

IMPROVING THE WETLAND ZOOPLANKTON INDEX FOR APPLICATION
TO GEORGIAN BAY COASTAL WETLANDS

By

STEPHANIE N. YANTSIS, B.Sc.

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AUTHOR: Stephanie N. Yantsis, B.Sc. (McMaster University)

SUPERVISOR: Dr. Patricia Chow-Fraser

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ABSTRACT: In 1996, the State of the Lakes Ecological Conference (SOLEC) stressed the need to create ecological indicators to monitor Great Lakes wetlands. Since then, a suite of indicators have been created by researchers throughout the Great Lakes using different environmental parameters such as water quality (Water Quality Index, WQI, Chow-Fraser 2006; Agriculture PC1, Danz et al. 2007), fish (Wetland Fish Index, WFI, Seilheimer and Chow-Fraser 2006, 2007), plants (Plant Index of Biotic Integrity, Rothrock and Simon 2006, 2008; Wetland Macrophyte Index, WMI, Croft and Chow-Fraser 2007) and zooplankton (Wetland Zooplankton Index, WZI, Lougheed and Chow-Fraser 2002). In a recent study, Seilheimer et al. (2009) found that the WQI had a significant linear relationship with both the WFI and WMI, but not with the WZI. They showed that the WZI was not able to discern the pristine nature of wetlands in Georgian Bay, Lake Huron, where there is minimal human disturbance. As the first objective of my thesis, I investigate three possible reasons for this poor performance. I investigated whether the lower than expected WZI scores associated with high-quality Georgian Bay sites could have been due to 1) inadequate sampling effort 2) inclusion of highly exposed sites or 3) lack of representation of Georgian Bay sites in the development of the WZI. Using data from the Chow-Fraser database, as well as analyzing additional samples from the zooplankton archive, zooplankton abundance data was used to analyze my hypotheses. Increasing sampling effort from 1 to 5 samples per wetland did not lead to significantly higher WZI scores, even though species richness increased

with sampling effort. Including Georgian Bay sites with high degree of exposure to wind and wave action did not significantly decrease WZI scores, although there was a trend towards lower overall abundance of zooplankton for exposed sites. I found strong support for the third reason, that the original development of the WZI had biased the index parameters against Georgian Bay sites. This was confirmed when I employed the same statistical approach to an expanded database that included 63 of the original 70 wetland-years along with 31 new wetland-years in Georgian Bay and 45 others in Lakes Erie and Ontario. Using the results of Partial Canonical Correspondence Analysis (pCCA), I made 5 modifications to the WZI optimum (U) and tolerance (T) values. Using an independent dataset, I found that the modified WZI (WZI₀₉) scores were linearly related to WQI ($r^2=0.283$; $P < 0.0001$; $n= 50$). The second objective of my thesis was to investigate whether or not aquatic macrophyte information was a stronger predictor of zooplankton community than water-quality information. I compared the percent fit of data from a co-correspondence analysis (CO-CA) of zooplankton abundance data and plant presence/absence data and a correspondence analysis (CCA) of zooplankton abundance and environmental data. Results indicated that plants were not a better predictor of zooplankton distribution than environmental variables (CO-CA: 12.8%, CCA: 13.3%, $n=107$). I therefore conclude that the modifications of the WZI₀₉ have resulted in an improved indicator that can be used in tandem with other indicators to determine wetlands health throughout the Canadian shoreline of the Great Lakes, including Georgian Bay.

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INTRODUCTION

Laurentian Great Lakes

North America is home to the world's largest freshwater ecosystem, the Laurentian Great Lakes. This system contains one fifth of the world's freshwater between the five lakes of Superior, Michigan, Ontario, Erie and Huron (containing Georgian Bay). These lakes are shared by one province in Canada and eight states in the USA. These lakes were created over 10,000 years ago by retreat of the glaciers during the Wisconsin glaciation. This retreat, along with natural weathering, have created the current shoreline by eroding and redistributing glacial bedrock. With a current shoreline spanning over 17,000 km, the Great Lakes range across diverse physical environments, with varying climate and geomorphology (Smith et al. 1991). Geologically, over a third of the lakes in the north and northwest regions lie on the Canadian Shield, a region of Precambrian granitic bedrock covered with a thin layer of soil. The remainder lie on the softer, more erosion-prone sedimentary rock such as shale, limestone and sandstone, deposited during the Palaeozoic period while the southern portion of the Great Lakes were covered by shallow seas. Climatic variation is also vast, ranging from humid continental conditions in the south to subarctic conditions in the north (Mayer et al. 2004).

Within the last 200 years, the landscape and use of the Great Lakes basin has changed dramatically due to settlement by Europeans. While over half the surrounding land remains forested, the majority of the land, especially in the

southern region, has been converted to urban, agricultural and commercial use. In this region, the bulk of the 33 million people that use the Great Lakes ecosystem can be found (Mayer, et al. 2004). This human disturbance has drastically changed land use, water quality as well as local and regional biodiversity.

Georgian Bay

Of particular importance to this study is the region of Georgian Bay. Georgian Bay is located in the eastern arm of Lake Huron and is the largest freshwater archipelago in the world. Also known as the “30,000 Islands”, Georgian Bay is also one of 530 UNESCO World Biosphere Reserves, due to its mosaic of ecological systems and high biodiversity. Wetland habitat is abundant along its highly complex shoreline. Georgian Bay is unique in that it contains both the geologic formations mentioned above with Pre-Cambrian Canadian Shield and the sedimentary rock of the Niagara Escarpment. Where the northern portion of Georgian Bay is dominated by granitic bedrock, southern Georgian Bay has combined effects from both rock types affecting sediment composition, trophic state and species composition. With the exception of the southern most tip, Georgian Bay contains very little human impact on its shoreline and surrounding watershed, giving its waters different chemistry and composition to the lower lakes, providing us with a unique baseline of data to use in comparisons and as a “pre-settlement” control site.

Great Lakes Coastal Wetlands

In 1996, the State of the Lakes Ecosystem Conference (SOLEC) defined Canadian wetlands as:

“...land that is saturated with water long enough to promote wetland or aquatic processes as indicated by poorly drained soils, hydrophytic vegetation and various kinds of biological activity adapted to wet environments.” (National Wetlands Working Group, 1988).

The great diversity of wetland types and subtypes differ in hydrology as well as vegetation communities and are divided into the five main types: marsh, shallow-water, swamp, fen and bog. Marshes are the most common type, defined as seasonally or permanently flooded areas that contain a transition from an aquatic submerged portion to a terrestrial upland portion (Herdendorf, 2004). This study focuses particularly on coastal marshes. These wetlands are hydrologically connected to the Great Lakes and fall within 2 km of the Great Lakes shoreline (as per Ontario Ministry of Natural Resources, 1994). Unlike inland wetlands, coastal marshes are affected and shaped by processes that affect the larger lake ecosystem, such as waves, wind tides and water-level fluctuations (Maynard and Wilcox 1996).

Wetlands are known to be one of the most productive ecosystems on earth with areas of exceptional biodiversity. Wetlands provide habitat for a variety of terrestrial and aquatic species including plants, fish, birds, reptiles, mammals, zooplankton and macroinvertebrates (Maynard and Wilcox 1996). Wetlands have also been estimated to provide trillions of dollars worth of ecosystem services annually through water supply, sediment control, flood storage, filtering, food

production, habitat, commercial as well as opportunities for research, education and recreation (Maynard and Wilcox 1996, Costanza et al. 1997). With over 70% of Canada's wetlands being lost since European settlement, it is clear there has been much anthropogenic impact on Great Lakes wetlands due to urban development, agriculture and lake level regulation. While most of the wetlands in the lower Great Lakes have seen a high level of anthropogenic disturbance, the majority of northern wetlands remain generally untouched by human disturbance. Georgian Bay on the north-eastern arm of Lake Huron is home to an abundance of pristine, unimpacted wetlands that provide us with a glimpse at conditions before human settlement and this can be contrasted with human disturbed wetlands.

Research on Great Lakes Coastal Wetlands

Due to the huge importance of the Great Lake and their coastal wetlands, many organizations exist to protect, monitor and research past and current conditions including, but not limited to: the International Joint Commission (IJC), Great Lakes Coastal Wetlands Action Plan (GLCWAP), Great Lakes Coastal Wetland Consortium (GLCWC), Great Lakes Ecological Indicators (GLEI), State of the Lakes Ecosystem Conference (SOLEC) and the International Association of Great Lakes Research (IAGLR). There are also many smaller groups that focus on particular regions or lakes. Of importance to our research are Georgian Bay Land Trust (GBLT), Georgian Bay Association (GBA) and Georgian Bay Forever (GBF, formerly GBA Foundation). The majority of coastal wetland

research has focused on improving knowledge of water quality, land use, water levels, macrophytes, fish and invertebrate composition in these coastal wetland, either on a per region or basin-wide level. Recently, due to a call by SOLEC in 1998, research in the Great Lakes has focused on the use and creation of indicators that will aid government and non-government parties in assessing the quality of the Great Lakes, with particular focus on wetland quality and tracking improvements and changes throughout the Great Lakes basin.

Ecological Indicators

An ecological indicator is used to provide information about an ecosystem as well as incorporate the impact of human disturbance on an ecosystem. In 1997, Karr and Chu stressed the importance and need for biological indicators to aid in ecological risk assessment. The most commonly used method of index creation in an Index of Biotic Integrity (IBI). Created and first used by J.R. Karr in 1981, an IBI is an integrative expression of site condition across at least seven different metrics and can be used to provide an easy to understand index value. A metric is an attribute that shows an empirical and predictable change in value along a gradient of human disturbance (Karr 1981). Examples of IBIs created for and/or utilized in Great Lakes wetlands include: Index of Biotic Integrity for fish assemblages (Minns et al. 1994), Index of Biotic Integrity for wetlands (Wilcox et al. 2002), Index of Marsh Bird Community (IMBCI; DeLuca et al. 2004), Invertebrate Index of Biotic Integrity (I-IBI; Uzarski et al. 2005), Index of Biotic

Integrity of summer polyhaline zooplankton (Carpenter et al. 2006) and Plant Index of Biotic Integrity (Rothrock and Simon 2006, 2008).

Previous investigators have used a variety of multivariate statistical methods and parameters (including biotic and abiotic factors) to create indices. Although the indices vary in the way they have been developed, few have been designed for basin-wide application. Examples of the indices developed in our laboratory for basin-wide application are presented chronologically as follows: *Wetland Zooplankton Index* (WZI, Lougheed and Chow-Fraser 2002), *Water Quality Index* (WQI, Chow-Fraser 2006), *Wetland Fish Index* (WFI, Seilheimer and Chow-Fraser 2006), *Wetland Macrophyte Index* (WMI, Croft and Chow-Fraser 2007) and *Wetland Exposure Index* (WEI, Wei 2007).

Zooplankton and the Wetland Zooplankton Index

Zooplankton are an important link in aquatic food-webs. They provide the link between primary producers and the higher trophic levels. They are affected by both top-down and bottom-up processes and as such any variation in nutrients, algae dynamics, fish populations and water quality changes can affect the zooplankton community composition and dynamics and make zooplankton good indicators of change (Gannon and Stemberger 1978, Schindler 1987, Attayde and Bozelli 1998). In wetlands, zooplankton are especially important to the fish community, since wetlands act as spawning habitat and the diet of larval fish is predominantly zooplankton (Leslie and Moore 1985). Zooplankton distribution

has also been shown to have an effect on nutrients and primary production by controlling algal growth and improving water clarity (Timms and Moss 1984, Lougheed and Chow-Fraser 2008; Schriver et al. 1995).

Zooplankton are also known to have strong relationships with aquatic vegetation (Quade 1969, Schriver et al. 1995, Stansfield et al. 1997, Perrow et al 1999, Kuczyn'ska-Kippen 2007). Zooplankton use submerged aquatic vegetation as a source of refuge from planktivorous fish (Timms and Moss 1984, Lougheed and Chow-Fraser 2002). Some zooplankton species are grazers that graze epiphyton that flourish in the presence of aquatic vegetation. A loss of submerged aquatic vegetation can alter zooplankton dynamics in wetlands and result in a community of grazers that are adapted to turbid, nutrient-rich, open-water systems (Lougheed and Chow-Fraser, 1998 & 2001). Also of importance, exposure has been shown to affect aquatic plant colonization (Keddy 1983 & 1985, Chamber 1987, Goforth and Carman 2005). Recent studies have used plant and exposure information together to examine the combined effects of plants and exposure on invertebrate distributions (Burton et al. 2002, 2004).

Within Great Lakes coastal wetlands, zooplankton research has shifted focus from zooplankton distribution and its changes (Norgady, 1989, Krieger and Klarer 1991, Krieger 1992, Hessen et al. 2006), to using that information to create indices to monitor wetland health (Wilcox et al. 2002, Uzarski et al. 2004). In 2002, Lougheed and Chow-Fraser published the Wetland Zooplankton Index (WZI) using data collected from 70 coastal and inland marshes from across all

five Great Lakes. The index was created with zooplankton abundance data and relied on the known relationship between water quality parameters and presence of submerged aquatic vegetation or macrophyte. The study set included sites that ranged from highly degraded, human-impacted sites dominated by emergent vegetation with little to no submerged plants, to a few high-quality marshes dominated by a diverse community of submergent, floating and emergent vegetation. Lougheed and Chow-Fraser (2002) used a method called Partial Canonical Correspondence Analysis (pCCA; ter Braak 1984; see Methods section for details) to ordinate zooplankton species along a gradient of water-quality degradation. The ordination produced pCCA axes scores that were subsequently used to develop species-specific metrics for the Wetland Zooplankton Index, with values ranging from 1 (to indicate poor quality, polluted sites) to 5 (high-quality, undisturbed sites) .

Other indices used in this study

The Water Quality Index (WQI, Chow-Fraser 2006), is a basin-wide Great Lakes wetlands indicator. It measures the degree of impairment by utilizing twelve water quality parameters. This impairment can be due to anthropogenic disturbance like altered land-use or increased nutrient load. It was developed from data collected at 110 sites across all five Great Lakes, 53 sites from the lower lakes (Erie and Ontario) and 57 sites from the upper lakes (Huron-Michigan and Superior). Since its development, the index has been applied to an additional

one hundred wetlands throughout Georgian Bay, the North Channel and Lake Erie, and was found to be highly and significantly related to total road-density (both all-season and seasonal-access roads; m/ha), which is a documented indicator of landscape-level disturbance (Danz et al. 2007; DeCatanzaro et al. 2009). The WQI scores tend to range from -3 ("highly degraded") to +3 ("excellent"), and Chow-Fraser (2006) has developed six "quality" categories at unit intervals that has the following interpretations: +3 to +2: Excellent, +2 to +1: Very Good, +1 to 0: Good, 0 to -1: Moderately Degraded, -2 to -1: Very Degraded, -3 to -2: Highly Degraded.

Despite its great utility, the WQI requires sample processing and analyses that make it difficult to be adopted for routine monitoring by environmental management agencies. Indices such as the WZI, that rely on collection and identification of biota to indicate water-quality conditions was therefore an attractive alternative. The development of the Wetland Fish Index (WFI) and Wetland Macrophyte Index (WMI) were predicated on the well-documented ecological relationships between plant and fish taxa and water-quality variables (e.g. nutrient concentrations, water clarity, etc), similar to that between zooplankton and water quality parameters as previously discussed.

The Wetland Exposure Index (WEI) was developed by Wei in 2007 specifically for coastal wetlands in Fathom Five National Marine Park where wind-swept conditions are prevalent year-round. The WEI combined a simplified Geomorphology Index (GI) and Relative Exposure Index (REI), and is a measure

of wave and wind action. This study uses a modified version of the WEI, called the Physical Disturbance Index (PDI) which was adapted by Cvetkovic (2008).

Thesis Objectives and Hypotheses

In a recent study, Seilheimer et al. 2009 compared the utility and cost-effectiveness of the WZI, WMI and WFI as surrogates of the WQI. Results from the study showed that the WMI and WFI both had positive linear relationships with the WQI, confirming that the WMI and WFI can be used to indicate similar wetland quality based solely on biotic sampling. Conversely, the WZI had a polynomial relationship with the WQI, making it impossible to use the WZI to discriminate between polluted and unpolluted sites. The two main objectives of my thesis are to first, uncover the reason(s) why the WZI is not linearly related to the WQI. A second objective is to investigate whether or not aquatic macrophyte information is a stronger predictor of zooplankton community than is water-quality information. This latter objective was prompted by the recent finding of Cvetkovic (2008) that the predictive ability of biotic factors (macrophyte assemblages) on fish distribution is stronger than abiotic factors (water quality) on both a regional (Georgian Bay) and Great-Lakes-basin scale.

To achieve my first objective, I investigated three possible reasons for the quadratic polynomial relationship between WZI and WQI that was documented by Seilheimer et al. (2009), indicating an intermediate optimum for WZI scores.

Previous research have demonstrated that most of the high-quality wetlands in the Great Lakes basin are found in eastern and northern Georgian Bay (Chow-Fraser 2006; Cvetkovic 2008; Croft and Chow-Fraser 2007; DeCatanzaro et al. 2009; Seilheimer et al. 2009). Croft and Chow-Fraser (2007) also showed that species richness of macrophytes in Georgian Bay wetlands is significantly higher compared with wetlands in human-disturbed wetlands of Lakes Erie and Ontario. Therefore, I hypothesize that the lower WZI scores in high-quality wetlands of Georgian Bay may be related to inadequate sampling of the amount species-rich wetlands of Georgian Bay. To address this, I will determine if there is a significant increase in WZI score with number of samples processed for Georgian Bay sites.

Another possible reason for low WZI score in Georgian Bay could be the degree of exposure in these wetlands. It has been shown in previous research that exposure can alter plant colonization in a wetland (Keddy 1983 & 1985, Chamber 1987, Goforth and Carman 2005), and thus indirectly affect the distribution of invertebrates associated with the plants (Burton et al. 2004). Since most of the wetlands in Loughheed and Chow-Fraser's original dataset are protected wetlands that occur in the lower lakes, whereas a number of wetlands in eastern Georgian Bay and the North Channel are exposed to wind and wave action (Wei 2007), I hypothesize that the more exposed wetland sites may be associated with lower WZI scores and that exclusion of these wetlands would yield a linear relationship between WZI and WQI.

Finally, if the previous two hypotheses prove false, I hypothesize that the development of the original WZI by Loughheed and Chow-Fraser (2002) was biased because of inadequate representation of high-quality sites from Georgian Bay since only seven of the 70 sites in the original dataset had been located in Georgian Bay. Also the WZI has a low-number of sites used in its creation; other indices use 100+ in their creation (WQI: 110, WFI: 100, WMI: 127). I therefore propose to modify the WZI by re-running analyses that include proportionately more Georgian Bay sites, along with Loughheed and Chow-Fraser's original data. By generating a new set of metrics that take into account the high-quality sites in Georgian Bay, I predict that this modified index (which I will call WZI₀₉) would yield proportionately higher WZI scores for Georgian Bay wetlands, and thus result in a linear relationship between WZI₀₉ and WQI.

My second objective is to determine if macrophyte information is a better predictor of zooplankton assemblage than is water-quality information. Recently, a study of plant-fish interactions by Cvetkovic (2008) showed that plants were statistically better predictors of fish species composition than was water quality. They compared the "percent fit" of two multivariate ordination techniques, Canonical Correspondence Analysis (CCA; ter Braak 1986) and the Co-Correspondance Analysis (CO-CA; ter Braak and Schaeffers 2004). The CCA uses water-quality variables to predict species distribution, whereas the CO-CA allows a direct comparison of two sets of species data. Cvetkovic (2008) showed that plants were consistently better at predicting the fish community than

were water-quality variables in three separate trials: all wetlands in the Great Lakes basin, all wetlands in Lakes Superior and Huron and all wetlands in Georgian Bay and the North Channel. In this study, I use the same approach to compare the use of plant information and water quality variables to see which set of parameters is a better predictor of zooplankton community. I hypothesize that similar to Cvetkovic et al. 2009, plants will be a better predictor of zooplankton community distribution, and should be used instead of water chemistry in further indices development involving zooplankton.

METHODS

Sample sites

Lougheed and Chow-Fraser (2002) developed the Wetland Zooplankton Index (WZI) index from 70 coastal wetlands found throughout the five Great Lakes, but few from eastern and northern Georgian Bay, where some of the most pristine wetlands are found (Chow-Fraser 2006; De Catanzaro et al. 2009). In reformulating the WZI (which I will refer to as WZI₀₉), I added to the original sites used by Lougheed and Chow-Fraser (2002) to total 139. I sampled 20 sites, analyzed 50 samples, re-analyzed 30 of Lougheed's samples and included data from previous studies by Lougheed in the Chow-Fraser lab. I specifically added 31 wetland-years from Georgian Bay, since pristine sites from this region were found to be under-represented in the original WZI (Figure 1). For a complete outline of all sites used in this study see Table 1. Besides geographic representation, I also ensured that there was a good distribution of sites according to Water Quality Index (WQI) scores, although I was not able to obtain equal distribution in all six WQI categories (see Table 2).

Four high-quality Georgian Bay sites, Pamplemousse, Rhodes Marsh, Green Island Channel and Green Island (exposed) were sampled more intensively in June and July 2008 to test hypotheses concerning the effect of sampling effort on WZI scores (Figure 2); they were chosen because of their relatively small size (so they could be sampled completely) and the high diversity of macrophyte assemblages throughout (Croft and Chow-Fraser 2007).

The sites included in Co-correspondence Analysis (CO-CA) analyses were fewer than those used in the Canonical Correspondence Analysis (CCA), because of limited availability of plant information that were associated with zooplankton samples. The number of sites were selected to cover as large a range as possible in WQI scores; in the end I was still able to include 107 wetland-years from three Lakes: Lakes Huron/Georgian Bay (67), Ontario (27) and Erie (13) (Figure 3). There were 33 sites used to test the effect of exposure to wind and wave action because of limited availability of data to calculate an index of site exposure (i.e. Physical Disturbance Index (PDI; Cvetkovic 2008) as well as WZI score (Figure 4). To eliminate possible confounding effects of differences due to geographic location, I only included sites in Georgian Bay in this analysis.

Sample collection

All of the zooplankton samples used to reformulate the WZI₀₉ had been collected in the same manner as that reported by Loughheed and Chow-Fraser (2002). Therefore, all samples were collected from June to August between 1998 to 2004 (including those used by in the formulation of the original WZI), in fair-weather conditions, at least 48 hours after a storm event to avoid effects of surface runoff (Krieger and Klarer, 1991; Chow-Fraser, 1999). All samples were collected with a clear Plexiglas 5-L Schindler-Patalas zooplankton trap. We only included samples taken within submersed aquatic vegetation (SAV) to be consistent with the original study. In samples with fewer than 100 animals, every

animal was identified to genus and species if possible. All other samples were thoroughly mixed and sub-sampled to obtain at least 100 animals. Rotifer identification was based on Chegalath et al. (1971) and Stemberger (1979). Cladoceran identification was based on Balcer et al (1984), Pennak (1989) and Thorp & Covich (1991). Abundances of zooplankton were enumerated (#/L) and sorted according to the taxonomic groups used in the WZI (Lougheed and Chow-Fraser 2002). I re-analyzed 20 of Lougheed's original samples to confirm that all species identification was uniform in all sets of data included.

In the four Georgian Bay wetlands where samples had been collected to test the effect of sampling effort, zooplankton were collected within macrophyte assemblages and in open water during June and July of 2008 (Figure 2). This is contrary to the recommendation of Lougheed and Chow-Fraser (2002) to sample only within vegetated areas, but since no significant statistical differences were found in WZI scores between samples collected in vegetation versus open water in Pamplemousse (t-test; $n=11$; $P>|t|=0.45$), I decided to group the data to increase the sample size.

Water quality information used in CCA and CO-CA analyses were selected from the archived database of Lougheed and Chow-Fraser (2002), Croft and Chow-Fraser (2007) and Cvetkovic (2008). All samples had been collected at the same time as zooplankton collection had been conducted. Water samples were collected with a horizontal 1-L Van Dorn sampler. Descriptions of the sample processing and analyses of nutrients and suspended solids have been well

documented (Lougheed and Chow-Fraser 2002; Chow-Fraser 2006; Croft and Chow-Fraser 2007). The environmental variables I included in this study are: Total Phosphorus (TP), Soluble Reactive Phosphorus (SRP), Total Nitrogen (TN), Total Ammonia-Nitrogen (TAN), Total-Nitrate Nitrogen (TNN), Total Suspended Solids (TSS), Inorganic Suspended Solids (ISS), Chlorophyll-*a* (CHL), and Turbidity (TURB).

The number of submerged plant species present in wetlands was determined as described by Croft and Chow-Fraser (2007). Plant information used in this study are part of a systematic plant survey of the entire wetland to determine presence of aquatic plant species from the wetland shoreline to a depth of about 2-m throughout the wetland (see Croft and Chow-Fraser for complete description). Emergent plant taxa were identified while walking in waders along representative stretches of the wetland shoreline. In ten to twelve transects, which had been selected to represent various habitat types (open water, dense submergent, floating, dense floating, etc.), all floating and submergent plant taxa encountered were identified. This was accomplished from a canoe or boat, and in deeper water, a rake was used to collect plants that could not be seen below the water surface. All plants were identified in the field to genus and species where possible according to Crow and Hellquist (2000) and Chadde (2002). More detailed plant collection information can be found in Croft & Chow-Fraser (2007).

Quantification of Exposure

The degree of exposure at the 33 sites used was estimated by calculating the Physical Disturbance Scores (PDI) that ranged from a score of 1 (protected) to >5 (highly exposed), see Figure 5. Cvetkovic (2008) modified the Wetland Exposure Index (WEI) of Wei (2007) to create the PDI, which only includes information on geomorphology and fetch as follows:

$$\text{PDI} = (\text{GI} + 1) * \text{Log} (\text{mean Fetch})$$

where GI represents the Geomorphology Index score calculated by the width of the wetland opening divided by its perimeter. Unlike the WEI, this calculation does not have a parameter that includes wind speed and direction. Direction of wind can have a great affect on distribution of zooplankton species depending on the wetlands shape and exposure level.

Statistical analyses

With the exception of the Partial Canonical Correspondence Analysis (pCCA) which was performed with CANOCO 4.0 (ter Braak and Smilauer 1998) and Co-Correspondance Analysis (CO-CA) which was performed using MATLAB (MATLAB 7.8, MathWorks Software), I performed all other statistical analyses using SAS JMP software (version 4, SAS Institute, North Carolina). Prior to the pCCA analysis, a Detrended Correspondence Analysis (DCA) was performed to ensure that the zooplankton data had a unimodal distribution (inertia > 4). Like Lougheed and Chow-Fraser (2002), a pCCA was used to account for

seasonal differences in species distribution since included samples were collected over a three-month period each season. This is achieved by including day of the year (Julian Day) as one of the environmental parameters. Environmental parameters used in the pCCA analysis included: latitude, longitude, depth, temperature, light extinction coefficient, pH, COND, TP, SRP, CHL-*a*, TN, ISS and number of submergent species. All environmental variables (Env Var) were least squares transformed to approximate normal distributions and zero mean. The least squares transformation was calculated with the following equation:

$$\text{Log}_{10} \frac{(\text{Env Var (units)} - \text{Mean (Env Var (units))})}{\text{Std Dev (Env Var (units))}} + 1$$

Co-correspondance analysis (CO-CA) was performed with MATLAB as described by ter Braak and Schaffers, 2004. Co-correspondance analysis (CO-CA) is a direct one-step symmetric ordination method used to compare the variance between sets of ecological community data from the same sites. This method maximizes the covariance by weighted average taxa scores that allows one to find similar distribution patterns within species presence/absence or abundance community data. In this study, the weighted average of zooplankton scores at each site were used to determine plant scores at each site and vice versa. Zooplankton abundance data and plant presence/absence data were used. I derived site scores using the weighted averages of each community data set. The example shown in ter Braak and Schaffers (2004) was followed. CO-CA site groups were determined with a cluster analysis (Ward's method) of CO-CA Axis 1 and 2 site

scores. All plots were created with SigmaPlot 10.0 graphing software (Systat Software Inc., 2006).

Index modification

Lougheed and Chow-Fraser (2002) used results of a zooplankton abundance data in a pCCA to derive “U” and “T” values for the WZI. “U” values were assigned to zooplankton taxa based on location of the taxon centroid along the first pCCA axis; these ranged from 1 to 5, where 1 was indicative of the most pollution tolerant taxa and 5 were the least pollution tolerant. “T” values were assigned to taxa based on the weighted standard deviations of the taxon scores along the pCCA axis 1. T values could be thought of as taxon niche-breadth or distribution and ranged from 1 (broad) to 3 (narrow). I employed the same method to assign U and T values as in the original study. The new locations of species along CCA axis 1 were used to determine new U values, while T values were reassigned using the newly calculated weighted standard deviations of each taxonomic group. The scores for both the original WZI and the newly modified WZI (WZI₀₉) were calculated with the tolerance weighted averages in the following equation (Zelinka and Marvan, 1961; Lennat 1993; Kelly and Whitton 1995; Lougheed and Chow-Fraser, 2002):

$$WZI = \frac{\sum_{i=1}^n Y_i T_i U_i}{\sum_{i=1}^n Y_i T_i}$$

where Y_i = abundance or presence of species, T_i = tolerance (1-3) and U_i = optimum (1-5). The index ranges from 1 (poor quality wetlands) to 5 (high quality wetlands).

RESULTS

Sampling effort

In the four Georgian Bay test wetlands, there was no significant relationship between WZI score and the number of samples analyzed from 1 to 5 (Figure 6a). Figure 6a shows WZI scores calculated by increasing the number of samples used (ie. 1 zooplankton sample used to calculate WZI score, 2 replicate samples used, etc.). Up to 5 pooled samples were used, and this number exceeds that recommended in Loughheed and Chow-Fraser's (2002) study. Though an increase in species richness was found as sampling effort increased (Figure 6b), WZI scores did not change significantly and this suggests that increased richness does not necessarily translate into difference in wetland quality. Though I did not sample exhaustively, increasing sampling effort to greater than 3 samples per site did not result in a higher mean WZI score and therefore the lower WZI scores

associated with high-quality sites in Georgian Bay could not be attributed to inadequate sampling effort.

Physical Disturbance and Exposure analysis

The 33 sites used to determine effect of exposure from Georgian Bay varied in wetland quality (WQI scores ranged from -0.65 to 2.79, with a mean of 1.32). PDI scores ranged from 1.98 to 5.35, with a mean of 2.88. WZI scores ranged from 2.68 to 4.88 with a mean of 3.58. I regressed WZI score against PDI score but found no significant relationship (Figure 7a; $R^2 = 0.0304$, $P = 0.3391$), as well as when regressed with total zooplankton abundance (Figure 7b; $R^2 = 0.0330$, $P = 0.3554$). General trends, however, showed that more exposed sites (higher PDI scores) had a lower overall abundance of zooplankton and were associated with higher variability in the zooplankton assemblage. Despite these trends, there was no evidence that lower WZI scores were attributed to higher site exposure.

Canonical Correspondence Analysis

The pCCA was based on 139 wetland-years throughout the Great Lakes basin, and included the wetland sites used in the creation of the original WZI along with 31 sites in a primarily undisturbed region of eastern and northern Georgian Bay. The relationship between the first two pCCA axes are shown in a biplot (Figure 8). Variation of environmental parameters within the first two axes explained 21.5% of the variation found in zooplankton distribution. The most

important predictors of zooplankton distribution, as shown by their correlation with pCCA axis 1 were latitude (-0.754), COND (0.814), TN (0.713), TP (0.698), and SRP (0.653). These associations helped to confirm an axis of degradation along pCCA axis 1 and is consistent with the results of Lougheed and Chow-Fraser (2002).

Alteration of Wetland Zooplankton Index

Using the collapsed taxonomic grouping listed in Table 4, I determined a new set of optimum (U) and tolerance (T) values from the pCCA output. Compared with the original set of U and T values from Lougheed and Chow-Fraser (2002), I found differences for 5 taxonomic groupings (see Table 4). U values for *Euchlanis* sp. and *D. Brachyurum* decreased from 4 and 5, respectively, to 3, while T values for both decreased from 2 to 1. By contrast, there was an increase in U value for *Keratella* from 3 and 5 and an increase in T value from 1 to 2. For *Bosminidae*, on the other hand, there was only an increase in T value from 1 to 2. Finally, the U value for *Kellicottia* species increased from 3 to 5 while the T value decreased from 3 to 1.

Comparisons of WZI, WZI₀₉ and WQI

I calculated WZI and WZI₀₉ scores for an independent dataset (n=50), and regressed them against corresponding WQI scores (n=50). Regressing WZI values (calculated with the original formulation by Lougheed and Chow-Fraser

2002) against WQI yielded a polynomial relationship ($R^2=0.3482$, $P=0.0013$) similar to that reported by Seilheimer et al. (2009); however, regression of the WZI₀₉ score (calculated from this study) showed a significant linear relationship with WQI score ($R^2=0.4085$; $P<0.0001$) (Figure 9). I compared differences between WZI and WZI₀₉ for three high-quality Georgian Bay sites as well as one good-quality site Lake Erie (Long Point Marsh), and one degraded wetland in Lake Ontario (Cootes Paradise Marsh). Using a paired t-test WZI₀₉ scores were found significantly higher for than WZI for Pamplemousse ($n=5$; $P>|t|=0.05$), and Cootes Paradise 2008 ($n=5$; $P>|t|=0.01$). Though not significant, WZI₀₉ scores averaged higher for Rhodes Marsh as well. WZI₀₉ scores showed an average increase of +0.668 across all sites. Also note, standard deviations were on average much lower for the reformulated WZI₀₉ (range 0.171-0.379; mean 0.28) compared with the original (range 0.461-0.867; mean 0.59).

Direct ordination of zooplankton and aquatic plants

Only 107 of 139 wetland-years used in the first pCCA had appropriate data to compare the power of water-quality parameters versus plant information for predicting zooplankton assemblages. For these 107 wetland-years, the percent fit of the pCCA for the first two axes was 13.3%, or in other words, 13.3% of the variation in zooplankton distribution was explained by the variation in environmental variables. The percent fit of the Co-Correspondence Analysis (CO-CA) based on zooplankton abundance and plant presence/absence was 12.8% for

the first two axes. The similar percent fits show that plants are equally important as environmental variables in relationship to zooplankton distribution, and there is no evidence that plant information is better than water-quality in this respect.

Site group characteristics

The CO-CA biplot (Figure 11a) shows the direct correspondence of zooplankton taxa (▼) and plant taxa (●) based on the covariance maximized over all 107 wetland-year sites (■). According to ter Braak and Schaffers (2004), the zooplankton and plant species that fall in similar positions relative to the origin are positively associated and those that jointly fall farther from the origin more strongly so.

Cluster analysis (Ward's method) was used to provide an objective grouping of sites based on similarities and dissimilarities among CO-CA Axis 1 scores for zooplankton and plants. There were five main groups as illustrated in Figure 11b. Groups 1 and 2 contained highly degraded wetlands that occur in Lakes Erie and Ontario. Group 3 contained sites from moderately degraded wetlands found in all three lakes. Group 4 contained the good quality sites of Georgian Bay, Lake Huron and Lake Ontario, and Group 5 contained the unimpacted sites found only in Georgian Bay. Figure 11b shows that the more degraded sites occurring to the left of the origin on CO-CA Axis 1 while unimpacted sites were grouped to the right, indicating an axis of increasing wetland degradation from left to right.

CO-CA score comparison with indices

To make some more concrete comparisons, CO-CA site scores were regressed against known indices of quality: Water Quality Index (WQI; Chow-Fraser, 2006), Wetland Macrophyte Index (WMI; Croft and Chow-Fraser, 2007), Wetland Zooplankton Index (WZI; Lougheed and Chow-Fraser, 2002). Average values for each site group are found in Table 5. Using this information, the WQI and WMI scores were regressed against CO-CA Axis 1 and the WZI scores against CO-CA Axis 2. Both the WQI and WMI showed significant positive linear relationships with CO-CA Axis 1 (Figure 12a). This information verifies the axis of degradation discussed above. When the WZI was linearly regressed against CO-CA Axis 1 there was no significant relationship; however, a significant positive relationship was found when the WZI was regressed with CO-CA Axis 2 (Figure 12b). This regression was performed due to a relationship of plant type (submerged vs. emergent) with CO-CA Axis 2. High quality zooplankton are known to be associated with a high abundance of submerged plants and WZI scores reflect this relationship.

DISCUSSION

Testing the WZI

The original Wetland Zooplankton Index (WZI) was created based on zooplankton distribution and their known associations with macrophytes and tolerance to pollutants. Using this information, the index was intended to be used to assess wetland quality across the Great Lakes basin. In recent years additional indices have been created with the same method using different species assemblages, eg. the Wetland Fish Index (WFI; Seilheimer and Chow-Fraser, 2007) and the Wetland Macrophyte Index (WMI; Croft and Chow-Fraser, 2007). Seilheimer et al. (2009) compared the three indices (WFI, WMI and WZI), using 32 wetlands across the Great Lakes that ranged in water quality conditions. Results showed that while the WFI and WMI could distinguish between high- and low- quality sites, the WZI could not. In particular, pristine sites in eastern Georgian Bay had very low WZI scores that erroneously indicated they were degraded.

This study was conducted to find potential reasons to explain the poor performance of WZI when applied to wetlands of Georgian Bay. I determined if the unexpectedly low WZI scores corresponding to the high-quality sites could be attributed to: 1) inadequate sampling; 2) inclusion of Georgian Bay sites that have a higher degree of exposure and exposure; or 3) underrepresentation of Georgian Bay sites in formulation of the original WZI.

To test the first hypothesis four high quality Georgian Bay wetlands were chosen. These sites varied in exposure but all contained diverse macrophyte assemblages while maintaining hydrological connection to the main Georgian Bay basin. Within each site, multiple samples were taken and WZI scores were recalculated as the number of replicates increased from 1 to 5. There was no significant change in WZI as sampling effort increased (Figure 3). Scores would increase or decrease depending on the addition of uncommon species that either indicated low or high tolerance to pollution. Past studies have suggested that in high-quality sites, planktivory by fish is more prevalent and as such increased sampling effort for zooplankton is necessary (Timms and Moss 1984, Seilheimer 2009). Therefore increased sampling effort is not required in Georgian Bay wetlands.

Our second hypothesis assumed the high degree of exposure in Georgian Bay wetlands was responsible for the lower-than-expected WZI scores. A significant negative relationship between WZI score and some quantitative measure of exposure would support this. Previous studies show that the distribution of invertebrate and zooplankton composition can vary along gradient of physical-disturbance (Burton et al 2002, 2004, 2009; Kostuk 2006). Exposure is also known to play a key factor in plant colonization in a wetland by diminishing abundance (Chambers 1987, Wei 2007) and affecting composition (Keddy 1983, 1985; Riis and Hawes 2003), and many high-quality zooplankton are dependent on the presence of these plants. A recent study by Cvetkovic

(2008) showed that the WMI was negatively related to the Physical Disturbance Index (PDI), a quantitative measure of the degree of exposure to wind and wave action for Georgian Bay wetlands. The negative relationship indicated that exposed sites were associated with low WMI scores that were indicative of a less healthy wetland, even though water-quality conditions and landscape variables indicate otherwise.

In this study, I did not find a significant negative relationship between WZI and PDI (Figure 7a). General trends showed more exposed sites having a lower overall abundance of zooplankton, but this cannot be related to WZI score. Therefore, I could not attribute the lower scores in Georgian Bay to inclusion of exposed sites.

After ruling out the first two hypotheses, I found support for the third hypothesis: underrepresentation of high-quality sites in the original formulation of the WZI may have caused some zooplankton taxa to be assigned inappropriate parameters (U and T values). I re-ran the pCCA with 139 wetland-years (which included 63 of the original wetland-years, and 31 new sites specifically chosen from a range of high-quality wetlands in the unimpacted area of Eastern Georgian Bay. 45 additional low-quality wetland-years were also included from throughout the Great Lakes basin. This resulted in a new set of pCCA axes used to determine optimum (U) and tolerance (T) values. Like Lougheed and Chow-Fraser (2002), I discovered that the pCCA axis 1 was again driven by environmental water-quality parameters.

Modification and improvement of the WZI

I found that 5 zooplankton taxa had to be modified with respect to their U and T values. The lower U values for the rotifer *Euchlanis*, and the cladoceran, *Diaphanosoma brachyurum*, in the reformulated WZI₀₉ indicates they have a higher tolerance to degradation than had been earlier determined. This was likely a consequence of having little to no representation of high-quality sites in Lougheed and Chow-Fraser's original dataset. Addition of the 10 Georgian Bay sites in the "Excellent" category clearly shifted the U and T values further down along the gradient towards higher degradation. In the reformulated WZI₀₉, T values for both taxa also decreased from 2 to 1, indicating a wider niche breadth. By comparison, U and T values for *Keratella* species increased. In the original 70 wetlands sampled, occurrences of *Keratella* were in "Good" to "Moderately Degraded" wetlands but in low abundances, and hence Lougheed and Chow-Fraser assigned this taxon a U value of 3. In this study, however, *Keratella* was found to be extremely abundant in the high-quality wetlands of Georgian Bay, and I therefore increased the U value to 5. Their increased abundance in the high-quality wetlands may be due to a lack of predatory cladocerans and copepods that are kept low by the presence of planktivorous fish (e.g. juvenile bass and pumpkinseeds). *Bosminidae*, on the other hand were also seen frequently in higher-quality wetlands, but not as abundantly as were *Keratella*. This resulted in no change to the U value, but an increase in the T value from 1 to 2, indicating a

more defined niche breadth in good-quality sites. Lastly, species of *Kellicottia* were associated with a higher U value of 5, compared with its original value of 3, primarily because they were found in over half of the new wetlands and were very abundant the best-quality sites.

Next, I tested for differences between WZI and WZI₀₉ scores using 50 selected wetlands that had not been included in the reformulation of the new index. When a linear regression was performed between the original WZI and the WQI (Figure 9a), no significant relationship was found (R-square = 0.0918, P=0.0816). A polynomial regression was run against the same data and this resulted in a significant regression (R-square = 0.3482, P=0.0013), similar to the findings of Seilheimer et al. (2009). When a linear regression was run on WZI₀₉ and WQI scores, however, I obtained a significant positive linear relationship (R-square = 0.4085; P < 0.0001). This confirms that the new modifications made to the WZI allowed it to discriminate between low- and high-quality sites.

To verify this conclusion, I compared WZI scores obtained from multiple samples within 7-wetland years, three from Georgian Bay sampled in 2008, and two each from Lakes Erie and Ontario, sampled ten years apart in 1998 and 2008. These comparisons showed that WZI₀₉ only increased in wetlands of high quality, but was statistically similar to the WZI for the good-quality and degraded sites such as Cootes Paradise Marsh. WZI₀₉ also tended to be accompanied by a much lower standard deviation as compared with the WZI.

Zooplankton-Macrophyte Associations

I also determined if aquatic macrophyte information would be better than water-quality information for predicting zooplankton distribution since zooplankton depend on macrophytes for food and to hide from predators. I achieved this by comparing the percent fit values of our CCA analysis of zooplankton abundance and environmental variables and our CO-CA analysis of zooplankton abundance and macrophyte presence data. I found that the CCA had a slightly stronger percent fit of 13.3% compared with 12.8% for the CO-CA. My results therefore indicate that water-quality parameters are equally important to plant taxonomic information for predicting zooplankton distribution.

Also, the CO-CA analysis assigned site scores that reflected an axis of degradation along CO-CA Axis 1 (Figure 12). This was confirmed when I regressed site scores against corresponding WQI and WMI scores. Interestingly, there was no significant linear relationship between CO-CA Axis 1 and WZI, but there was one between CO-CA Axis 2 and WZI. One of the reasons for this may be that groups of aquatic plants are ordinated along CO-CA Axis 2, from submergent to emergent, and WZI scores are higher in wetlands where submergent vegetation dominates. Further studies must be carried out to determine if these relationships are spurious or are ecologically meaningful.

CONCLUSIONS AND FUTURE RESEARCH

This study has led to the reformulation of the Wetland Zooplankton Index (WZI₀₉) that can be applied throughout the Canadian shoreline of the Great Lakes basin, including Georgian Bay. I have confirmed that WZI score does not increase with sampling effort from 1 to 5 replicates, even though species richness increases with number of samples analyzed. I have also shown that WZI scores did not vary significantly with degree of exposure as indicated by the Physical Disturbance Index. My results are consistent with the hypothesis that the polynomial relationship between the WQI and WZI shown in Seilheimer et al. 2009 is likely a result of under-representation of high-quality sites in the original formulation. The WZI₀₉ now has the ability to differentiate between wetlands with that are dominated by pollution-tolerant zooplankton taxa and those dominated by taxa requiring a diverse and abundant community of submersed aquatic vegetation.

Though exposure did not have a significant effect on WZI scores, it may still may an important role in determining the distribution of zooplankton because of the trend in reduced numbers of zooplankton with degree of exposure. Future studies should be conducted in controlled experiments where other environmental variables (e.g. plant and fish community) can be held constant. More research also needs to be done to determine the role of planktivorous fish on the increased abundance of certain zooplankton taxa (e.g. *Keratella* and *Bosmina*) in high-quality wetlands.

Wetland monitoring has become increasingly important in the last decade. Many researchers have been attempting to create easy to use cost-effective monitoring programs to maintain and conserve the wetlands that are left in the Great Lakes. Though Seilheimer et al. (2009) concluded that the Wetland Zooplankton Index developed by Loughheed and Chow-Fraser (2002) is not cost-effective compared with the WMI or the WFI, I believe that the WZI₀₉ should now be added to the suite of indicators used to predict habitat quality in coastal wetlands across the Great Lakes basin. Since zooplankton is known to change their distribution and adapt to the environment faster than many other aquatic species (Gannon and Stemberger 1978, Schindler 1987, Attayde and Bozelli 1998), the WZI should be able to detect rapid changes in wetland quality associated with climate change. Besides their usefulness for calculating WZI scores, zooplankton survey information can also be used to help ecologists understand the dynamics of the lower food-web and to help predict energy transfer from both a top-down and bottom-up direction.

Another advantage of the WZI over plant- or fish-based indices is that very few archives of plant and fish specimens exist because they are difficult to preserve, whereas it is relatively simple and cheap to preserve zooplankton samples for many decades. Therefore, one can collect zooplankton from wetlands during routine sampling and calculate WZI₀₉ at a later date when there is more time and resources. I suggest that a good monitoring strategy should include a

combination of indices in order to provide information about both wetland health and food-web dynamics, and to create long-term data sets.

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Table 1: Outline of sites used in this different portion of this study.

Portion of Study	Number and type of sites	Location	Date Collected	Assoc. Table(s) or Figure(s)
Sampling Effort	4 wetlands; multiple sampled taken from different locations in wetlands	Georgian Bay	July-August 2007	Figure 1 Figure 2 Figure 4
Open vs. vegetation sampling	4 wetlands; multiple sampled taken from different locations in wetlands	Georgian Bay	July-August 2007	Table 2
Exposure	33 wetlands	Georgian Bay	June-August 2006-2008	Figure 4 Figure 7
pCCA for WZI ₀₉	139 wetland-years	All 5 Great Lakes	June-August 1994-2006	Table 3 Figure 1 Figure 8
WZI vs. other indices	50 wetlands	All 5 Great Lakes	June-August 1998-2006	Figure 9
WZI vs. WZI ₀₉ comparison	7 wetlands	Georgian Bay, Lakes Erie and Ontario	June-August 1998, 2007, 2008	Figure 10
CCA/CO-CA comparison	107 wetland-years	Lakes Huron/Georgian Bay, Erie, Ontario	June-August 1998-2006	Table 5 Figure 11 Figure 12

Table 2: Distribution of sites used in the reformulation of the WZI₀₉ according to WQI category (after Chow-Fraser 2006).

WQI Category	Range in WQI score	# Occurrences
Highly Degraded	$\leq - 2$	9
Very Degraded	-2 to -1	14
Moderately Degraded	-1 to 0	25
Good	0 to +1	49
Very Good	+1 to +2	32
Excellent	$> +2$	10

Table 3: WZI scores for samples collected in four Georgian Bay sites. Unbolded scores correspond to samples collected in open water, **bolded** scores to samples collected in vegetation.

	Pamplemousse	Rhodes Marsh	Green Island Channel	Green Island Exposed
	3.41	3.86	2.86	4.26
	3.72	2.74	3.24	3.78
	3.32	3.42	2.74	3.22
	3.90	2.89	4.26	3.25
	3.79	3.80	3.25	4.10
	3.65			
	4.23			
	3.33			
	3.56			
	3.51			
	4.38			
Integrated score from all samples	3.600	3.217	3.100	4.109
Open AVG	3.631 ± 0.224	3.299 ± 0.796	3.050 ± 0.270	---
Veg AVG	3.803 ± 0.470	3.371 ± 0.461	3.414 ± 0.774	3.871 ± 0.541
Overall AVG	3.603 ± 0.495	3.342 ± 0.516	3.268 ± 0.598	3.722 ± 0.475

Table 4. Zooplankton U and T values of the WZI, derived from pCCA using abundance data. From Lougheed and Chow-Fraser (2002). Modified U & T values from this study in brackets.

ROTIFERS	U	T	CLADOCERANS	U	T
<i>Anuropsis</i> sp.	3	1	CHYDORIDAE	4	2
<i>Ascomorpha</i> sp.	1	1	<u>Except</u>		
<i>Asplanchna</i> sp.	2	1	<i>Kurzia latissima</i>	3	3
<i>Brachionus</i> sp.	2	1	<i>Leydigia leydigii</i>	1	1
<i>Cephalodella</i> sp.	3	1	<i>Monospius dispar</i>	2	1
<i>Colletheca</i> sp.	5	2			
<i>Conochiloides</i>	4	2	MACROTHRICIDAE	5	3
<i>Euchlanis</i> sp.	4 (3)	2 (1)	<u>Except</u>		
<i>Filinia</i> sp.	1	1	<i>Illiocryptus sordidus</i>	1	1
<i>Gastropus</i> sp.	2	1			
<i>Hexarthra</i> sp.	1	1	DAPHNIDAE		
<i>Kellicottia</i> sp.	3 (5)	3 (1)	<i>Ceriodaphnia</i> sp.	4	2
<i>Keratella</i> sp.	3 (5)	1 (2)	<i>Daphnia</i> sp.	2	2
<i>Lecane</i> sp.	5	2	<i>Megafenestra</i> sp.	2	1
<i>Lepadella</i> sp.	4	2	<i>Scapholeberis</i> sp.*	4	2
<i>Lophocaris</i> sp.	2	1	<i>Simocephalus</i> sp.	5	3
<i>Macrochaetus</i> sp.	4	1			
<i>Monostyla</i> sp.	5	2	SIDIDAE		
<i>Mytilina</i> sp.	5	3	<i>Diaphanosoma birgei</i>	1	2
<i>Notholca</i> sp.	3	1	<i>D. brachyurum</i>	5 (3)	2 (1)
<i>Platylus</i> sp.	4	2	<i>Latona parviremis</i>	5	1
<i>Ploesoma</i> sp.	4	2	<i>Latonopsis occidentalis</i>	5	1
<i>Polyarthra</i> sp.	3	1	<i>Sida crystallina</i>	5	3
<i>Pompholyx</i> sp.	1	1			
<i>Scardium</i> sp.	5	1	BOSMINIDAE	3	1 (2)
<i>Testudinella</i> sp.	4	2			
<i>Trichocerca</i> sp.	4	2	Additional Species		
<i>Trichotria</i> sp.	5	2	<i>Polyphemus</i> sp.	5	3
			<i>Leptodora kindti</i>	1	1
			<i>Moina</i> sp.	1	1
			<i>Holopedium gibberum</i>	5	1

* The original publication has this indicated as *Scapholeberis kingi*, which only occurs in South America (Dumont and Pensaert, 1983) as such it has been changed to the genus level.

Table 5. Average index values for site groups in CO-CA analysis: Water Quality Index (WQI; Chow-Fraser, 2006), Wetland Macrophyte Index (WMI; Croft and Chow-Fraser, 2007), Wetland Zooplankton Index (WZI; Lougheed and Chow-Fraser, 2002)

Group #	Symbol	Average WQI	Average WMI	Average WZI ₀₉
1	●	-1.453	1.868	2.567
2	○	-0.447	1.761	3.611
3	▼	0.135	2.036	4.164
4	△	0.301	2.738	3.683
5	■	1.187	3.186	3.157

Figure 1: Map of the Great Lakes region with Georgian Bay inset, showing the location of the original 70 wetlands (●) used to create the WZI (Lougheed and Chow-Fraser, 2002), new sites (⊙) added in this study and four Georgian Bay test sites (⊗).

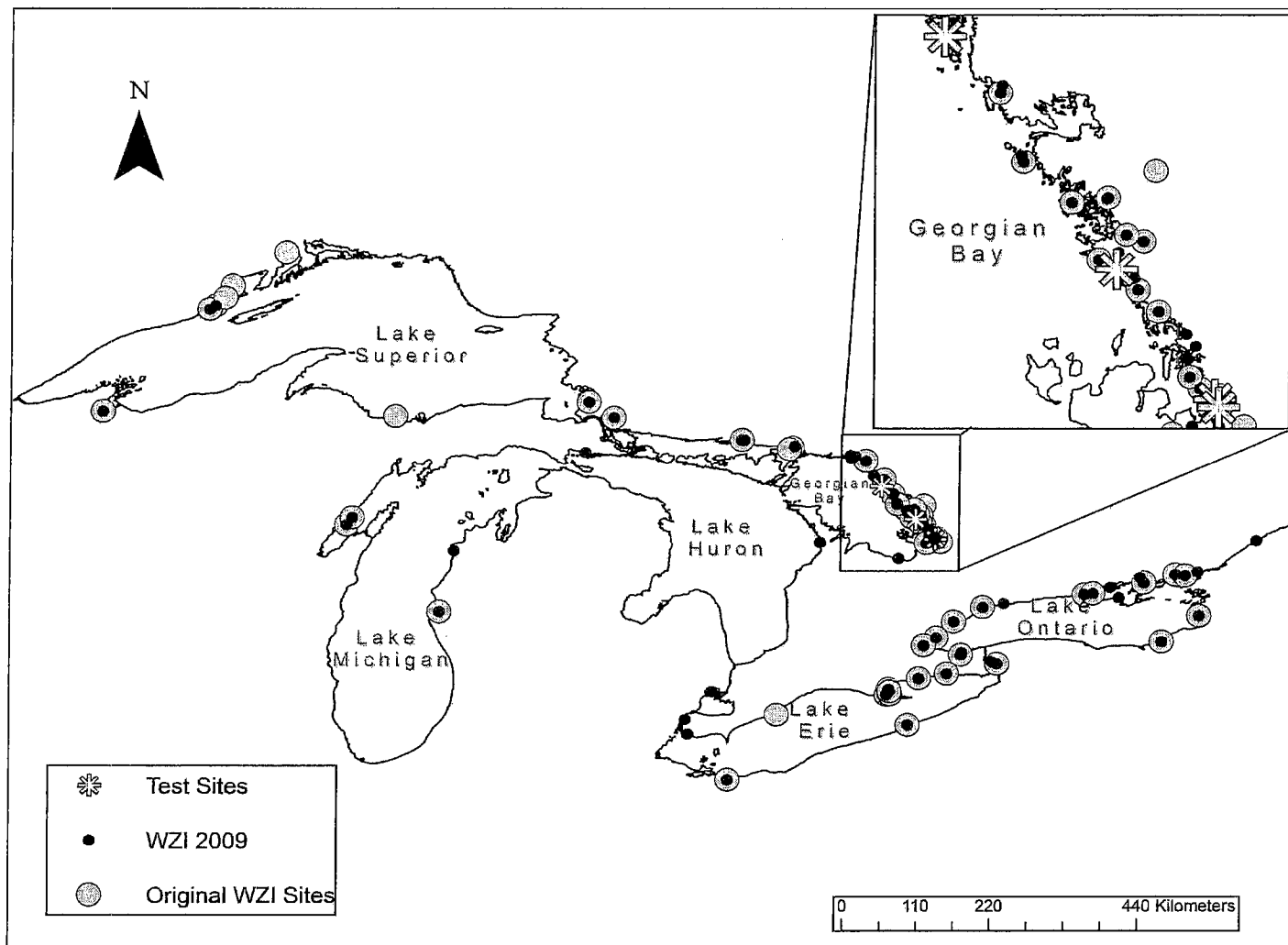
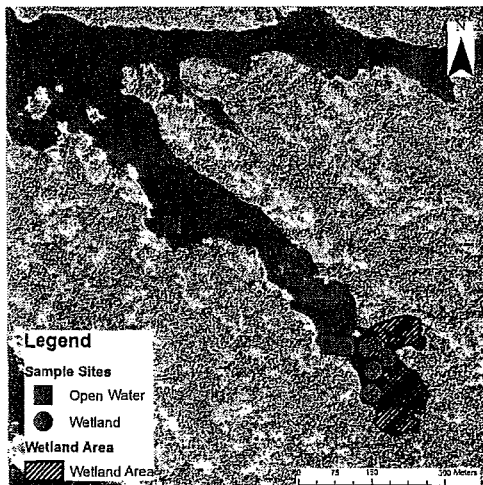
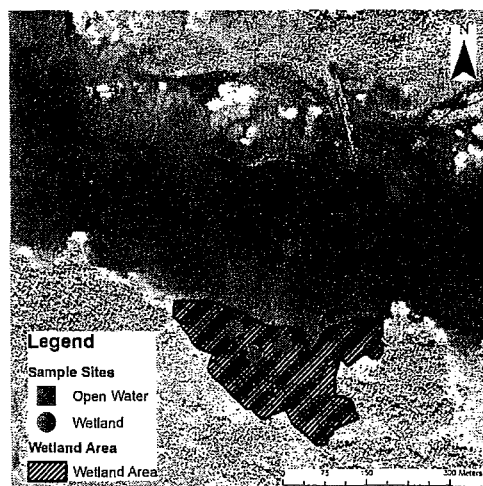
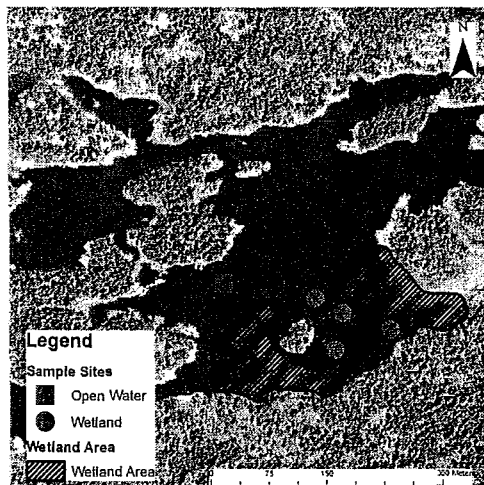


Figure 2. Maps of four high quality Georgian Bay test sites used to verify sampling effort and confirm sampling location. a) Pamplémousse;
b) Rhodes Marsh;c) Green Island Channel; d) Green Island Exposed

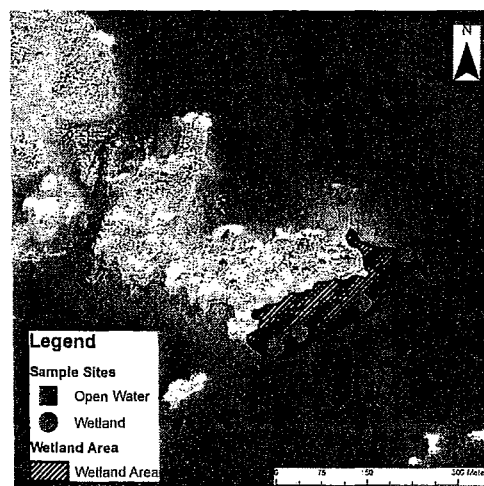
A



B



C



D

Figure 3. Map of the location of the 107 sites used in CO-CA analysis. All sites were located in Lakes Huron, Erie and Ontario.

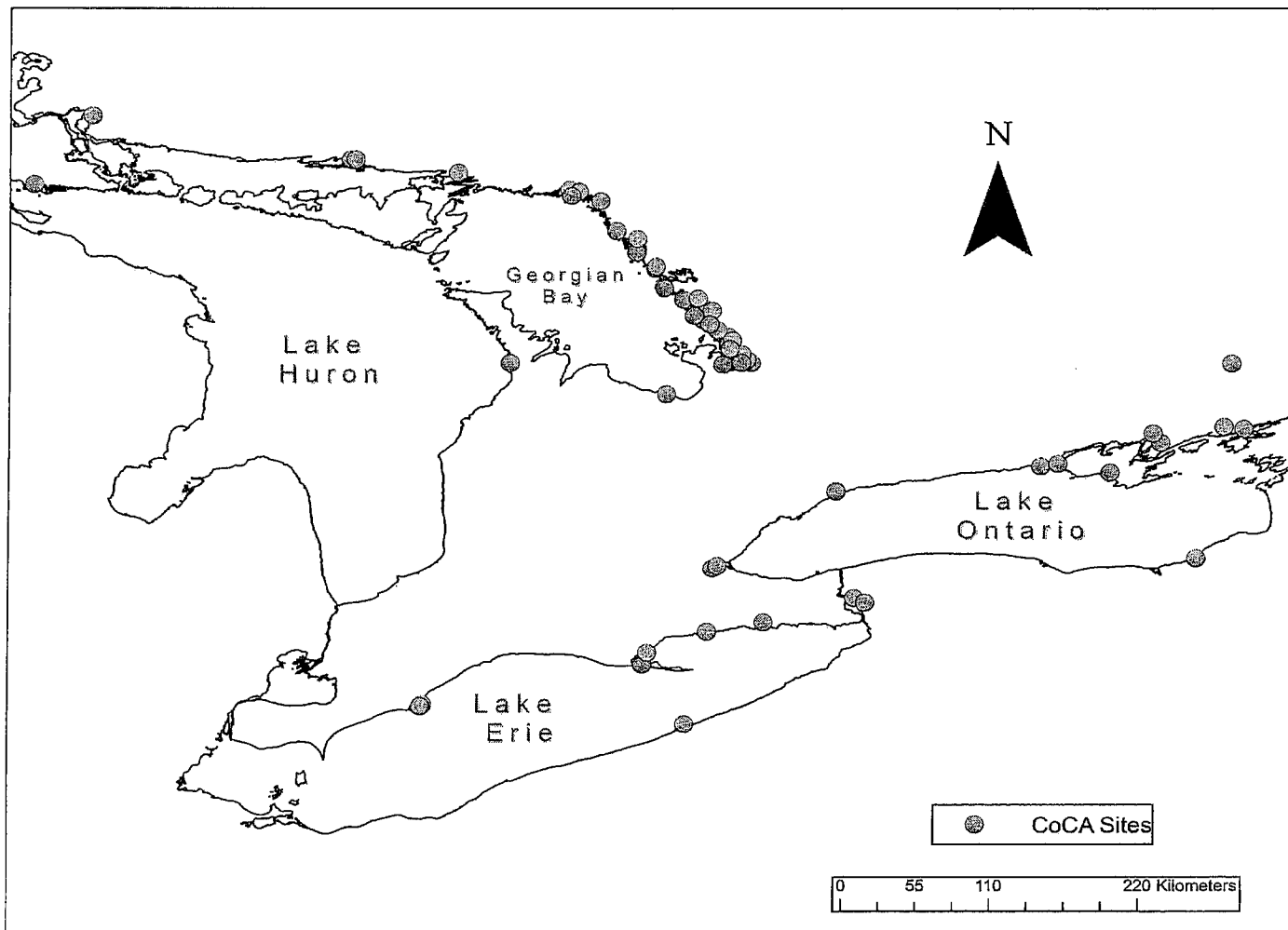


Figure 4. Map of the location of the 33 sites used in Exposure analysis using PDI scores in Georgian Bay.

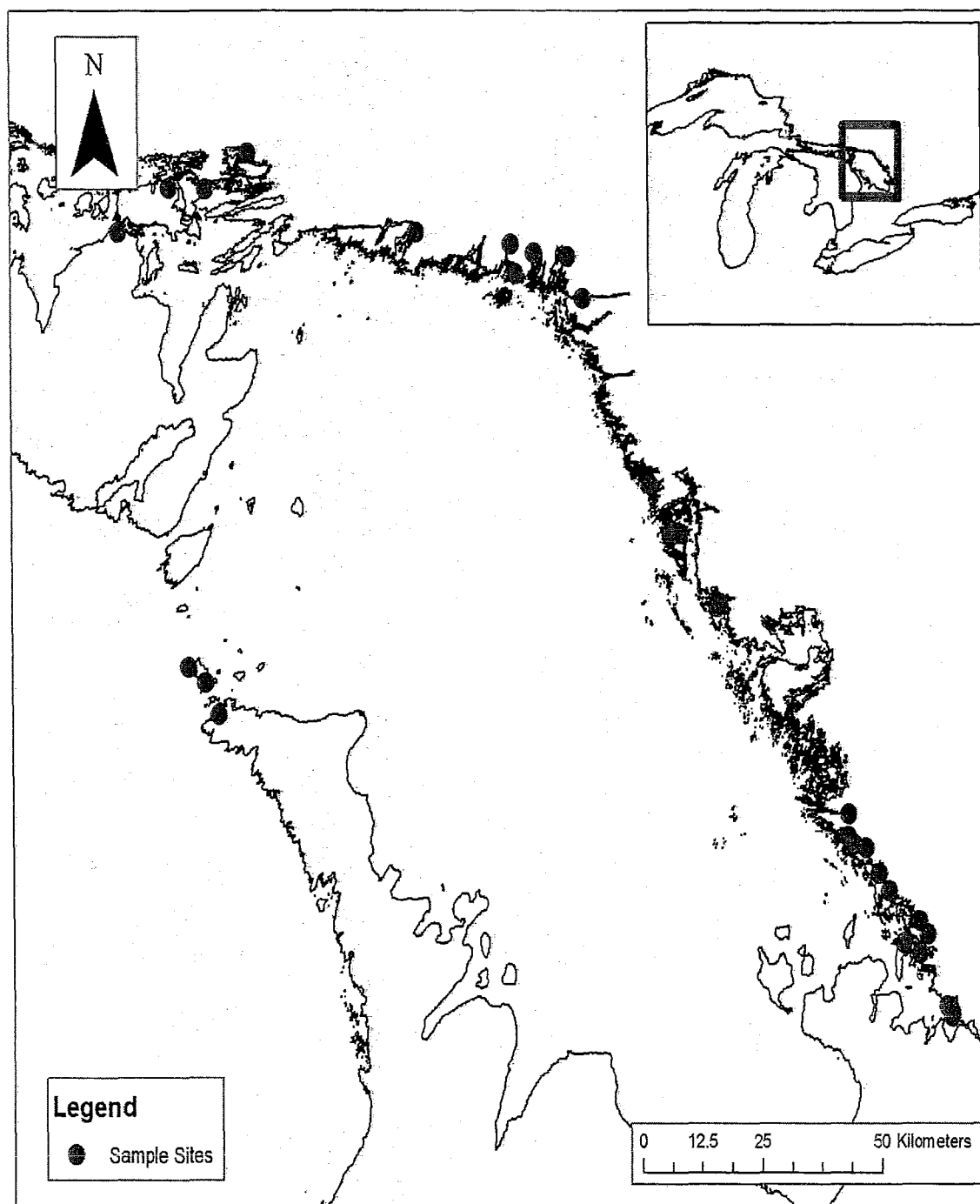


Figure 5: Scale of wetland exposure using the Physical Disturbance Index (PDI)
using three examples.

1 ← 3 → 5

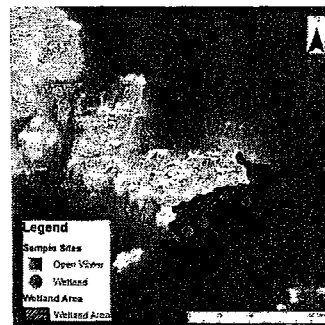
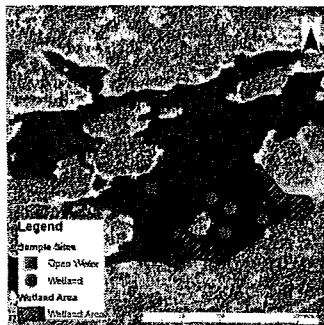
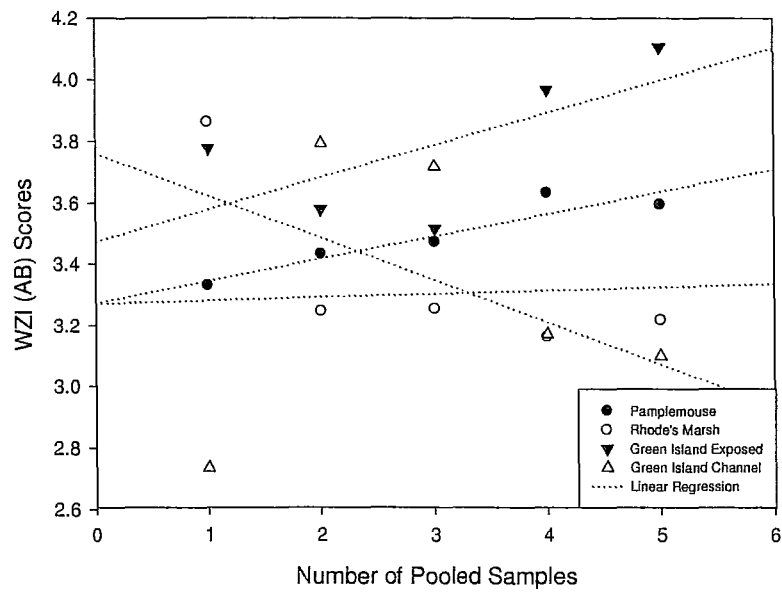


Figure 6. Sampling effort in the four high quality Georgian Bay Wetlands: Green Island Channel (●); Green Island Exposed (○); Rhodes Island (▼); Pamplemousse (Δ). In (a), WZI abundance scores were calculated for multiple single samples from the same site. In (b), species richness is shown for each site as the number of samples increases.

a)



b)

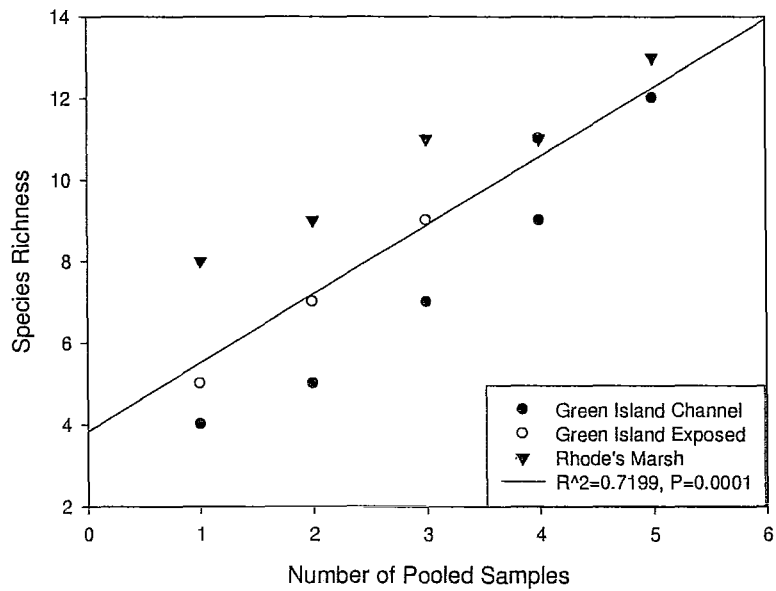
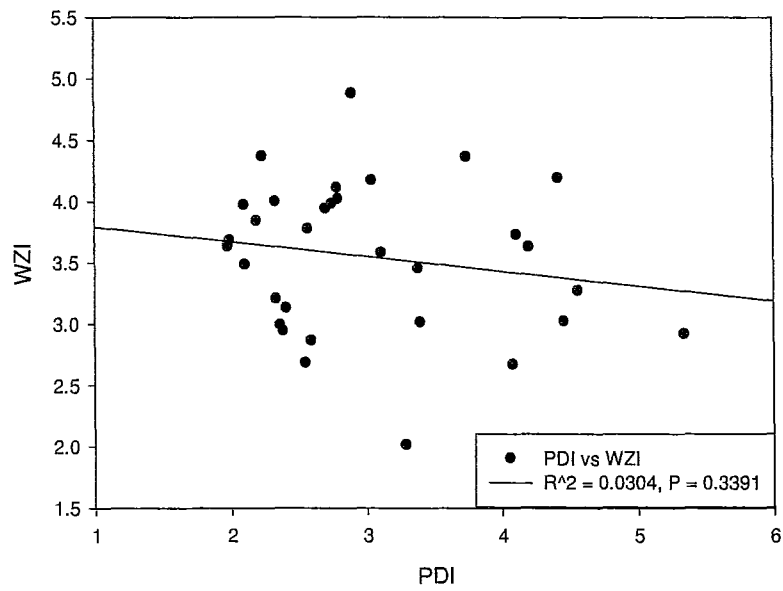


Figure 7. Scatter plot of Exposure data. (a) WZI vs. PDI and (b) Total

Zooplankton Abundance vs. PDI. Linear regression was not significant for either data set.

a)



b)

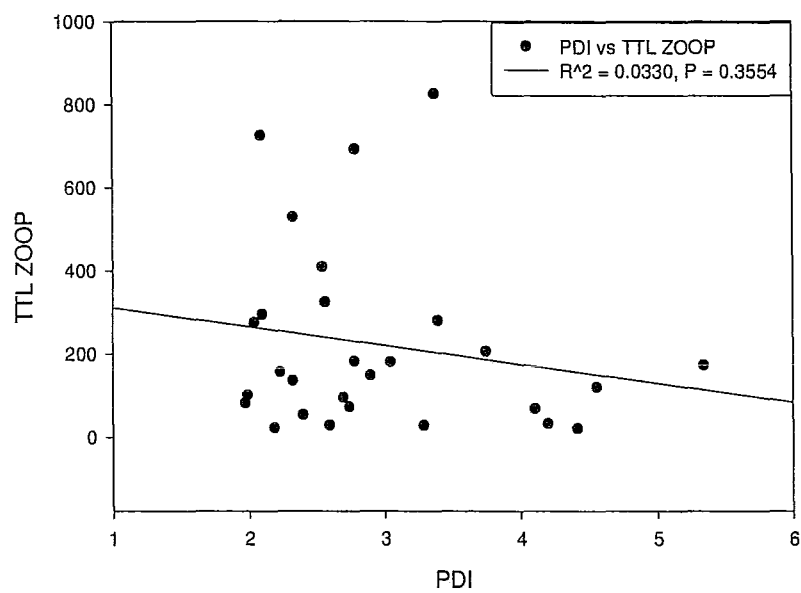


Figure 8. Bi-plot of the pCCA (axis 1 vs. axis 2). For explanation of zooplankton species abbreviations see Appendix A.

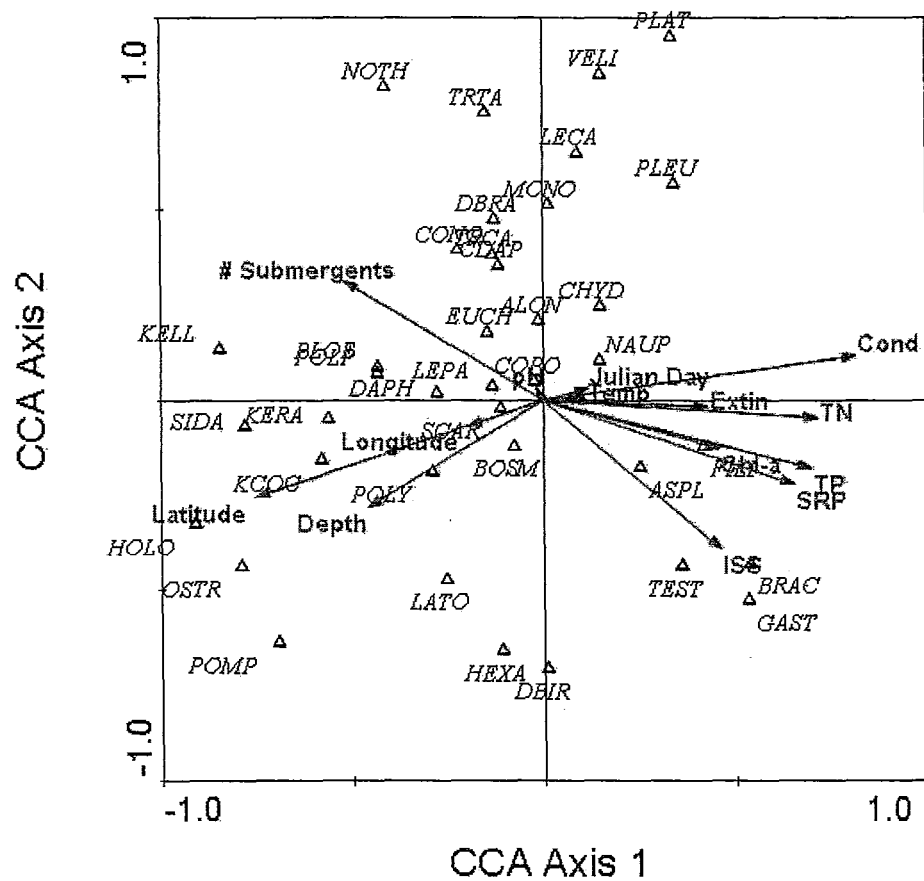


Figure 9. WZI and WZI₀₉ abundance scores compared with the WQI.

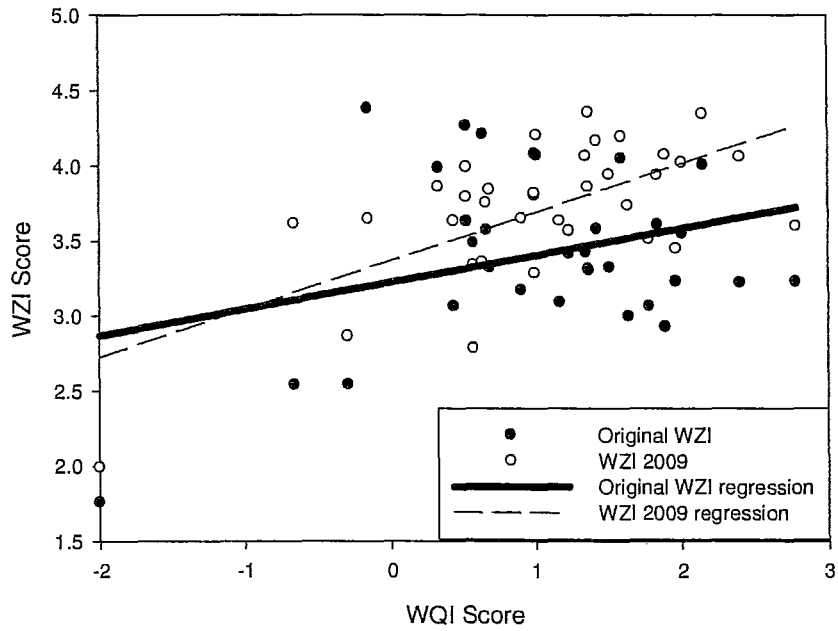
a) Linear Regression: WZI₀₉ vs. WQI ($R^2 = 0.4085$; $P < 0.0001$);

WZI vs. WQI ($R^2 = 0.0918$, $P = 0.0816$)

b) Polynomial regression: WZI vs. WQI ($R^2 = 0.3482$, $P = 0.0013$);

WZI₀₉ vs. WQI ($R^2 = 0.4943$, $P < 0.0001$)

a)



b)

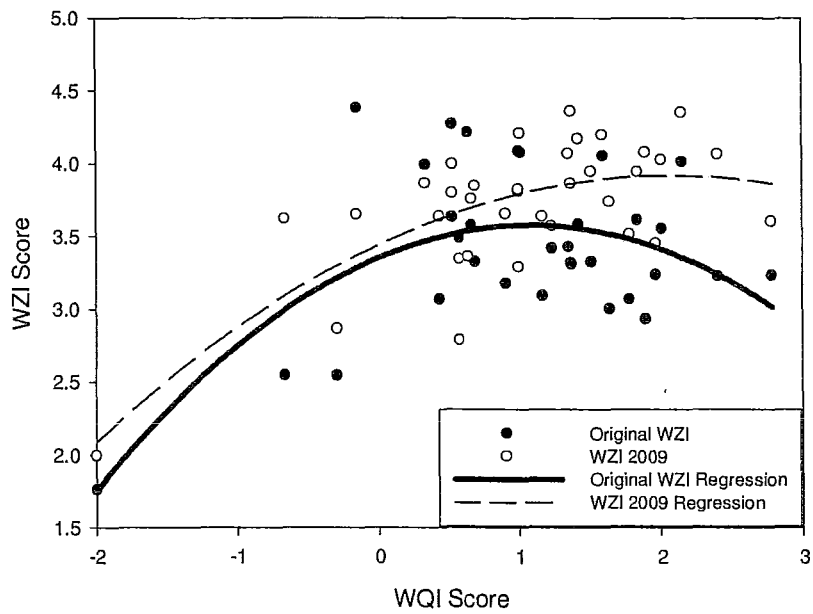


Figure 10. Comparison of average WZI scores over multiple samples taken within SAV at three Georgian Bay test sites (PP-Pamplemousse; RM-Rhodes Marsh; GIC- Green Island Channel), Long Point Provincial Park (LP) in Lake Erie and Cootes Paradise Marsh (CP) in Lake Ontario. Average original WZI scores are seen in black and WZI₀₉ scores seen in grey, with standard deviation bars.

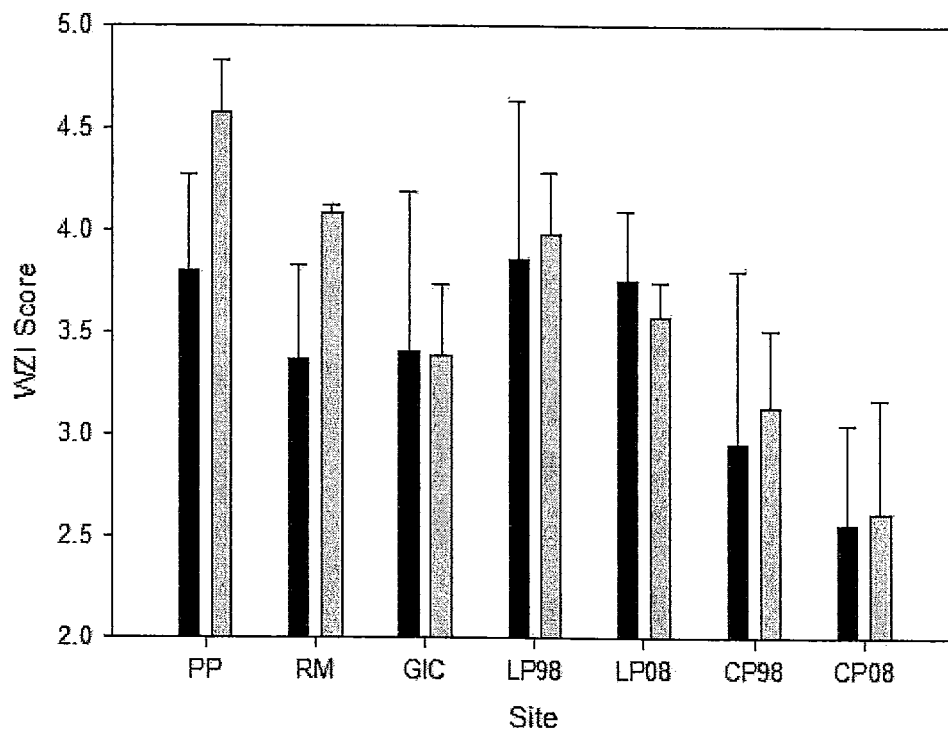


Figure 11. Bi-plot of CO-CA Axis 1 and CO-CA Axis 2

a) Scores plotted for each parameter: Plants (●); Zooplankton (▼);

Sites (■)

b) Sites groups from Ward's Cluster Analysis:

Site Group 1 (●): Low quality Ontario sites

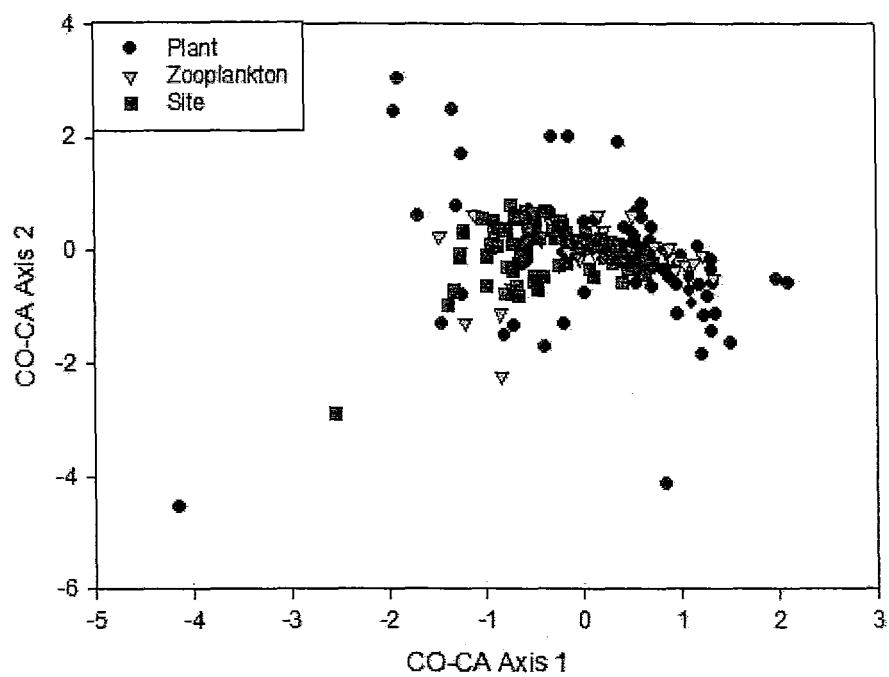
Site Group 2 (○): Low quality Erie and Ontario sites

Site Group 3 (▼): Low to mid range quality sites from all lakes

Site Group 4 (Δ): Mid quality Ontario and Georgian Bay sites

Site Group 5 (■): High quality sites, mostly Georgian Bay

a)



b)

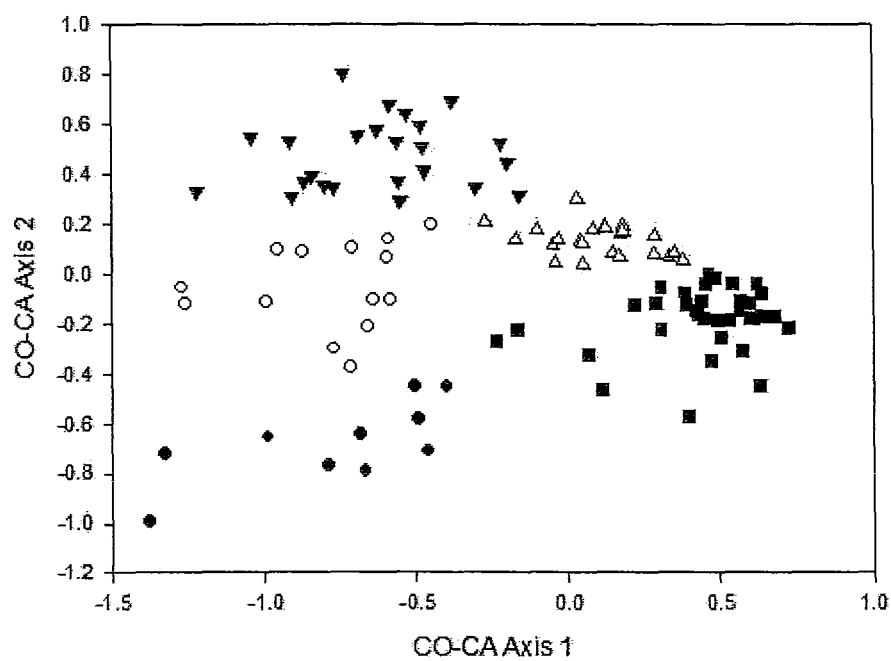
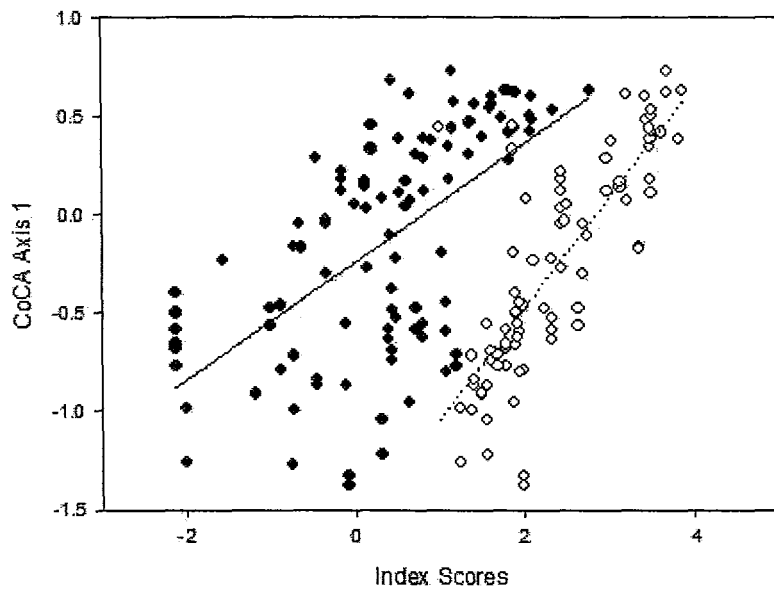
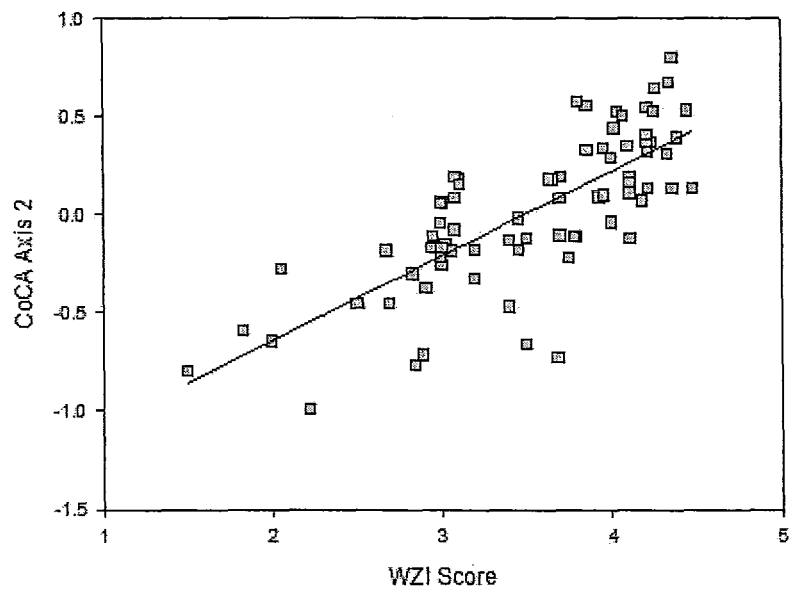


Figure 12. a) Plot 1 (●): CO-CA Axis 1 vs. WQI ($R^2 = 0.3847$, $P < 0.0001$); Plot 2 (○): CO-CA Axis 1 vs. WMI ($R^2 = 0.6136$, $P < 0.0001$); Linear regression confirms axis of wetland degradation ; b) CO-CA Axis 2 vs. WZI: Linear regression shows zooplankton of better quality in submerged vegetation ($R^2 = 0.5773$, $P < 0.0001$)

a)



b)



Appendix A: Zooplankton Species Abbreviations

ROTIFERS		CLADOCERANS	
<i>Anuropsis</i> sp.	ANUR	CHYDORIDAE	CHYD
<i>Ascomorpha</i> sp.	ASCO	<u>Except</u>	
<i>Asplanchna</i> sp.	ASPL	<i>Kurzia latissima</i>	KURZ
<i>Brachionus</i> sp.	BRAC	<i>Leydigia leydigii</i>	LEYD
<i>Cephalodella</i> sp.	CEPH	<i>Monosiplus dispar</i>	MONS
<i>Collethea</i> sp.	COLL		
<i>Conochiloides</i>	CONO	MACROTHRICIDAE	MACR
<i>Euchlanis</i> sp.	EUCH	<u>Except</u>	
<i>Filinia</i> sp.	FILI	<i>Illicryptus sordidus</i>	ILLI
<i>Gastropus</i> sp.	GAST		
<i>Hexarthra</i> sp.	HEXA	DAPHNIDAE	
<i>Kellicottia</i> sp.	KELL	<i>Ceriodaphnia</i> sp.	CDAP
<i>Keratella</i> sp.	KERA	<i>Daphnia</i> sp.	DAPH
<i>Lecane</i> sp.	LECA	<i>Megafenestra</i> sp.	MEGA
<i>Lepadella</i> sp.	LEPA	<i>Scapholeberis</i> sp.	SCAP
<i>Lophocaris</i> sp.	LOPH	<i>Simocephalus</i> sp.	SIMO
<i>Macrochaetus</i> sp.	MACR		
<i>Monostyla</i> sp.	MONO	SIDIDAE	
<i>Mytilina</i> sp.	MYTI	<i>D. birgei</i>	DBIR
<i>Notholca</i> sp.	NOTH	<i>D. brachyurum</i>	DBRA
<i>Platyus</i> sp.	PLAT	<i>Latona parviremis</i>	LATP
<i>Ploesoma</i> sp.	PLOE	<i>Latonopsis occidentalis</i>	LATO
<i>Polyarthra</i> sp.	POLY	<i>Sida crystallina</i>	SIDA
<i>Pompholyx</i> sp.	POMP		
<i>Scardium</i> sp.	SCAR	BOSMINIDAE	BOSM
<i>Testudinella</i> sp.	TEST		
<i>Trichocerca</i> sp.	TRCA	Additional Species	
<i>Trichotria</i> sp.	TRTA	<i>Polyphemus</i> sp.	POLP
OTHER - not in WZI		<i>Leptodora kindti</i>	LEPT
<i>Cercopages</i> sp.	CERC	<i>Moina</i> sp.	MOIN
Copepod	COPO	<i>Holopedium gibberum</i>	HOLO
Nauplis	NAUP		
Ostracod	OSTR		
Veliger	VELI		