G_1:1	G_2:2
008A	---	G_1:1	G_2:2
009B	---	G_1:1	G_2:2
010B	---	G_1:1	G_2:2
011A	---	G_1:1	G_2:2
012A	---	G_1:1	G_2:2
013A	---	G_1:1	G_2:2
014B	---	G_1:1	G_2:2
015C	---	G_1:1	G_2:2
016C	---	G_1:1	G_2:2

Appendix III. Continued.

Site ID	1	2
017B	G_1:1	G_2:2
019B	G_1:1	G_2:2
022B	G_1:1	G_2:2
023C	G_1:1	G_2:2
024C	G_1:1	G_2:2
025B	G_1:1	G_2:2
027B	G_1:1	G_2:2
029C	G_1:1	G_2:2
030ABC	G_1:1	G_2:2
036C	G_1:1	G_2:2
037B	G_1:1	G_2:2
042C	G_1:1	G_2:2
043ABC	G_1:1	G_2:2
044A	G_1:1	G_2:2
045B	G_1:1	G_2:2
048C	G_1:1	G_2:2
049A	G_1:1	G_2:2
052B	G_1:1	G_2:2
055C	G_1:1	G_2:2
057C	G_1:1	G_2:2
058C	G_1:1	G_2:2
059ABC	G_1:1	G_2:2
060B	G_1:1	G_2:2
064B	G_1:1	G_2:2
065C	G_1:1	G_2:2
066A	G_1:1	G_2:2
067B	G_1:1	G_2:2
068B	G_1:1	G_2:2
069A	G_1:1	G_2:2
070B	G_1:1	G_2:2
072A	G_1:1	G_2:2
074B	G_1:1	G_2:2
075A	G_1:1	G_2:2
076ABC	G_1:1	G_2:2
077B	G_1:1	G_2:2
078B	G_1:1	G_2:2
079C	G_1:1	G_2:2
080C	G_1:1	G_2:2
081B	G_1:1	G_2:2
082A	G_1:1	G_2:2
083B	G_1:1	G_2:2

Appendix III. Continued.

Site ID	1	2
084A	G_1:1	G_2:2
085C	G_1:1	G_2:2
086A	G_1:1	G_2:2
088C	G_1:1	G_2:2
089A	G_1:1	G_2:2
090B	G_1:1	G_2:2
091C	G_1:1	G_2:2
092ABC	G_1:1	G_2:2
093C	G_1:1	G_2:2
094C	G_1:1	G_2:2
095A	G_1:1	G_2:2
096A	G_1:1	G_2:2
097ABC	G_1:1	G_2:2
098C	G_1:1	G_2:2
099C	G_1:1	G_2:2
100C	G_1:1	G_2:2
101C	G_1:1	G_2:2
102B	G_1:1	G_2:2
103A	G_1:1	G_2:2
104C	G_1:1	G_2:2
105C	G_1:1	G_2:2
106B	G_1:1	G_2:2
107C	G_1:1	G_2:2
108B	G_1:1	G_2:2
111C	G_1:1	G_2:2
115ABC	G_1:1	G_2:2
116B	G_1:1	G_2:2
119B	G_1:1	G_2:2
123A	G_1:1	G_2:2
124A	G_1:1	G_2:2
125A	G_1:1	G_2:2
126A	G_1:1	G_2:2
127B	G_1:1	G_2:2
128B	G_1:1	G_2:2
129A	G_1:1	G_2:2
130A	G_1:1	G_2:2
131B	G_1:1	G_2:2
132B	G_1:1	G_2:2
133B	G_1:1	G_2:2
134C	G_1:1	G_2:2
135A	G_1:1	G_2:2

Appendix III. Continued.

Site ID	1	2
136B	G_1:1	G_2:2
137A	G_1:1	G_2:2
138B	G_1:1	G_2:2
139B	G_1:1	G_2:2
140C	G_1:1	G_2:2
141B	G_1:1	G_2:2
142C	G_1:1	G_2:2
143B	G_1:1	G_2:2
144B	G_1:1	G_2:2
145B	G_1:1	G_2:2
146B	G_1:1	G_2:2
147A	G_1:1	G_2:2
148B	G_1:1	G_2:2
149C	G_1:1	G_2:2
150B	G_1:1	G_2:2
16FB	G_1:1	G_2:2
18FB	G_1:1	G_2:2
198FB	G_1:1	G_2:2
1FB	G_1:1	G_2:2
200FB	G_1:1	G_2:2
21FB	G_1:1	G_2:2
221FB	G_1:1	G_2:2
223FB	G_1:1	G_2:2
224FB	G_1:1	G_2:2
22FB	G_1:1	G_2:2
23FB	G_1:1	G_2:2
24FB	G_1:1	G_2:2
26FB	G_1:1	G_2:2
2FB	G_1:1	G_2:2
34FB	G_1:1	G_2:2
35FB	G_1:1	G_2:2
37FB	G_1:1	G_2:2
39FB	G_1:1	G_2:2
40FB	G_1:1	G_2:2
42FB	G_1:1	G_2:2
43FB	G_1:1	G_2:2
44FB	G_1:1	G_2:2
45FB	G_1:1	G_2:2
46FB	G_1:1	G_2:2
48FB	G_1:1	G_2:2
51FB	G_1:1	G_2:2

Appendix III. Continued.

Site ID	1	2
5FB	G_1:1	G_2:2
6FB	G_1:1	G_2:2
70FB	G_1:1	G_2:2
71FB	G_1:1	G_2:2
73FB	G_1:1	G_2:2
75FB	G_1:1	G_2:2
83FB	G_1:1	G_2:2
84FB	G_1:1	G_2:2
85FB	G_1:1	G_2:2
8A	G_1:1	G_2:2
A	G_1:1	G_2:2
A23	G_1:1	G_2:2
A27	G_1:1	G_2:2
A28	G_1:1	G_2:2
A29	G_1:1	G_2:2
A58	G_1:1	G_2:2
A6	G_1:1	G_2:2
A66	G_1:1	G_2:2
B	G_1:1	G_2:2
D	G_1:1	G_2:2
DBC2	G_1:1	G_2:2
DCE3	G_1:1	G_2:2
DJC2	G_1:1	G_2:2
E	G_1:1	G_2:2
H	G_1:1	G_2:2
I	G_1:1	G_2:2
J	G_1:1	G_2:2
L	G_1:1	G_2:2
M	G_1:1	G_2:2
MCE2	G_1:1	G_2:2
N	G_1:1	G_2:2
P	G_1:1	G_2:2
S100	G_1:1	G_2:2
S15	G_1:1	G_2:2
S16	G_1:1	G_2:2
S27	G_1:1	G_2:2
S27(5)	G_1:1	G_2:2
S3	G_1:1	G_2:2
S41	G_1:1	G_2:2
S42	G_1:1	G_2:2
S45	G_1:1	G_2:2

Appendix III. Continued.

Site ID	1	2
S46	G_1:1	G_2:2
S47	G_1:1	G_2:2
S48	G_1:1	G_2:2
S5	G_1:1	G_2:2
S56	G_1:1	G_2:2
S61	G_1:1	G_2:2
S62	G_1:1	G_2:2
S63	G_1:1	G_2:2
S66	G_1:1	G_2:2
S78	G_1:1	G_2:2
S80	G_1:1	G_2:2
S84	G_1:1	G_2:2
S85	G_1:1	G_2:2
S89	G_1:1	G_2:2
S93	G_1:1	G_2:2
S94	G_1:1	G_2:2
S96	G_1:1	G_2:2
S97	G_1:1	G_2:2
S98	G_1:1	G_2:2
UBC1	G_1:1	G_2:2
UCC1	G_1:1	G_2:2
UCE1	G_1:1	G_2:2
UJC1	G_1:1	G_2:2
018A	G_2:2	G_1:1
021B	G_2:2	G_1:1
031A	G_2:2	G_1:1
033ABC	G_2:2	G_1:1
034C	G_2:2	G_1:1
035C	G_2:2	G_1:1
047ABC	G_2:2	G_1:1
050B	G_2:2	G_1:1
054B	G_2:2	G_1:1
071B	G_2:2	G_1:1
10FB	G_2:2	G_1:1
113B	G_2:2	G_1:1
114B	G_2:2	G_1:1
117ABC	G_2:2	G_1:1
118A	G_2:2	G_1:1
121C	G_2:2	G_1:1
12FB	G_2:2	G_1:1
13FB	G_2:2	G_1:1



Appendix III. Continued.

Site ID	1	2
14FB	G_2:2	G_1:1
15FB	G_2:2	G_1:1
17FB	G_2:2	G_1:1
186FB	G_2:2	G_1:1
189FB	G_2:2	G_1:1
195FB	G_2:2	G_1:1
199FB	G_2:2	G_1:1
19FB	G_2:2	G_1:1
222FB	G_2:2	G_1:1
240FB	G_2:2	G_1:1
25FB	G_2:2	G_1:1
27FB	G_2:2	G_1:1
28FB	G_2:2	G_1:1
33FB	G_2:2	G_1:1
36FB	G_2:2	G_1:1
41FB	G_2:2	G_1:1
49FB	G_2:2	G_1:1
50FB	G_2:2	G_1:1
53FB	G_2:2	G_1:1
62FB	G_2:2	G_1:1
9FB	G_2:2	G_1:1
C	G_2:2	G_1:1
F	G_2:2	G_1:1
G	G_2:2	G_1:1
K	G_2:2	G_1:1
O	G_2:2	G_1:1
S13	G_2:2	G_1:1
S64	G_2:2	G_1:1
S83	G_2:2	G_1:1
S87	G_2:2	G_1:1
S90	G_2:2	G_1:1
S95	G_2:2	G_1:1

## Appendix IV

Much of the potential contaminant toxicity in the Lake Huron-Lake Erie Corridor may be associated with the variables summarized by PC2 in the principal component analysis (RDC approach). If so, a stronger relationship might exist between ZCI (biological condition) and sediment contamination if reference and degraded conditions were based solely on the scores for PC2. To assess and confirm whether PC2 might dominate the toxicity stress gradient, I reanalyzed the data following the RDC approach described in Chapter 2.

A site was classified as “reference site” if its “PC2 score” placed it within the lowest quintile (lowest 20 percent) of the frequency distribution of all sites. A site was classified as “degraded site” if its “PC2 score” placed it within the highest quintile of the gradient of all sites. All other sites were classified as “test sites”.

Cluster analysis identified two groups of reference sites based on relative abundances of 16 zoobenthic taxa (Figure IV-1). The DFA model distinguished groupings on the basis of water depth (Table IV-1 and IV-2), Bray-Curtis ordination and multiple regression analyses were then performed to describe the strongest association between zoobenthic community composition and sediment contamination score for each cluster (Figure IV-2 and IV-3 and Table IV-3). These analyses indicated that the relationships between ZCI and sediment contamination based solely on PC2 were indeed stronger than those based on the SumRel measure that incorporated all for PC factors. This is consistent with the idea that PC2-associated compounds account for much of the stress-response relationship between ZCI and sediment contamination score. At the same time, the results of multiple regression analysis indicated that the same taxa served as indicators of reference and degraded

conditions as were identified in the analysis employing the SumRel measure of sediment contamination.

Ultimately, the decision on whether to use an empirical approach to quantify the stressor gradient (SumRel of all sets of statistically independent compound variables) or an approach based on best professional judgment (in this case, PC2 scores) may depend on the nature and prior knowledge of the system under investigation.

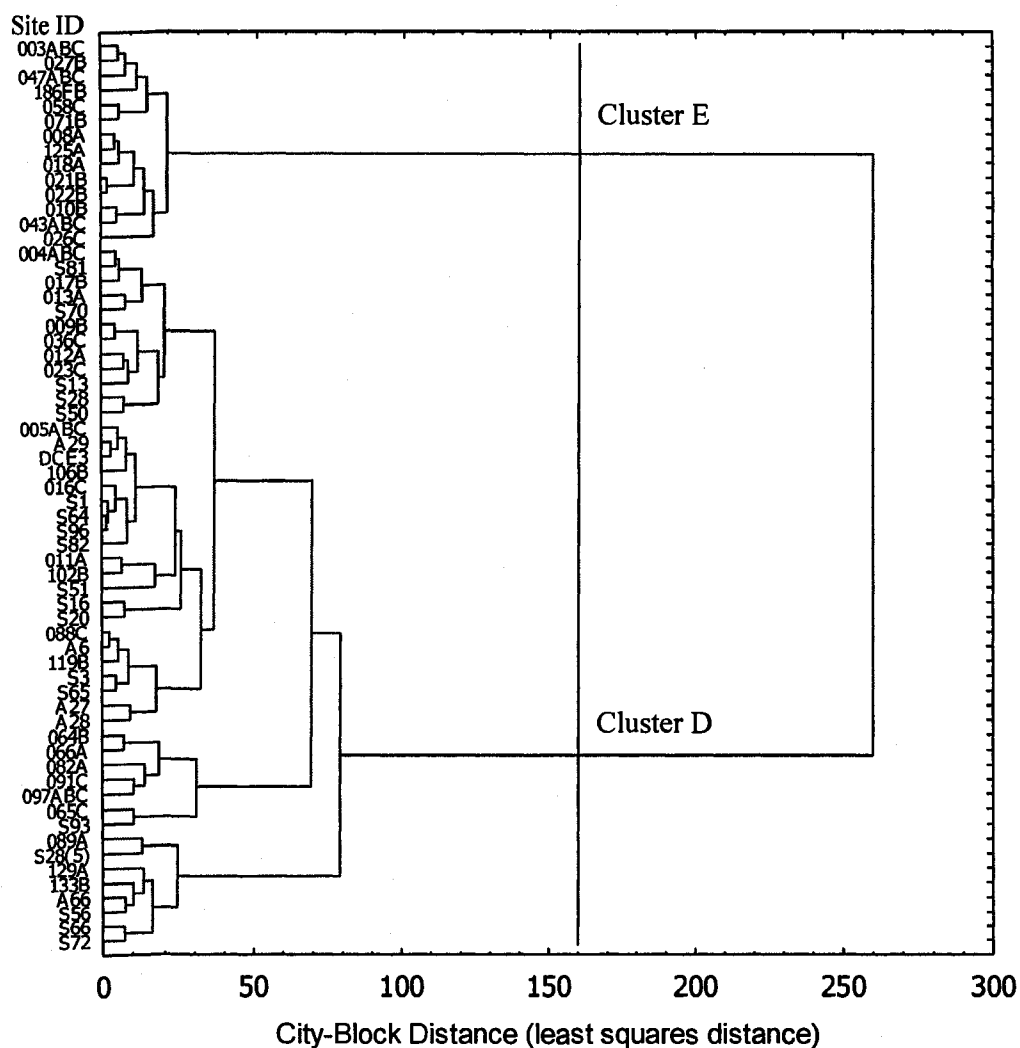


Figure IV-1. Dendrogram of REF sites ( $n = 62$ ) grouped according to similar zoobenthic community composition in the 1991, 1999 and 2004/5 Lake Huron-Lake Erie Corridor (Ward's method clustering city-block distances of octave-transformed relative abundances of zoobenthic taxa). REF sites were selected solely based on the second principal component factor (PC2). Site locations corresponding to site labels are summarized in Appendix I

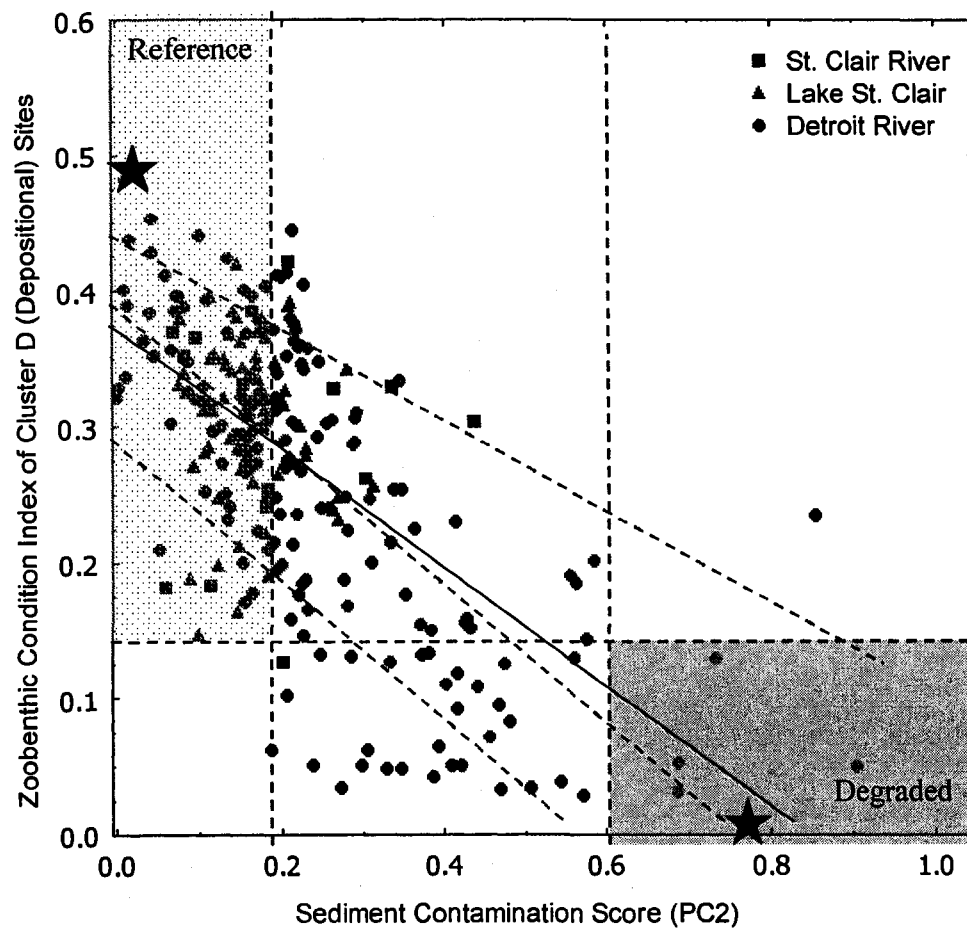


Figure IV-2. Relationship between Zoobenthic Condition Index (ZCI; Bray-Curtis zoobenthic relative abundance ordination scores) and the sediment contamination score (PC-2) for sites in cluster D.  $n = 253$  sites. The site with black star indicates the REF endpoint (high ordination score together with low PC-2 score); the site with grey star indicates the DEG endpoint (low ordination score together with high PC-2 score). Solid line indicates the least square fit line; dashed lines indicate 0.9, median and 0.1 quantile linear regression lines, respectively. The horizontal and vertical lines separate the samples into sectors as would be identified by piecewise quantile regression. All sites with PC-2 scores  $\leq 0.19$  have a ZCI score of 0.14 or greater. All sites with PC-2 scores  $\geq 0.59$  have a ZCI score of  $< 0.14$ . Accordingly, depositional (D) sites with ZCI scores  $> 0.14$  cannot be said to be degraded

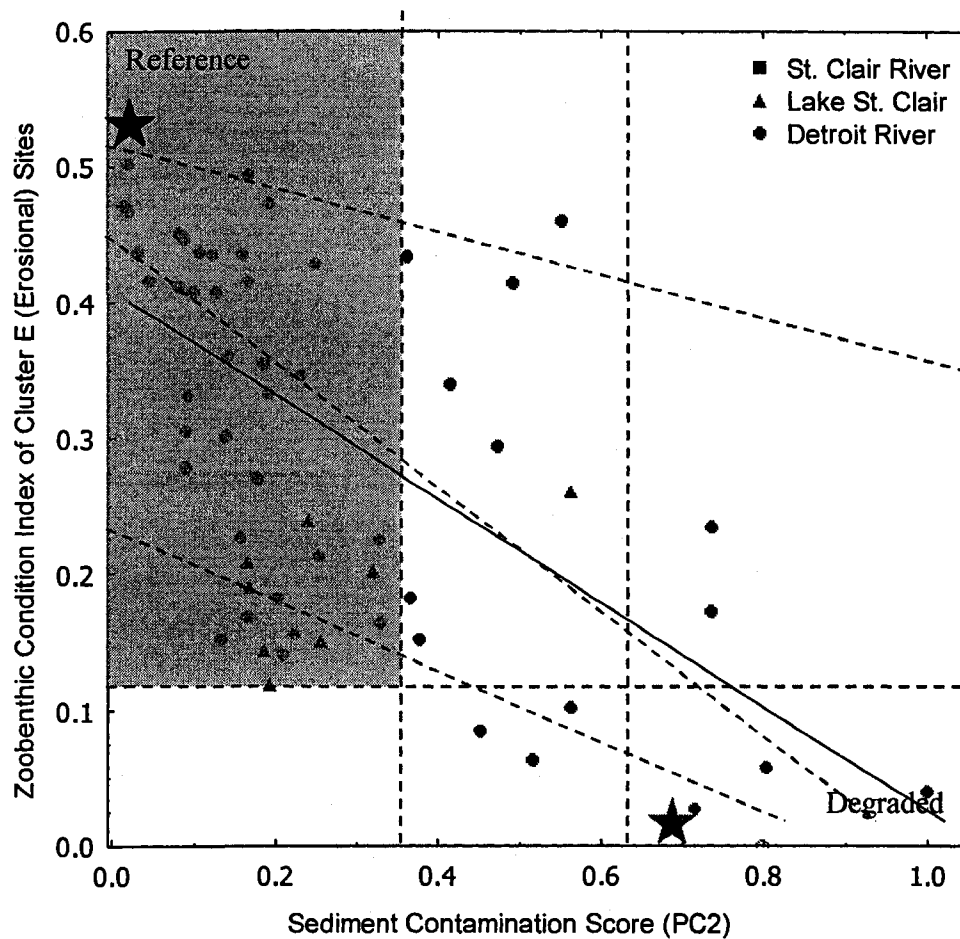


Figure IV-3. Relationship between Zoobenthic Condition Index (ZCI; Bray-Curtis zoobenthic relative abundance ordination scores) and the sediment contamination score (PC-2) for sites in cluster E.  $n = 58$  sites. The site with black star indicates the REF endpoint (high ordination score together with low PC-2 score); the site with grey star indicates the DEG endpoint (low ordination score together with high PC-2 score). Solid line indicates the least square fit line; dashed lines indicate 0.9, median and 0.1 quantile linear regression lines, respectively. The horizontal and vertical lines separate the samples into sectors as would be identified by piecewise quantile regression. All sites with PC-2 scores  $\leq 0.41$  have a ZCI score of 0.10 or greater. All sites with PC-2 scores  $\geq 0.72$  have a ZCI score of  $< 0.10$ . Accordingly, depositional (D) sites with ZCI scores  $> 0.10$  cannot be said to be degraded

Table IV-1. Summary of observed number of Lake Huron-Lake Erie Corridor sites in each cluster (columns) identified by zoobenthic taxa relative abundances and membership predicted (rows) by discriminant function classification on the basis of habitat characteristics measured at those sites

Group	Observed		
	% Correct	Cluster D	Cluster E
Cluster D	96	46	2
Cluster E	86	2	12
Total	94	48	14

Table IV-2. Habitat variables accepted into the DFA model describing discriminant functions and their mean ( $\pm$  1SE) in the 62 REF sites. Variables with bold face were determined by DFA model as significant in classifying REF site cluster membership. Asterisk (\*) indicates significance level: \*\*\* highly different

---

Habitat variables	Significance level
<hr/>	
<b>Water Depth (m)</b>	<b>p &lt; 0.001 ***</b>
Lake or River	p > 0.05
Median Particle Size (Phi)	p > 0.06
Water Temperature (°C)	p > 0.05
Longitude	p > 0.05

---



Table IV-3. Forward stepwise multiple regression of relative abundances of 16 taxa vs. ZCI scores for cluster D sites.  $F_{[5,247]}=638.31$   $p<0.0001$   $R^2= 0.93$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.189 ± 0.011	17.873	0.000	
<b>Chironomidae</b>	0.036 ± 0.001	37.318	0.000	0.644
<b>Oligochaeta</b>	-0.020 ± 0.001	-13.831	0.000	0.174
<b>Nematoda</b>	0.016 ± 0.001	15.738	0.000	0.062
<b>Amphipoda</b>	0.011 ± 0.001	7.672	0.000	0.032
ZCI (Depositional) = 0.189 + 0.036*Chironomidae + 0.016*Nematoda + 0.011*Amphipoda - 0.020*Oligochaeta				

Table IV-4. Forward stepwise multiple regression of relative abundances of 13 taxa vs. ZCI scores for cluster E sites.  $F_{[4,53]} = 248.72$   $p < 0.0001$   $R^2 = 0.95$

	<b>B ± 1SE</b>	<b>t</b>	<b>p</b>	<b>Partial R<sup>2</sup></b>
<b>Intercept</b>	0.358 ± 0.021	17.218	0.000	
<b>Dreissena</b>	0.020 ± 0.003	7.584	0.000	0.622
<b>Hydrozoa</b>	-0.032 ± 0.003	-11.869	0.000	0.213
<b>Oligochaeta</b>	-0.029 ± 0.003	-9.968	0.000	0.097
ZCI (Erosional) = 0.358 + 0.020*Dreissena - 0.032*Hydrozoa - 0.029*Oligochaeta				

Appendix V. Classifications of 213 Detroit River sites based on discriminant function analysis (DFA) ('G' refers to group/cluster number). The 'Observed' column contains the grouping of the REF sites based on sediment contamination. Column '1' contains the grouping of test sites based on the DFA analysis with 8 habitat variables. Sites with asterisk (\*) were misclassified by the DFA model. When the classification probability was near 0.5, a "best judgment" method was used to assign the sites to proper group based on the zoobenthic community composition

Site ID	Observed	1	2	3
*004ABC	G_1:1	G_3:3	G_1:1	G_2:2
005ABC	G_1:1	G_1:1	G_3:3	G_2:2
011A	G_1:1	G_1:1	G_2:2	G_3:3
016C	G_1:1	G_1:1	G_2:2	G_3:3
019B	G_1:1	G_1:1	G_3:3	G_2:2
023C	G_1:1	G_1:1	G_2:2	G_3:3
025B	G_1:1	G_1:1	G_3:3	G_2:2
*033ABC	G_1:1	G_3:3	G_1:1	G_2:2
*036C	G_1:1	G_3:3	G_1:1	G_2:2
097ABC	G_1:1	G_1:1	G_3:3	G_2:2
121C	G_1:1	G_1:1	G_3:3	G_2:2
122B	G_1:1	G_1:1	G_3:3	G_2:2
*S100	G_1:1	G_3:3	G_1:1	G_2:2
*S81	G_1:1	G_2:2	G_3:3	G_1:1
S82	G_1:1	G_1:1	G_3:3	G_2:2
S96	G_1:1	G_1:1	G_2:2	G_3:3
073C	G_2:2	G_2:2	G_3:3	G_1:1
*1FB	G_2:2	G_3:3	G_1:1	G_2:2
39FB	G_2:2	G_2:2	G_3:3	G_1:1
*5FB	G_2:2	G_3:3	G_1:1	G_2:2
*E	G_2:2	G_1:1	G_2:2	G_3:3
G	G_2:2	G_2:2	G_1:1	G_3:3
H	G_2:2	G_2:2	G_3:3	G_1:1
S97	G_2:2	G_2:2	G_1:1	G_3:3
S99	G_2:2	G_2:2	G_3:3	G_1:1
010B	G_3:3	G_3:3	G_2:2	G_1:1
026C	G_3:3	G_3:3	G_1:1	G_2:2
030ABC	G_3:3	G_3:3	G_1:1	G_2:2
035C	G_3:3	G_3:3	G_2:2	G_1:1
057C	G_3:3	G_3:3	G_1:1	G_2:2
058C	G_3:3	G_3:3	G_1:1	G_2:2
060B	G_3:3	G_3:3	G_2:2	G_1:1
104C	G_3:3	G_3:3	G_1:1	G_2:2
109C	G_3:3	G_3:3	G_1:1	G_2:2

Appendix V. Continued.

Site ID	Observed	1	2	3
125A	G_3:3	G_3:3	G_1:1	G_2:2
*133B	G_3:3	G_2:2	G_1:1	G_3:3
27FB	G_3:3	G_3:3	G_2:2	G_1:1
47FB	G_3:3	G_3:3	G_2:2	G_1:1
A	G_3:3	G_3:3	G_1:1	G_2:2
C	G_3:3	G_3:3	G_1:1	G_2:2
*S101	G_3:3	G_1:1	G_3:3	G_2:2
S102	G_3:3	G_3:3	G_2:2	G_1:1
S95	G_3:3	G_3:3	G_1:1	G_2:2
007ABC	---	G_1:1	G_3:3	G_2:2
008A	---	G_1:1	G_3:3	G_2:2
009B	---	G_1:1	G_3:3	G_2:2
017B	---	G_1:1	G_2:2	G_3:3
027B	---	G_1:1	G_3:3	G_2:2
042C	---	G_1:1	G_2:2	G_3:3
044A	---	G_1:1	G_2:2	G_3:3
045B	---	G_1:1	G_2:2	G_3:3
054B	---	G_1:1	G_3:3	G_2:2
059ABC	---	G_1:1	G_3:3	G_2:2
067B	---	G_1:1	G_2:2	G_3:3
070B	---	G_1:1	G_2:2	G_3:3
079C	---	G_1:1	G_2:2	G_3:3
080C	---	G_1:1	G_2:2	G_3:3
081B	---	G_1:1	G_3:3	G_2:2
082A	---	G_1:1	G_3:3	G_2:2
084A	---	G_1:1	G_3:3	G_2:2
085C	---	G_1:1	G_3:3	G_2:2
086A	---	G_1:1	G_3:3	G_2:2
088C	---	G_1:1	G_3:3	G_2:2
089A	---	G_1:1	G_3:3	G_2:2
091C	---	G_1:1	G_2:2	G_3:3
092ABC	---	G_1:1	G_3:3	G_2:2
095A	---	G_1:1	G_2:2	G_3:3
096A	---	G_1:1	G_3:3	G_2:2
099C	---	G_1:1	G_3:3	G_2:2
100C	---	G_1:1	G_3:3	G_2:2
101C	---	G_1:1	G_3:3	G_2:2
105C	---	G_1:1	G_3:3	G_2:2
107C	---	G_1:1	G_3:3	G_2:2
108B	---	G_1:1	G_2:2	G_3:3
111C	---	G_1:1	G_3:3	G_2:2

Appendix V. Continued.

Site ID	1	2	3
115ABC	G_1:1	G_2:2	G_3:3
119B	G_1:1	G_3:3	G_2:2
123A	G_1:1	G_3:3	G_2:2
124A	G_1:1	G_3:3	G_2:2
126A	G_1:1	G_3:3	G_2:2
127B	G_1:1	G_3:3	G_2:2
130A	G_1:1	G_3:3	G_2:2
131B	G_1:1	G_3:3	G_2:2
135A	G_1:1	G_2:2	G_3:3
138B	G_1:1	G_2:2	G_3:3
140C	G_1:1	G_3:3	G_2:2
142C	G_1:1	G_2:2	G_3:3
146B	G_1:1	G_3:3	G_2:2
149C	G_1:1	G_2:2	G_3:3
186FB	G_1:1	G_3:3	G_2:2
18FB	G_1:1	G_2:2	G_3:3
199FB	G_1:1	G_2:2	G_3:3
53FB	G_1:1	G_3:3	G_2:2
6FB	G_1:1	G_3:3	G_2:2
B	G_1:1	G_3:3	G_2:2
S85	G_1:1	G_3:3	G_2:2
S89	G_1:1	G_3:3	G_2:2
S94	G_1:1	G_2:2	G_3:3
S98	G_1:1	G_2:2	G_3:3
012A	G_2:2	G_1:1	G_3:3
013A	G_2:2	G_1:1	G_3:3
014B	G_2:2	G_1:1	G_3:3
015C	G_2:2	G_3:3	G_1:1
052B	G_2:2	G_3:3	G_1:1
064B	G_2:2	G_1:1	G_3:3
065C	G_2:2	G_1:1	G_3:3
066A	G_2:2	G_1:1	G_3:3
068B	G_2:2	G_1:1	G_3:3
069A	G_2:2	G_1:1	G_3:3
071B	G_2:2	G_3:3	G_1:1
072A	G_2:2	G_1:1	G_3:3
075A	G_2:2	G_3:3	G_1:1
077B	G_2:2	G_1:1	G_3:3
078B	G_2:2	G_1:1	G_3:3
093C	G_2:2	G_3:3	G_1:1
102B	G_2:2	G_1:1	G_3:3

Appendix V. Continued.

Site ID	1	2	3
10FB	G_2:2	G_3:3	G_1:1
12FB	G_2:2	G_3:3	G_1:1
134C	G_2:2	G_1:1	G_3:3
136B	G_2:2	G_1:1	G_3:3
137A	G_2:2	G_1:1	G_3:3
139B	G_2:2	G_1:1	G_3:3
13FB	G_2:2	G_3:3	G_1:1
15FB	G_2:2	G_1:1	G_3:3
17FB	G_2:2	G_3:3	G_1:1
195FB	G_2:2	G_3:3	G_1:1
198FB	G_2:2	G_1:1	G_3:3
200FB	G_2:2	G_3:3	G_1:1
21FB	G_2:2	G_3:3	G_1:1
224FB	G_2:2	G_3:3	G_1:1
22FB	G_2:2	G_1:1	G_3:3
23FB	G_2:2	G_3:3	G_1:1
240FB	G_2:2	G_1:1	G_3:3
24FB	G_2:2	G_1:1	G_3:3
25FB	G_2:2	G_1:1	G_3:3
33FB	G_2:2	G_1:1	G_3:3
34FB	G_2:2	G_3:3	G_1:1
35FB	G_2:2	G_1:1	G_3:3
36FB	G_2:2	G_3:3	G_1:1
37FB	G_2:2	G_3:3	G_1:1
40FB	G_2:2	G_3:3	G_1:1
41FB	G_2:2	G_3:3	G_1:1
42FB	G_2:2	G_1:1	G_3:3
43FB	G_2:2	G_1:1	G_3:3
44FB	G_2:2	G_3:3	G_1:1
46FB	G_2:2	G_3:3	G_1:1
50FB	G_2:2	G_1:1	G_3:3
51FB	G_2:2	G_1:1	G_3:3
62FB	G_2:2	G_3:3	G_1:1
71FB	G_2:2	G_1:1	G_3:3
84FB	G_2:2	G_1:1	G_3:3
8A	G_2:2	G_3:3	G_1:1
F	G_2:2	G_3:3	G_1:1
I	G_2:2	G_3:3	G_1:1
J	G_2:2	G_3:3	G_1:1
K	G_2:2	G_3:3	G_1:1
L	G_2:2	G_1:1	G_3:3

Appendix V. Continued.

Site ID	1	2	3
M	G_2:2	G_3:3	G_1:1
N	G_2:2	G_1:1	G_3:3
O	G_2:2	G_3:3	G_1:1
P	G_2:2	G_3:3	G_1:1
S83	G_2:2	G_3:3	G_1:1
S93	G_2:2	G_1:1	G_3:3
003ABC	G_3:3	G_1:1	G_2:2
018A	G_3:3	G_1:1	G_2:2
021B	G_3:3	G_1:1	G_2:2
022B	G_3:3	G_1:1	G_2:2
024C	G_3:3	G_1:1	G_2:2
029C	G_3:3	G_1:1	G_2:2
031A	G_3:3	G_1:1	G_2:2
034C	G_3:3	G_2:2	G_1:1
037B	G_3:3	G_2:2	G_1:1
043ABC	G_3:3	G_1:1	G_2:2
047ABC	G_3:3	G_1:1	G_2:2
048C	G_3:3	G_1:1	G_2:2
049A	G_3:3	G_1:1	G_2:2
055C	G_3:3	G_2:2	G_1:1
083B	G_3:3	G_1:1	G_2:2
090B	G_3:3	G_1:1	G_2:2
094C	G_3:3	G_1:1	G_2:2
098C	G_3:3	G_1:1	G_2:2
103A	G_3:3	G_1:1	G_2:2
106B	G_3:3	G_1:1	G_2:2
113B	G_3:3	G_2:2	G_1:1
114B	G_3:3	G_1:1	G_2:2
116B	G_3:3	G_2:2	G_1:1
117ABC	G_3:3	G_2:2	G_1:1
118A	G_3:3	G_2:2	G_1:1
128B	G_3:3	G_1:1	G_2:2
129A	G_3:3	G_1:1	G_2:2
141B	G_3:3	G_1:1	G_2:2
143B	G_3:3	G_1:1	G_2:2
144B	G_3:3	G_1:1	G_2:2
145B	G_3:3	G_2:2	G_1:1
148B	G_3:3	G_2:2	G_1:1
14FB	G_3:3	G_2:2	G_1:1
150B	G_3:3	G_1:1	G_2:2
189FB	G_3:3	G_2:2	G_1:1

Appendix V. Continued.

Site ID	1	2	3
19FB	G_3:3	G_2:2	G_1:1
221FB	G_3:3	G_2:2	G_1:1
222FB	G_3:3	G_2:2	G_1:1
223FB	G_3:3	G_2:2	G_1:1
28FB	G_3:3	G_1:1	G_2:2
2FB	G_3:3	G_1:1	G_2:2
45FB	G_3:3	G_1:1	G_2:2
48FB	G_3:3	G_2:2	G_1:1
49FB	G_3:3	G_2:2	G_1:1
70FB	G_3:3	G_2:2	G_1:1
9FB	G_3:3	G_2:2	G_1:1
D	G_3:3	G_1:1	G_2:2
S84	G_3:3	G_2:2	G_1:1
S87	G_3:3	G_2:2	G_1:1
S90	G_3:3	G_2:2	G_1:1



Appendix VI. Number of chironomid larvae of 43 genus examined from the 12 zones of the Lake Huron-Lake Erie Corridor, 2004/5. Genera with bold face were considered "common"

Taxa / Site ID	1ASR	2CSR	3ASR	4CSR	1LSC	2LSC
<b>Polypedilum</b>	<b>206</b>	<b>167</b>	<b>20</b>	<b>77</b>	<b>11</b>	<b>434</b>
<b>Dicrotendipes</b>	<b>25</b>	<b>45</b>	<b>9</b>	<b>134</b>	<b>9</b>	<b>69</b>
<b>Chironomus</b>	<b>70</b>	<b>25</b>	<b>3</b>	<b>2</b>	<b>17</b>	<b>30</b>
<b>Phaenopsectra/Tribelos</b>	<b>111</b>	<b>8</b>	<b>1</b>	<b>17</b>	<b>39</b>	<b>32</b>
<b>Tanytarsus</b>	<b>5</b>	<b>1</b>	<b>1</b>	<b>10</b>	<b>89</b>	<b>34</b>
<b>Procladius</b>	<b>35</b>	<b>11</b>	<b>4</b>	<b>5</b>	<b>13</b>	<b>16</b>
Cricotopus	100	53	2	10	0	6
Cryptochironomus	38	54	19	23	13	60
Pseudochironomus	0	0	0	0	55	45
Paratanytarsus	2	2	0	1	1	8
Ablabesmyia	15	4	6	4	9	22
Stictochironomus	17	6	1	0	6	5
Harnischia	1	2	5	1	14	12
Coelotanypus	0	0	0	0	8	9
Rheotanytarsus	10	2	1	12	0	1
Demicryptochironomus	1	9	0	2	1	20
Cryptotendipes	0	0	1	3	1	24
Paralauterborniella	0	2	0	1	3	15
Paratendipes	8	1	0	0	0	2
Cladotanytarsus	3	3	0	2	0	3
Psectrocladius	1	2	0	0	5	14
Monodiamesa	7	0	0	0	0	19
Thienemannimyia	0	3	0	0	2	0
Potthastia	0	2	1	1	1	8
Epoiccladius	0	0	1	0	2	5
Apsectrotanypus	3	0	0	0	0	1
Clinotanypus	0	0	0	0	1	2
Parachironomus	1	0	0	1	4	0
Nanocladius	0	0	0	0	0	0
Labrundinia	0	0	0	0	3	1
Synendotendipes	0	4	0	0	0	0
Thienemanniella	0	0	0	1	0	0
Pentaneura	0	0	0	0	0	2
Tanypus	0	0	0	0	1	0
Nilothauma	0	0	0	0	1	1
Apedilum	0	0	0	0	0	0
Paracladopelma	2	0	0	0	0	0
Larsia	1	0	0	0	0	0
Axarus	0	1	0	0	0	0
Cladopelma	0	0	0	0	0	0
Microtendipes	0	0	0	0	0	1
Xenochironomus	0	0	0	0	0	1
Stempellina	0	0	0	0	0	1
unknown	0	0	1	0	1	1

## Appendix VI. Continued

Taxa / Site ID	3LSC	4LSC	1ADR	2CDR	3ADR	4CDR
Polypedil	346	51	86	44	0	8
Dicrotend	217	41	4	13	2	1
Chirono	98	22	5	86	5	25
Phaenops	30	55	16	4	4	5
Tanytars	66	88	1	7	6	4
Procladiu	19	23	8	0	3	2
Cricotopu	62	8	2	0	1	0
Cryptochi	75	39	20	10	9	2
Pseudochi	6	11	0	1	0	0
Paratanyta	59	10	0	0	1	0
Ablabesm	56	5	11	2	1	1
Stictochir	33	42	0	2	0	0
Harnischi	10	28	24	5	1	3
Coelotany	16	39	9	3	0	0
Rheotanyt	27	2	3	22	0	1
Demicrypt	14	3	2	0	4	0
Cryptoten	6	10	3	1	0	2
Paralauter	12	14	2	0	0	1
Paratendip	31	1	0	1	0	0
Cladotany	17	4	2	9	0	1
Psectrocla	15	3	1	2	0	0
Monodia	3	0	0	1	0	0
Thienema	20	3	1	0	0	0
Potthastia	4	4	0	1	0	0
Epoicladi	4	3	5	0	0	0
Apsectrot	2	2	4	3	0	0
Clinotany	2	8	0	0	0	0
Parachiro	1	5	0	1	0	0
Nanocladi	6	1	0	0	1	0
Labrundin	0	1	0	0	0	0
Synendote	0	1	0	0	0	0
Thienema	4	0	0	0	0	0
Pentaneur	2	0	0	0	0	0
Tanypus	3	0	0	0	0	0
Nilothaum	0	1	0	0	0	0
Apedilum	2	0	0	0	0	0
Paraclado	0	0	0	0	0	0
Larsia	0	0	0	0	0	0
Axarus	0	0	0	0	0	0
Cladopel	0	0	0	0	1	0
Microtend	0	0	0	0	0	0
Xenochiro	0	0	0	0	0	0
Stempelli	0	0	0	0	0	0
unknown	2	0	0	3	0	0

Appendix VII. Description and number of mentum deformities in six selected chironomid taxa

Genus	Deformity	Description of Deformity	Number of Observances
<i>Chironomus</i>			
	Missing Teeth	Missing right lateral	3
		Missing left lateral	9
		Missing median	4
	Extra Teeth	Extra median	1
	Köhn gap		5
<i>Dicrotendipes</i>			
	Missing Teeth	Missing right lateral	6
		Missing right and left lateral	1
		Missing left lateral	6
	Extra Teeth	Extra left lateral	1
		Extra median	1
<i>Phaenopsectra / Tribelos</i>			
	Missing Teeth	Missing right lateral	2
		Missing left lateral	1
		Missing median	2
	Extra Teeth	Extra median	1
<i>Polypedilum</i>			
	Missing Teeth	Missing right lateral	5
		Missing left lateral	11
		Missing median	5
	Extra Teeth	Extra left lateral	1
		Extra median	7
<i>Procladius</i>			
	Extra Teeth	Extra Ligula Teeth	3
<i>Tanytarsus</i>			
	Extra Teeth	Extra right lateral	1

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