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Independent and combined effects of land-use stress on macroinvertebrate community condition at Great Lakes coastal margins

Jasmine Isabel St. Pierre

University of Windsor

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By

Jasmine St. Pierre

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by

Jasmine St. Pierre

APPROVED BY:

_____

E. Tam

Department of Civil and Environmental Engineering

_____

K.E. Kovalenko

NRRI University of Minnesota, Duluth

_____

K. Drouillard

Department of Biological Sciences

_____

J.J.H. Ciborowski, Advisor

Department of Biological Sciences

September 14 2016

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Abstract

Coastal ecosystems of the Laurentian Great Lakes provide many of the services upon which society most depends and yet they are the most highly threatened by anthropogenic stress. Several means of quantifying anthropogenic stress in the Great Lakes have been developed, and measures of expected impact exist for watersheds across the entire basin. However, it is still unknown whether the biological communities present at coastal margins reflect the environmental condition expected from the level of stress in each watershed. Using data previously collected for the Great Lakes Environmental Indicators collaboration, the goal of this thesis was to assess both independent and combined effects of land-use stress on macroinvertebrate community condition.

Macroinvertebrate community condition was measured by using multivariate analysis to derive a composite Zoobenthic Assemblage Condition Index (ZACI), which employs the Reference-Degraded Continuum approach. The variation in ZACI scores was then related to two classes of human land use- agriculture and urban development via surrogate environmental gradients. The combined effect of land-use was evaluated by three different methods in a proof of concept. These methods included 1) comparing predicted single-stress ZACI scores to combined stress ZACI scores, 2) response-surface modelling using non-parametric multiplicative regression, and 3) mapping of isopleths distinguishing reference from non-reference conditions. A cluster analysis of biota at site subjects to minimal stress identified 5 distinct invertebrate assemblages which could be classified by environmental attributes (3 in the north, 2 in the south). ZACI condition scores showed distinct but varying trends among groups as a function of land-use stress. Levels of agricultural land-use were found to continuously constrain biological condition in one group, while condition of other groups exhibited potential threshold changes with increasing development-associated stress. In combination, both agriculture and development stress were observed to have significant negative effects on macroinvertebrate assemblages at Great Lakes coastal margins, and development stress is the more severe stressor.

To Matt and Ash, my boys.

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CHAPTER 1

GENERAL INTRODUCTION

Coastal margins, especially wetlands are an ecologically, socially and economically important part of the Great Lakes basin. Ecological functions of coastal wetlands include flood storage, sediment trapping, water quality filtration, and erosion buffers (deGroote et al. 2012) as well as vital habitat for many biological assemblages (e.g. plants, fishes, birds and other wildlife). Coastal wetlands are also highly productive and are hot spots of biodiversity (Costanza et al. 1997a). Wetlands provide $2 billion worth of ecosystem services each year (Sierszen et al. 2012) such as storm protection, the transformation, removal and storage of nutrients, fishing, hunting and recreation. A high proportion of the Great Lakes fish assemblage depends on coastal wetlands for some part of their life cycle (Jude and Papas, 1992). Being small in area, the coastal wetlands are thereby disproportionately important to the lakes as a whole. With lake dependent commerce in the U.S. alone providing 1.5 million jobs in 2010 (Vaccaro and Read, 2011), the condition of coastal wetlands is also of great economic importance.

The coastal margins of the Great Lakes serve as the land-water interface, (Mitsch and Gosselink, 2000), making them especially vulnerable to impact as they can be exposed to both watershed and lake based human activity (Bedford, 1992; Wilcox, 1995; Allan et al. 2013). However, despite their obvious importance, two thirds or more of the original coastal wetlands present prior to European settlement have been lost due to land drainage, commercial and industrial land use, dyking and dredging (Whillans, 1982). Great Lakes coastal wetlands are threatened by human activities such as land use, climate change, point and non-point source pollution, aquatic invasive species, atmospheric deposition and shoreline modifications (Danz et al. 2005).

Quantifying Anthropogenic Stress in the Great Lakes

The number of studies addressing environmental 'stress' and 'stressors' is increasing in ecological literature, but the use of these terms remains ambiguous (Borics et al. 2013). Often, the term 'stress' is used interchangeably with 'disturbance' and 'perturbation', with definitions

depending on the field of study and researcher perspective (see Borics et al. 2013; Rykiel, 1985). The term disturbance usually refers to a factor or event that disrupts important ecological factors, such as community structure, changes in resource availability, or the physical environment (Paine et al. 1998; Pickett et al. 1989). Some authors refer to disturbances as infrequent, destructive events but many ecologists now consider disturbances to be normal events that occur within a range of natural variability (e.g. Berger and Hodge, 1998; White and Pickett 1985; USEPA, 2016). Additionally, the terms 'disturbance' or 'stress' are used by some to refer to the causes of ecological change, whereas others allude to the response of the system, with 'stressor' referring to the cause (Rapport et al. 1985). Recent frameworks for describing biological condition in aquatic ecosystems recognize that disturbances do not necessarily lead to stress unless the disturbances exceed the range of natural variability and exert 'pressure' (USEPA, 2016; European Environmental Agency, 1999). "Pressures" are human-related activities that alter ecosystem processes and generate "stressors", which are the proximate factors that adversely impact biological condition (Niemi and MacDonald, 2004). In this thesis, 'anthropogenic stress' refers to human induced changes to ecosystem processes or environmental factors resulting in the creation of "stressors" that cause changes in biological condition ("responses").

Quantifying and describing the level of stress applied to an ecosystem comes with many technical challenges. A single source of stress can create multiple stressors that can co-occur in time and space. Simultaneously-acting stressors have the potential to interact with each other (see section on Cumulative Stress) resulting in unpredictable cumulative impacts. In addition, stressors can operate at different spatial and temporal scales and their impacts may be expressed at different levels of biological organization, e.g. individual, population or community level (Pickett et al. 1989; Glasby and Underwood, 1996).

Despite the challenges of deriving and documenting gradients of stress, several means of quantifying anthropogenic stress in the Great Lakes have been developed. Chow Fraser (2006)

derived the "Water Quality Index" (WQI) to describe wetland characteristics from principal component axis scores that summarized co-variation among 12 water chemistry variables such as temperature, pH, Chlorophyll-*a,* and nutrient concentrations. Other approaches use mapping of large scale variables, such as the type and amount of land use within a watershed, to summarize the distribution of stress over large regions (Danz et al. 2005; Allan et al. 2013). The Great Lakes Environmental Indicators (GLEI) collaboration (Niemi et al. 2007) developed a geographical information system (GIS) database with 186 spatially outlined variables that were summarized into 5 categories of anthropogenic stress that are prominent in watersheds draining into the basin: agriculture, atmospheric deposition, human population, land cover, and point source pollution (Danz et al. 2007, Niemi et al. 2007). Subsequently, to quantify the distribution of overall stress in the basin, the above main classes of stress were further summarized in terms of two independent surrogate measures of anthropogenic stress represented by human land-use - percent agricultural land cover and an index of urban development stress called MaxRel (Host et al., 2005, Host et al. in review). Such broad-scale composite measures of anthropogenic stress serve as proxies for the effects of stressors at finer scales because many local-scale stressors have common causes and similar spatial domains (Boughton et al. 1999). Thereby, large-scale measures of human activity can be used as summary measures of the overall risk of impact to biological communities.

Although environmental stress has been quantified in the Great Lakes by the GLEI consortium as well as by various other schemes (Allan et al. 2013; Host et al. 2005, US EPA, 2016, Environment Canada and US EPA, 2014), the degree to which biological communities are influenced by the level of stress present in each watershed is still largely unknown. There is evidence that biota at coastal margins may be greatly influenced by anthropogenic land use at the watershed scale, even from a considerable distance upstream (Peterson et al. 2007; Niemi et al. 2011). For example, Peterson et al. (2007) reported a direct link between watershed based human land use and coastal biota (plankton and macroinvertebrates) was established using $\delta^{15}N$

enrichment as an indicator of exposure. The $\delta^{15}N$ of plankton and benthos increased linearly with an agricultural landscape gradient, with the expression of landscape disturbance being stronger in embayment and wetland habitats compared to open nearshore (Peterson et al. 2007). Marked changes in community composition of multiple assemblages have also been shown to occur at threshold levels of watershed agriculture and urban development in the Great Lakes (Kovalenko et al. 2014).

Land use may indirectly influence biological communities in coastal habitats through changes in water quality and sediment characteristics and hydrologic regimes (DeCantanzaro et al. 2009, Crosbie and Chow-Fraser 1999; Lee et al. 2006)). Tributaries receive both agricultural and urban runoff carrying nutrients, contaminants, and sediments (Environmental Canada and U.S. EPA, 2014), which are deposited at coastal zones. The amount of agricultural land in watersheds drained by 22 Ontario marshes was positively correlated with inorganic solids and phosphorus content in sediment (Crosbie and Chow-Fraser, 1999). Road density may influence specific conductance, concentration of total suspended solids, and may be positively correlated with concentrations of total phosphorus and total nitrate nitrogen (DeCatanzaro et al. 2009).

Quantifying Community Condition and the Concept of Reference Condition

Early water quality assessment programs largely involved chemical and physical monitoring and reporting (Karr, 1981). However, this type of assessment is considered to be insufficient to detect the full spectrum of human impacts currently affecting aquatic ecosystems (e.g. land use changes, species invasions, etc.) (Karr and Dudley, 1981). Assessment of the biological assemblages ("bioassessment") conducted in conjunction with chemical monitoring is argued to capture the full, cumulative impacts of environmental stress (Karr, 1991) . Many different types of biota have been used in the assessment of Great Lakes aquatic ecosystem health including birds (e.g. Howe et al. 2007), macrophytes (e.g. Grabas et al. 2012) and most commonly, fishes and macroinvertebrates (e.g. Uzarski et al. 2005; Bhagat et al. 2007; Burton et

al 1999). Macroinvertebrate based bioindicators are a valuable and successfully used part of many stream and river assessment programs (e.g. Barbour et al. 1999) because benthic organisms are ubiquitous, relatively sedentary, easy to sample and are capable of responding to a wide variety of stressors at a variety of scales (Johnson et al., 2007). However, when applied to bioassessment of wetland systems the use of macroinvertebrate indicators has been more challenging, with little consensus surrounding how invertebrates are ecologically controlled in wetlands and difficulties developing reference standards (Batzer, 2013; Wilcox et al. 2002). However, macroinvertebrate indicators of wetland condition have had some success in limited areas of the Great Lakes with the development of indices of biological integrity (IBIs) in lacustrine wetlands of Lake Huron (Burton et al. 1999; Uzarski et al. 2004) and Georgian Bay (Kostuk, 2006). See further discussion in Chapter 2.

Fundamental to the use of bioindicators for ecosystem assessment and monitoring is the establishment of a 'regional reference condition'. The reference condition is generally defined as the environmental condition of a group of sites that are minimally disturbed (and by inference the biological community that occurs under those conditions). The suite of reference sites is grouped by some method (e.g. biological, physical or chemical classification) so that the biota at test sites can be matched to a group of reference sites expected to be similar in their physical, chemical or biological characteristics (Reynoldson et al. 1997; Bailey et al. 2004). It is important that this classification places reference sites into groups exhibiting similar habitat and physical characteristics because the use of the reference condition in bioassessment relies on the assumption that similar site attributes support similar invertebrate communities in the absence of disturbance.

Two approaches for establishing the reference condition are currently employed - multimetric and multivariate approaches. Typically, when a multimetric approach is used, reference (i.e. minimally disturbed) sites are assigned to groups a priori by the establishment of

ecoregions and subecoregions based on professional judgement and geophysical attributes (e.g. climate, soil, water chemistry). This approach assumes that reference sites in the same ecoregion will have similar invertebrate assemblages. However, there is evidence that invertebrate communities may not be homogeneous within a region (Corkum, 1990, 1991; Richards et al. 1993). Multivariate approaches such as RIVPACS (River Invertebrate Prediction and Classification System, Wright 1995) or BEAST (Benthic Assessment of SedimenT, Reynoldson et al. 1995) models classify reference sites into groups using clustering methods based on species composition, thereby making no a priori assumptions about assemblage similarity based on a locale's environmental or physical attributes. While multimetric methods assume that the environmental characteristics of test-sites are congruent with those of groups of reference sites, multivariate methods use a predictive model based on the association between reference sites' habitat attributes and community composition to determine the probability that a test site should be classed with a group of reference sites.

A key step in assessing biological condition involves measuring biological attributes and combining suites of measurements into a single, composite number (i.e. index). As in establishing reference conditions, indices can be created using *a priori* criteria (multimetric approaches) or empirically (multivariate approaches). Multimetric indices (MMIs), such as the IBI (index of biological integrity) developed by Karr (1981) are used frequently in streams of the United States in programs such as the US EPA's Rapid Bioassessment Protocols for streams (Barbour et al., 1999). MMIs involve the definition of an array of measures (or metrics) representing different attributes of biological assemblages such as community structure and function that, when integrated (summed), are thought to provide an additive measure of overall biological condition. Commonly used metrics include those related to richness (e.g. total number of taxa, diversity indices), composition (e.g. percentage of a sample made up of Ephemeroptera, Plecoptera, Trichoptera), feeding behaviour (e.g. % filterers), and habit (e.g. % clingers). Traditionally,

metrics are selected from a list of candidates based on expert knowledge of what reflects a "healthy" system (Karr, 1981). However, where systems and their stressors aren't well-known, metrics most sensitive to some measure of human disturbance are selected empirically (Schoolmaster et al. 2013). Deriving a multi-metric index empirically is often highly predictive because it is highly correlated with human disturbance (Uzarski et al. 2004, Seilheimer and Chow-Fraser 2006).The downside is that they are less general than those indices based on causal knowledge. In addition, IBI construction is largely based on researcher expectations of changes in community attributes (Barbour et al. 1996; Schoolmaster 2013; Whittier et al. 2007; Karr, 1981). Only the attributes that seem to match expectation (i.e. those that exhibit monotonic changes with respect to a stress measure) are used in developing the indicator model (e.g., Cooper et al. In revision). Furthermore, metrics initially chosen for testing are often those deemed important by researchers.

In current multivariate approaches, test sites may be evaluated in two ways: 1) by comparing test sites and reference sites in taxa ordination space (e.g. BEAST, Reynoldson et al. 1995) or 2) by comparing expected probabilities of occurrence of individual taxa (based on their prevalence at reference sites) to observed occurrences at test sites (e.g RIVPACs, Wright 1995; Hawkins et al. 2000). In the first method, assessment of biological communities at test-sites occurs by using a 90% confidence ellipse plotted for reference sites (Bowman and Somers, 2005). Test-site assemblages that fall outside the ellipse have diverged from reference (presumably as a consequence of environmental disturbance) and are classified as "not equivalent to reference". With the second method, the severity of disturbance at test-sites is evaluated based on how much the number of taxa observed at a site deviates from the number expected in that habitat. Multivariate methods are cited as being more complex, requiring specialized knowledge with results that are difficult to convey to managers and the public. However, the predictive models developed from multivariate approaches are more precise and accurate than multimetric indices

(Reynoldson et al. 1997) and can be incorporated into computer systems for ease of use, e.g. Canadian Aquatic Biomonitoring Information Network (CABIN, Strachan et al. 2014).

Cumulative Effects of Stressors

Often, derived stress-response relationships may be statistically significant but their explanatory power is typically low, with test sites exhibiting broad ranges of index scores at any one value on the disturbance gradient (e.g. Bhagat et al. 2007; Seilheimer and Chow-Fraser, 2007; Buckley, 2015; Buckley et al. In prep.). Many indices may have limited explanatory power because the stress-response relationships are assumed to be linear. However, biota are subject to multiple classes of stress simultaneously (Allan 2004; Yates et al., 2010) and the cumulative effect of multiple stressors may be difficult to discern because of potential interactions among stressors. Multiple stressors in combination may have unpredictable effects on biota (i.e. result in "ecological surprises"; Paine et al. 1998) that are greater or less than the sum of their individual effects (i.e. synergism or antagonism, respectively).

Although not all stressors may interact, non-additive effects (i.e. synergies or antagonisms) may be more common than simple additive effects (Darling and Cote, 2008). Smith et al. (in review) reported that among 65 studies of multiple stressor effects in the Laurentian Great Lakes, non-additive effects were more common (49% synergies, 42% antagonisms) than additive effects. Therefore, it is reasonable to expect that biological response to anthropogenic disturbance is likely non-linear (Davies and Jackson 2006). For example, there is increasing evidence that assemblages of organisms may collectively exhibit threshold responses to environmental stress due to synchrony in stress tolerance across taxa (King and Baker, 2010; Kail et al. 2012). Wang et al. (1997) detected complex relationships between stream fish IBI scores and agriculture and urban land use with possible thresholds at 50% agriculture and 10-20% urbanization. In Great Lakes coastal wetlands, assemblages of a variety of guilds (fishes, plants, invertebrates, birds and amphibians) exhibited marked changes in community composition at two

levels of watershed development stress – one at which many sensitive species became reduced in abundance or extirpated, and another at which 'tolerant' species became increasingly abundant (Kovalenko et al. 2014).

Study Objectives

In light of the variety of anthropogenic threats to the condition of Great Lakes coastal wetlands and their ecological and societal value, it becomes increasingly important to understand how multiple human activities interact to influence the biological condition at Great Lakes coastal margins. Focused on benthic macroinvertebrates, this thesis uses a multivariate approach of identifying indices of ecological condition by ordinating the relative abundances of assemblages of aquatic invertebrates collected from wetland samples against several proxies of anthropogenic stress - agricultural stress (percent agriculture in a watershed), urban stress (measures of urban development in a watershed), and a composite of the two measures.

In Chapter 2, I derive a composite, multivariate index of biological condition using benthic macroinvertebrate relative abundance data at 141 coastal wetlands in the U.S. portion of the Great Lakes basin. I then relate that index to two land-use stressor gradients and a composite gradient developed by GLEI (Great Lakes Environmental Indicators) and threshold estimates of each land-use stressor at which discontinuities in the index scores occur across a gradient are observed. I hypothesized that benthic macroinvertebrate condition exhibits a negative, non-linear threshold response to both agriculture and urban development associated land-use stresses.

In Chapter 3, I use several statistical multivariate procedures to investigate how the cumulative joint effects of the two classes of stress influence the composite indices derived in Chapter 2. These include 1) evaluation of 2-dimensional scatterplots comparing predicted ZACI scores of samples exposed to individual sources of stress, to ZACI scores of samples exposed to both types of stress, 2) using an occupancy modeling software program called HyperNiche (MjM

Software Group) to fit Nonparametric Multiplicative Regression (NPMR) models of the macroinvertebrate ZACI scores as a response to multiple environmental predictors, simultaneously (i.e. Agriculture and Development stress gradients), and 3) examining the shape of isopleths distinguishing reference from non-reference conditions relative to the two classes of stress (isopleth mapping). Using the above three approaches as proofs of concept, I assess the evidence for possible interactive effects of agriculture and development stress on macroinvertebrate assemblage condition. I hypothesize in this chapter that, agriculture and development stress will have a greater effect on macroinvertebrate biological condition in combination, than independently. I also expect to find evidence of a synergistic interaction.

Chapter 4 is a discussion of the findings from the previous two chapters and provides recommendations for the development of cumulative stressor indices for biological assessment at Great Lakes coastal margins. The results of this research contributes to the understanding of how human activity in the watershed influences biological communities in Great Lakes coastal wetlands. The ability to detect measurable changes at the assemblage level, including the existence of possible thresholds will also aid in setting restoration and mitigation targets for the management of Great Lakes coastal ecosystems.

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CHAPTER 2

DEVELOPMENT OF A MULTIVARIATE ZOOBENTHIC ASSEMBLAGE
CONDITION INDEX (ZACI) FOR GREAT LAKES COASTAL MARGINS

Introduction

Wetlands are highly productive, valuable ecosystems that have experienced great losses worldwide (Davidson, 2014). The degradation of wetlands is exacerbated by anthropogenic activities that impair ecological function (deGroot et al. 2012). In the Great Lakes region, coastal wetlands experience the greatest amount of anthropogenic stress because of the tendency for urban centers and agricultural/industrial activity to be focused at coastal margins. Therefore, monitoring and assessment of nearshore areas is a current objective of the amended Great Lakes Water Quality Agreement 2012 (Governments of Canada and the United States, 2012). Assessment and consequent management requires reliable methods of monitoring wetland condition and quantifying impacts of increasing anthropogenic activity.

Historically, chemical and physical attributes of aquatic environments have been measured to assess the degree to which ecosystems have been disturbed. However, water and sediment quality monitoring alone can fail to detect many impacts of human activity such as habitat alteration and hydrologic modification, species invasions, etc. (Karr and Dudley, 1981). Biological assemblages should be monitored in conjunction with chemical monitoring because the ultimate, cumulative impacts of environmental stress are only expressed by the biota (Karr, 1991). The use of biota as indicators (i.e. "bioindicators") of aquatic ecosystem condition is now widely accepted and used in programs such as the European Water Framework Directive (Directive 2000/60/EC, 2000), the State of the Great Lakes Ecosystem Conference (SOLEC; Environment Canada and the U.S. EPA, 2014), the Canadian Aquatic Biomonitoring Information Network (CABIN; Strachan and Reynoldson, 2014; http://www.ec.gc.ca/rcba-cabin/), and various U.S. EPA Biological Assessment programs (US EPA, 2002).

Fishes and macroinvertebrates are the most commonly used groups in freshwater biomonitoring and assessment, especially in streams. Benthic macroinvertebrates are especially useful indicators of ecosystem health due to their crucial position in the food web (Batzer and

Wissinger, 1996) and their ability to reflect site-specific, cumulative impacts (Fore et al. 1996). They are an important food source for many ecologically and economically important taxa higher in the food web (e.g. fishes, birds, amphibians), and protocols for their collection are well established.

The use of macroinvertebrate bioindicators is widespread and largely successful in lotic systems; but efforts to develop macroinvertebrate-based indices of ecosystem condition in wetlands have had limited success. This seems largely due to a lack of consensus on how invertebrate assemblages are controlled in wetlands, with studies in the same systems producing contrasting or unexpected results (see review by Batzer, 2013). However, in Great Lakes coastal wetlands there have been several promising attempts to develop multimetric "indices of biological integrity" (IBIs, Barbour et al. 1995; Karr, 1981). Early work by Burton et al. (1999) proposed IBIs based on plant zonation in lacustrine wetlands of northern Lake Huron, which were later validated by Uzarski et al. (2004) and Gathman and Burton (2011). These studies identified a number of promising invertebrate metrics including the proportion of a sample comprised of Crustacea, Mollusca, Gastropoda, Odonata, and Simpson indices of diversity and evenness. Kashian and Burton (2000) tested invertebrate metrics adapted from stream literature in two Northern Lake Huron wetlands. They also identified metrics that were able to distinguish between a pristine and an impaired wetland, including the proportion of samples dominated by Ephemeroptera + Trichoptera, Isopoda, filterers, and predators. Kostuk (2006) used a multivariate cluster analysis approach to group wetlands *a priori* using a combination of water-quality information as well as plant community characteristics. The cluster analysis distinguished the most highly degraded (i.e. eutrophic) sites from all others, as well as separated moderately-degraded (mesotrophic) from most pristine wetlands (oligotrophic). Metrics proposed by Kostuk (2006) were consistent with those proposed by Burton et al. (1999) and Kashian and Burton (2000). For example, the proportions of Odonata, Ephemeroptera + Trichoptera, and Gastropoda

were progressively reduced in wetlands subject to increasing amounts of disturbance in all three studies. Also consistent with Kashian and Burton (2000), the proportions of a sample made up of Gammaridae and % Chironomini + Orthocladinae were highest in more impacted sites while samples most greatly dominated by Hyalellidae and Tanypodinae + Tanytarsini chironomids were associated with more pristine sites.

Wilcox et al. (2002) proposed Great Lakes coastal wetland metrics based on fishes, macrophytes and macroinvertebrates, and found some promising invertebrate metrics for barrier-beach wetlands in Lake Superior (including median # of taxa, # of adult Trichoptera, median number of individuals as Crustacea) but suggested a number of limitations of bioindicators for Great Lakes wetlands. First, variation in hydrology, exposure, sediment transport and deposition, and latitudinal gradients may require even wetlands in the same lake to be evaluated separately, limiting the pool of comparable sites for index development and validation. This also limits any future use of an IBI to those sites from which it was developed. Wilcox et al. (2002) also argued that dramatic changes in lake levels would yield different results year to year, even if human disturbance remained the same thereby invalidating metric scores. This question was also addressed by Gathman and Burton (2011). However, Uzarski et al. (2004) argued that invertebrate IBIs could be used for a wide range of lake levels if they included metrics calibrated individually for deep and shallow-water plant zones.

Other concerns with invertebrate IBIs previously developed for Great Lakes wetlands relate to the small samples sizes (i.e. number of wetlands) and the spatial scales used in their development. Wilcox et al. (2002) developed IBIs for individual Great Lakes from only 6 sites in each Lake, and the study by Kashian and Burton (2000) compared a single reference wetland to a single impacted wetland. Burton et al. (1999) developed IBIs from sites only on Lake Huron with all test sites being in the South and all reference sites in the North, introducing a possible latitudinal confounding. Another limitation to these studies is that results cannot truly be

compared due to different sampling methods. For example, Burton et al. (1999) and Kashian and Burton (2000) used D-Net sweeps to collect sediment and macrophyte associated macroinvertebrates, while Kostuk (2006) and Wilcox et al. (2002) used funnel traps to collect both micro- and macroinvertebrates. The examples mentioned also do not employ the reference condition approach as described in Chapter 1.

The approach used in this study is an extrapolation of the BEAST model of assessing biological communities ordinated across the full range of a stressor gradient (the Reference-Degraded Continuum approach (Ciborowski et al. 2013)).The BEAST model uses multivariate analyses to characterize the range of natural variability of benthic invertebrate abundance and community composition in samples collected from sites in the reference condition (Reynoldson et al. 1995). Groups of reference sites with similar zoobenthic community composition (identified by cluster analysis) are then discriminated on the basis of environmental attributes that distinguish the biologically distinct clusters of sites. Consequently, test sites can be matched to the most appropriate group of reference sites using independent data (i.e. environmental or habitat data) for subsequent bioassessment. Bioassessment using the reference-condition approach (RCA) typically results in a binary classification of test sites as either "equivalent to reference" or "Not equivalent to reference`` (Reynoldson et al., 1997).

The Reference-Degraded Continuum (RDC) approach used in this study extends the reference condition approach (RCA) by establishing both a "Reference" and a complementary "Degraded" biological condition. The degraded condition is characterized by the range of variability in biological condition at test sites subject to the highest amount of anthropogenic disturbance in the system of interest. In the BEAST model of the RCA, bioassessment is based on a comparison of biota at test sites and reference sites in taxa ordination space using 90% confidence ellipses. The RDC approach uses a two end-point ordination to delineate the extremes of the reference and degraded conditions, i.e. establish a reference-degraded continuum and

assesses the position of test site biota along the continuum. With the RDC approach, test sites can be classified as "Equivalent to Reference", "Not Equivalent to Reference", or "Equivalent to Degraded", allowing managers to set more efficient restoration priorities and even make predictions (Ciborowski et al. 2010).

In this study I derive a Zoobenthic Assemblage Condition Index (ZACI) for Great Lakes coastal areas using the Reference-Degraded Continuum approach. I subsequently relate the ZACI to two GLEI stressor gradients (i.e. % agriculture and MaxRel Development gradients) to identify changes in benthic community composition that accompany increasing levels of independent watershed-scale anthropogenic stress. The stress gradients used in this thesis are composite measures representing "pressures", with many associated single stressors. Consequently, there are likely many important factors influencing macroinvertebrate community condition that are associated with agricultural or urban land use. Therefore, I expect that macroinvertebrate assemblage condition will be constrained as a function of agriculture and development stress, rather than exhibit simple linear trends (i.e. stress-controlled relationship).

Methods

Study Area

The Great Lakes basin covers more than 760,000 km^2 with the coastal region bordering 8 States in the U.S. and the Province of Ontario, Canada. The basin is comprised of two ecoprovinces that differ in climate and physiography - the northern Laurentian Mixed Forest (LMF) in and the southern Eastern Broadleaf Forest (EBF) (Omernick 1987). The basin contains around 10% of the US population and 30% of the Canadian population (U.S. EPA and Environment Canada, 1995), with most human activity occurring in the southern ecoprovince. The northern portion is mostly forested. In contrast, much of the landscape of the southern province is characterized by agricultural activity and urban development.

Sampling Sites

Sampling units were selected from a GIS-derived delineation of each second-order or higher watershed draining into the Great Lakes coastal margins, termed a "segment-shed" (Danz et al., 2005; Hollenhorst et al., 2007). Segment-sheds were created by defining the shoreline boundaries at the midpoint between the drainage basins of adjacent second order or higher streams. Delineating the drainage areas associated with these segments created the segment-sheds. A total of 762 segment-sheds were delineated for the U.S. portion of the basin. The Canadian coastal margin was not assessed at this time because the research was limited to the US portion of the Great Lakes. Coastal sites located within segment-sheds were selected to span the full range of anthropogenic stress in the basin (Danz et al. 2005; Brazner et al. 2007). The sampling design also took into account the hydrogeomorphic type of each wetland according to the classification system of Keough et al. (1999) - high-energy shoreline (HE), embayments (EM), and three wetlands types- coastal wetlands (CW), riverine wetlands (RW) and barrier protected wetlands (PW).

Stressor Gradients

Values of 207 variables relating to six classes of human disturbance (agriculture, atmospheric deposition, land cover, point source pollution, human population density, and shoreline alteration) were collected at county-level resolution from publicly available web-based data sources and were spatially transformed using weighted-averaging to fit the segment-sheds (Danz et al. 2005). This study focuses on two major classes of watershed scale anthropogenic stress: agriculture and urban development. The agricultural stress category was originally comprised of 21 variables characteristic of stressors associated with agricultural activities, including measures of nutrient runoff, erosion, and fertilizers. Agricultural land cover *per se* was included in the Land Cover category but was found to be highly correlated to all other agricultural stress variables (Danz et al. 2005). Therefore, the percentage of a segment shed occupied by

agricultural land cover now serves as a simple proxy for all disturbances related to agriculture (Host et al. 2005).

The urban development stress gradient uses a composite measure of three variables associated with human development: population density, road density, and the percentage of a segment shed's area classified as developmental land cover. Although the three components were correlated across the whole basin, it was clear that single components dominated in different areas. For example, in areas with low population density, the dominant stress consisted of roads that influenced the movement of water and biota between a wetland and the open lake (i.e. road density). In other areas, developed land along the shoreline was the dominant stressor, whereas other watersheds simply had high population densities. Therefore, the composite score was developed by assigning the single maximum relative value (dubbed MaxRel) of the above three development-associated variables (Host et al., 2005, Johnson et al. 2015). This was done under the assumption that the most prevalent development variable within a particular segment-shed would exert the greatest effect on biota.

Both percent agriculture and the MaxRel development gradients are surrogate measures, or environmental indicators of the risk of potential human impact influencing wetland biota at the mouth of a particular watershed. Arguably, some would refer to % agriculture and MaxRel as pressures rather than individual stressors, but in this thesis I operationally define them as stressors because they have been constructed as quantitative measures of impact, recognizing that the stress exerted takes many forms.

Values of the two independent stress gradients were combined into a composite gradient called "AgDev" representing overall anthropogenic stress in the Great Lakes (Host et al. in review; Johnson et al. 2015). AgDev scores `were calculated as the Euclidean distance from the (0,0) origin to each site x,y coordinate of scaled % agriculture and MaxRel indices (Figure 2.1).

Macroinvertebrate Sampling and Processing

Benthic macroinvertebrate samples were collected in 2002 and 2003 at 141 coastal margin sites allocated across the 762 segment sheds of the U.S. Great Lakes basin using D-framed dip nets with 500 µm-mesh (Niemi et al. 2006; Brazner et al. 2007). Samples were collected at two depth zones along multiple transects within each site. Two transects were delineated for each dominant shoreline type within a site, extending perpendicularly from shore towards open water. The depth zones sampled along a transect corresponded to the approximate position of emergent vegetation (0.3-0.5 m depth) and submergent vegetation (0.5-1 m depth) expanses. Ten 1-m D-net sweeps comprised a single composite sample at the emergent and submergent vegetation depth zones, capturing invertebrates from within the water column (including those clinging to macrophytes) as well as the benthos.

In the lab, samples were washed through a stacked series of soil test sieves, separating materials into size fractions (4 mm, 1 mm, 0.5 mm, 0.25 mm) to increase the efficiency of sorting. Invertebrates were removed from debris beneath a dissecting microscope and identified to the lowest possible taxonomic level, typically genus except for Chironomidae and Oligochaeta, which were left at the family and class levels, respectively.

Point-level abundance data were used for index development and subsequent analyses - i.e. each composite sample collected at each depth zone along each transect were treated as individual units. Samples that contained 20 or fewer invertebrates were deemed too small to be included in analyses and were removed (approximately 30% of samples had 20 or fewer individuals). Rare taxa (those that occurred in <5% of samples within an ecoprovince) were either excluded from analyses or were combined with other related genera and treated as a group representing a higher taxonomic level. Relative abundances of each taxon in a sample were calculated ([number of individuals in a group]/[total number of individuals in a sample (including discarded taxa)]). Values were then transformed using natural logarithms [log_e(percentage+1)]

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(Gauch 1982). Further detail on the structure of this data set and the results of matrix formatting can be found in Appendix A.

Environmental Variables

A suite of environmental variables was also measured concurrently with biological sampling at each site. At each point where invertebrate samples were collected, variables such as presence and density of dominant vegetation types, dominant vegetation growth forms, dominant substrate characteristics and shoreline features were recorded (detailed in Olker et al 2015).

Theoretical Approach to Index Development: Reference-Degraded Continuum

Defining Reference Condition: We defined the reference condition as a set of environmental attributes that characterize sites that are minimally affected by human disturbance (Stoddard et al. 2006). Operationally, we defined the reference condition as wetlands or shoreline areas into which drain watersheds with levels of agriculture and development less than threshold values previously identified by Kovalenko et al. (2014). These threshold levels of stress were estimated using Threshold Indicator Taxa Analysis, or TITAN (Baker and King, 2010) and correspond to levels of each stressor at which there was a marked decrease in the abundance of 'sensitive' invertebrate taxa (reference/non-reference threshold). Although the confidence in threshold estimates for macroinvertebrate assemblages was only high along the development stress axis in Kovalenko et al. (2014), we decided to use their threshold estimate for agriculture as well, for consistency. Reference samples in the northern ecoprovince (LMF) were identified as those taken from sites that drained watersheds with <30% agricultural land cover and <0.08 MaxRel development stress, resulting in 83 reference samples. Reference samples in the southern ecoprovince (EBF) were identified as those taken from sites that drained watersheds with <55% agricultural land cover and MaxRel development stress scores of < 0.10, resulting in 112 reference samples.

Identifying Biologically Similar Groups of Reference Sites: Variation in macro-invertebrate community composition among reference samples within each ecoprovince was characterized using hierarchical tree cluster analysis in Statistica 7.0 (StatSoft Inc., Tulsa, Oklahoma). Each cluster identified represents a group of reference samples with distinct fauna characteristic of reference communities (i.e. similar macroinvertebrate assemblages). Samples were clustered using Ward's method of amalgamation and a Euclidean distance measure. The species contributing the most to distinguishing the clusters was determined by calculating F-statistics from the ratio of among-group Mean Squares to within-group Mean Squares for each taxon (Green and Vascotto, 1978). Taxa that were most discriminant were those with the largest F-ratios. F-statistics were calculated for cluster groupings at each bifurcation of the dendrogram.

Identifying Environmental Characteristics of Biologically Distinct Reference Samples: Following cluster analysis, forward stepwise discriminant function analysis (DFA) was used to determine the habitat and environmental variables that best distinguished the clusters of samples representing the unique reference community types (Reynoldson et al. 1997). The habitat/environmental variables included in each DFA analysis were a suite of dummy variables indicating lake of origin, hydrogeomorphic classification, primary sediment texture, presence/absence of aquatic habitat types (e.g. emergent, submergent, open water), aquatic plant growth forms, aquatic vegetation types (e.g. floating leaf) and shoreline habitat types (e.g. muddy bank, vegetated bank). Forward stepwise DFA analysis was performed in Statistica 7.0. We then used the DFA model to assign each non-reference site (i.e. test sites) to one of the reference groups based on the values of each test site's habitat/environmental variables.

Ordinating Macroinvertebrate Assemblages with Respect to Stress: Once test sites had been assigned to a reference type, suites of reference + test sites were ordinated relative to their position along a Reference-Degraded Continuum (stress axis) based on their benthic community composition (Ciborowski, 2010). If anthropogenic stress has an effect on the macroinvertebrate

community, then the most stressed sites within each cluster should support different community composition compared to the least stressed sites. Bray-Curtis polar ordination using subjective endpoints was used to ordinate test sites with environmental characteristics corresponding to each reference cluster (PC-ORD version 6.0 software; McCune and Mefford, 2011). The two subjective endpoints created for each ordination were the centroids of macroinvertebrate species composition at the 10% of sites exhibiting the lowest stress scores (reference centroid) and the 10% of sites with the highest stress scores (degraded centroid). An ordination of samples in species space was conducted relative to subjective endpoints created using agriculture and MaxRel stress scores independently for each cluster of samples (i.e. two ordinations per cluster). Further detail on the construction of centroids and ZACI axes is provided in Appendix B. The result of each Bray-Curtis ordination denoted the position of each sample relative to the reference and degraded endpoint scores created above based on its biological community composition. The ordination scores can therefore be used as a composite index of biological condition (the Zoobenthic Assemblage Condition Index (ZACI)). Forward stepwise multiple regression was then used to determine the taxa loading most importantly on each ZACI axis. Important and significant taxa were used to develop functions of the form:

$$ZACI = B_0 + B_1X_1 + B_2X_2 \ldots B_nX_n,$$

where B_0 is the intercept of the multiple regression model, Bi is the regression coefficient of each taxon i in the model and Xi is the $Ln(i+1)$ transformed relative abundance of each taxon i in the model. The above function can be used to determine ZACI scores for other test-samples.

The ZACI scores for samples in each cluster were subsequently plotted relative to the environmental stress scores of the two disturbance gradients (%Ag and MaxRel Development). Least-squares linear regression and quantile regression analysis (Cade and Noon, 2003) at several percentiles (0.05, 0.10, 0.20, 0.50, 0.80, 0.90 and 0.95) were used to determine the relationship between ZACI and environmental stress for each cluster of sites and for each stress type.

Results

Species Assemblages

Three groups of sampling points with distinct biological assemblages were identified in the northern ecoprovince as a result of the cluster analysis (Figure 2.2). The first bifurcation separated Cluster N3 from the other two clusters. Cluster N3 was the largest group of reference data (38 samples), having no single dominant taxon. Rather, Cluster N3 samples were comprised of on average, 9% more amphipods (5% more *Gammarus*, 4% more *Hyalella*), 15% more gastropods (7% more Hydrobiidae, 4% more *Physella*, 4% more *Gyraulus*) and 2% more of both *Baetis* (mayflies) and Aeshnidae (dragonflies) than Clusters N1 and N2 combined (Table 2.1.). Cluster N1 and N2 samples were much less even, with Chironomidae or Oligochaeta dominating and very low relative abundance (i.e. ≤ 3%) of all other taxa. Cluster N1 had 21 samples that were comprised of on average, 60% Chironomidae (35% more than Cluster N2 and 49% more than Cluster 3-N). Cluster N2 had 24 samples that were comprised of on average, 50% Oligochaeta (40% more than Cluster N1 samples and 41% more than Cluster N3 samples) (Table 2.2.).

Two distinct reference assemblages were identified in the Southern Ecoprovince, named Cluster S1 (54 samples) and Cluster S2 (58 samples) (Figure 2.3). Cluster S1 reference samples were comprised of on average, 19% *Hyalella* (15% more than Cluster 2-S), 4% Leptoceridae (3% more than Cluster S2), and 2% Coenagrionidae and *Neoplea* (2% and 1% more than Cluster S2, respectively). Cluster S2 reference samples were comprised of on average, 48% Oligochaeta (36% more than Cluster S2 samples) (Table 2.3.).

Group membership was effectively predicted by DFA models for samples in both the northern and southern ecoprovinces. In the northern ecoprovince the DFA model correctly classified 60 of 77 reference samples (Table 2.4), with 66% of samples being classified with ≥ 60% probability of group membership. Six reference samples could not be included in model

development due to missing data related to presence of vegetation growth forms and aquatic vegetation types. Variables included in the classification model were those related to presence/absence of vegetation types and growth forms, hydrogeomorphology, and the Lake from which the samples were taken (Table 2.5). See Table 2.6 for descriptions of variable codes.

In the southern ecoprovince the DFA model correctly classified 75 of 104 samples, with 70% of samples being classified with ≥ 60% probability of group membership (Table 2.7). Eight samples could not be included in the DFA analysis due to missing habitat data (including presence of aquatic habitat types, presence of aquatic vegetation types and shoreline habitat variables). Variables included in the classification model were those related to coastal wetland hydrogeomorphology, shoreline habitat and presence of submergent vegetation and sandy substrate (Table 2.8). A summary of resulting sample sizes at each preliminary stage of index development can be found in Appendix A.

The classification models generated for the north and south were used to subsequently assign the non-reference sampling points to the most appropriate reference clusters. In the northern ecoprovince, 35 of the 249 test samples could not be classified into one of the reference groups due to missing plant growth form data. In the southern ecoprovince, 36 of the 228 test samples could not be classified because of missing shoreline habitat data. Classification functions used to classify test sites can be found in Table 2.9.

Stressor Influences- Agricultural Land Use
Agriculture ZACI Axis Structure

The ZACI axes derived for agricultural stress (Ag-ZACI axes) in the northern ecoprovince extracted 32%, 21% and 12% of the original distance matrices in Clusters N1, N2 and N3, respectively. Chironomidae was included in regression models as an indicator taxon in samples collected at sites at the reference end of Ag-ZACI axes in Clusters N1 and N2 and towards the degraded end of Cluster N3 (Table 2.10). *Gammarus* was also a significant taxon in

all three clusters, being most dominant in samples towards the degraded ends of the Ag-ZACI

axes. The most important reference indicator taxa (based on highest standardized regression

coefficients ± 1SE) were Chironomidae in Cluster N1 (ß = 0.52 ± 0.04), Oligochaeta in Cluster

N2 (ß = 0.57 ± 0.06) and Hydrobiidae in Cluster N3 (ß = 0.28 ± 0.05). The most important

degraded indicator taxa were *Gammarus* in Cluster N1 and N3 (ß = -0.44 ± 0.04 and ß = -0.57 ±

0.05; respectively) and Hydrobiidae in Cluster N2 (ß = -0.36 ± 0.05).

The Ag-ZACI axes derived for clusters in the southern ecoprovince extracted 21% of the

original distance matrices in Cluster S1 and 14 % in Cluster S2. Oligochaeta was the most

important indicator taxa in samples towards the degraded end of Ag-ZACI axes in both Cluster S1

and S2 (ß = -0.40 ± 0.04 and ß = -0.59 ± 0.06; respectively). The most important reference

indicator taxa were *Hyalella* in Cluster S1 samples (ß = 0.62 ± 0.05) and Hydrobiidae in Cluster

S2 samples (ß = 0.29 ± 0.06; Table 2.10).

Ag-ZACI vs. Watershed Agricultural Land Use

Simple least-squares linear regression relating Ag-ZACI scores to stress from all

northern clusters identified highly significant, but relatively non-predictive negative relationships

(Cluster N1: $R^2 = 0.41$, $p < 0.00002$; Cluster N2: $R^2 = 0.25$, $p < 0.0002$; Cluster N3: $R^2 = 0.32$, $p <$

0.00001). Cluster N1 and N2 Ag-ZACI scores were highly variable at reference levels of %

agriculture (i.e. < 30%), ranging from 0.04 to 1.0 in Cluster N1 and from 0.30 to 1.0 in Cluster N2

(Figure 2.4a and 2.5a). However, the upper values of Ag-ZACI reference site scores (those at

wetlands with <30% agriculture) in Cluster N1 and N2 were all high - above 0.65 in Cluster N1

and above 0.60 in Cluster N2. LOWESS curves fitted to data in Cluster N1 and N2 show

discontinuities in the relationship between Ag-ZACI scores and % agriculture in the watershed.

Quantile regression slopes fitted through a subset of samples in Cluster N1 and N2 exposed to <

30% agriculture were not significantly different from zero (N1: $p > 0.50$, N2: $p > 0.13$) and were

not different from each other (N1: $p = 0.69$, N2: $p = 0.38$). However, there was a lack of samples

with agriculture scores above 30% in these clusters so quantile regression-derived trends and potential thresholds could not be evaluated at non-reference levels of agriculture. Cluster N3 Ag-ZACI scores appeared to exhibit a somewhat wedge-shaped distribution across the % agriculture gradient (Figure 2.6a). Quantile regression slopes at upper percentiles (i.e. tau = 0.80, 0.90, 0.95) were similar to each other (p = 0.98) and differed significantly from the lower percentile slopes (p < 0.001) (Figure 2.7).

Least squares regression relating Ag-ZACI scores to stress from both southern clusters identified highly significant, but non-predictive negative relationships (Cluster S1: R^2 = 0.29, p < 0.00001; Cluster S2: R^2 = 0.34, p < 0.00001). Cluster S1 and S2 Ag-ZACI scores were highly variable but LOWESS curves fitted to data in each cluster show discontinuities in the relationship between Ag-ZACI scores and % agriculture in the watershed (Figure 2.8a, 2.9a). Quantile regression slopes fitted through a subset of samples in Cluster S1 exposed to < 55% agriculture were positive but not significantly different from zero (Figure 2.10a), and were similar to each other (p = 0.85). Above 55% agriculture in Cluster S1, quantile regression slopes became negative but again, they were not significantly different from zero, and were similar to each other (p = 0.30; Figure 2.10b). In Cluster S2, quantile regression slopes were negative throughout but below 55% agriculture they were all highly significant (p < 0.001) and not significantly different from one another (p = 0.36), whereas above 55% agriculture the slopes were mostly not significantly different from zero or from each other (p = 0.18; Figure 2.11a,b).

Stressor Influences- Development Stress

Development ZACI Axis Structure

The ZACI axes derived for development stress (i.e. Dev-ZACI axes) in the northern ecoprovince extracted 7.6%, 21%, and 18% of the original distance matrix in Clusters N1, N2 and N3 respectively. Chironomidae was included in regression models as the most important indicator taxon in samples collected at sites at the reference end of the Dev-ZACI axes in Cluster N1 (ß =

0.50 ± 0.11) and towards the degraded end of both Cluster N2 and N3 ($ß = -0.33 \pm 0.05$ and $ß = -0.45 \pm 0.05$ respectively; Table 2.11). Oligochaeta also appeared as a significant taxon in Cluster N1 being most dominant in samples towards the degraded end of the Dev-ZACI axis and as the most important reference taxon in Cluster N2 ($ß = 0.74 \pm 0.05$). Hydrobiidae was a significant taxon in samples towards the reference end of the Dev-ZACI axis in Cluster N1 and was the most important taxon in samples at the reference end of the Dev-ZACI axis in Cluster N3 ($ß = 0.50 \pm 0.05$).

The Dev-ZACI axes derived for clusters in the southern ecoprovince extracted 13% of the original distance matrix for Cluster S1 and 13% for Cluster S2. Only two taxa were important indicators in samples along the Dev-ZACI axis for Cluster S1: *Hyalella* towards the reference end ($ß = 0.83 \pm 0.05$) and Hydrobiidae towards the degraded end ($ß = -0.33 \pm 0.05$; Table 2.10b). Hydrobiidae also appeared as the most important taxon in samples from Cluster S2 towards the *reference* end of the Dev-ZACI axis. Chironomidae was the most important indicator taxon in samples towards the degraded end of the Cluster S2 Dev-ZACI axis (Table 2.11).

Dev-ZACI vs. Watershed Development Stress

Least-squares regression indicated significant but non-predictive negative relationships between biological condition and the MaxRel development stress gradient in all northern clusters (Cluster N1: $R^2 = 0.13$, $p < 0.04$; Cluster N2: $R^2 = 0.41$, $p < 0.00001$; Cluster N3: $R^2 = 0.23$, $p < 0.00001$). LOWESS curves fitted to plots in all clusters showed that trends were non-linear between Dev-ZACI scores and MaxRel stress (Figure 2.4-2.6b). Slopes of the quantile regression lines fitted through data in all clusters showed significant negative relationships with development stress at most of the percentiles tested. MaxRel stress appeared to limit the biological condition of samples in Cluster N3. The scatterplot showed a wedge-shaped relationship, and the slopes of the quantile regression lines differed significantly ($p < 0.01$) between the upper (0.90, 0.95) and lower (0.05, 0.10) percentiles (Figure 2.12). Slopes of the upper quantile lines (0.90, 0.95) were not

significantly different than the 50th quantile slope (p > 0.10) but the standard errors of the upper

slopes were high, likely due to the sparsity of data at the upper quantiles. The slope of the quantile

line through the 80th percentile had a much lower standard error and was found to be significantly

different than the slope at the 50th percentile (p = 0.037).

Visual inspection of each ZACI-stress plot suggests that there is a potential

reference/non-reference threshold along the MaxRel stress gradient in each cluster located around

the operational reference threshold of 0.08 MaxRel stress (Figures 2.4-2.6b). Above 0.08 MaxRel

the minimum ZACI score shifts downward towards zero and the maximum ZACI score does not

extend above 0.80 in any of the clusters. In addition, the scatterplot of Dev-ZACI scores in

Cluster N2 appeared to show a stair-step pattern, with a second potential change-point around

0.12-0.15 MaxRel, above which the maximum ZACI score was limited to 0.20 or less (Figure

2.5b). In the subset of data below 0.12 MaxRel, slopes of quantile regression lines were not

significantly different from zero, and were similar to each other (p > 0.10; Figure 2.13a). When

fitted through data above 0.12 MaxRel, quantile regression slopes were still negative but were

mostly non-significant, specifically the upper and lower quantile slopes). (Figure 2.13b).

Dev-ZACI scores from both southern clusters were significantly negatively related to the

MaxRel development stress gradient as indicated by least squares regression (Cluster S1: R^2 =

0.18, p < 0.0034; Cluster S2: R^2 = 0.35, p < 0.00003). However, these relationships were non-

predictive and LOWESS curves fitted to both Cluster S1 and S2 showed non-linear trends

between Dev-ZACI scores and MaxRel stress (Figure 2.8b, 2.9b). Scatterplots did not show

wedge-shaped patterns and potential thresholds could not be evaluated due to large gaps between

data points above the reference cut off. However, it should be noted that Dev-ZACI scores

remained below 0.60 for samples collected from sites where MaxRel stress exceeded 0.20.

Stressor Influences- Combined Stress

AgDev ZACI Axis Structure

The ZACI index for combined stress was only developed for samples in Cluster N3 in the northern ecoprovince. The AgDev ZACI extracted only 9% of the original distance matrix. *Gammarus* accounted for most of the variation in AgDev ZACI scores and was the most important contributor to low ZACI scores (ß = -0.70 ±0.05, p < 0.000). *Physella* and *Hyalella* both contributed to high ZACI scores, with *Physella* being the most important (ß = 0.35 ±0.21, p < 0.001).

AgDev ZACI vs. Combined Stress

The AgDev ZACI was significantly negatively related to the combined stress gradient (R^2 = 0.41, p < 0.000) but a LOWESS showed that the relationship is actually non-linear below 0.30 on the AgDev scale before decreasing linearly at higher levels of composite stress (Figure 2.14). Quantile regression slopes were all highly significantly different from zero (p < 0.001) but quantile lines through the upper and lower quantiles were similar to each other (p > 0.10; Figure 2.15). The relationship between the independent stress gradients (% agriculture and MaxRel) and the composite AgDev ZACI axis was determined using multiple regression. Results showed that % agriculture gradient contributed the most to the composite ZACI (ß = -0.56, p < 0.001) and was the only stressor significantly correlated with AgDev ZACI. This was due to the shorter development stress axis (0.049 – 0.47 MaxRel) compared to the % agriculture stress axis (0 – 82% agriculture). This resulted in % agriculture being the only stressor contributing to high composite AgDev scores.

Discussion

Species Assemblages

A cluster analysis of reference samples revealed distinct invertebrate assemblages among reference samples in both the northern and southern ecoprovinces. For example, in the northern ecoprovince samples from Cluster N1 were distinguished by a dominance of midge larvae (Chironomidae), which may indicate that these points were unsuitable for other taxa (either because of a lack of vegetation cover or unsuitable substrate). However, chironomids are a ubiquitous group that includes a variety of functional groups, including case building filterers/collectors, grazers, and predators (Merritt et al. 2008) so it is also possible that the chironomids in these samples simply outnumber the other taxa, which may be present at typical densities. In contrast, samples in Cluster N2 and S2 were dominated by oligochaete worms, which are burrowing detritivores. No single taxon dominated samples in Cluster N3 but samples in this cluster had a greater relative abundance of several taxa, including amphipods (gatherers)), snails (grazers) and mayflies of the genus *Baetis* (swimming and clinging gatherers). Gatherers and detritivores dominated samples in many of the reference Clusters (e.g. Cluster N2, N3, S1 and S2) implying that these sites had a significant amount of vegetation supplying detrital food. Cluster N2 and Cluster S2 samples had few taxa other than Oligochaeta, indicating a predominance of depositional habitat and organic substrate.

Environmental Variables

Northern Ecoprovince

The dominant taxa in samples from each reference cluster have functional traits and habitat requirements that are dependent on local substrate and vegetation conditions. It was therefore expected that measured variables such as dominant substrate characteristics and presence of different vegetation types would be more important for distinguishing between clusters compared to larger scale variables such as lake or shoreline habitat. In the absence of human disturbance, hydrology and sedimentation are two main physical drivers in coastal

wetlands (Lee et al. 2006), influencing the types of substrates and plant communities that can occur (Keough et al. 1999). Brazner et al. (2007) also found that most of the variation in indicators of ecological condition derived from macroinvertebrate assemblages was explained by wetland type (as opposed to Great Lake, ecoprovince and human disturbance). So in addition to the proximate substrate and vegetation variables, it was expected that hydrogeomorphology may also be an important factor distinguishing the reference assemblages.

The DFA model developed effectively distinguished among the groups of reference assemblages on the basis of habitat and environmental variables. Variables included in the discriminant model distinguishing reference clusters in the northern ecoprovince were hydrogeomorphic and vegetative in nature, as expected. However, dominant substrate characteristics were not identified in the DFA model for this ecoprovince. Whether or not broad-leaf emergent vegetation was present near the sampling point was the most important variable in the model for distinguishing among the three unique reference assemblages. Other variables important in the DFA model included those related to the absence of aquatic vegetation (e.g. presence of open water habitat) as well as those related to the presence of certain plant growth forms (e.g. broad-leaf emergent and ribbon-like floating plants). Structural complexity and density of macrophytes have a positive influence on macroinvertebrate species richness (Burton et al. 2004; St. Pierre and Kovalenko, 2014) and invertebrate communities have been shown to differ significantly among types of plant zones (Burton et al. 2002; Uzarski et al. 2004; Gathman and Burton 2011).

The high energy hydrogeomorphic variable was also included and indicates coastal sites subject to a high degree of wave exposure, resulting in habitats with relatively little organic matter, and sandy or rocky substrates (e.g. boulder, cobble) (Burton et al. 2002). The macrophyte community at these sites is either non-existent (beach or rocky shoreline) or is composed of species able to resist wave disturbance (e.g. *Typha* and *Scirpus* spp.; Keough et al. 1999). Cluster

N1 contained the majority of the high energy sites and was very clearly distinguished from Clusters N2 and N3 by this variable. Samples in this cluster were dominated by Chironomidae, corroborating previous research stating exposed wetland zones are dominated by filtering-collectors (Burton et al. 2002).

Variables specifying lake of samples' origin were also present in the model, indicating that invertebrate assemblages may be indirectly influenced by geography even within an ecoprovince. In a study of geographic and geomorphic influences on Great Lakes wetland multi-assemblage indicators, Brazner et al. (2007) found that lake had the strongest influence across all indicators (compared with wetland type and ecoprovince).

Southern Ecoprovince

In the southern ecoprovince, one of the most important variables in the model was the open coastal wetland hydrogeomorphic classification. Similarly to high energy shoreline, wetlands in this class have a direct, open connection to the lake but are characterized by the presence of distinct zones of submergent marsh, emergent marsh and wet meadow vegetation zones (Keough et al. 1999). Cluster S2 was composed almost entirely of sites located within open coast wetlands, whereas Cluster S1 was comprised of sites situated in barrier protected and drowned river-mouth wetlands. This is further evidence for the importance of hydrology and geomorphology on shaping wetland invertebrate communities. In contrast to open coast and high energy wetlands, wetlands protected from wave exposure accumulate organic matter resulting in organic sediments and, potentially, lower dissolved oxygen concentrations. Sandy substrate was a significant variable in the DFA model and was associated with Cluster S1 (open coast wetlands), supporting the concept that wetlands exposed to greater wave action are less prone to accumulating thick layers of organic substrate.

The most important variable distinguishing Cluster S1 from S2 was the type of shoreline habitat, specifically vegetated bank. Shoreline type has been suggested to influence nearshore substrate composition and stability, thereby potentially having an effect on nearshore assemblages. However, Goforth and Carman (2005) found no difference in benthic assemblage structure among Great Lakes shoreline types and stated that shoreline type alone may not be enough to predict nearshore substrate features. The presence of a vegetated bank was highly predictive of sites in Cluster S2 and may be associated with the high number of riverine wetlands in this cluster.

Stressor Influences

Ag-ZACI Axis Structure

Each ZACI axis represents a gradient of macroinvertebrate assemblages scored relative to two end-point assemblages representing reference and degraded assemblages. Multiple regression analysis identified taxa that were the important contributors to the Ag and Dev-ZACI scores for samples in each cluster. In all northern clusters, assemblages more similar to "degraded" endpoints had a greater abundance of *Gammarus* relative to other taxa. This was also true along the AgDev ZACI axis for Cluster N3. Previous studies have also found the proportion of amphipods to be indicative of a disturbed condition (Burton et al. 1999, Uzarski et al. 2004). Proportion of the family Gammaridae has been associated with more nutrient rich wetlands while proportion of Hyalellidae has been associated with more oligotrophic sites (Kostuk, 2006). Towards the reference end of the AgDev axis, *Hyalella* was a contributor to high ZACI scores, along with relative abundance of *Physella* a gastropod genus sensitive to dissolved oxygen concentrations (Brown, 2001).

Chironomidae and Oligochaeta are often considered to be tolerant of disturbance and are often indicators of disturbed or eutrophic conditions. This is in contrast to my results in the northern ecoprovince where samples at the reference end of the Ag-ZACI axis had a high relative

abundance of Chironomidae in Cluster N1 and Oligochaeta in Cluster N2. However, %
Tanytarsini and % Tanypodinae have been reported to decrease with eutrophy in some studies
while % Chironomini and Orthocladinae tended to increase with eutrophic conditions (Kostuk
2006; Kashian and Burton, 2000). It may be that the dominant chironomid subfamilies in samples
from Clusters N1 are less tolerant to nutrient loading and this could explain the decrease in
relative abundance with degraded Ag-ZACI scores. Since Chironomidae were not identified past
family level in this study we could not formally investigate this hypothesis.

Similarly, Oligochaeta were the most dominant taxon in reference assemblages in Cluster
N2, contributing the most to high Ag-ZACI scores; but in the southern ecoprovince (Clusters S1
and S2) they contributed the most to low Ag-ZACI scores. Oligochaete taxa vary in their
tolerance to pollution and this concept has been used to develop an oligochaete based index
(Oligochaete Trophic Index-OTI) which is used by EPA and SOLEC to assess trophic status of
Great Lakes ecosystems (Millbrink, 1983). Increased eutrophication causes a shift in oligochaete
communities to greater proportions of organic pollution-tolerant species, reflected in an increase
in the OTI (Howmiller and Scott, 1977). OTI values calculated for nearshore Lake Erie were
positively correlated with agricultural activity in the watershed in a recent study (Scharold et al.
2015). It may be that reference assemblages in Cluster N2 are able to support pollution sensitive
species while degraded assemblages in the southern ecoprovince are reflecting a shift to pollution-
tolerant assemblages at high levels of agricultural stress.

Dev-ZACI Axis Structure

Taxa contributing the most to high Dev-ZACI scores (indicative of reference
assemblages) were the same as those contributing to high Ag-ZACI scores. This should be
expected since reference centroids were constructed from samples with minimal levels of both
agriculture and development stress. However, important indicator taxa in assemblages towards the
degraded end of Dev-ZACI axes differed from those at the degraded end of the Ag-ZACI axes.

For example, among clusters in the northern ecoprovince, *Gammarus* and Hydrobiidae were the most important contributors to low Ag-ZACI scores but Chironomidae and Nematoda were the most important contributors to low

Ag-ZACI vs. Agriculture Stress

For each cluster, Bray-Curtis ordination was used to develop a Zoobenthic Assemblage Condition Index with which to assess the quality of samples (in terms of macroinvertebrate community composition) along two stressor gradients measuring the extent of anthropogenic land-use at the watershed scale. No threshold patterns were identified between the Ag-ZACI and percent agriculture in the watershed for any clusters in either ecoprovince. This is consistent with findings of Kovalenko et al. (2014) who did not find sufficient evidence of a threshold change along the agriculture stress axis for macroinvertebrate assemblages in coastal wetlands of the Great Lakes. Agricultural land cover in the watershed does not act directly on biological communities but rather indirectly through proximate drivers such as sedimentation of substrate, nutrient enrichment and reduced water quality (Riseng et al. 2011; Allan 2004). The effect of these proximate drivers may be expressed as a more gradual function of increasing agriculture than is observed with the effects of urban land use in the watershed, which often includes direct habitat destruction, shoreline modification, and an increasing extent of impervious surfaces (Allan 2004, Lee et al. 2006). For this reason, perhaps community thresholds do not exist for macroinvertebrate assemblages along percent agriculture gradients in Great Lakes wetlands. In addition, the southern ecoprovince is characterized by longer history of agriculture and development compared to the northern ecoprovince (US EPA and Environment Canada,1995), which may explain the large variation in Ag-ZACI along the agriculture gradient in those clusters.

Although no threshold responses were identified, non-linear negative relationships between the Ag-ZACI and percent agriculture were detected in the watersheds of both ecoprovinces. In the southern ecoprovince, the scatterplot of Cluster S2 samples showed a non-

linear trend in which Ag-ZACI decreased linearly until about 55% agriculture, after which the slope of quantile regression lines decreased. This indicates that assemblages in Cluster S2 change steadily (in a linear fashion) at levels of agriculture below 55% land cover in the watershed. Above 55%, biological condition continues to decrease but at a slower rate. This implies that most of the changes to macroinvertebrate assemblages due to agriculture stress occur when less than 55% of a watershed contains agricultural land cover, and levels of agriculture above 55% do not contribute much additional effect on macroinvertebrate assemblages in this cluster.

The scatterplot of Cluster N3 samples showed a wedge-shaped pattern in relation to % agriculture, and slopes of the quantile regression lines in upper quantiles were significantly different from the slope in lower percentiles. This indicates that the upper limit of possible ZACI scores decreases as % agriculture in the watershed increases, but at low levels of agriculture it is possible to have a wide range of ZACI scores, likely due to other (unmeasured) habitat or stressor variables (see Cade et al. 1999).

Dev-ZACI vs. Urban Development Stress

Cluster N3 ZACI-Dev scores also exhibited a wedge-shaped pattern in relation to the development stress axis. There is previous empirical evidence for wedge-shaped relationships between urban land use and invertebrate metrics in streams (Baker and King, 2010; Kail et al., 2012). For example, Kail et al. (2012) found that ecological status of macroinvertebrate assemblages in European rivers was limited by increasing urban land use in the upstream catchment.

In all clusters, changes in community composition were evident at relatively low levels of MaxRel stress (<0.10), with Cluster N2 showing a possible stair-step threshold response (Brendan et al., 2008) with ZACI-Dev scores limited to ≤0.20 at levels of development above 0.15. This is consistent with threshold values reported for other aggregate community variables in

the literature, which seem to range from 8-16% urbanization (Stepenuck et al., 2002; Hilderbrand et al., 2010; King et al., 2011; Kail et al., 2012). However, taxon-specific threshold analyses indicate that many invertebrate taxa begin to decline in abundance at much lower thresholds (i.e. 0.5%-5% development) in lotic environments (e.g. King et al., 2011; Kail et al., 2012). This sensitivity to development seems to hold true for taxa in Great Lakes coastal wetlands as well. Kovalenko et al. (2014) used Threshold Indicator Taxon Analysis (TITAN; Baker and King, 2010) to identify community change points along a gradient of % developed land in the watershed and found congruent thresholds among wetland assemblages at 4-6% developed land.

ZACI-Dev scores in the southern ecoprovince were negatively related to the MaxRel stressor gradient in a non-linear fashion in both clusters. All points above 0.20 MaxRel stress in Cluster S1, although sparse in number, remained below 0.60 on the ZACI-Dev axis, indicating a possible stair-step threshold response pattern similar to Cluster N2. However, whether threshold or wedge-shaped relationships exist in either Cluster S1 or S2 could not be determined because of a lack of data along the MaxRel gradient, where sites with intermediate stress scores were not sampled.

Although some macroinvertebrate indices for assessing biological condition have been developed for Great Lakes coastal wetlands (e.g. Uzarski et al. 2004, Wilcox et al. 2002) they are mainly multimetric indices and are applicable to only limited areas. In this study I was able to use macroinvertebrate assemblages collected from across the U.S. Great Lakes basin to develop a multivariate index (ZACI) that can be theoretically be applied across the entire Great Lakes basin. The ZACI was used to assess how macroinvertebrate communities are affected by large-scale anthropogenic stress (represented by % agriculture in the watershed and a composite urban development stress gradient). Because the indices are based on both biological samples and environmental data collected across the full range of stress, they can be applied throughout the

Great Lakes basin, and results of this study show that the ZACI is capable of capturing variable responses of distinct assemblages to large-scale anthropogenic stress.

It was expected that biological condition as measured by the ZACI would decrease non-linearly in relation to increasing land-use stress in both the north and south portions of the basin. This prediction was supported in a number of clusters where macroinvertebrate assemblage scores exhibited non-linear changes as a function of the amount of both agriculture and development sourced anthropogenic stress. Clusters representing distinct types of macroinvertebrate assemblages were identified and the responses to each stress gradient varied among clusters. Cluster N2 showed the only potential threshold relationship in this study, exhibiting a stair-step pattern that indicates a shift to a degraded condition at relatively low levels of development stress. This type of result can be especially valuable for management that seeks to prevent degradation at vulnerable sites and restoration activities seeking to reverse degradation (Host et al. in review).

The other notable patterns were wedge-shaped relationships between ZACI and stress in Cluster N3 as evidence that watershed land use can act as a limiting factor for macroinvertebrate community composition in some wetlands. In addition, despite a long history of agriculture and development in the southern ecoprovince, a clear non-linear relationship was found between biological condition and % agriculture in one cluster (Cluster S2). It has been suggested that inferring community response aggregate community metrics confounds the synchronous yet variable responses of individual taxa (King and Baker, 2010; King et al., 2011), underestimating or masking thresholds. However, one could argue that responses derived from aggregate metrics and indices could be interpreted as changes in ecosystem function, while taxon-specific changes serve as thresholds for species conservation (Kail et al. 2012, Kovalenko et al. 2014).

In a number of clusters in the northern ecoprovince, evaluation of the stress-ZACI relationship was limited by a lack of samples with intermediate and high levels of either stress type. The GLEI consortium evaluated land use variables for over 6000 segment-sheds across the

Great Lakes basin (Host et al. 2005). When plotted against their agriculture and development stress scores, sites do exist in the northern ecoprovince that have intermediate and high stress scores (Figure 2.16). Future surveys that propose to use the ZACI in the northern ecoprovince may need to focus efforts on including samples from segment-sheds subject to intermediate and high levels of agriculture and development stress. In addition, the Euclidean composite stress gradient was biased towards agricultural stress in the cluster of samples examined (Cluster N3) and the AgDev ZACI was derived using a similar degraded endpoint to the Ag-ZACI. Consequently, the AgDev ZACI may not have captured the contributing effect of development stress in this cluster. The combined effect of agriculture and development stress on the macroinvertebrate assemblages in Cluster N3 is assessed further in Chapter 3.

Multivariate analysis of samples collected across the reference-degraded continuum was able to generate indices that change significantly as a function of anthropogenic stress related to agriculture and development. The key indicator taxa were Chironomidae, Oligochaeta, Hydrobiidae, and amphipod genera (*Gammarus* and *Hyalella*). The novelty of this suite of indices is that, unlike previous studies that were geographically restricted, they can be used to assess all coastal margins of the U.S. Great Lakes. However, further data are needed at intermediate levels of stress. Data recently collected through the Great Lakes Coastal Wetland Monitoring Program (Uzarski et al. 2016) cover these levels of stress and may ultimately be able to provide confirmatory data.

Table 2.1. Mean relative abundance of significant taxa delineating reference clusters in the northern ecoprovince at the first bifurcation of a cluster analysis dendrogram (see Figure 2.2). F-values were calculated from the ratio of among-group Mean squares to within-group Mean squares for each taxon. Column N1+N2: Mean relative abundance of each taxon in samples from Clusters N1 and N2 combined. Column N3: Mean relative abundance of each taxon in samples from Cluster N3.

		Mean Relative Abundance	
Taxa	F-Ratio	N1+N2	N3
Chironomidae	59.48	0.41	0.12
Oligochaeta	29	0.29	0.087
Gammarus	12.8	0.010	0.063
Baetis	10.51	0.0024	0.026
Physella	10.41	0.0024	0.045
Hydrobiidae	9.62	0.017	0.084
Aeshnidae	8.73	3.4E-05	0.016
Hyalella	7.61	0.0094	0.047

Table 2.2. Mean relative abundance of significant taxa delineating reference clusters in the Northern Ecoprovince at the second bifurcation of a cluster analysis dendrogram (see Figure 2.2). F-values were calculated from the ratio of among-group Mean squares to within-group Mean squares for each taxon.

		Mean Relative Abundance	
Taxa	F-Ratio	N1	N2
Oligochaeta	132.49	0.095	0.50
Chironomidae	49.76	0.60	0.26

Table 2.3. Mean relative abundance of significant taxa delineating reference clusters in the Southern Ecoprovince (see Figure 2.3). F-ratios were calculated from the ratio of among-group Mean squares to within-group Mean squares for each taxon.

Taxa	F-Ratio	Mean Relative Abundance		% difference
		S1	S2	
Oligochaeta	218.6	0.12	0.48	36
Hyalella	40.2	0.19	0.03	16
Coenagrionidae	17.8	0.02	0.00	2
Leptoceridae	12.98	0.04	0.01	3
Neoplea	7.2	0.02	0.01	1

Table 2.4. Summary of the number of sites from the northern ecoprovince predicted to be in each cluster by discriminant function model based on 12 habitat variables (see Table 2.5).

		Observed			
	% Correct	Cluster N1	Cluster N2	Cluster N3	Total
Cluster N1	67	12	3	3	18
Cluster N2	70	3	16	4	23
Cluster N3	89	2	2	32	36
Total	78	17	21	39	77

Table 2.5. Habitat variables included in a DFA model for classification of samples from the northern ecoprovince into three clusters (N = 76). Significant variables are in bold. All variables included in classification models are binary variables representing a presence (value of one) or absence (value of zero) of each habitat/environmental feature. For a description of variable abbreviations see Table 2.6.

Variables	Wilks' Lambda	F-ratio	Significance Level
gformE-BL	**0.49**	**4.7**	**p < 0.01**
Michigan	0.46	2.73	p < 0.07
Superior	0.46	2.64	p < 0.08
aqvegOP	0.46	2.54	p < 0.09
gformNONE	0.46	2.32	p < 0.10
gformFL-R	0.46	2.30	p < 0.11
habitatEM	0.46	2.25	p < 0.11
He	0.46	2.10	p < 0.13
aqvegEM	0.45	1.92	p < 0.16
aqvegSU	0.44	1.31	p < 0.27
gformS-CD	0.44	0.91	p < 0.41
habitatOW	0.44	0.76	p < 0.47

Table 2.6. Habitat variable abbreviations and their descriptions. Note: this table does not contain every habitat variable measured, it only shows variables that were included in classification models.

Category	Abbreviation	Description
Aquatic Habitat	habitatEM	Presence/absence of emergent aquatic habitat.
	habitatOW	Presence/absence of open water aquatic habitat.
	habitatMES	Presence/absence of mixed submergent and emergent aquatic habitat.
	habitatFL	Presence/absence of floating leaf aquatic habitat.
	gformE-BL	Presence/absence of broad-leaf emergent vegetation growth forms. E.g. Typha
	gformNONE	Absence of aquatic vegetation growth forms.
	gformFL-R	Presence/absence of floating ribbon vegetation growth forms. E.g. Valisneria
	gformS-CD	Presence/absence of canopy-forming, dissected leaf submergent growth forms. E.g. Myriophyllum.
	aqvegOP	Presence/absence of open water within a surrounding 5m radius.
	aqvegEM	Presence/absence of emergent vegetation within a surrounding 5m radius.
	aqvegSUB	Presence/absence of submergent vegetation within a surrounding 5m radius.
	Sand	Primary sediment characteristic is sand.
	Mud	Primary sediment characteristic is mud.

Table 2.7. Summary of the number of reference samples from the southern ecoprovince predicted to be in each cluster by a discriminant function model based on 4 habitat variables (see Table 2.8).

	Observed		
	% Correct	Cluster S1	Cluster S2	Total
Cluster S1	96	51	2	53
Cluster S2	47	27	24	51
Total	72	78	26	104

Table 2.8. Habitat variables included in a DFA model classifying samples from the southern ecoprovince into two clusters (N = 99). Significant variables are in bold. All variables included in classification models are binary variables representing a presence (value of one) or absence (value of zero) of each habitat/environmental feature. See Table 2.6 for variable descriptions.

Variable	Wilks' Lambda	F-ratio	Significance Level
slhabVB	0.88	27.1	$p < 0.00001$
Cw	0.86	24.2	$p < 0.00003$
slhabHUMAN	0.80	16.0	$p < 0.0001$
Sand	0.73	5.62	$p < 0.02$

Table 2.9. Classification function coefficients used to classify test sites to a reference assemblage cluster on the basis of habitat and environmental variables (See Table 2.6 for variable descriptions). To classify new samples, calculate probability of group membership ('P') for each cluster by plugging values from the table into the following formula: $P = C_0 + C_1X_1 + C_2X_2 + ...C_nX_n$; where C_i are the coefficients for each variable in the model and X_i is the observed value of each variable. Blank cells indicate where each variable was not included in the classification model.

	Northern Ecoprovince			Southern Ecoprovince	
Cluster	N1	N2	N3	S1	S2
Constant	-17.5	-19.9	-16.6	-4.4	-7.3
Superior	11.1	14.3	13.4		
Michigan	10.2	12.5	10.1		
Ontario				2.3	0.7
He	6.3	19.5	7.9		
Cw				-0.6	-5.4
habitatOW	9.0	7.7	6.4		
habitatEM	9.1	11.9	11.9		
habitatMES				-0.5	0.7
habitatFL				0.9	-0.7
gformE-BL	-1.1	-4.1	-4.8		
gformFL-R	4.0	6.0	3.7		
gformNONE	15.3	10.6	12.7		
gformS-CD	3.3	1.9	2.4		
aqvegOP	-4.1	0.8	0.6		
aqvegSU	15.7	17.4	15.4	1.4	3.2
aqvegEM	2.1	-0.4	1.3		
slhabVB				4.6	9.3
slhabHUMAN				4.7	8.3
Mud				0.2	1.5
Sand				3.9	5.7

Table 2.10. Raw regression coefficients (± 1 SE) of important taxa loading on ZACI axes for use in predicting ZACI scores based on **agriculture** stress for new datasets. Entries in bold are taxa contributing the most to high ZACI scores (i.e. positive coefficients) and low ZACI scores (i.e. negative coefficients). Note: Coefficients should be multiplied by the Ln-transformed relative abundance of each taxon on the sample.

	Northern Ecoprovince			Southern Ecoprovince	
	ZACI-N1	**ZACI-N2**	**ZACI-N3**	**ZACI- S1**	**ZACI-S2**
Constant	0.49 ± 0.03	0.40 ± 0.05	0.52 ± 0.03	0.52 ± 0.04	0.69 ± 0.05
Chironomidae	**0.77 ± 0.07**	0.44 ± 0.09	-0.70 ± 0.08	-0.32 ± 0.08	
Gammarus	**-1.0 ± 0.09**	-0.87 ± 0.20	**-0.80 ± 0.07**	-0.42 ± 0.09	
Caecidotea	-0.92 ± 0.12				1.24 ± 0.56
Oligochaeta		**0.76 ± 0.08**		**-0.70 ± 0.08**	**-0.97 ± 0.11**
Hydrobiidae		**-1.0 ± 0.15**	**0.62 ± 0.11**		**3.07 ± 0.60**
Hyallela			0.63 ± 0.14	**1.11 ± 0.08**	
Corixidae			0.37 ± 0.12	-0.46 ± 0.09	
Physella			0.75 ± 0.28		
Aeshnidae			1.61 ± 0.43		
Acari					1.04 ± 0.29
Leptoceridae					3.40 ± 1.21
Caenis					-0.57 ± 0.17
Planorbidae					1.16 ± 0.30
Neoplea					1.77 ± 0.55

Table 2.11. Regression coefficients (± 1 SE) of indicator taxa for use in predicting ZACI scores based on **development** stress for new datasets. Entries in bold are taxa contributing the most to high ZACI scores (i.e. positive coefficients) and low ZACI scores (i.e. negative coefficients). Note: Coefficients should be multiplied by the Ln-transformed relative abundance of each taxon on the sample.

	Northern Ecoprovince			Southern Ecoprovince	
	ZACI-N1	**ZACI-N2**	**ZACI-N3**	**ZACI- S1**	**ZACI-S2**
Constant	0.39 ± 0.09	0.33 ± 0.04	0.63 ± 0.02	0.23 ± 0.02	0.77 ± 0.04
Chironomidae	**0.74 ± 0.17**	**-0.54 ± 0.08**	**-0.44 ± 0.05**		**-0.94 ± 0.14**
Nematoda	**-2.15 ± 0.48**				-1.53 ± 0.29
Hydrobiidae	2.11 ± 0.72		**0.88 ± 0.09**	**-1.71 ± 0.29**	**4.1 ± 0.68**
Oecetis	-9.90 ± 2.61				
Oligochaeta	-0.31 ± 0.15	**1.10 ± 0.07**			
Physella		-1.39 ± 0.29	1.22 ± 0.20		
Caenis		0.55 ± 0.23			
Hyallela				**1.65 ± 0.11**	-1.19 ± 0.27
Gammarus					-0.69 ± 0.24
Lymnaeidae					49.8 ± 13.2
Helobdella					20.4 ± 7.64
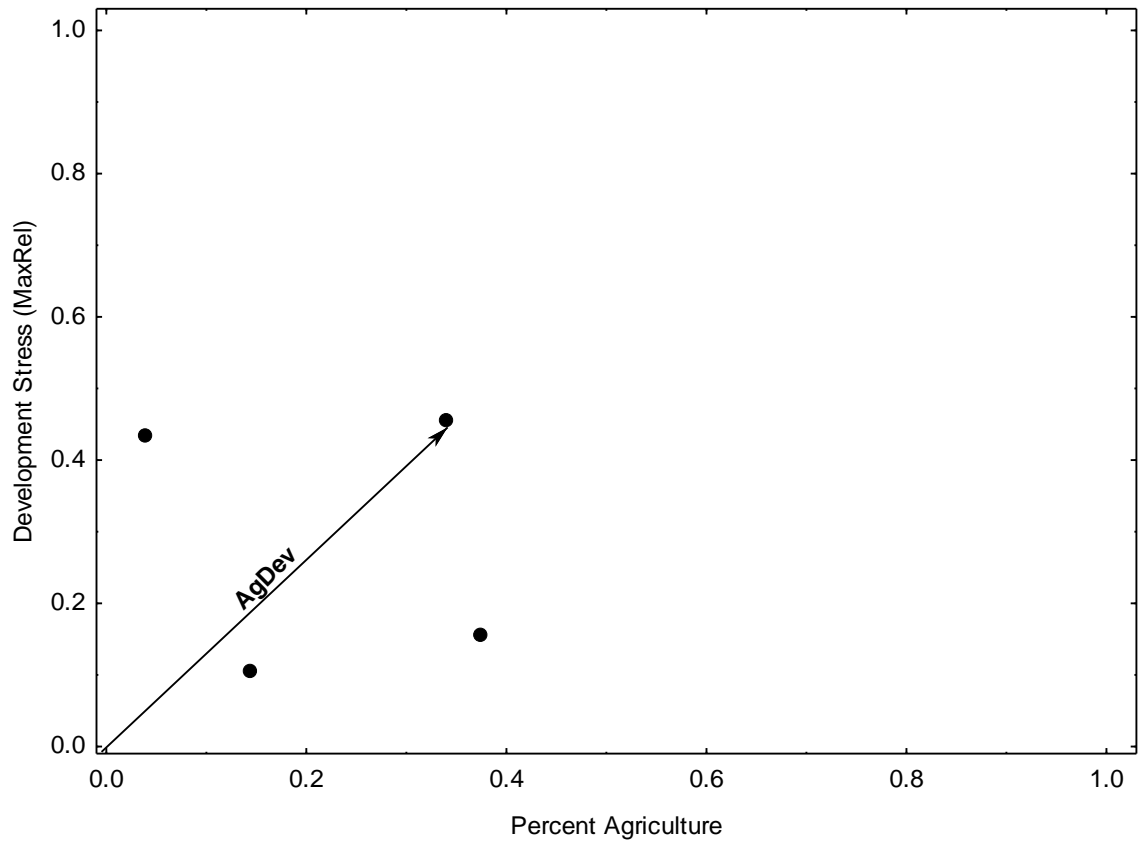
Figure 2.1. Example of how the composite stress index AgDev is calculated. The AgDev index is calculated as the Euclidean distance from the origin to any given point on the x,y plane of % agriculture and MaxRel development stress indices. i.e. $AgDev = \sqrt{Ag^2 + Dev^2}$.
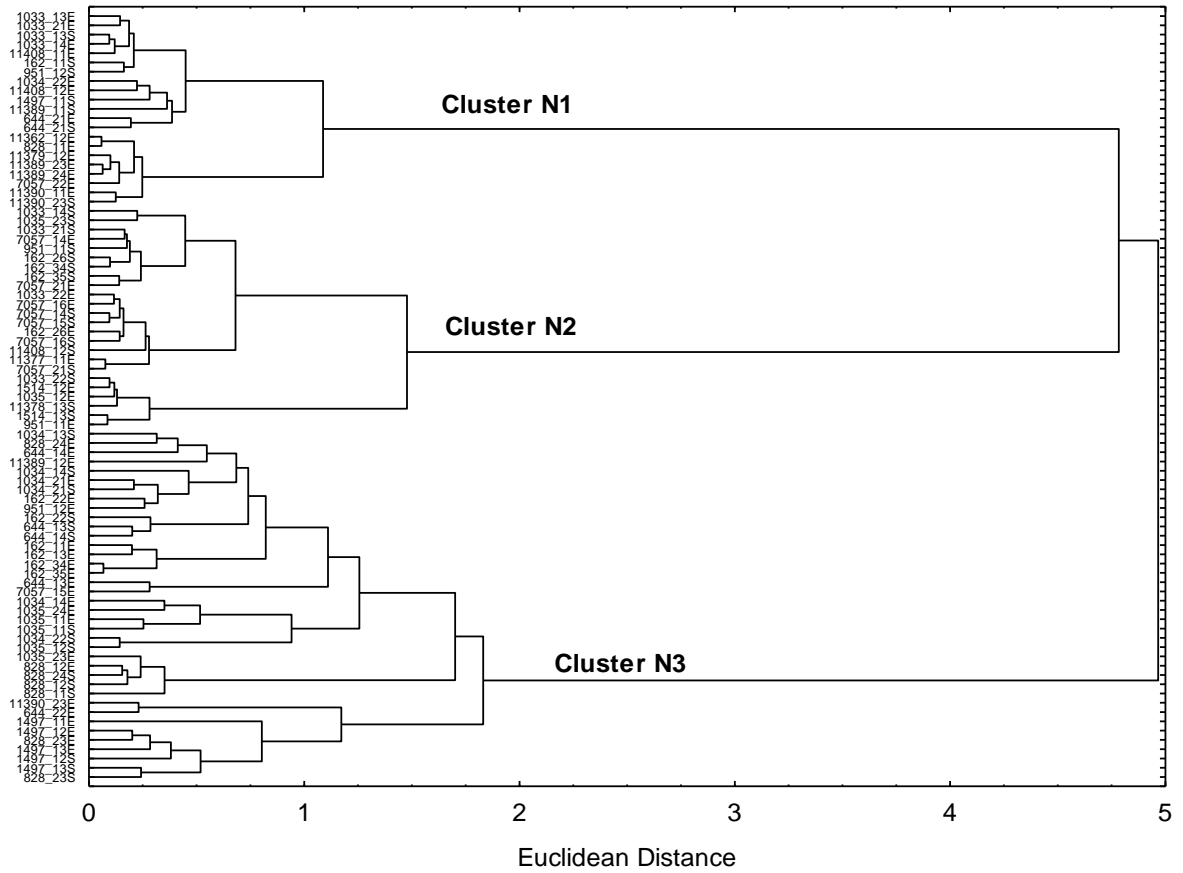
Figure 2.2. Dendrogram of reference sampling points (n = 83) from Ecoprovince 212 grouped according to Ln-transformed relative abundances of invertebrate species using Ward`s method of amalgamation and a Euclidean distance measure.
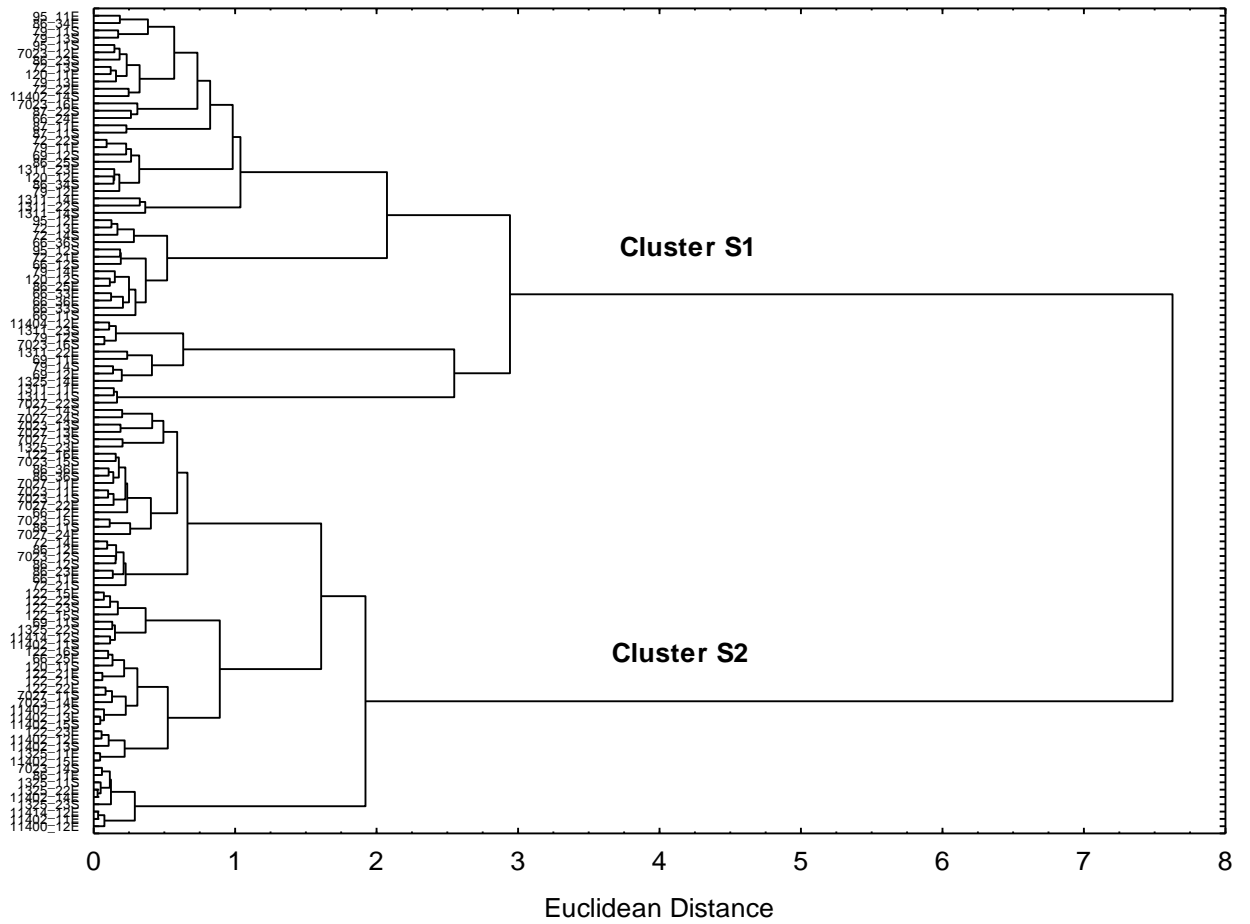
Figure 2.3. Dendrogram of reference sampling points from ecoprovince 222 (n = 112) grouped according to Ln-transformed relative abundances of invertebrate species using Ward`s method of amalgamation and a Euclidean distance measure.

Figure 2.4. Scatterplots of samples in Cluster N1 showing the relationship between the agriculture and development Zoobenthic Assemblage Condition Index (ZACI) scores and land use stress indices. A) Ag-ZACI in relation to percent agriculture in the watershed for sampling points with development stress below 0.10 MaxRel (n= 37). B) Dev-ZACI in relation to MaxRel development stress for sampling points with % agriculture below 10% (n= 32). Low ZACI scores indicate a biological assemblage most similar to the assemblage at the 'degraded' centroid, where stress is greatest. High ZACI scores indicate an assemblage more similar to the 'reference' centroid, where percent agriculture is lowest. Vertical dashed lines indicate the operational reference cut-offs. Least squares regression functions indicated by solid lines (A: Ag-ZACI = 0.92-1.60*(%Ag); B: Dev-ZACI = 0.66-0.61*(Dev)) and LOWESS curves indicated by dotted lines.

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Figure 2.5. Scatterplots of samples in Cluster N2 showing the relationship between the agriculture and development Zoobenthic Assemblage Condition Index (ZACI) scores and land use stress indices. A) Ag-ZACI in relation to percent agriculture in the watershed for sampling points with development stress below 0.10 MaxRel (N= 43). B) Dev-ZACI in relation to MaxRel development stress for sampling points with % agriculture below 10% (N= 40). Low ZACI scores indicate a biological assemblage most similar to the assemblage at the 'degraded' centroid, where stress is greatest. High ZACI scores indicate an assemblage more similar to the 'reference' centroid, where stress is lowest. Vertical dashed lines indicate the operational reference cut-offs. Least squares regression functions indicated by solid lines (A: Ag-ZACI = 0.80-0.77*(%Ag); B: Dev-ZACI = 0.63-1.37*(Dev)) and LOWESS curves indicated by dotted lines.
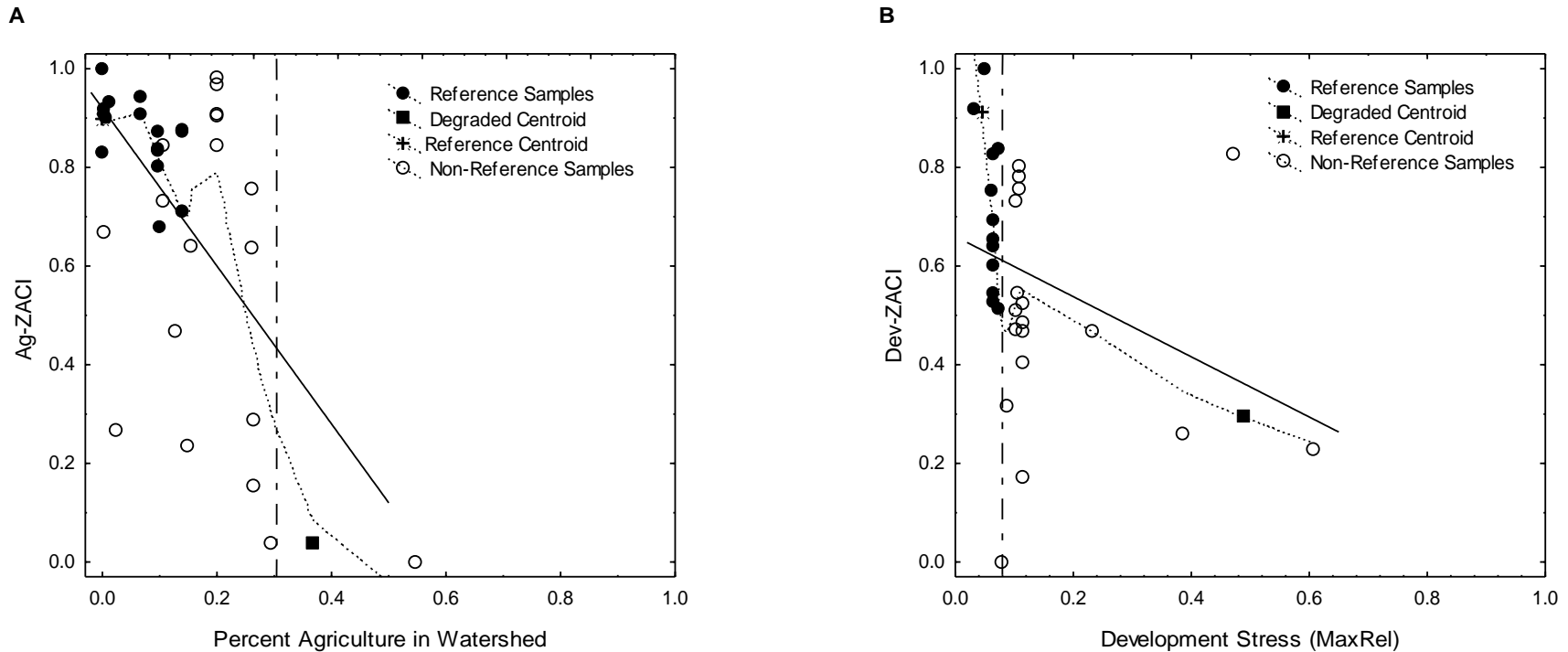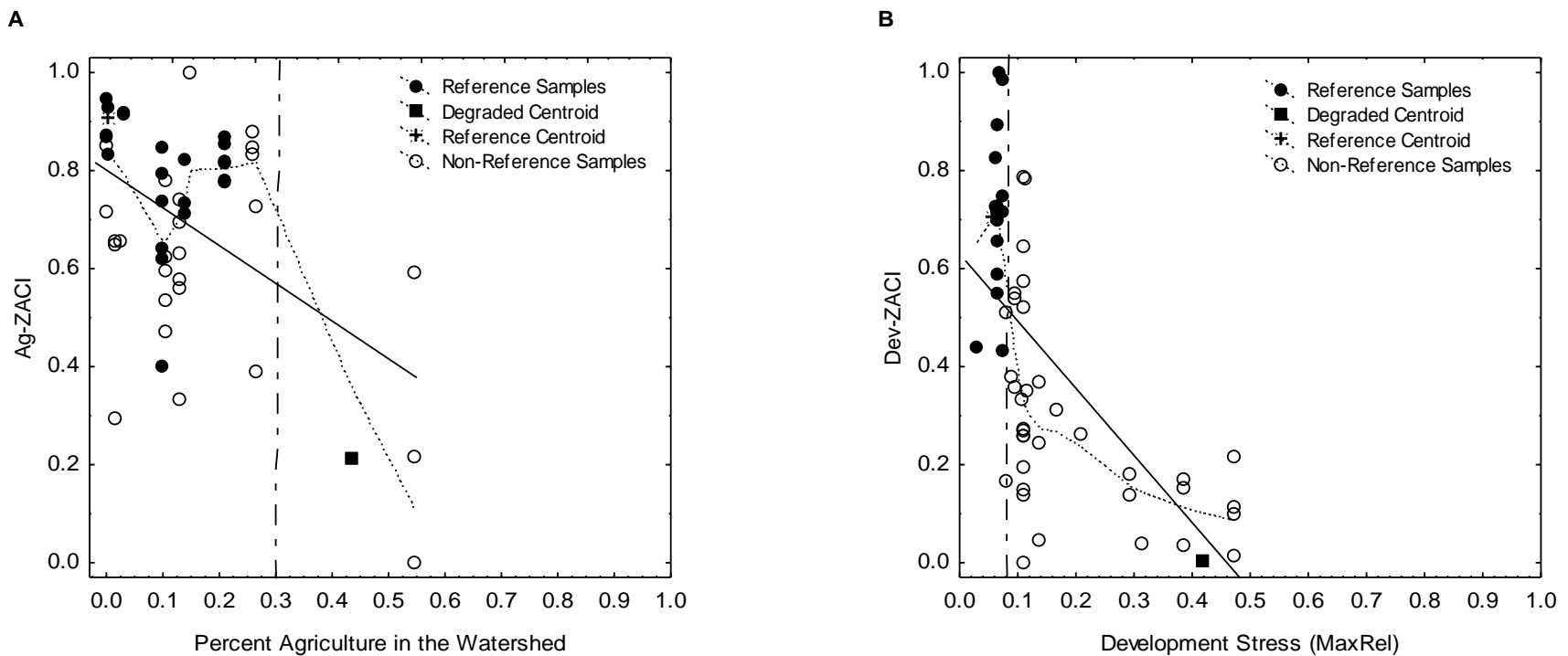
Figure 2.6. Scatterplots of samples in Cluster N3 showing the relationship between the agriculture and development Zoobenthic Assemblage Condition Index (ZACI) scores and land use stress indices. A) Ag-ZACI in relation to percent agriculture in the watershed for sampling points with development stress below 0.10 MaxRel (N = 100). B) Dev-ZACI in relation to MaxRel development stress for sampling points with % agriculture below 10% (N= 96). Low ZACI scores indicate a biological assemblage most similar to the assemblage at the 'degraded' centroid, where stress is greatest. High ZACI scores indicate an assemblage more similar to the 'reference' centroid, where stress is lowest. Vertical dashed lines indicate the operational reference cut-offs. Least squares regression functions indicated by solid lines (A: Ag-ZACI= 0.52-0.78*(%Ag); B: Dev-ZACI = 0.46-1.0*(Dev)) and LOWESS curves indicated by dotted lines.

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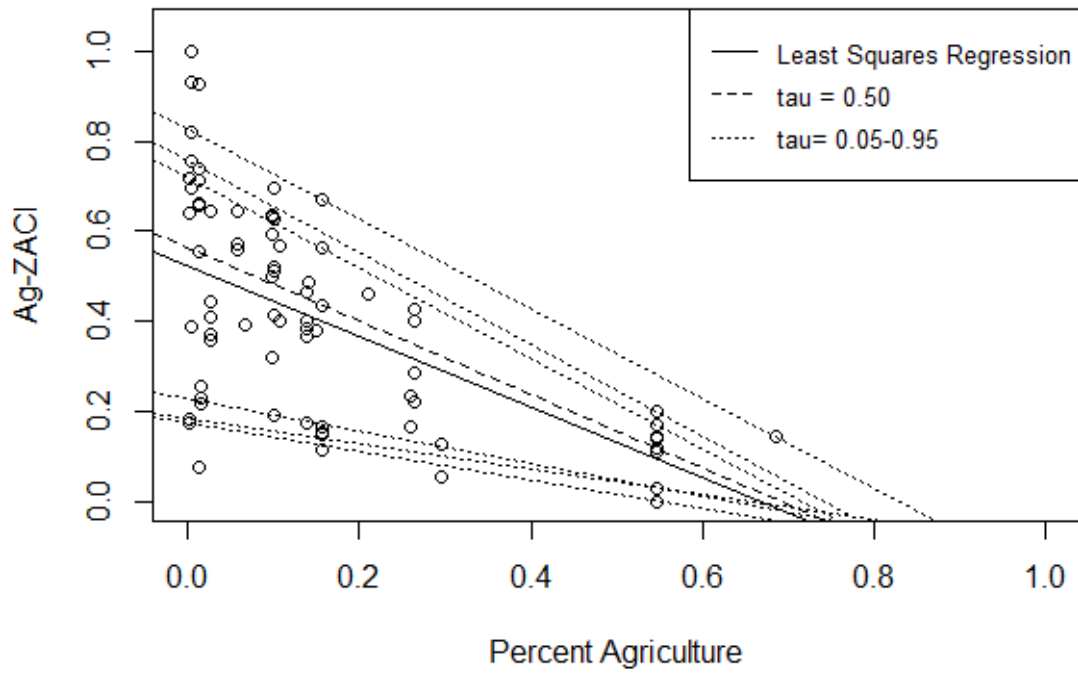
Figure 2.7. The same Ag-ZACI scores (y) as Figure 2.6A as a function of % agriculture, but the lines plotted are (from the bottom) 5th, 10th, 20th, 50th, 80th, 90th and 95th quantile regression estimates as well as the least squares regression line. Slopes for regression quantiles (± 1SE) range from b(0.05) = - 0.32 ± 0.11 to b(0.95) = -0.100 ± 0.17.
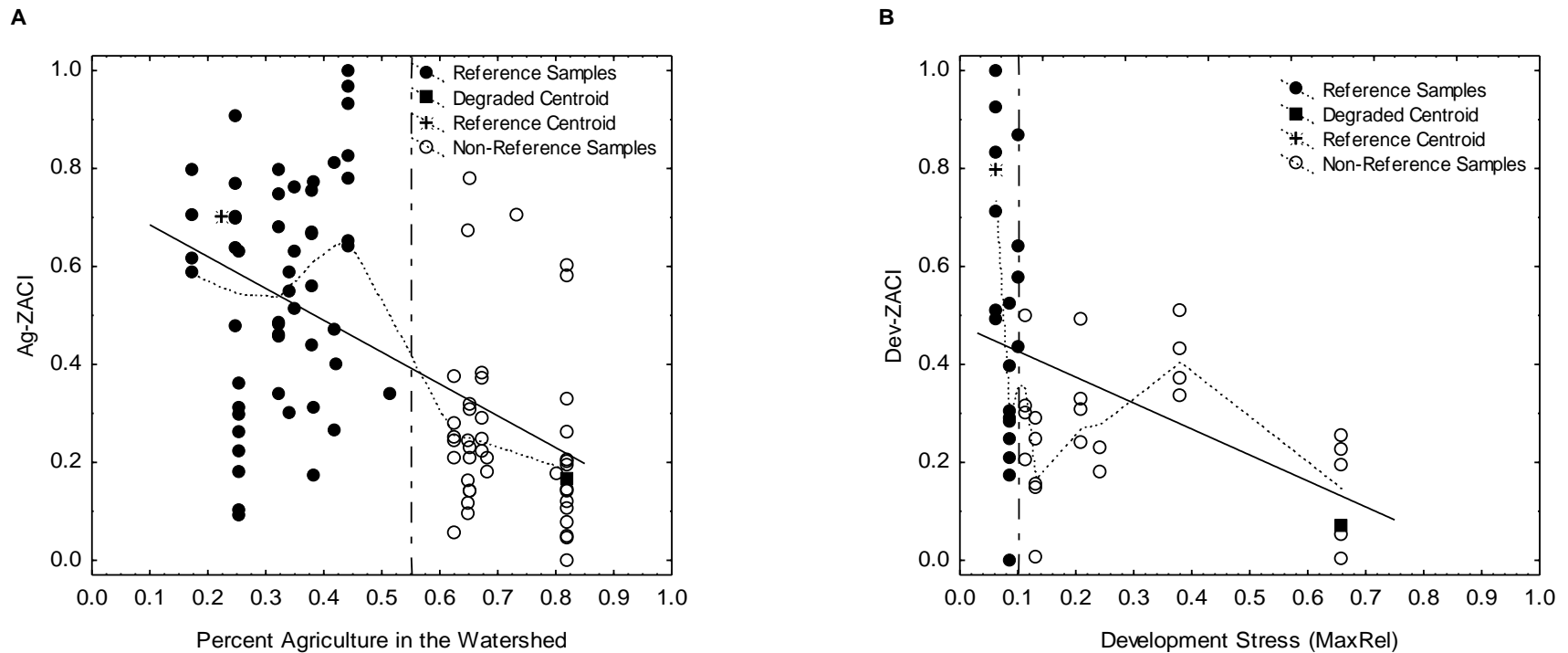
Figure 2.8. Scatterplots of samples in Cluster S1 showing the relationship between the agriculture and development Zoobenthic Assemblage Condition Index (ZACI) scores and land use stress indices. A) Ag-ZACI in relation to percent agriculture in the watershed for sampling points with development stress below 0.10 MaxRel (N= 102). B) Dev-ZACI in relation to MaxRel development stress for sampling points with % agriculture below 30% (N= 37). Low ZACI scores indicate a biological assemblage most similar to the assemblage at the 'degraded' centroid, where stress is greatest. High ZACI scores indicate an assemblage more similar to the 'reference' centroid, where percent agriculture is lowest. Vertical dashed lines indicate the operational reference cut-offs. Least squares regression functions indicated by solid lines (A: Ag-ZACI = 0.75-0.65*(%Ag); B: Dev-ZACI = 0.48-0.53*(Dev)) and LOWESS curves indicated by dotted lines.
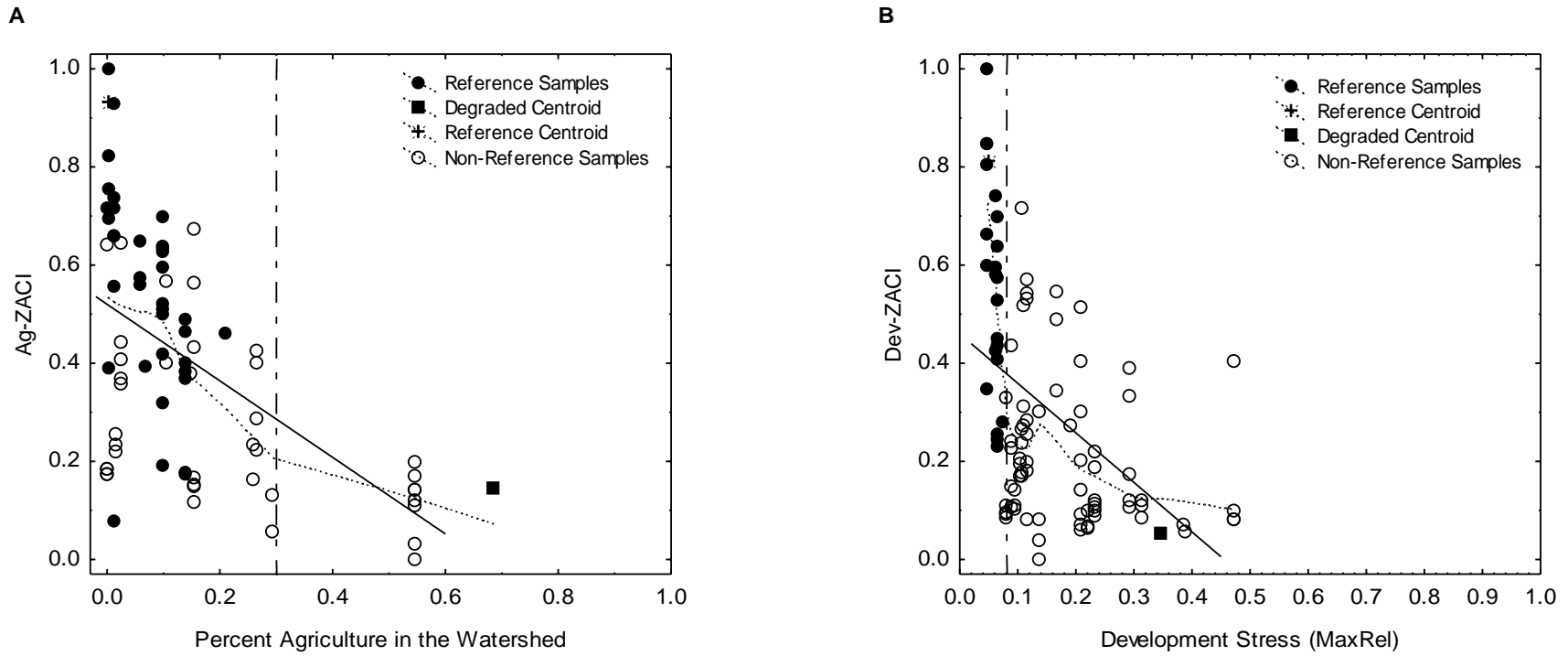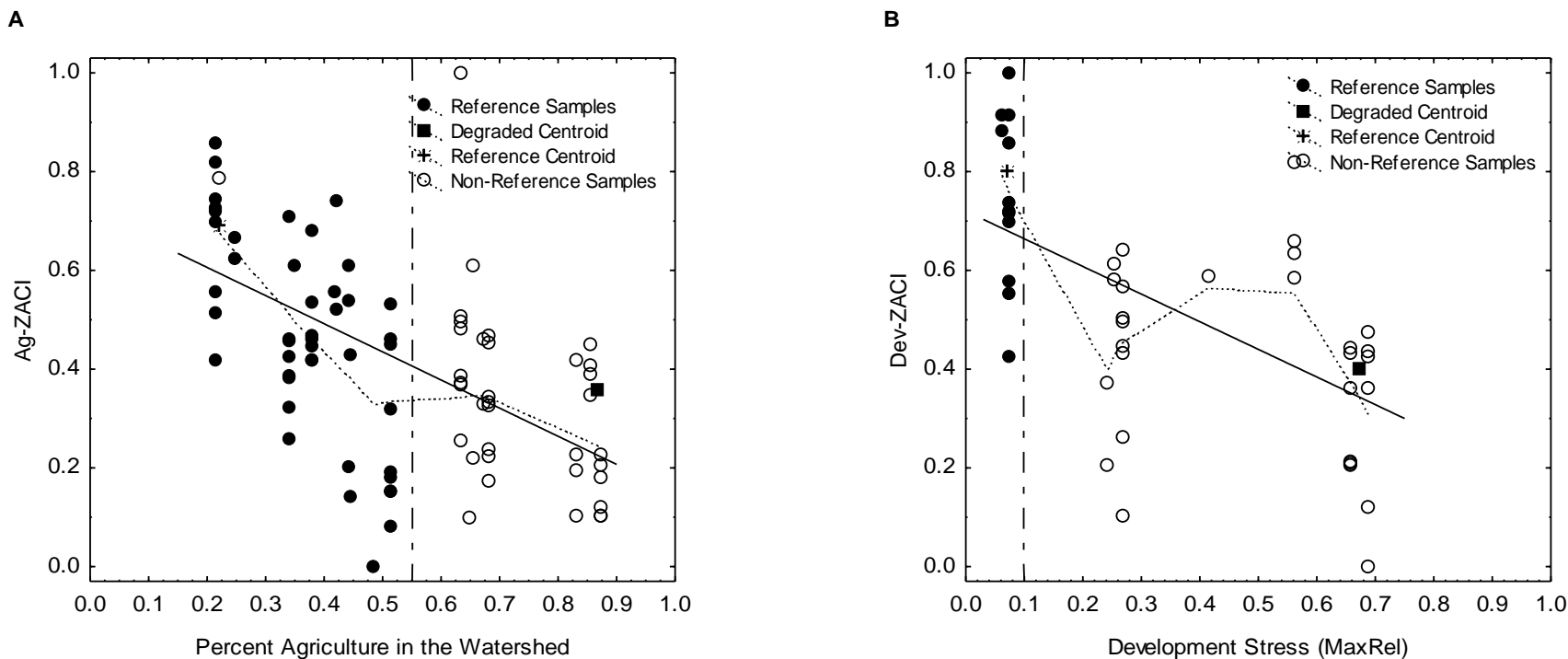
Figure 2.9. Scatterplots of samples in Cluster S2 showing the relationship between the agriculture and development Zoobenthic Assemblage Condition Index (ZACI) scores and land use stress indices. A) Ag-ZACI in relation to percent agriculture in the watershed for sampling points with development stress below 0.10 MaxRel (N= 68). B) Dev-ZACI in relation to MaxRel development stress for sampling points with % agriculture below 30% (N= 51). Low ZACI scores indicate a biological assemblage most similar to the assemblage at the 'degraded' centroid, where stress is greatest. High ZACI scores indicate an assemblage more similar to the 'reference' centroid, where percent agriculture is lowest. Vertical dashed lines indicate the operational reference cut-offs. Least squares regression functions indicated by solid lines (A: Ag-ZACI = 0.72-0.57*(%Ag); B: Dev-ZACI = 0.72-0.56*(Dev)) and LOWESS curves indicated by dotted lines.
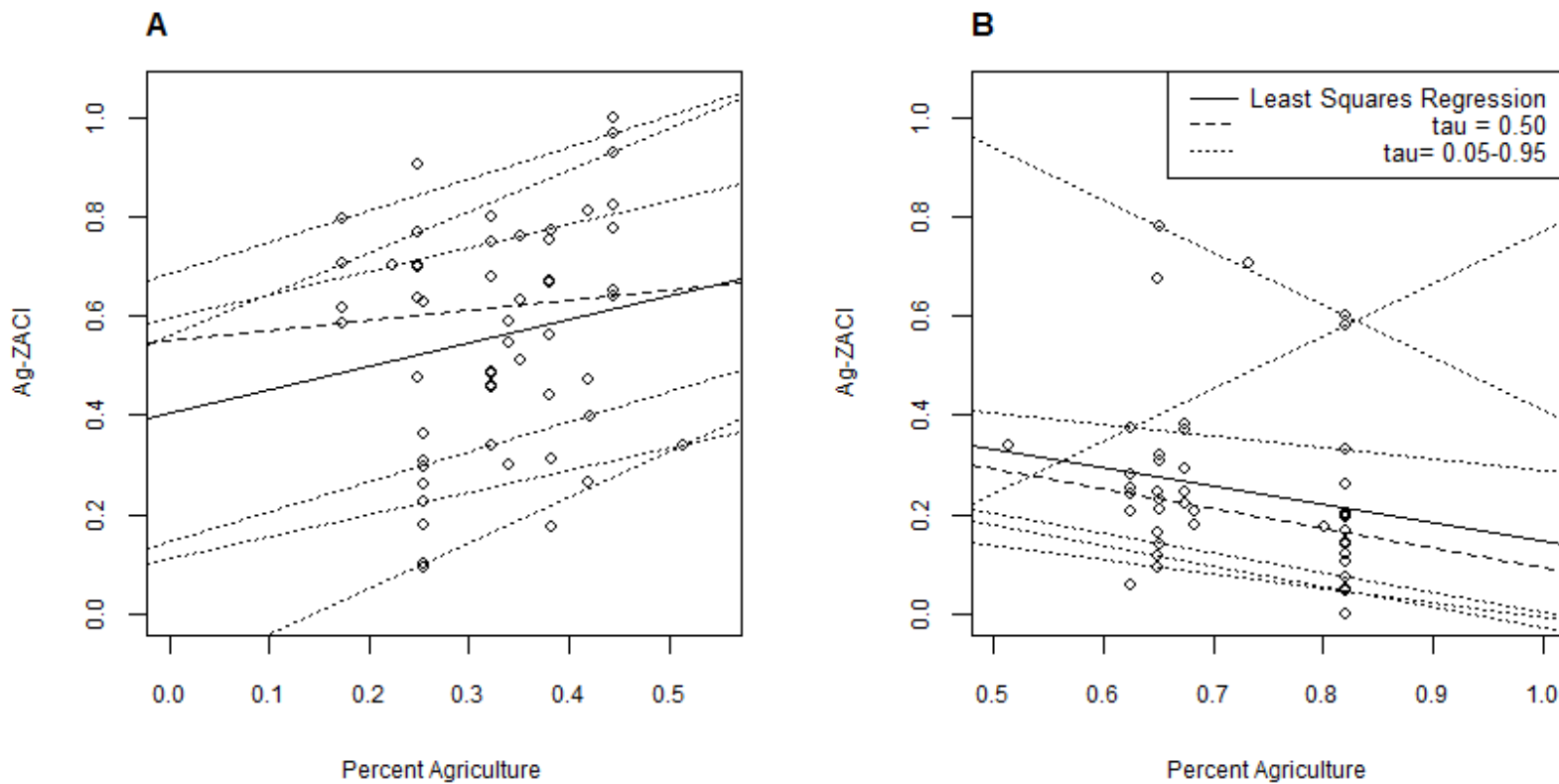
Figure 2.10. Relationship between Ag-ZACI and % agriculture for two subsets of the 102 samples from Cluster S1 with % agriculture < 0.55 (A) and % agriculture > 55% (B). Lines plotted are quantile regression estimates for the 5th, 10th, 20th, 50th, 80th, 90th, and 95th percentiles of data, as well as the least-squares regression line. Slopes of quantile lines ($\pm$ 1SE) range from A) $b(0.05) = 0.99 \pm 0.7$ to $b(0.95) = 0.63 \pm 0.34$ and B) $b(0.05) = -0.55 \pm 0.26$ to $b(0.95) = -1.05 \pm 0.96$.
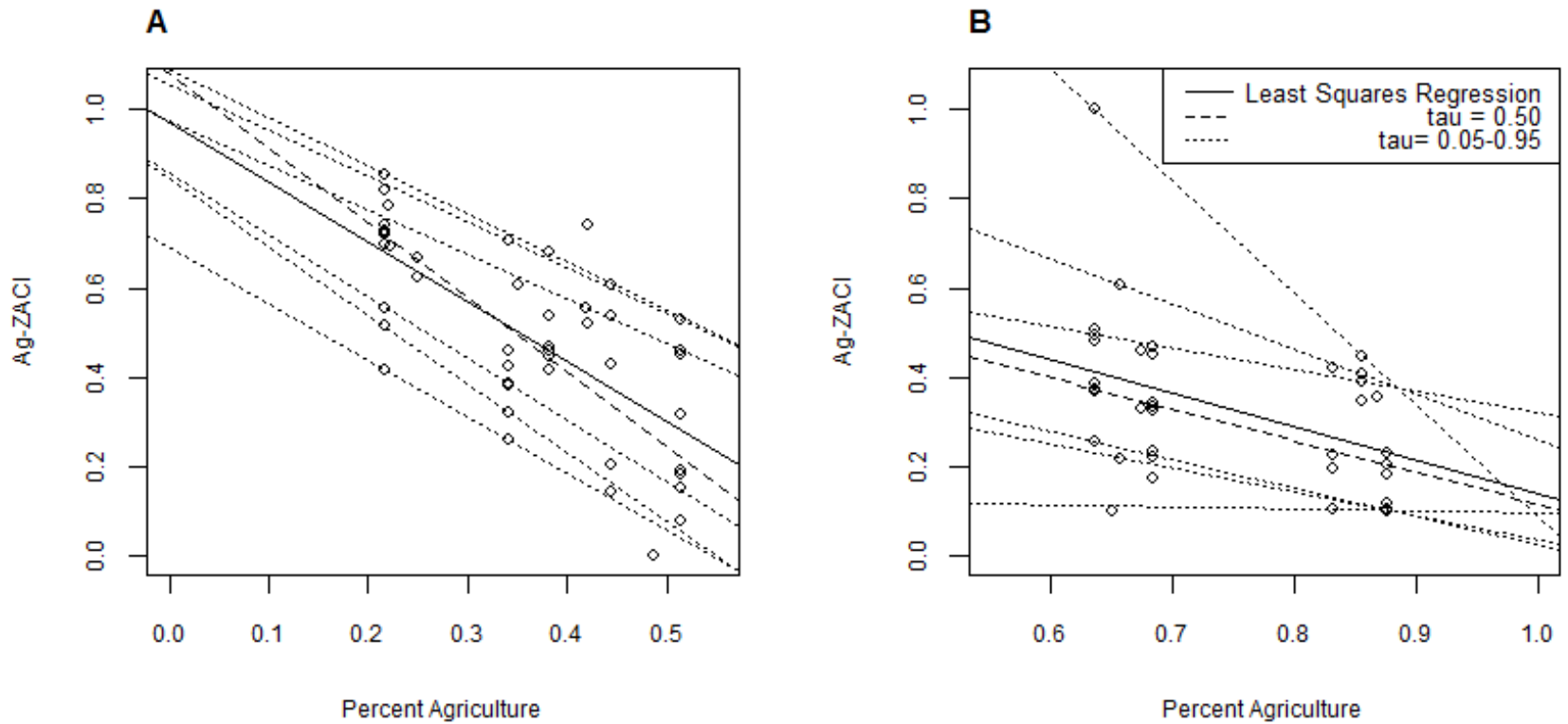
Figure 2.11. Relationship between Ag-ZACI and % agriculture for two subsets of the 68 samples from Cluster S2 with % agriculture < 0.55 (A) and % agriculture $> 55\%$ (B). Lines plotted are quantile regression estimates for the 5th, 10th, 20th, 50th, 80th, 90th, and 95th percentiles of data, as well as the least-squares regression line. Slopes of quantile lines ($\pm$ 1SE) range from A) $b(0.05) = -1.27 \pm 0.38$ to $b(0.95) = -1.07 \pm 0.29$ and B) $b(0.05) = -0.05 \pm 0.30$ to $b(0.90) = -1.01 \pm 0.84$.
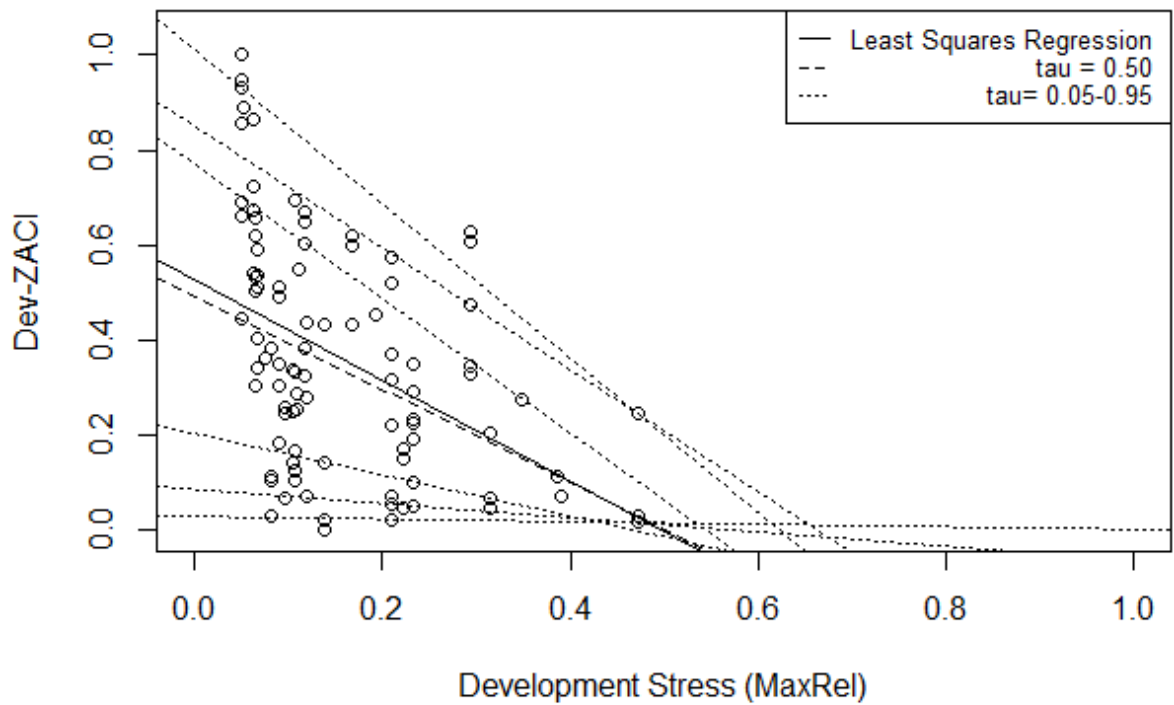
Figure 2.12. The same Dev-ZACI scores (y) as Figure 2.6B as a function of the MaxRel stress gradient, but the lines plotted are (from the bottom) 5th, 10th, 20th, 50th, 80th, 90th and 95th quantile regression estimates as well as the least squares regression line. Slopes for regression quantiles ($\pm$ 1SE) range from b(0.05) = - 0.14 $\pm$ 0.08 to b(0.95) = -1.70 $\pm$ 0.40.
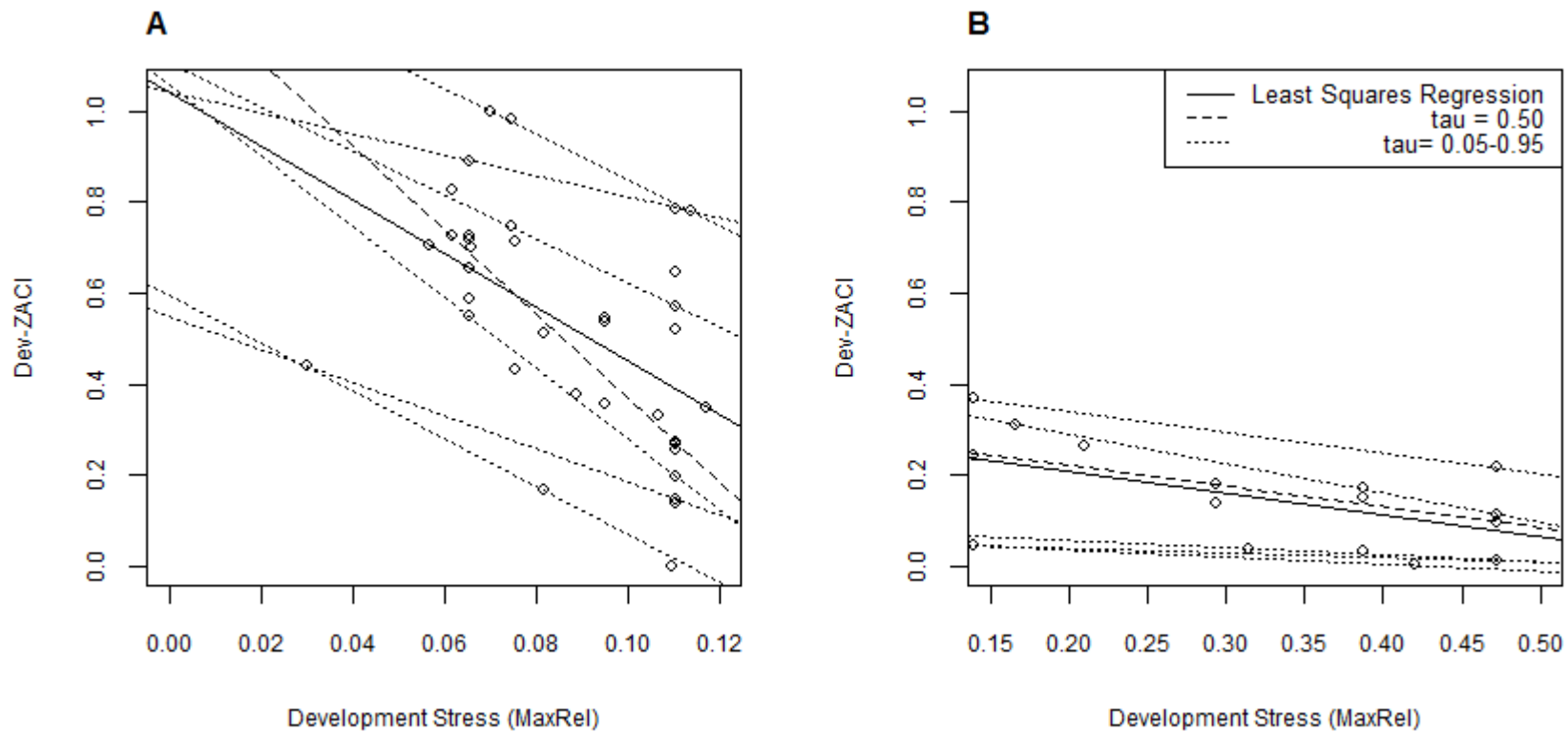
Figure 2.13. Relationship between Dev-ZACI and MaxRel stress for two subsets of the 53 samples from Cluster S2 with MaxRel stress < 0.12 (A) and MaxRel > 0.12 (B). Lines plotted are quantile regression estimates for the 5th, 10th, 20th, 50th, 80th, 90th, and 95th percentiles of data, as well as the least-squares regression line. Slopes of quantile lines (± 1SE) range from A) b(0.05) = - 5. 26 ± 3.22 to b(0.95) = - 5.03 ± 2.66 and B) b(0.05) = - 0.15 ± 0.37 to b(0.95)= - 0.46 ± 0.21.
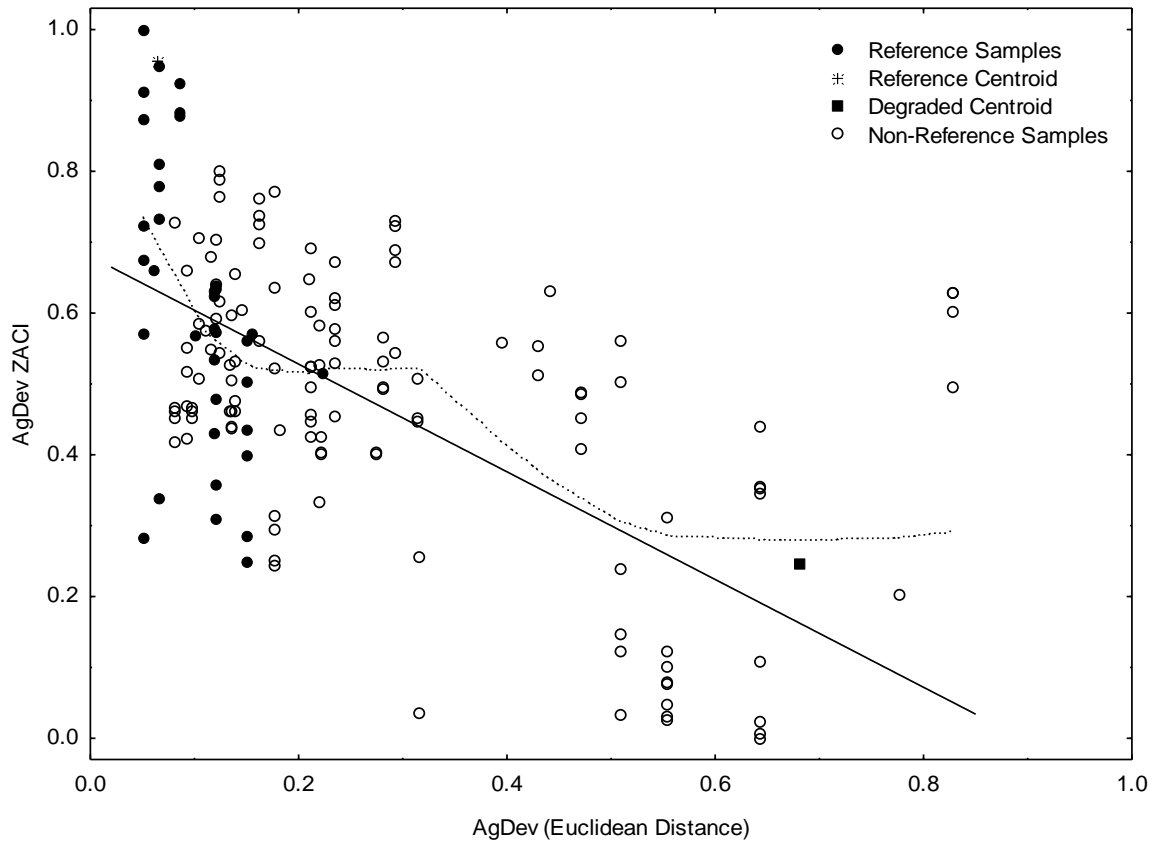
Figure 2.14. AgDev ZACI in relation to the composite stress index for sampling points in Cluster N3 (N = 100). Low ZACI scores indicate a biological assemblage most similar to the assemblage at the 'degraded' centroid, where stress is greatest. High ZACI scores indicate an assemblage more similar to the 'reference' centroid, where stress is lowest. Least squares regression function is indicated by the solid line (AgDev ZACI = 0.68-0.76*(AgDev)) and LOWESS curve indicated by the dotted line.
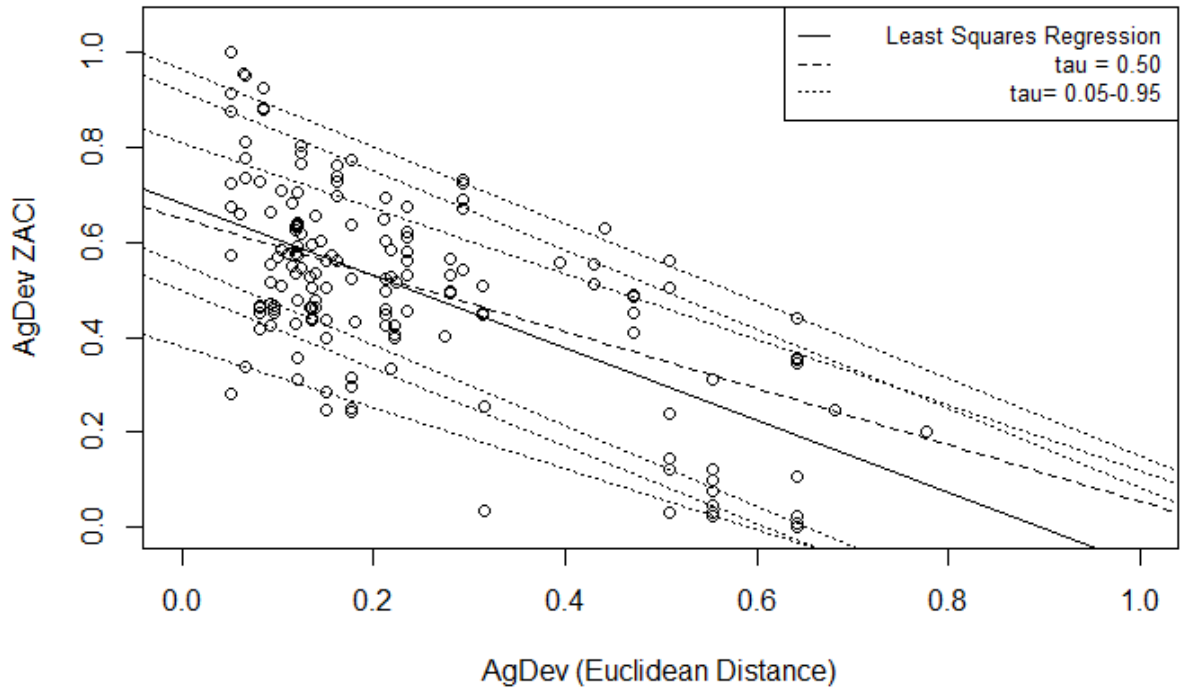
Figure 2.15. The same AgDev ZACI scores (y) as Figure 2.14 as a function of the composite AgDev stress gradient, but the lines plotted are (from the bottom) 5th, 10th, 20th, 50th, 80th, 90th and 95th quantile regression estimates as well as the least squares regression line. Slopes for regression quantiles ($\pm$ 1SE) range from b(0.05) = - 0.64 $\pm$ 0.11 to b(0.95) = - 0.81 $\pm$ 0.10.
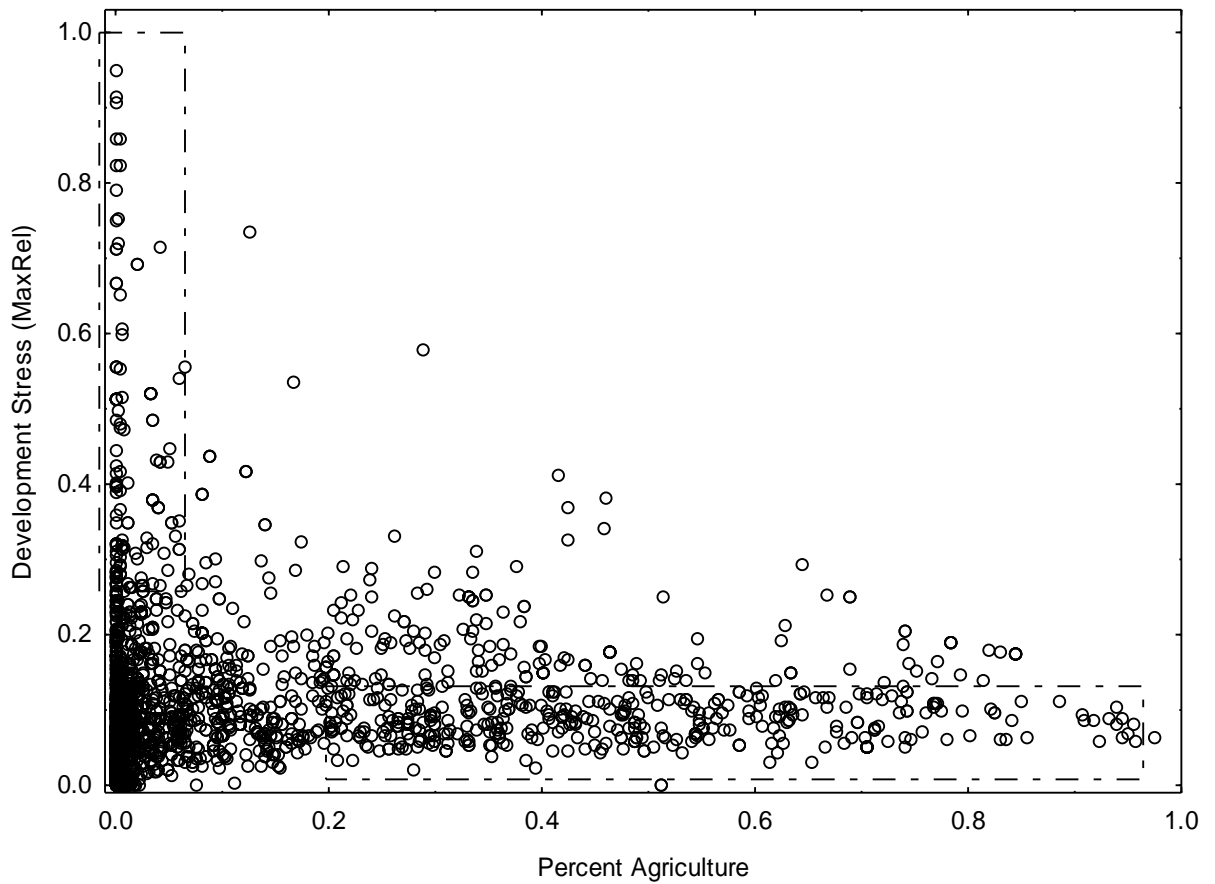
Figure 2.16. Development (MaxRel) and % agriculture stress index scores generated by the GLEI project (Johnson et al. 2015) for 2423 GLEI segment-sheds in the northern ecoprovince of the U.S. Great Lakes basin. Sites with intermediate to high stress scores (denoted with dashed boxes) exist along the % agriculture gradient as well as the MaxRel gradient.

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CHAPTER 3

ASSESSING COMBINED EFFECTS OF LAND-USE STRESSES ON MACROINVERTEBRATE COMMUNITY CONDITION AT GREAT LAKES COASTAL MARGINS

Introduction

Coastal ecosystems of the Great Lakes are subject to a variety of stressors (Brazner et al. 2007, Danz et al. 2005). In order to effectively manage coastal ecosystems in the face of increasing anthropogenic stress, there is a need for the ability to accurately describe and reliably predict the effects of multiple stressors on biological condition. This has prompted a shift of focus from studies of individual stressors, to those of cumulative impacts of multiple, simultaneous stressors with more recent attempts to combine the effects of watershed-based stressors (Environment Canada and US EPA, 2014; Brown et al. 2016; Smith et al. 2015). However, our current capacity to predict the effects of multiple stressors is limited (Downes, 2010). Assessing multiple stressor impact is made difficult by the sheer number of stressors affecting any one ecosystem, as well as potential interactions among stressors that result in unexpected effects. In the Great Lakes alone, dozens of stressors from different classes of human impact co-occur in the basin (Danz et al, 2005; Allan et al. 2013; Smith et al. 2015). This translates to the potential for hundreds of two-way or higher level interactions to influence the way that organisms respond to these stressors (Christensen et al. 2006).

The effects of stressor interactions can be classified as synergies or antagonisms. A synergy is an interaction where the combined impact of two or more stressors on a response variable is more than an expected combined effect calculated from the estimated impacts of the individual stressors alone. Synergies are of particular concern to researchers because of the potential for this interaction to magnify stressor impacts, accelerating degradation of ecosystems (Paine et al. 1998). An interaction is classified as an antagonism when the net effect of multiple stressors produces a response that is smaller than the effect estimated from individual sources of stress.

Predicting cumulative impacts and identifying interactions among multiple stressors is further complicated by the fact that different null models can be used to calculate the expected

cumulative effect. The interpretation of multiple stressor interactions (and the operational definitions of synergism and antagonism) depends on which of the null models is assumed. Three general models can be used to evaluate interactions among multiple stressors: the additive, multiplicative and comparative effects models (Folt et al. 1999).

Comparative effects model- A comparative effects model predicts that the cumulative effect of multiple stressors is equal to the effect of the strongest or dominant stressor (Bruland et al. 1991). Leibig's law of the minimum describes a similar situation for effects of limiting nutrients on plant growth (Haefner, 2005). Only the nutrient that is the most limiting affects growth at any one time. Similarly, when the strongest stressor is exerting an effect, lesser stressors have no additional impact. When assuming the comparative effects model, synergism and antagonism occur when the cumulative effect is greater or less than, respectively, the effect of the single greatest stressor.

Additive effects model- This is the most commonly assumed model in studies of multiple stressor interactions. If the stressors in a set are independent (i.e. they do not interact) then the cumulative effect should be the sum of the individual effects. However, if there are interactions among multiple stressors the cumulative effect may be greater than or less than the sum of their individual effects (i.e. synergism and antagonism, respectively).

Multiplicative effects model- This model is applicable when stress from one source can be further operated upon by another source. The multiplicative null model predicts that the combined effect is approximately equal to the *product* of the individual effects. Synergism and antagonism occur when the combined effect is greater than or less than the product of individual effects, respectively.

The type of interactions identified can vary depending on which of the above models is used to calculate the null effect. For example, the impact of multiple stressors predicted by a

comparative effects model tends to be smaller than the predicted impact under an additive model. Therefore, the combined effect of two stressors could be labeled as an antagonism under the additive model when in reality the combined effect is comparative. This could be an important consideration for conservation and management assessments since the response is actually driven by a single, important stressor.

Previous work to assess the influences of multiple stressors includes many factorial experiments investigating the combined effect of two or more specific stressors on a variety of ecological responses. Examples include the combined effects of high temperature, toxins and low food on zooplankton populations (Folt et al. 1999), effluent concentration and low DO on mayflies (Lowell and Culp, 1999), nutrients and organic matter on food web properties (O'Gorman et al. 2012) and water quality, thermal regime and invasive species on zooplankton communities (Palmer and Yan, 2013) to name just a few.

Instead of trying to identify and label the outcome of every multiple stressor combination (a limitless task), a focus on identifying generalities about ecosystems and stressor responses could be more useful for conservation and management. For example, identifying responses to stressors that require different management interventions or are relevant to different conservation goals (Pearsall et al. 2013) would be a more useful exercise. Clearly, describing the combined effect of two specific stressors requires enough knowledge of the system and the mechanisms in order to choose an appropriate null model. Misspecification of the null model can lead to misinterpretation of stressor interactions. Many researchers assume the additive null model without stating support for the decision (Folt et al. 1999), including the examples mentioned above. Focusing on a subset of data from Chapter 2, this chapter uses three approaches as a proof of concept to assess how sources of watershed land-use stress may combine and interact to influence macroinvertebrate community condition. The influence of combined stress on the ZACI was visualized using scatterplots in Approach 1 and response surface estimation in Approach 2.

The third approach was used to identify whether there is evidence of interaction between the two major sources of stress summarized by the Ag and MaxDev indices. I expected the combined effects of agriculture and development stress on macroinvertebrate community composition to be greater than the effects of each stress type alone; in particular I expected to find evidence of a synergistic interaction. If effective, these approaches could be used to diagnose when cumulative effects occur and ultimately provide information useful for mitigating the effects of agriculture and development on biota at Great Lakes coastal margins.

Methods of Assessment

Approach 1: Comparison of ZACI-index scores at sites with single-stress to sites subject to combined stress

This approach involves observing how ZACI scores are influenced when samples collected from sites exposed to a single stressor are compared to scores for samples collected from locations having both stresses. For example, the Ag-ZACI index developed in Chapter 2 was derived from samples collected from locations with agriculture only (low development stress, or MaxRel scores < 0.10). The question is, how do Ag-ZACI scores differ for a particular level of agriculture if the site is also subject to development stress? To answer this question I used the Ag-ZACI index coefficients (see Table 2.10) to calculate Ag-ZACI scores for samples collected from Cluster N3. I then classified sites as being affected by agriculture only (those with MaxRel stress scores <0.10) and sites subject to MaxRel stress > 0.10. The classified sites were then plotted on a graph of Ag-ZACI vs. % agriculture. The same procedure was repeated with the Dev-ZACI scores to determine how Dev-ZACI scores change when % agriculture is > 10 in the watershed. If the ZACI scores of samples from locations having both agriculture and development stress are generally lower than the ZACI score for samples with individual stressor types this may indicate the presence of possible additive or non-additive (i.e multiplicative) negative cumulative effects. If one observes greater ZACI scores at samples with combined stress, this could indicate a

possible antagonism. If there are no differences in ZACI scores for sites that occur in locations with combined stress, this could also indicate antagonism or a possible comparative effect.

Approach 1 Results

There were marked differences in Ag-ZACI scores of samples that have MaxRel scores < 0.10 and samples that have MaxRel scores > 0.10 (Fig. 3.4). The Ag-ZACI scores vs. % agriculture in watershed for samples with MaxRel development stress > 0.10 were lower (i.e. more degraded) than were the scores for samples from locations with an equivalent amount of agricultural stress but < 0.10 MaxRel stress. Below the agriculture reference threshold the Ag-ZACI scores for samples with > 0.10 MaxRel remained below 0.60 for all levels of % agriculture. However, the converse was not true. When Dev-ZACI scores were plotted on a graph of Dev-ZACI vs. MaxRel stress, samples with > 10% agriculture remained within the distribution of samples with < 10% agriculture (Fig. 3.5).

Approach 2: ZACI score Surface-response estimation using Non-Parametric Multiplicative Regression

Non-parametric multiplicative regression (NPMR) models were used to estimate community response (measured by ZACI scores) to the simultaneous effects of two predictors - amount of agriculture (percent) and development in the watershed (MaxRel stress). Non-parametric multiplicative regression estimates a two-dimensional response surface using a multiplicative kernel smoother with a local mean estimator and Gaussian weighting function (McCune, 2006). The goal of this analysis was to determine the extent to which including a multiplicative estimator could describe the ZACI response surface and whether changes in ZACI scores were gradual or showed evidence of thresholds in 3-dimensional space (Lintz et al. 2011).

Individual response surface values are estimated using information borrowed from observed response values nearby in the predictor space. The observed values are weighted (using a Gaussian weighting function) by their proximity to the target point. Weights for each observed value are calculated by taking the product of weights for individual predictors, thereby

automatically including interactions. The final estimate of the response at a target point is the average of the weighted observed values. The tolerance of a response variable to a continuous predictor is a measure of how broadly we need to borrow information from nearby in the predictor space. The neighborhood size is the amount of data contributing to each estimate of the response variable. A minimum neighborhood size can be set to protect against estimates where there is insufficient data.

NPMR in HyperNiche

The software HyperNiche (McCune and Mefford, 2009) performs NPMR as an iterative search to select predictors and maximize fit. Predictor variables are added in forward stepwise fashion and the software does a grid search at each step. Leave-one-out cross validation is always included in model fitting and evaluation to reduce overfitting. Other overfitting controls include setting a minimum acceptable average neighborhood size, setting an improvement criterion, and setting a minimum data:predictor ratio. Larger minimum average neighborhood sizes (producing stiffer curves) are needed with small data sets or clumped data distributions. Improvement criterion refers to a percentage improvement in model fit when a new predictor is added and setting a minimum data: predictor ratio prevents fitting models with too many predictors compared to sample units.

Model Evaluation

After a free search of all possible NPMR models, the fit of each model is evaluated by the size of the residual sum of squares (RSS) compared to the total sum of squares (TSS) (McCune, 2006), resulting in a cross-validated R^2 (i.e. xR^2):

$xR^2 = 1- (RSS/TSS)$

The relative importance of each predictor in the chosen model can be evaluated with a sensitivity analysis. This involves nudging values of each predictor up or down and measuring the resulting change in response variable. The greater the sensitivity (denoted with 'Q') the more

influence that predictor has in the model. A sensitivity of $Q = 1.0$ means that on average, nudging a predictor results in a change in response of equal magnitude. A sensitivity of $Q = 0$ means that the predictor has no influence on the response variable in the model (McCune, 2006).

Statistical significance of a selected model is evaluated by randomization tests where the response values are shuffled and the same model fitting procedure is applied and the resulting fit is calculated. The procedure tests the null hypothesis that the fit of the selected model is no better than could be obtained by chance alone, given an equal number of predictors (McCune, 2006). The proportion of randomization runs that result in an equal or better fit is used as the p-value for the test.

The response variables used in this analysis were Ag-ZACI and Dev- ZACI scores for samples in Cluster N3 from the northern ecoprovince. Each ZACI response variable was modeled separately in response to two simultaneous predictors- % agriculture and development (MaxRel) stress gradients.

Approach 2 Results

Ag-ZACI Response to Multiple Stressors

A free search for all possible models was conducted using conservative over-fitting controls, including a large minimum acceptable average neighborhood size of 28.4 and an 8% improvement criterion. The free search resulted in a list of 277 models. The best model fitted included both of the stressor predictor variables (%Ag and MaxRel) and had a cross-validated R^2 (xR^2) of 0.40, which is relatively weak. Tolerance of the % agriculture and MaxRel predictors was 0.288 and 0.021, respectively. Results of the sensitivity analysis revealed that on average, MaxRel was a more influential predictor ($Q = 2.47$) than was % agriculture ($Q = 0.20$). A randomization test was performed with 40 runs and none of the randomization runs had a fit equal to or better than the observed fit ($p = 0.024$).

A contour plot of the estimated response of Ag-ZACI shows that Ag-ZACI scores decline with increasing amounts of both agriculture and MaxRel development stress (Fig. 3.6.). However, Ag-ZACI appears to decrease more sharply in response to the MaxRel stress gradient. Slices through the data at several levels of % agriculture show that around 0.10 MaxRel the rate of decrease actually slows when % agriculture is above 40%. However, as MaxRel increases above 0.12 the decline in Ag-ZACI is greater with increasing % agriculture (Figure 3.7).

Dev-ZACI Response to Multiple Stressors

A free search with minimum acceptable average neighborhood size of 31.20 and an 8% improvement criterion resulted in 263 possible models. The best model fitted to both predictors had an xR^2 of 0.22 and predictor tolerances of 0.37 and 0.021 for % agriculture and MaxRel respectively. The sensitivity analysis revealed that on average, % agriculture was much less influential in the model compared to MaxRel (Q = 0.091 and Q = 2.53, respectively). A 40-run randomization test revealed that the selected model did not occur by chance alone (p = 0.024).

Visualized on a contour plot, the model estimates a decrease in Dev-ZACI scores with increasing MaxRel development stress (Figure 3.8.). When slices of the model are examined there seems to be a potential antagonism at levels of development between 0.08 and 0.15 where increasing levels agriculture cause an increase in Dev-ZACI (i.e. an increase in estimated biological condition). However, above 0.15 MaxRel there is a sharp decrease in Dev-ZACI, at all levels of % agriculture (Figure 3.9).

Approach 3: Ordination approach to delineating Isopleths distinguishing Reference from Nonreference conditions.

This approach involves two stages of assessment: the first follows the Reference Condition Approach (RCA) for evaluating the boundaries of the range of natural variation (reference condition) and then determining the degree of deviation of a test site from the Reference Condition (Bailey et al. 2004). Taxa relative abundance in samples collected from

reference and test locations corresponding to Cluster N3 were combined into a single data set, comprised of 161 sampling points (36 of which were reference sites and 125 of which were test sites) and Ln-transformed relative abundances of 48 taxa. A Bray-Curtis polar ordination was conducted in species space along two axes. The variance-regression method of endpoint selection was used because it tends to exclude outliers. The Sorensen (Bray-Curtis) distance measure was used and both axis projection geometry and residual distances were measured in Euclidean distances. A scatterplot of the Axis 1 and 2 ordination scores for each sample was created, and an 80% confidence ellipse was drawn around the reference samples. The choice of an 80% confidence ellipse was subjective, but it provided the polygon that best enclosed all reference sites while excluding test sites.   If samples fall within the 80% confidence ellipse the assemblage in those samples is within the range of assemblages characteristic of the Reference Condition and are classified as "equivalent to reference". Samples situated outside the range of variability in reference assemblages (i.e. outside the ellipse) are classified as "not equivalent to reference". The purpose of this part of the approach is to determine which test samples have assemblages that are equivalent to and not equivalent to the assemblages in reference samples.

The samples thus classified were then plotted on an agriculture (X-axis) vs. MaxRel (Y-axis) stress graph to illustrate the degree to which the two classes of samples (reference/ nonreference) are separated or interspersed with respect to the stress gradients. Plotting the sample classes on the stress axes allows one to determine if there is a consistent graph-spatial distinction between sites that are equivalent-to-reference vs. not-equivalent-to-reference that can be explained by levels of anthropogenic stress. If one can identify an isopleth that separates the two "states" along the stress axes, one may be able to infer whether there is evidence of interactions by the shape of reference-nonreference isopleth. If the isopleth is a straight line (Figure 3.1a) it would imply the stressors combine independently to exert their effect. If the isopleth function is convex, this implies antagonism since the boundary between ref/non-ref is

farther away from the origin than occurs if the stress effects are additive (Figure 3.1b). Similarly, a concave line isopleth implies that there is a synergism because it takes less of each stressor for communities to become non-reference (Figure 3.1c).

Approach 3 Results

The 2-axis polar ordination extracted a total of 33% of the variation in the original distance matrix with Axis 1 extracting 22% and Axis 2 accounting for 11% of variation. Axis 1 represented a gradient of samples ranging in composition from those with high relative abundance of Chironomidae or Oligochaeta to samples with high relative abundance of *Gammarus*. Axis 2 represented a gradient of samples with high relative abundance of Chironomidae to samples with high relative abundance of *Hyalella* and Corixidae. Of 125 test samples, 54 fell within the 80% confidence ellipse encircling the reference samples (Figure 3.2) and thereby were classified as "equivalent to reference".

When classified samples were plotted on the agriculture-development stress graph, it was found that multiple samples collected from within the same site overlapped because stress scores are assigned at the site level (i.e. samples from the same site all have the same stress scores). Therefore, any separation between samples classified as reference and those classified as non-reference may be hidden by this overlap. After examining the classifications of samples taken from the same sites it was found that the majority (e.g. 2/3, 4/5, etc.) of samples at a site had the same classification. Consequently, the predominant classification within each site was illustrated in the plot of % agriculture vs. MaxRel (Fig 3.2).

An isopleth line was drawn by eye to separate the group of sites closest to the origin that consisted entirely of "equivalent to reference sites" (Figure 3.3). All sites that were subject to agriculture levels less than 30% and had MaxRel score of 0.08 or less were classified as equivalent to reference because these sites were the TITAN reference sites designated a priori. Above 0.08 MaxRel and 22% agriculture, site classifications are mixed but there were more sites

classified as "equivalent to reference" than "non-reference". Sites located in watersheds subject to high levels of stress (above 0.30 MaxRel and 45% agriculture) were all classified as "not equivalent to reference". A second isopleth was drawn to enclose all the reference sites and to connect the levels of each stressor at which the state of all sites became "not equivalent to reference". The shape of both hand drawn isopleths tended towards convex (Figure 3.1b), implying the effects of combined land use are antagonistic rather than independent. However, there were too few sites draining watersheds with a significant combination of both agriculture and development to allow a formal statistical test to be performed.

<div align="center">

Discussion

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Approach 1: Combined Stress ZACI Plots

Samples from watersheds with > 0.10 MaxRel development had lower Ag-ZACI scores than samples from watersheds with the same degree of agricultural stress but with < 0.10 MaxRel development. This suggests that the existence of significant amounts of development in a watershed further reduces zoobenthic community condition above an effect of agriculture alone. In contrast, the addition of > 10% agriculture did not lower the Dev-ZACI scores below the range of samples with <10% agriculture. Together, these results indicate that agriculture and development follow a comparative effects model for assemblages in this cluster, whereby the presence of agriculture in a watershed has no additional detrimental effect when significant amounts of development are present. This finding has significant implications for managing watersheds in which both agriculture and development are present. If a watershed has a development score of over 10% it may be most effective to implement management practices that mitigates the impacts of development, even if there is a greater amount of agricultural land cover in the watershed (Walsh et al. 2005, Withey et al. 2012; Brown et al. 2014). This adds support to other studies that have also found urban development to be the more severe stressor compared to agricultural land use (Kovalenko et al. 2014; King and Baker, 2011). Development stress can be

more severe (especially for coastal zones) because of direct habitat destruction and degradation and shoreline modification (Lee et al. 2006).

Approach 2: Nonparametric Multiplicative Regression

The NPMR model estimating Ag-ZACI scores in response to both stressors revealed that Ag-ZACI was broadly tolerant to % agriculture in the watershed (tolerance = 0.288) compared to MaxRel (tolerance = 0.021). This, in addition to the sensitivity analysis indicated differences in MaxRel scores to have more influence on the Ag-ZACI than % agriculture. This was also true for the Dev-ZACI model. These results corroborate the findings of the previous approach and suggest that although agriculture by itself has an effect on macroinvertebrate communities, development stress has a greater impact when it is present.

Contour and slice plots indicated that both agriculture and development have an effect on ZACI scores only at levels of development below 0.20 MaxRel. Above 0.20 MaxRel stress, agriculture had no additional effect on either Ag or Dev ZACI scores. Interestingly, at certain levels of MaxRel stress (~ 0.10-0.15) there seems to be a mitigating effect of % agriculture. In particular, the Dev-ZACI response model (Figure 3.8) predicted greater values of the Dev-ZACI index at greater % agriculture values when MaxRel scores were in the range of 0.10-0.15. However, examination of the group of samples likely contributing to the estimate of the response at high levels of % agriculture suggests that the positive effect of agriculture on Dev-ZACI between 0.10 and 0.15 MaxRel could be an artifact of the different indicator taxa used for calculating ZACI scores. Analyses presented in Chapter 2 (Table 2.11), indicate that the taxon contributing the most to high Dev-ZACI scores in Cluster N3 was Hydrobiidae, while Chironomidae contributed the most to low Dev-ZACI scores. From Table 2.10, the taxon contributing the most to low Ag-ZACI scores (i.e. degraded Ag-ZACI scores) was *Gammarus*. The samples with levels of MaxRel between 0.10-0.15 and high levels of agriculture were composed of high relative abundances of *Gammarus* followed by Hydrobiidae, with low relative

abundances of Chironomidae. Therefore, these sites may have scored low Ag-ZACI scores because of a predominance of *Gammarus* but the higher Dev-ZACI scores may reflect the presence of Hydrobiidae and a low relative abundance of chironomids.

Approach 3: Reference/Non-Reference Isopleths

In this approach, I identified two potential cumulative stress thresholds denoted by isopleths in Figure 3.2. The isopleth, closest to the origin, separated low-stress locations within which all faunal assemblages were equivalent-to-reference sites from higher- stress locations (some of which supported equivalent-to-reference assemblages and others of which contained fauna characteristic of non-reference locations). The TITAN thresholds for single classes of stress used in this study were identified by Kovalenko et al. (2014) as estimated levels of stress at which abundance of many sensitive species began to decline. The first isopleth might be representative of the sensitive species threshold, beyond which the abundance of sensitive species continues to decline but the condition has not yet become fully degraded. As sensitive species continue to be replaced by stress tolerant species, the assemblages approach a second threshold (the second isopleth), which is the TITAN-identified point at which assemblages become dominated by tolerant species (degraded). At levels of stress above 0.30 MaxRel and 45% agriculture, all sites were classified as "not equivalent to reference". Whether these samples are truly degraded is unknown because the degree to which assemblages deviated from the reference condition was not quantified.

The limited number of sites that are subject to combined levels of stress also made identifying interactions difficult, and the isopleths were drawn arbitrarily. However, identifying points at which communities are no longer reference and points at which communities become degraded is useful for managing the effect of cumulative stress before specific interactions are teased out. The level of combined stress at which declines in abundance of sensitive taxa begin to occur would be of use for the conservation of biodiversity or specific taxa (Johnson et al. 2015).

In addition, the point at which all communities are no longer reference is an important threshold for setting restoration targets and priorities for sites that are in danger of crossing the non-reference/degraded threshold (Johnson et al. 2015).

Summary

This chapter used three approaches for assessing effects of cumulative stress to determine how watershed scale agriculture and development combine to influence macroinvertebrate communities at Great Lakes coastal margins. Each approach provided a different subset of information regarding multiple stressor effects. The first approach used the ZACI developed for individual stressors in Chapter 2 and assessed changes in the ZACI scores when both stress types are present. Approach 1 compared the values of ZACI scores from singly-stressed sites with those subject to combined stress. This approach was expected to illustrate the combined effects of the two stress classes. Findings using this approach indicated that ZACI index score patterns were consistent with a comparative effects model, with urban development being the dominant or more severe source of stress. If both Ag-ZACI and Dev-ZACI scores had been reduced in samples subject to combined stress it would have indicated a greater effect than the individual stressors alone. However, the presence of development generally lowered the Ag-ZACI scores, but the presence of agriculture did not influence Dev-ZACI scores. When one does not know how stressors may act in combination, or which null model to assume, this approach can be used to eliminate some of the possibilities and help to develop expectations. However, this approach can only indicate the direction of combined effect, i.e. positive (antagonism) or negative (additive, multiplicative, synergy) and could not distinguish additive from synergistic effects, for example.

The second approach, NPMR, is a promising tool for exploring the effect of multiple stressors because it is able to estimate the response to multiple predictors simultaneously, without making any a priori assumptions about the shape of the response surface. This is especially important when knowledge of the system is limited. A disadvantage of multiplicative kernel

smoothers is that results do not include a functional equation of the model but the pattern of the resulting NPMR model can aid in determining a suitable parametric model. Furthermore, mathematical approaches for quantifying thresholds from n-dimensional response surfaces have been developed (e.g. Lintz et al. 2011). However, both the absence of clear thresholds and the sparsity of data representing combined manifestations of stress prevented our use of the methodology proposed by Lintz et al. (2011). This method was able to portray the relationship between macroinvertebrate assemblage condition and land use stress in finer detail than was evident in the first approach. Results of this approach confirmed the finding of Approach 1 that development stress is a more severe source of stress for assemblages in Cluster N3. Although development was the more influential predictor, % agriculture continued to add to the combined effect, even interacting antagonistically between 0.10 and 0.20 MaxRel. Once MaxRel stress reached 0.20, it became the dominant predictor of both the Ag-ZACI and Dev-ZACI. The relative contribution of each type of stress and at which point a stressor becomes dominant was not evident from the analysis using Approach 1.

However, the fit of NPMR models was relatively poor. This may be because sample-level ZACI values were used as a response to site-level predictor variables. In other words, more than one response value corresponded to a single combination of predictor values. Consequently, the response values (ZACI scores) at any one observed point in the predictor space was sometimes quite variable and could not be explained well by the stress gradients.

Approach 3 was able to provide the level of each individual stressor that may result in a change of status from "reference" to "not equivalent to reference" or from "not equivalent to reference" to "degraded". These thresholds of condition are valuable for multiple sets of conservation goals such as biodiversity conservation or ecological restoration. The pattern of isopleths meant to indicate stressor interactions were most consistent with a convex function (Figure 3.1B). However there is a need for more data at intermediate levels of both stress types.

This approach depends on having a sample size large enough not only to span the range of each independent stress axis, but also to include sufficient data points that reflect exposure to both stressors simultaneously. This is difficult to achieve in terms of land-use in the Great Lakes because the amounts of agriculture and development in a watershed tend to be inversely correlated (Kovalenko et al. 2014). For example, watersheds with significant proportions of agricultural land cover often have only small amounts of urban development.

Conclusions

Evidence for a dominant stressor effect (following the comparative effects model) was found with two out of three approaches employed. This is in contrast to the hypothesized additive effect with a possible synergism. Most multiple stressor studies assume that stressors combine additively, and define any resulting interactions in relation to an additive null model (Cote et al. 2016). However, multiple stressor effects can exhibit complex behaviour with varying response patterns of biota in different locations in the predictor space (Lintz et al. 2011). This was demonstrated in Approach 2 where development stress was found to be more severe than agriculture but development isn't completely dominant until after 0.20 MaxRel. Linear additive models may not have detected those changes in response. Overall, each of these three approaches appear to have value in assessing the relative importance of independent stressors on the zoobenthic composite indices derived for Cluster N3. Consequently, there is value in applying these values to other indices, or for data in other Great Lakes regions for which sufficient data exist.

Figure 3.1.A) Demonstration of isopleth functions of the linear form distinguishing between reference and non-reference, as well as between non-reference and degraded thresholds in relation to agriculture and development stress. An observed linear isopleth implies that the two sources of stress combine independently of one another (Ciborowski, unpublished).
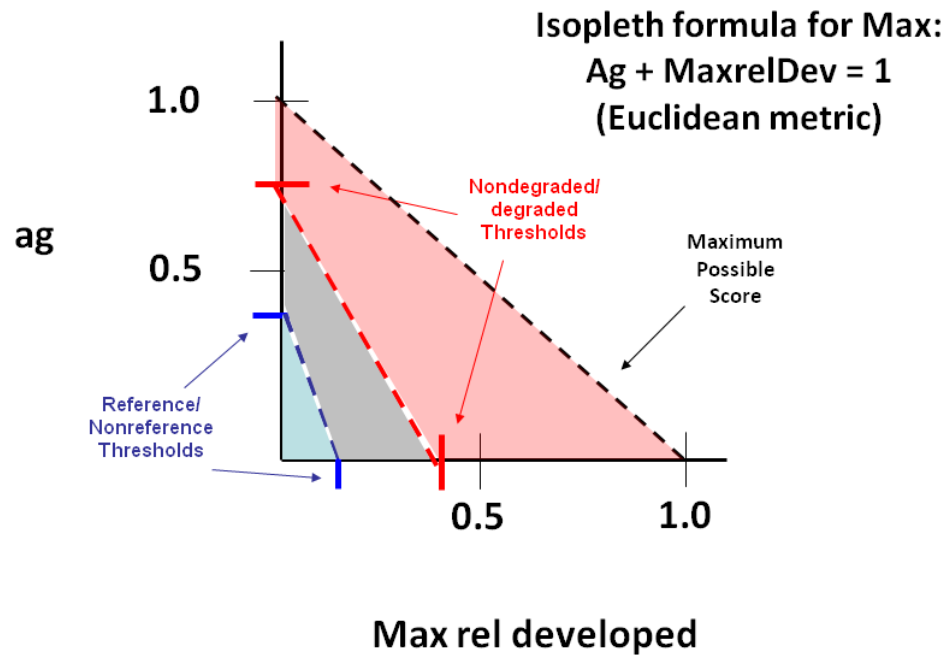
Figure 3.1.B) Demonstration of isopleth functions of the convex form distinguishing between reference and non-reference, as well as between non-reference and degraded thresholds in relation to agriculture and development stress. Convex forms of the isopleths indicate antagonistic effects of combined stress (Ciborowski, unpublished).
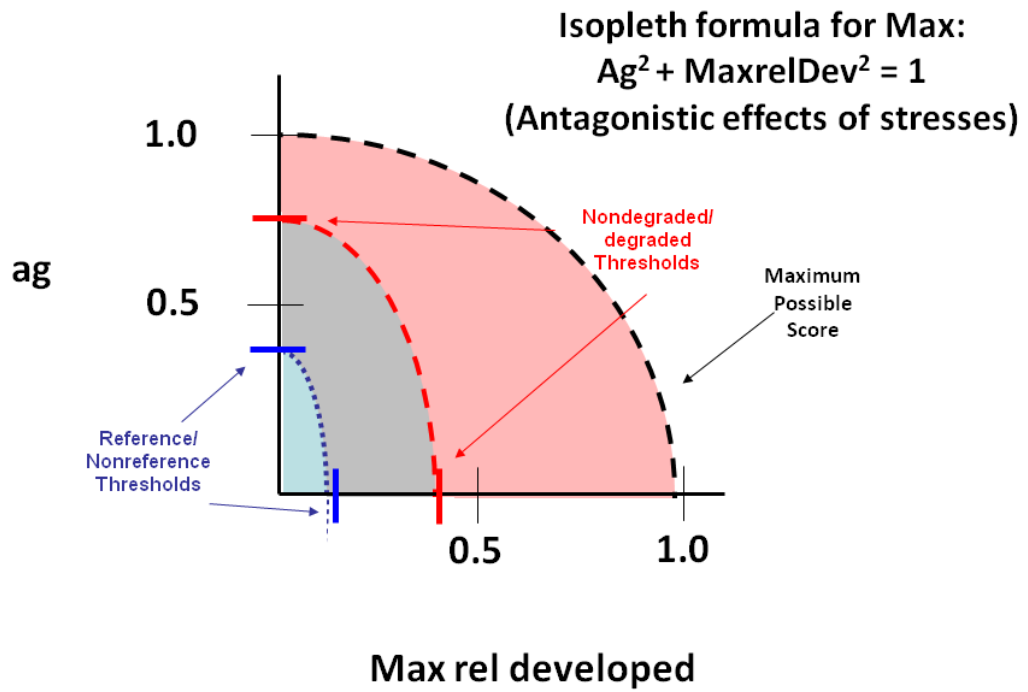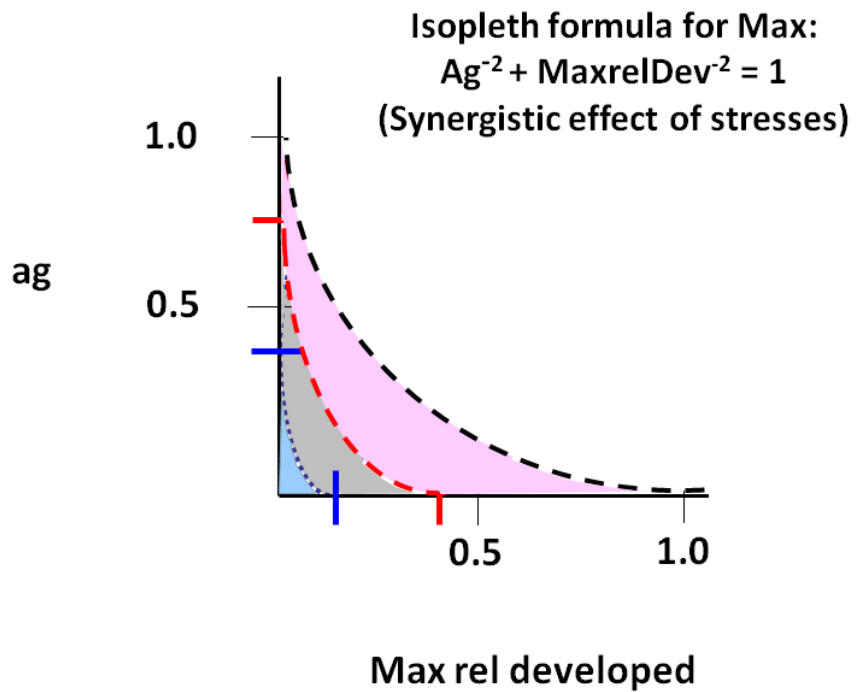
Figure 3.1.C) Demonstration of isopleth functions of the concave form distinguishing between reference and non-reference, as well as between non-reference and degraded thresholds in relation to agriculture and development stress. Concave forms of the isopleths indicate a possible synergistic effect of combined stress (Ciborowski, unpublished).

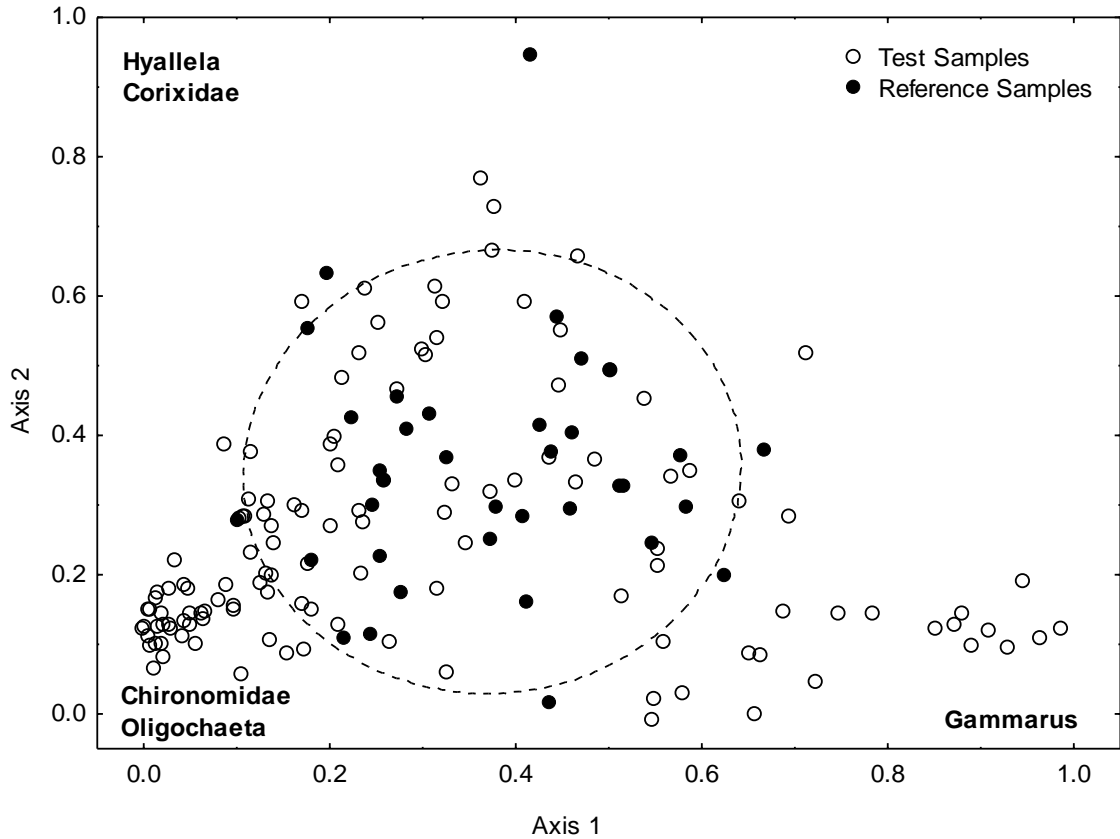**Figure 3.2.** Bray-Curtis polar ordination of reference samples (filled circles) and test samples (open circles) in Cluster 3N along two axes in species space. The dashed line is the 80% confidence interval of the reference samples. Test samples falling within the ellipse are classified as "*equivalent to reference*" on the basis of their community composition. Important taxa loadings on each axis are labeled.
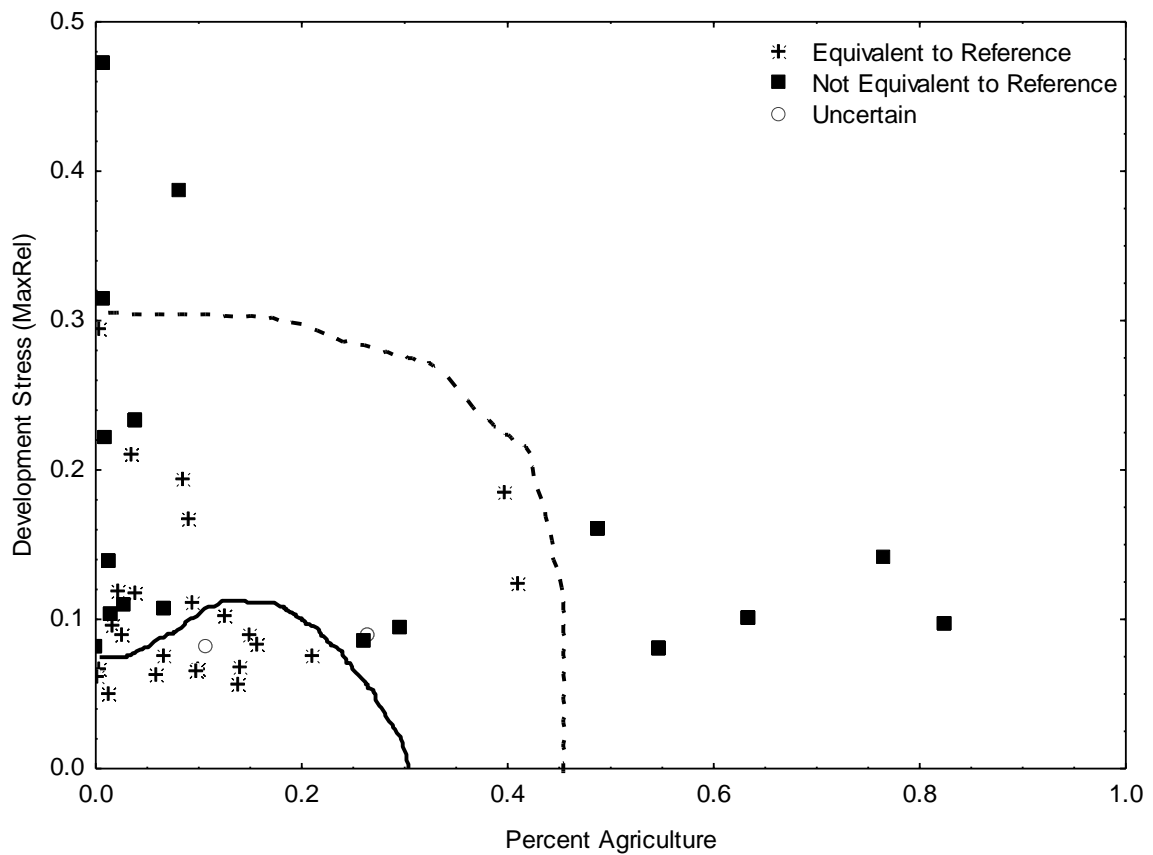
Figure 3.3. Sites plotted according to their levels of watershed agriculture and development and classified as reference or non-reference assessed from a Bray-Curtis polar ordination (see Fig. 3.1). Isopleths are mapped to demonstrate potential interactions between agriculture and development stress. The first isopleth (solid line) is entirely composed of "equivalent to reference" sites whereas the second isopleth (dashed line) is the biggest line encompassing the reference sites.
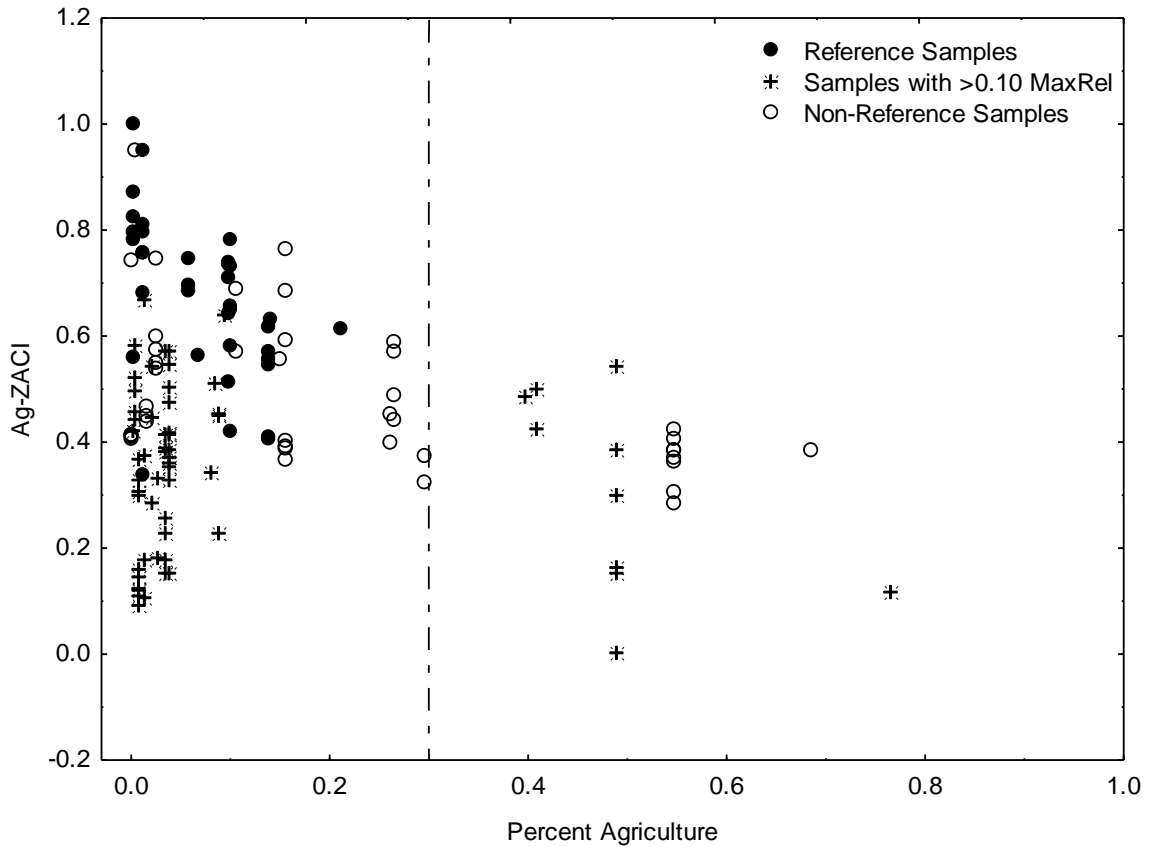
Figure 3.4. Agriculture based ZACI scores derived from samples in Cluster 3N plotted against the % agriculture stress gradient. Black circles (open and closed) represent reference and test samples from locations having MaxRel development scores < 0.10. Ag-ZACI scores were predicted for samples from locations with > 0.10 MaxRel development stress using the Ag-ZACI function for Cluster 3N found in Table 2.10 and are plotted here as stars. The vertical dashed line represents the operational reference threshold of 30% agriculture identified by Titan analysis (Kovalenko et al. 2014).

Figure 3.5. Development-based ZACI scores derived from samples in Cluster 3N plotted against the MaxRel development stress gradient. Black circles (open and closed) represent reference and test samples from locations having percent agriculture < 10% in the watershed. Dev-ZACI scores were predicted for samples from locations with > 10% agriculture using the Dev-ZACI function for Cluster 3N found in Table 2.11 and are plotted as open triangles. The vertical dashed line represents the operational reference threshold of 0.08 MaxRel identified by Titan analysis (Kovalenko et al. 2014).
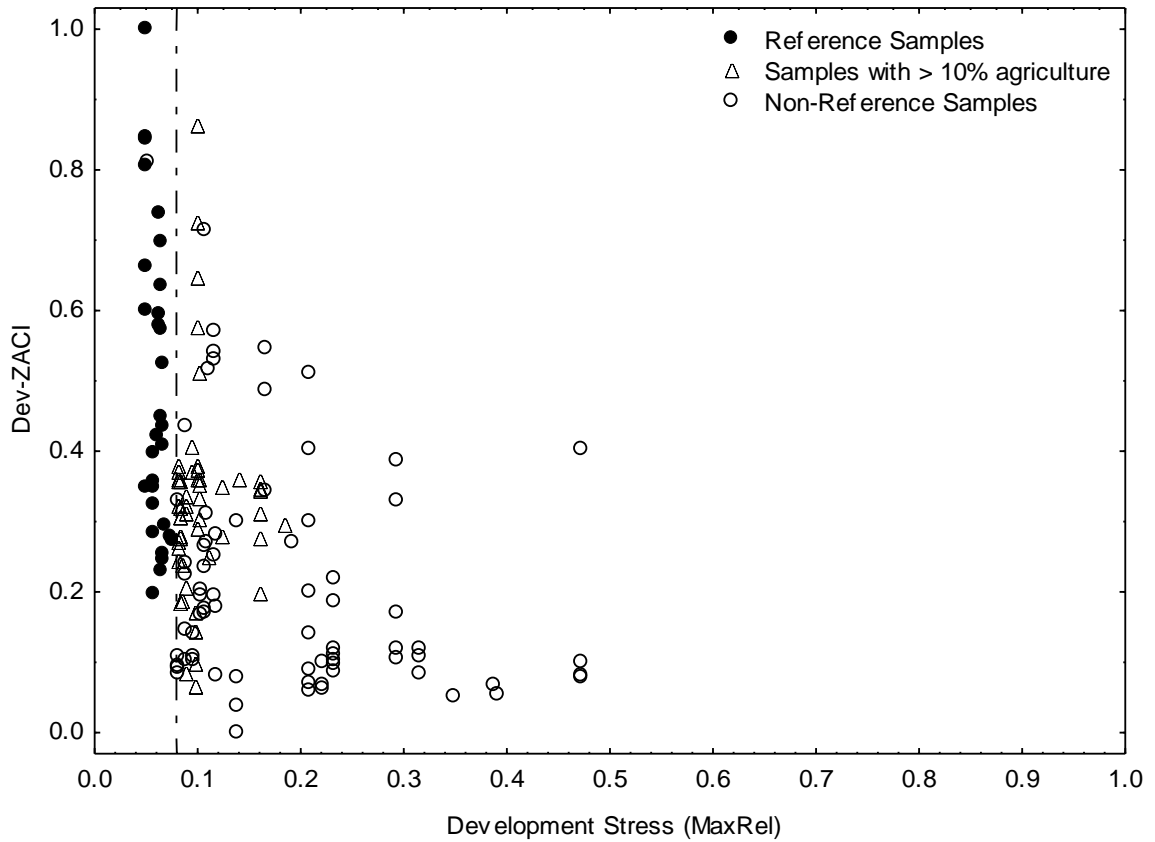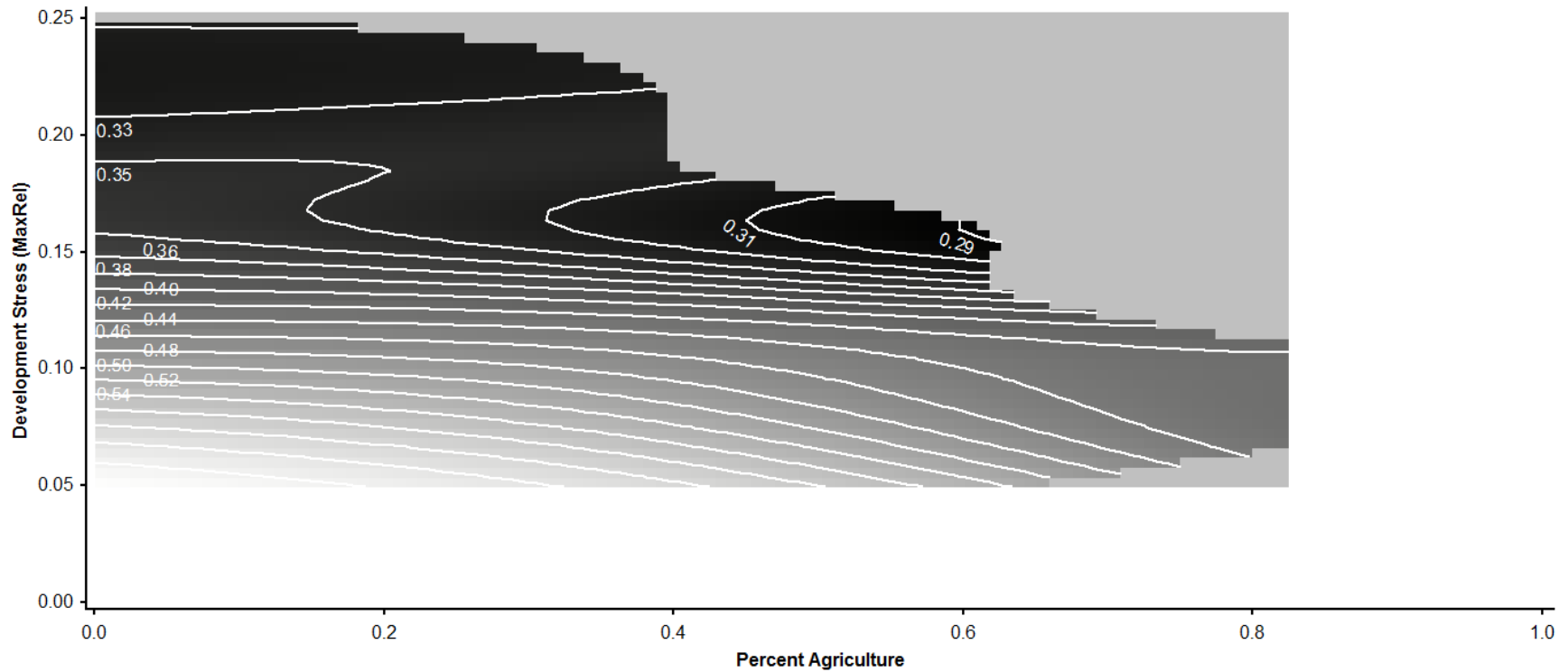
Figure 3.6. Non-parametric multiplicative regression model of Ag-ZACI scores as a function of % Agriculture and MaxRel stress (cross-validated $R^2 = 0.40$). Estimates of response were calculated using a local mean estimator with a Gaussian weighting function. Observed Ag-ZACI scores from Cluster 3N were used in model development. Countour lines connect points with equal Ag-ZACI scores (indicated with numerals). Lighter shading indicates higher Ag-ZACI scores and therefore, an assemblage more similar to a reference condition. The shading gradient is more pronounced, and spacing between isopleths is narrower in the vertical (Development) dimension than the horizontal dimension (Agriculture) implying that a unit of development stress has a greater effect on Ag-ZACI scores than a unit of agricultural stress.

Figure 3.7. Estimated biological condition (as measured by Ag-ZACI) as a function of the MaxRel development stress gradient at different 10 different levels of % agriculture in the watershed modeled using non-parametric multiplicative regression.
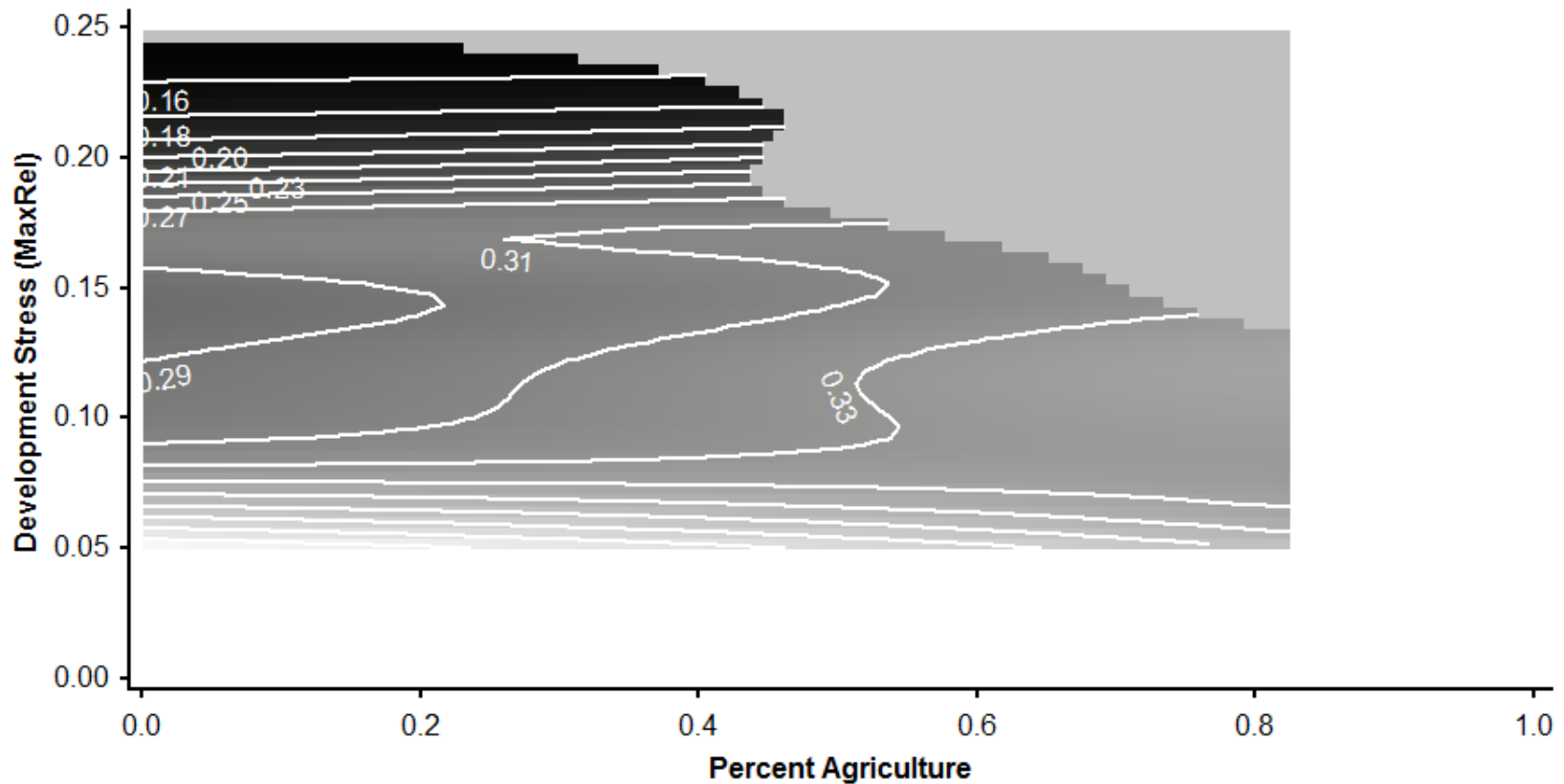
Figure 3.8. Non-parametric multiplicative regression model of Dev-ZACI scores predicted by % agriculture and MaxRel development (cross-validated $R^2 = 0.48$). Estimates of response were calculated using a local mean estimator with a Gaussian weighting functions. Observed Dev-ZACI scores from Cluster 3N were used in model development. Lighter shading indicates higher Dev-ZACI scores and therefore, an assemblage more similar to a reference condition. The shading gradient is more pronounced, and spacing between isopleths is narrower in the vertical (Development) dimension than the horizontal dimension (Agriculture) implying that a unit of development stress has a greater effect on Ag-ZACI scores than a unit of agricultural stress.
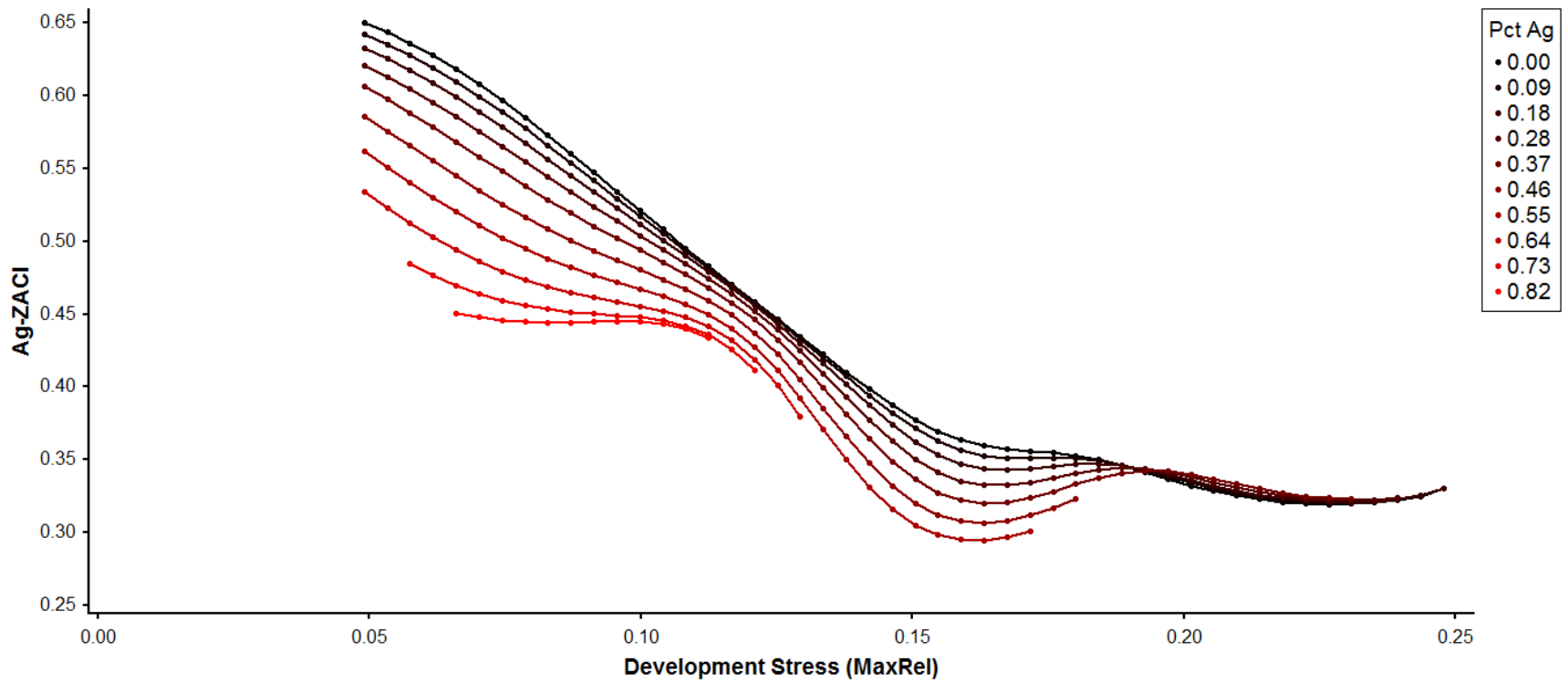
Figure 3.9. Estimated biological condition (as measured by Dev-ZACI) as a function of the MaxRel development stress gradient at 10 different levels of % agriculture in the watershed modeled using non-parametric multiplicative regression.
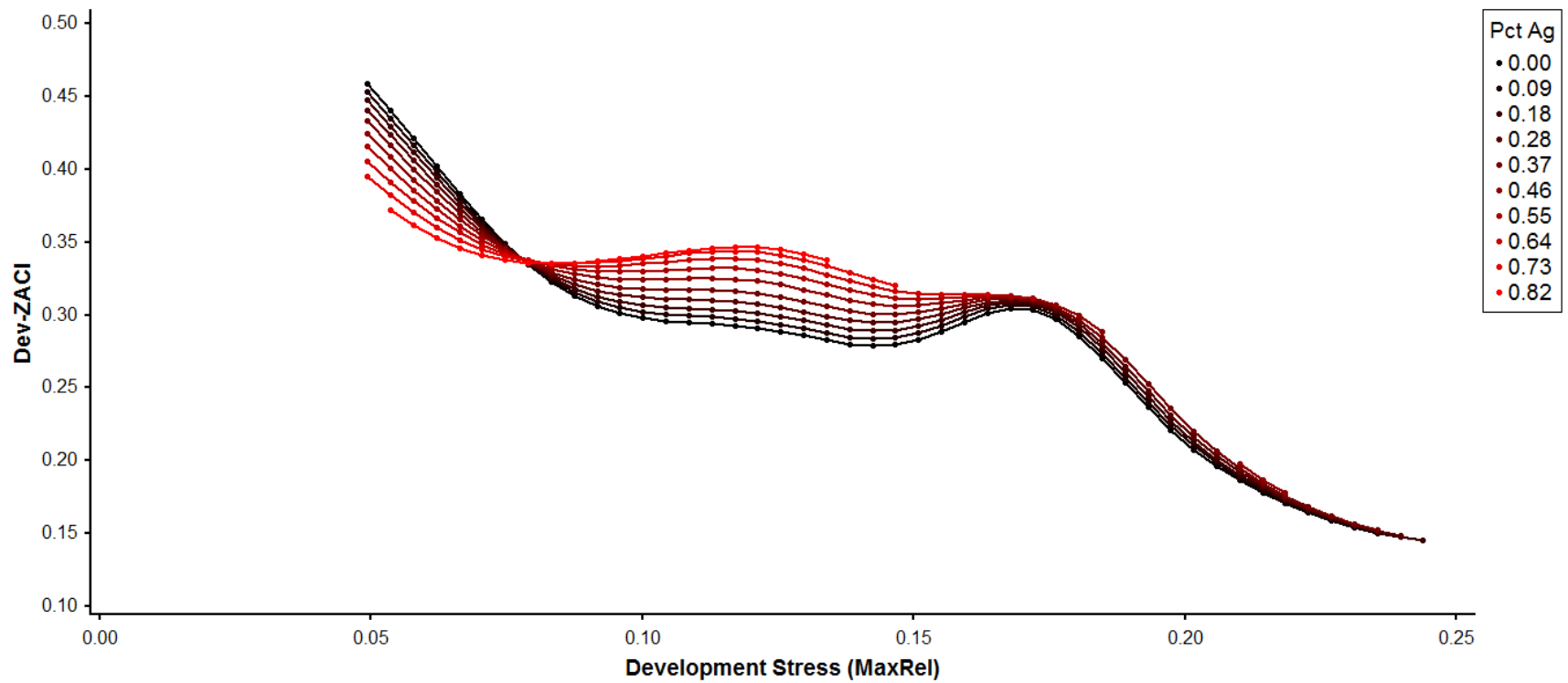
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CHAPTER 4

GENERAL DISCUSSION

The goal of this thesis was to assess the independent and combined effects of watershed land-use stress on macroinvertebrate community condition at Great Lakes coastal margins. I derived a new composite, multivariate Zoobenthic Assemblage Condition Index with which to relate assemblage composition to two classes of land-use- agriculture and urban development.

Multivariate techniques for the assessment of aquatic communities in streams are well established in countries such as the U.K. (RIVPACS, Wright, 1995), Australia (AUSRIVAS, Parsons and Norris, 1996; Nichols et al. 2014) and Canada (BEAST, Reynoldson et al. 1995; Strachan et al. 2014). These techniques employ the reference condition approach (Karr 1981; Bailey et al. 2004) whereby test sites are compared to a suite of sites in the reference condition locations representing the range of variability in biological condition at locations subject to the least amount of disturbance. Deviation in community composition (or surrogate measures thereof) from the reference condition is used as a measure of impact. However, in the absence of a complementary degraded condition, these techniques are only able to determine deviation from reference, and not proximity to degraded (Ciborowski et al. 2013; Johnson et al. 2015). The Reference-Degraded Continuum (RDC) approach applied in this study extends the RCA by establishing a complementary "degraded" biological condition defined as the range of variability in biological condition at test sites subject to the *highest* amount of disturbance. The two extremes form a continuum along which the position of test site biota can be assessed (Ciborowski et al. 2013). This approach provides information on the position of each assemblage relative to both the reference and degraded endpoints, allowing an ordinal rather than probability-based evaluation of biological condition.

In this study the use of the RDC approach generated indices that varied significantly as a function of watershed agriculture and development related stress. Macroinvertebrate indices previously developed for Great Lakes coastal wetlands include multimetric IBIs developed in limited geographic ranges and for specific plant zones (Burton et al. 1999; Uzarski et al. 2004;

Gathman et al. 2011). Other assessments of macroinvertebrates such as the National Coastal

Condition Assessment (NCCA, Scharold et al. 2015) pertain to the entire Great Lakes basin but

relate to benthic communities of nearshore zones rather than wetlands or coastal margins. The

indices generated in this thesis are unique in that they can theoretically be applied to coastal

margins across the U.S. Great Lakes and can likely be extrapolated to coastal margins in

Canadian waters. It was expected that macroinvertebrate community condition would be

negatively affected by both agriculture and development stress but that responses would be non-

linear. Many bioassessment studies relate biotic indices and metrics to disturbance using some

form of linear model (e.g. Lunde and Resh, 2012; Minns et al. 1994; Karr, 1991; Danz et al.

2007). Findings from Chapter 2 corroborated expectations, with several distinct assemblages

exhibiting negative, non-linear relationships with both % agriculture and development stress in

the watershed. These findings have implications for the appropriateness of linear-based models

for relating community condition to disturbance gradients.

Land-use stress was also observed to limit biological condition in one cluster of

assemblages (Cluster N3) eliciting wedge-shaped patterns of index scores. The land-use stress

gradients used in this study are surrogates rather than the stressors *per se* (Danz et al. 2005;

Brazner et al. 2007; Johnson et al. 2015). Accordingly, other unmeasured variables representing

proximal sources of stress are likely influencing biological condition, e.g. water quality, wave

action, water level fluctuation. (Cooper et al. 2014). Limiting factor relationships (those creating

wedge-shaped stress-effect patterns) are of interest to managers because they imply that large-

scale pressures such as watershed land-use potentially constrain the effects of restoration

measures (Palmer et al. 2010) rather than directly control them.

In contrast to expectations, strong threshold relationships were not apparent between

macroinvertebrate assemblage composition and land-use stress, although one assemblage

exhibited some evidence of a change-point along the development stress gradient. There is

previous evidence that composite response variables (metrics and indices) are unable to distinguish the large, synchronous decline in sensitive taxa from the gradual increase in tolerant taxa that occurs along a disturbance gradient (Baker and King, 2010). Evidence for synchronous declines in the abundance of sensitive taxa at points on the development stress gradient have been reported for assemblages in wetlands of the U.S. Great Lakes, especially in the northern ecoprovince (Kovalenko et al. 2014). The ZACI, being a composite community index may not have reflected these taxon-specific changes.

In Chapter 3 we investigated the utility of three approaches as proofs of concept to assess how the combined influences of watershed-based agriculture and urban development may affect the macroinvertebrate communities at coastal margins. It was expected that combined stress would reduce biological condition more than independent effects of each stressor alone (i.e. additive or synergistic effects). Contrary to expectations, two of three approaches displayed patterns suggesting that cumulative land-use stress follows a model similar to the comparative effects model (Folt et al. 1999) where a dominant, single stress type (development) was responsible for most or all of the effect on biological condition. Urban development exerts severe impacts on stream communities (Walsh et al. 2005), and negative threshold effects have been observed at very low levels of impervious cover and urban land use (King et al. 2011; Kail et al. 2012). Urban development stress commonly affects coastal wetlands through modification of hydrological processes and direct habitat alteration (Lee et al. 2006). Disturbances related to urban development can also introduce additional drivers of stress. For example, fragmentation of forested wetland habitat not only causes changes in native plant community composition, but also creates the opportunity for invasive plant species to become established (Faulkner, 2004). In contrast, agriculture exerts impacts through less direct mechanisms (e.g. non-point source pollution and sedimentation) that could be mediated if best-management practices are in place.

Where there is a greater amount of agriculture compared to urban development in a watershed, it is easy to assume that agriculture stress is dominant and therefore should receive most of the mitigation efforts. However, our findings in Chapter 3 suggest that the most spatially extensive stress type is not necessarily the most severe. Non-parametric multiplicative regression models predicted strong effects of development, even when much of the watershed was allocated to agriculture in the watershed. This supports our use of the TITAN identified thresholds which estimated changes in abundance of sensitive taxa at lower levels of watershed development compared to agriculture (Kovalenko et al. 2014). The severity of impact from urban development may also be related to the location of the stress in the watershed. Whereas agriculture may occur throughout a watershed, urban centers may be more concentrated at river mouths and shorelines thereby exerting greater amounts of stress per unit area relative to agriculture. Therefore, when setting management priorities it may be important to consider whether or not the really predominant source of stress is in fact the most severe or else risk wasting time and resources.

This also has implications for the development of cumulative stress indices. Current attempts to combine measures of stress in for the Great Lakes include additive indices (e.g. Watershed Stress Index, Host et al. 2011), cumulative impact mapping (Allan et al. 2013) and other models such as the application of Euclidean distance calculate the AgDev index (Johnson et al. 2015). A simple sum of individual stress scores may not accurately reflect the true impact on biota if the stresses do not combine additively. If a single stress type is dominant (i.e. comparative effects- additional stresses have little to no effect), an additive index would overestimate the expected impact on biota. The AgDev composite index developed by the Great Lakes Environmental Indicators consortium (GLEI, Johnson et al. 2015) uses Euclidean distance, which more closely mimics the comparative effects model by giving greater weight to the stress with the higher independent stress score. Therefore, it may be a more accurate representation of expected impact than simple summation when one stress type dominates the effect. However, in cases

where both stresses affect biological condition but one is much more severe (as in our NPMR models) independent stress indices may need to be weighted according to their relative influence when being combined.

The use of point-level abundance data in this study was a source of added variability in ZACI scores that was not explained well by the land-use stress gradients. Assemblages collected from the same wetland site (therefore having the same stress scores) often had varying ZACI scores, in part reflecting the local habitat differences within the wetland at which they were collected. This is further evidence that macroinvertebrate communities are sensitive to within-site variation in habitat (e.g. plant zonation, Uzarski et al. 2004; Gathman and Burton, 2011). However, the added variability likely contributed significantly to the relatively poor fit of the least squares regression functions in Chapter 2 and the NPMR models in Chapter 3. A further limitation was an insufficient amount of data available from sites situated at intermediate and high levels of stress. This was a problem predominantly in clusters from the northern ecoprovince but also for values of the MaxRel development gradient in the southern ecoprovince. Watershed agriculture and urban development land-use are two of the main sources of anthropogenic stress in the Great Lakes (Danz et al. 2005). However, many fundamentally different types of stress exist in the Great Lakes that are not captured by the % agriculture and MaxRel stress gradients, such as local effects of invasive species or stress applied from the lake (e.g. wave action).

Future surveys focusing on the use of the ZACI for bioassessment should emphasize sampling of non-reference (intermediate stress) and degraded (high stress) areas. A valuable addition to this study would be to collect comparable independent data with which to test the ZACI-stress relationships. Cross-validation by leaving out a portion of data was considered for this study but it was felt that doing so would leave insufficient data points for model derivation in the individual clusters. Validation of the indices and cumulative effect assessments undertaken in

this study would greatly benefit from datasets that provided more homogeneous coverage of the range of stresses.

In addition, this study was conducted using existing assemblage and environmental data collected during the first phase of the GLEI project (between the years 2001-2003; Niemi et al. 2006) so there may have been changes in the levels of stress over time. However, data collection for the second part of the GLEI project (GLEI-2) during the years 2010 to 2015 was designed to determine whether the biota are different at sites subject to changing stress. It was found that the changes in stress scores assessed at the watershed scale over the 10-year period between projects were very small (Johnson et al. 2015).

Despite these limitations, the approaches used in this study demonstrate that the independent stresses caused by agricultural and development activities in Great Lakes watersheds are manifested as differences in the relative abundances of macroinvertebrates at coastal margins. The effects appear to be more pronounced in wetlands of the northern ecoprovince than in the south, possibly because reference conditions in the north more closely approximate the complete absence of anthropogenic stressors than is observed in the southern ecoprovinces. The indices developed provide quantitative measures of zoobenthic assemblage condition that, once validated, could be used to assess both status of previously un-sampled locations and trends at sites that are repeatedly monitored. The proof of concept approaches used to assess combined effects of the two classes of stress suggest that development exerts stronger effects than agriculture, and that the combined effects are less than additive.

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Appendix A:

Description of data set and samples used in analyses

Nine hundred and eighty-one samples were collected from 141 coastal sites on the U.S. side of the Great Lakes basin. After removing samples with less than 20 invertebrates, there were 673 sampling points remaining - 332 in the Northern ecoprovince and 341 in the Southern ecoprovince. In the Northern ecoprovince individuals were recorded in 221 taxonomic groups with chironomid larvae, oligochaete worms, Acari (mites), and amphipod genera (*Hyalella* and *Gammarus*) being the most frequent and abundant taxa recorded. In the Southern ecoprovince individuals were recorded in 216 taxonomic groups, with Chironomidae, Oligochaeta and Acari being the most frequent taxa. The most abundant taxa in the South included Oligochaeta, Chironomidae, and Amphipod genera (*Hyalella* and *Gammarus*). After removing rare species, 51 taxa remained in the North and only 32 taxa in the South. Among the rare taxa deleted in the North were Bivalvia, representatives of various dipteran families (e.g. Tipulidae, Tabanidae, Culicidae) and leeches. In the South, removing rare species mostly involved moving single individuals of a species to a higher taxonomic level (e.g. most often family).
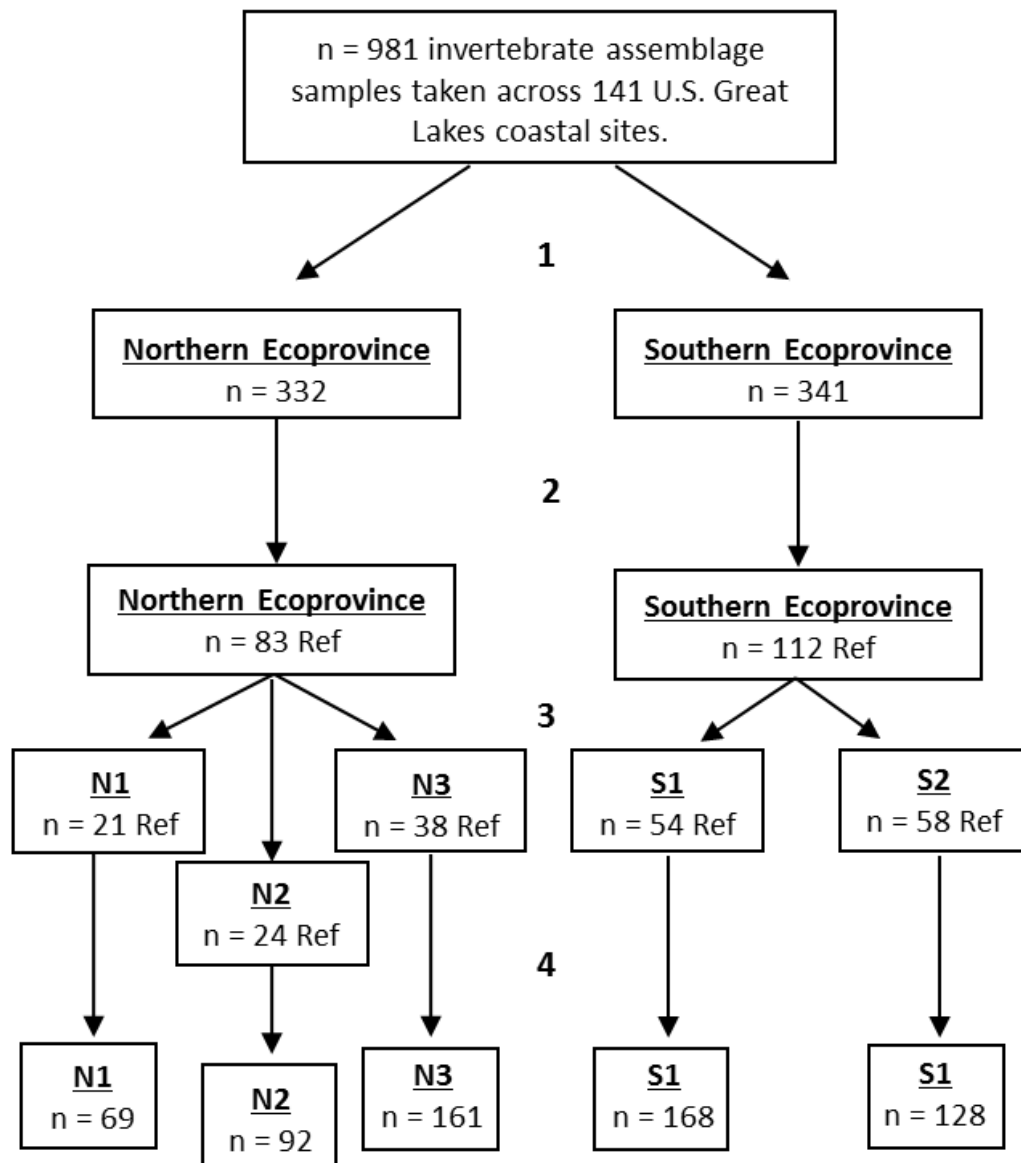
Figure A-1. Flowchart depicting sample sizes used at each stage of development as follows: 1) Removal of samples comprised of < 20 individuals, split by ecoprovince; 2) Reference sample selection; 3) Cluster analysis of reference samples; 4) Classification of test samples into reference clusters based on habitat variables.

Appendix B:

Construction of Reference-Degraded Continuum Axes

Cluster N1 consisted of 69 samples (of which 18 were reference samples). Thirty-seven samples with low development stress scores (<0.10 MaxRel) were used to derive the ZACI axis for agricultural stress using Bray-Curtis Ordination (including 17 reference samples and 2 constructed 'centroid' samples). Centroid samples were constructed using 3 samples each. Thirty two samples with low % Ag scores (≤10% agriculture) were used to derive the ZACI axis for development stress (including 13 reference samples and 2 centroid samples). The development centroid samples were built using 3 samples each.

Cluster N2 consisted of 92 samples (of which 23 were reference samples). Fifty samples with low development scores (≤0.10 MaxRel) were used to derive the ZACI axis for agricultural stress (including 22 reference samples and 2 constructed 'centroid' samples). The centroids were constructed using 5 samples each. Fifty three samples with low % Ag (≤10) were used to derive the ZACI axis for development stress (including 15 reference samples and 2 centroid samples). The reference and degraded development centroids were built using 5 and 8 samples respectively.

Cluster N3 consisted of 161 samples (of which 36 were reference samples). Eighty one samples with low development stress (≤0.10 MaxRel) were used to derive the ZACI axis for agricultural stress (including 36 reference samples and 2 constructed 'centroid' samples). Centroids were constructed using 8 samples each. Ninety five samples were used to derive the development ZACI axis (22 reference, 2 centroid samples). The development centroid samples were built using 9 samples each.

Cluster S1 consisted of 168 samples (of which 53 were reference points). Ninety seven samples with low development stress (≤0.10 MaxRel) were used to derive the agriculture ZACI

axis (including 53 reference samples and 2 constructed 'centroid' samples). The reference centroid sample was constructed using 10 samples while the degraded centroid was constructed using 15 samples. Forty five samples were used to derive the development ZACI axis (19 reference samples and 2 centroid samples). The reference and degraded centroid samples were developed using 6 and 5 samples, respectively.

Cluster S2 consisted of 128 samples, including 51 reference samples. Eighty three samples with low development stress (≤ 0.10 MaxRel) were used to derive the agriculture ZACI axis (including 46 reference samples and 2 constructed 'centroid' samples). The reference centroid was developed using 12 samples, while the degraded centroid was developed using 10 samples. Forty three samples were used to derive the development ZACI axis (including 12 reference samples and 2 centroid samples). The development centroid samples were built using 12 samples each.

Vita Auctoris

NAME:	Jasmine St. Pierre
PLACE OF BIRTH:	Windsor, ON
YEAR OF BIRTH:	1990
EDUCATION:	University of Windsor, B.Sc. (Hons)- Biological Sciences, Windsor, ON, 2013
	University of Windsor, M.Sc. – Biological Sciences, Windsor, ON, 2016
PUBLICATIONS:	St. Pierre, J. I., and K. E. Kovalenko. 2014. Effect of habitat complexity attributes on species richness. Ecosphere 5(2):22. http://dx.doi.org/10.1890/ES13-00323.1