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Declaration of Originality

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Abstract

Wetlands are an ecotone between terrestrial and aquatic environments and therefore support a diverse and unique flora and fauna. Macroinvertebrates make up a substantial portion of the biodiversity. The relationship between aquatic invertebrate community composition and the association with submergent and emergent macrophyte biomass is relatively well documented. However, the constraints imposed by conditions in wet meadow zones – areas that are intermittently flooded but whose soils are typically water-saturated and anoxic are less well understood. I investigated the relative importance of dissolved oxygen along the water depth gradient and its influenced on invertebrate community composition in comparison to the uniform vegetation found in the wet meadow zones of 10 wetlands in Lake Huron of the Laurentian Great Lakes.

In 2017, I evaluated macrophyte community composition, sampled zoobenthos and fishes, and recorded diel dissolved oxygen trends along multiple transects in 10 coastal wetland wet meadows varying in geomorphology and exposure to agricultural activity in the contributing watersheds. The duration of hypoxia (DO < 4.0 mg/L) was a negative function of water depth along 30-m transects varying from 30-100 cm deep within each wet meadow site. Differences in the environmental factors were reflected in the relative abundance of oxygen-sensitive zoobenthos, being greater in areas that experienced a shorter duration of diel hypoxia and anoxia. However, overall invertebrate community composition was most greatly influenced by the major environmental differences between ecoregions and among wetlands within ecoregions. Thus, wet meadow community composition can be inferred from synoptic benthic samples collected from a wetland without concern for biases related to sampling depth.

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General Introduction:

My research describes invertebrate community composition along a gradient of varying depth within the wet meadow zone of Great Lakes coastal wetlands. In addition, I describe the diel variation in oxygen concentration in relation to a location's relative position within the wet meadow zone, and its putative influence on invertebrate community composition throughout the wet meadow.

Coastal Wetland Structure and Function:

Coastal wetlands are an important part of lacustrine and river ecosystems. Wetlands are an ecotone between terrestrial and aquatic environments and therefore provide refuge to a diverse and unique flora and fauna (Kirkman, 2012, Mitsch and Gosselink, 2015). Over 1400 species of plants, birds, fishes, reptiles, mammals and invertebrates have been estimated to populate North America's Lake Huron coastal wetlands alone (Burton and Uzarski 2009). Aquatic macroinvertebrates make up a substantial portion of the biodiversity within individual wetlands (Williams et al. 2004, Davies et al. 2008). Although Great Lakes wetlands support many economically important resources, such as the recreational fishery, valued at seven billion dollars annually (Allan 2013), active management and restoration of these ecosystems is a relatively recent initiative (US EPA, 1994. Environment and Climate Change Canada, 2016). Over the past two centuries, anthropogenic alteration has removed two-thirds of the Great Lakes coastal wetlands (Mayer, 2004), and these changes have been accompanied by changes to many aspects of the Great Lakes ecosystem, including altered

water chemistry and fisheries (Krieger 1992). Wetland ecological services include features such as pollutant removal, floodwater storage, microclimate regulation, as well as protecting the coastline from wave action and erosion (Costanza 1989, Allan 1997, McLaughlin 2013, Sierszen et al. 2012).

Coastal wetlands found around the Great Lakes do not show the typical signs of senescence usually seen with freshwater wetlands that are found inland. The senescence or aging process of inland wetlands, such as marshes and shallow lakes, tends to start with open ponds that proceed to densely vegetated marshes and finally end up as dry fields (Sierszen et al. 2012). The fluctuating water levels of the Great Lakes prevent the senescence of coastal wetlands through periodic rejuvenation to the wetland communities (Herdendorf 1990, Keough et al. 1999). The rejuvenation is a product of the well-established relationship between the fluctuation of water levels interacting with the extensive seed banks of the coastal wetlands. Coastal wetland plant communities are resilient because the annual and multiple-year cycling periods of low and high waters allows diverse wetland communities to persist despite multiple disturbances (Kowalski et al. 2009, Frieswyk and Zedler 2006).

Coastal wetlands such as those found around the Great Lakes are defined as "wetlands under substantial hydrologic influence from Great Lakes waters" (McKee et al. 1992). According to the Great Lakes Coastal Wetland Consortium, coastal wetlands can be classified into three hydrogeomorphic categories based on their hydrologic connectivity, geomorphic position, and dominant hydrologic source (Albert et al. 2005). The three primary hydrologic system classifications outlined by Albert et al. (2005) are lacustrine, riverine and barrier-protected. These three hydrologic system classifications

can be further described based on their geomorphic type - connecting channel, delta, lagoon, open, protected, river mouth, or swale (Albert et al. 2005; Fig. 1).

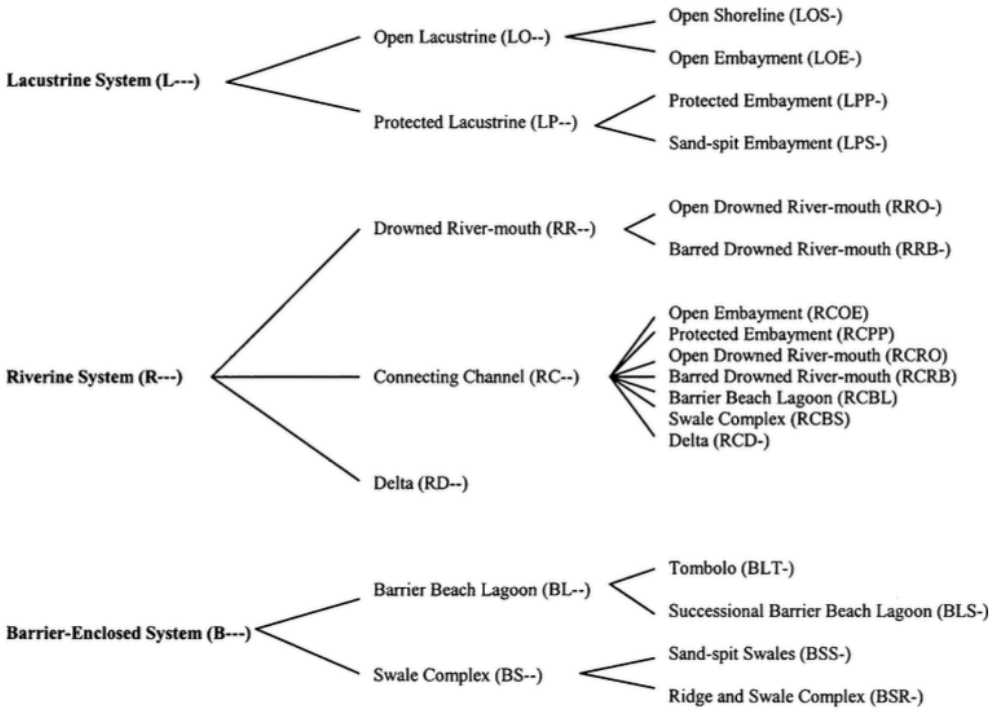

Figure 1.1 Classification of Great Lakes Coastal wetlands as described by Albert et al. (2005).

Great Lakes Coastal Wetland Formation:

The Great Lakes were formed from glacier depressions that were gradually filled with melt water from the receding glaciers. As the ice continued to melt, the land began to rebound as the weight of the glacier disappeared. The glacial rebound forced water into the Great Lakes southwest, flooding river mouths and lowlands to create today's coastal wetlands (Van Steeg 1935). Coastal wetlands in the Great Lakes can also be found at river mouths around the Great Lakes, where sediments have been transported downstream to form a suitable substrate for plants to take root (Van Steeg, 1935).

Wetland and Wet Meadow Characteristics:

Wetlands are incredibly diverse when it comes to vegetation, and this is due to fluctuation water levels, which will periodically drown or dehydrate the dominant vegetation and allow other subdominant genera and species in the seed bank to grow once their optimal conditions occur (Keddy and Reznicek 1986). Although there are many species of macrophytes in these complex ecosystems, the habitats of Great Lakes wetlands can be broadly subdivided based on vegetation into four basic zones: wet meadows, strands/inner emergent vegetation, marsh/outer emergent vegetation and aquatic (Keddy and Reznicek 1986). Each vegetation zone has a unique relationship with flooding or water levels (Keddy and Reznicek 1986).

Strands or the inner emergent vegetation zone exist in areas where seasonal water fluctuations and waves sometimes reach and might cause erosion. These areas are usually dominated by plants in the genera *Bidens*, *Juncus* and *Polygonum* (Keddy and Reznicek 1986).

Marshes or the outer emergent vegetation zone, occur in areas that are flooded with water all year to a depth of up to 1.5 meters, though many of the plants present in this zone do better in shallower water depths (Keddy and Reznicek 1986). The dominant genera in this zone are usually *Typha*, *Phragmites* and *Scirpus* (Keddy and Reznicek 1986).

Aquatic vegetation can occur in shallow and deeper water so long as it is flooded all year (Keddy and Reznicek, 1986). In shallower water, genera that are likely to be

present are *Potamogeton* and *Elodea*, whereas floating-leaf plants (*Nymphaea*, *Nuphar* and *Brasenia*) are likely to be found in deeper areas (Keddy and Reznicek 1986).

Wet Meadows are often found on flooded deltas, with vegetation ranging from cattail to grass or sedge dominated areas growing in deep organic soils ranging from 30-100 cm in depth (Albert 2005). However, most often wet meadows are dominated by fine textured, hummock-creating sedges, grasses and rushes (DeKeyser 2003). The wet meadows sampled for this thesis were indeed sedge and hummock dominated areas, with 42% of sites being dominated by *Carex* spp, and 18% being dominated by *Calamogrostis* (appendix A).

Distribution of Aquatic Invertebrate among Wetland Habitats:

The factors that influence invertebrate community composition in coastal wet meadow habitats around the Great Lakes are poorly understood. This limits our ability both to infer the ecological condition of wetlands and to propose effective restoration and management strategies for these diverse habitats if they are affected by anthropogenic activities. Studies such as those by Uzarski et al. (2004) have suggested that the relationships between the macrophytes and macroinvertebrates within the emergent vegetation zones of wetlands provide a framework from which to develop indices of biotic integrity (IBI) robust enough to be applied throughout the Great Lakes. However, Gathman and Burton (2011) observed that macroinvertebrates quickly colonize newly flooded areas, despite the macrophytes in such habitats not being the same as the flora of the emergent vegetation zones. Wilcox et al. (2002) also found that the reliability of wetland IBIs deteriorated when used for wetlands subject to hydrologic variability. Gathman and Burton (2011) suggested that dissolved oxygen concentration (DO) might

be an important factor to consider with regards to aquatic macroinvertebrate community composition.

The potential predator-prey interactions occurring in these coastal wetlands should also be taken into consideration. Aquatic organisms are often limited in distribution due to predatory pressure (Cook, 1984). The complex habitats made up of submerged macrophytes are a place of refuge for aquatic invertebrates from foraging vertebrate predators, (fishes; Diehl 1988). Though fishes are undoubtedly an important factor when it comes to organizing the invertebrate community structure, the impact of predatory invertebrates also needs to be considered (Blaustein 1998) especially in areas where fish may not be present.

Great Lakes Coastal Wetlands Monitoring Program:

Assessing the range of natural variation of conditions among wetlands around the Great Lakes is crucial for detecting environmental degradation and tracking trends through time, and being able to predict how this giant freshwater ecosystem will be affected by factors such as agricultural practices (Goldsborough 2015) and climate change (Mortsch 1998). The Great Lakes Coastal Wetland Monitoring Program (CWM; Uzarski et al. 2017) is a collaborative, basinwide, binational ecological assessment program whose co-investigators use standardized sampling techniques to assess wetland condition and create a database that is shared among the researchers and the sponsoring organizations (US EPA 2017). The CWM program both collects and summarizes these data in terms of a variety of biological indices that characterize the condition of different guilds of biota within each wetland (US EPA 2017). These summaries provided by the CWM program offer researchers, governments, regional conservational organizations and

other stakeholders a means to effectively assess local to lakewide trends, and to guide appropriate conservation or restoration strategies (Great Lakes Coastal Wetlands Consortium 2008).

Various composite indices of environmental condition have been developed, including the Water Quality Index (WQI; Chow-Fraser et al. 2006), Wetland Macrophyte Index (WMI; Croft and Chow Fraser 2007) and Wetland Fish Index (WFI; Seilheimer et al. 2006), and have shown comparable results when used to document the ecological condition of a wetland (Seilheimer et al. 2009). By contrast, proposed wetland indices for benthic invertebrates (WII; Kashian and Burton, 2000), and zooplankton (WZI) (Lougheed and Chow Fraser 2002) have been found to be less sensitive at detecting degradation, presumably because of the complex interactions that take place between the macrophyte community and predatory fishes (Kashian and Burton 2000; Burton et al. 2002, Seilheimer, et al. 2009). The lack of sensitivity is especially apparent when applied to minimally impacted wetlands (Seilheimer et al. 2009).

Further investigation and the creation of a new multivariate index (ZACI; the Zoobenthic Assemblage Condition Index; St. Pierre 2016) has shown that significant macroinvertebrate trends are evident at the regional scale, supporting the idea that large-scale disturbance creates constraints on community condition. The combined impacts of multiple stressors, such as agricultural and land-development activities as studied by St. Pierre (2016) can lead to complex response patterns of biota in different locations in the predictor space (Lintz et al. 2011), as was the case when the macroinvertebrate assemblages were found to vary widely among locations at a single coastal site (St. Pierre 2016). Once a system is fully understood, effective and efficient sampling protocols can

be used from which standard index scores can be calculated.For now however, our limited understanding of the interactions that influence the trophic base of Great Lake coastal wetlands limits the application of these standard indices. The University of Windsor has been an integral part of the monitoring efforts of CWM, especially with respect to identifying and documenting the aquatic invertebrate species present.

My research addresses the invertebrate community composition within a relatively restricted portion of coastal wetland habitat (wet meadows), but one that is essential for maintaining wetland resilience (Albert et al. 2005, Goldsborough 2015, Keough et al. 1999). In particular, my research assesses the variation observed along a depth/DO gradient through the wet meadow zone of wetlands found along the Canadian shore of Lake Huron. My thesis poses three main questions:

1) Can variation in environmental variables be detected along a relatively short depth gradient transect within the wet meadow zone of a wetland?
2) Can variation in invertebrate community composition be detected along the environmental/depth gradient within the wet meadow zone?
3) What environmental variables influence the aquatic invertebrate community composition?

Thesis Outline:

My research assessed how strongly the concentration of dissolved oxygen (DO) influences local aquatic macroinvertebrate diversity, abundance, and community composition. If DO concentrations are an important determinant of aquatic invertebrate community composition, I expected to observe distinct groups of invertebrates inhabiting

different locations along the DO gradient within wet meadows sampled. On the other hand, if DO concentration-associated factors are not important determinants of aquatic invertebrate community composition I expected the distribution of invertebrate assemblages to be uniform within the wet meadow zone. I tested these predictions by sampling the wet meadow zones of 10 wetlands along the Canadian shoreline of Lake Huron.

This research was designed to further the understanding of the basic relationships between the wet meadow invertebrates and factors influencing their community composition within the immediate habitat.

Chapter 2 of this thesis focuses on the major water chemistry-associated environmental variables and their variation within wet meadow zones of Lake Huron. Water temperature and DO variables can vary along a depth gradient as well as throughout the course of the day (Nielsen 2013). Because they are such dynamic variables within the wet meadow zone, they were made the focus of this chapter. Temperature and DO concentrations were measured at 15-min intervals over a 24-h sampling period at four different points along the depth gradient extending between the lakeward edge of the wet meadow and the shore in each wetland. Water temperature and DO variables are also likely to be very important to an aquatic organism's choice of habitat (Davis 1975). Other environmental covariates associated with habitat characteristics were also measured at each DO and biological sampling point (specific conductance, pH, water depth, organic sediment depth, and ORP).

Chapter 3 of my thesis identified invertebrate assemblages and their distribution along the DO/depth gradient within wet meadow zones of the 10 Lake Huron wetlands

described in chapter 2. Invertebrate communities were assessed and compared to the measured environmental gradients to determine the extent to which the invertebrate community was structured by the variables associated with the depth gradient.

My final chapter summarizes and integrates my findings, recognizes the studies limitations and proposes potential future research questions.

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Chapter 2:
Spatial patterns of Key Water Chemistry Factors within Lake Huron Coastal Wet Meadows.

Introduction

Aquatic invertebrate distribution in wetlands is influenced by a number of water-quality characteristics (Cardinale, 1997) of which dissolved oxygen (DO) and temperature are most important because they directly affect an organism's metabolic and respiratory functions. Dissolved oxygen availability is especially important to consider in organically enriched ecosystems such as wetlands (Spieles, 2003). Light-dependent primary production, continuous microbial respiration, and the exchange of oxygen between the water and air account for the predictable, diel DO concentration patterns found in lakes and streams (Nielsen, 2013). These recurrent diurnal fluctuations in DO indicate system metabolism and have been used to calculate net primary productivity and respiration in a wide variety of ecosystems (Cornell, 2008). Hypoxia is likely a major constraint on habitat use by both macroinvertebrates and fishes (Davis, 1975). Measuring DO using high-frequency records provided by *in-situ* data loggers can contribute substantial ecological information about the likely habitat use of aquatic biota and their ecological interactions. Furthermore, an understanding of the relationship between land-use related eutrophication and the degree and duration of hypoxia can contribute to the development of management plans of these valuable coastal wetlands. The objective of this study is to document the variation in DO and several associated key environmental variables thought to influence macroinvertebrate and fish community composition.

Physical processes

Oxygen is essential to most aquatic biota and especially fishes. Thus, understanding the spatial and temporal distribution of oxygen within the body of water of interest is crucial understanding the distribution of organisms, especially when they arestudied to assess the ecological condition of a particular location. Atmospheric oxygen diffuses slowly into water; the rate depends on water temperature, atmospheric pressure, and salinity (Wetzel, 2001). Temperature has a nonlinear relationship with the solubility of oxygen in water, with dissolved-oxygen concentrations increasing considerably with cooler water temperatures (Wetzel, 2001; Mortimer, 1981; Benson and Krause, 1980). Dings-Avery (2019) confirmed this relationship by logging diel dissolved oxygen variation in an open-topped "reference tub" of distilled water left outside over a 24-h period. Although dissolved-oxygen concentration rarely varied by more than 1 mg/L, it increased at night when water temperatures were lower, and declined during daylight hours, when the water temperature was higher.

Atmospheric pressure and salinity also influence oxygen solubility in water (Wetzel, 2001). Pressure differences due to differences in the altitude of spatially separated bodies of water are more pronounced than local short-term variation in atmospheric pressure associated with weather. Salinity, (often measured as specific conductance) has also often been used as a tracer to determine the amount of mixing taking place between different bodies of water (Cardinale, 1997; Gaudet and Roy, 1995). In macrophyte beds, conductivity increases along a gradient extending from the open water edge of the vegetation stand, where it will be lowest, towards shore, where the conductivity will be greatest (Cardinale, 1997).

Wind is also an important factor to consider in aquatic ecosystems. Fluctuations in water level, water column mixing and sediment resuspension result from wave action and wind-induced seiche events (Deng, 2018; Bachmann, 2000; Trebitz, 2006). Variation in wind direction is also important to consider. Scully (2010) showed that the volume of hypoxic water within Chesapeake Bay depended on the direction of the wind, and that even the slightest deviation from the prevailing wind direction had dramatic effects on the oxygen dynamics due to the bay's geometry and bathymetry. Wind direction determined where the deeper hypoxic water would be pushed along the bottom of the bay (Scully, 2010). Winds from the south resulted in a net flux of oxygen for Chesapeake Bay. Because those winds would cause upwelling of the deeper hypoxic water, it could be ventilated and later sink back as newly oxygenated water once the winds died down (Scully, 2010). A west wind however, would not move water into shallower areas and thus ventilating the deep hypoxic water was not as effective (Scully, 2010).

The attenuation of waves by vegetation found in coastal wetlands protects shoreline from erosion and personal property from damage due to storm surges (Costanza, 1989). Waves lose energy as they travel through vegetation beds due to the drag imparted onto the surface water, thus reducing wave amplitude and velocity (Dalrymple et al. 1984; Cardinale, 1997). Wave height has been observed to decrease by as much as 20% per meter (Anderson et al., 2011), and water flow rate can be reduced by 98% within the first 10-15 m (Losee and Wetzel, 1993) of the lakeward edge of a vegetation bed. Generalizing wave-vegetation interactions however, is extremely difficult as the dissipation of wave energy depends on vegetation biomechanics and wave characteristics (Anderson et al., 2011).

Biological processes

Biological processes (photosynthesis and respiration) account for most of the diel DO fluctuations observed in wetlands (Spieles, 2003; Cornell 2008). The marked extent of DO concentration fluctuations found in wetlands reflects the high density of plants, animals, benthic algae and plankton (Reeder, 2011) as well as the presence of oxidizing compounds in the sediment (Wetzel, 2001). Dissolved oxygen concentrations areexpected to vary throughout a wetland, reflecting spatial patterns of the plant community (Frodge et al. 1990, Carpenter and Lodge 1986, Chimney et al. 2006). Water in dense *Typha* patches, for example, is often hypoxic (<4 mg /L) and varies little over a 24-h period (Chimney et al., 2006). This reflects microbial respiration due to the large amounts of plant material decomposing in the sediments (Carpenter and Lodge, 1986), as well as the reduced physical mixing of the water.

Various water quality-related gradients exist along transects in coastal-wetland zones that extend from the outer, lakeward edge of a wetland to shore (Suzuki, 1995), and the composition of zooplankton, macroinvertebrates and fish communities varies along this gradient (Cardinale, 1998; Gathman and Burton, 2011). This thesis chapter assesses the variation in several key environmental variables thought to influence macroinvertebrate and fish community composition, specifically within the 15-30 m wide ecotone between the open water/submergent aquatic vegetation zone and the wet meadow zone of Lake Huron coastal wetlands. My objectives in this chapter were to

1. Describe the temporal and spatial patterns in environmental characteristics among sample locations within the wet meadow zone of multiple wetlands.

2. Describe how 24-h dynamics of dissolved oxygen vary along a depth gradient within wet meadow zones relative to the respiratory needs of fishes and invertebrates

Methods and Materials

Wetland Selection:

Using the Great Lakes Coastal Wetland Consortium's (CWM) Coastal Wetland Monitoring Program Mapping Tool (http://www.GreatLakesWetlands.org) and Database, suitable wet-meadow sampling sites around Lake Huron were identified for the 2017 field season. Wetland selection was based primarily on the agricultural stress scores calculated by Danz et al. (2007) as part of the Great Lakes Environmental Indicators (GLEI) initiative that corresponded with the documented plant community composition described by CWM crews for each wetland site sampled in the previous 5 years (2011-2015). The agricultural stress score ranged from 0-1 (southern region: n= 1065, mean=0.416, s=0.281, northern region: n= 2423, mean=0.129, s0.206). Aerial photos of sites containing a wet meadow were then examined to confirm their suitability. However, photo analysis of sites was supplemented by first-hand knowledge from researchers who had visited these wetlands more recently than the date on which the aerial photos were taken. Information on the amount of agricultural stress (areal percentage of land in a watershed used for agriculture) found in the contributing watershed of such wet meadows was also a strong factor in the site selection process (Danz et al. 2007, Host et al., 2019).

Each of the Lake Huron wet-meadow sampling sites were first classified as either being part of the North Channel or Bruce Peninsula region of Lake Huron. The North Channel region is part of the Canadian Shield (granitic bedrock) and has relatively little agriculture and anthropogenic disturbance. In contrast, the Bruce Peninsula of Lake Huron is carbonaceous and is subject to agricultural activity – largely pasture land. Each wetland was then also classified as either being minimally used for agriculture (i.e., a reference site), or considerably affected by agricultural activity (stressed site), (n=10 wetlands, 5 minimally stressed, 5 more highly stressed). Host et al. (2019) classified Great Lakes basin wetlands having a Euclidean distance score (combination of Agriculture and Development stress scores ranging from 0-1, Table 2.1) of 0.05 or less to be in a least-disturbed state and affecting the biota minimally, and sites with scores at or greater than 0.95 to be degraded. Sites with Euclidean distance scores between 0.05 and 0.95 were categorized as being "at risk" of becoming degraded (Host et al., 2019).

Finally, after each candidate wetland had been classified, wetlands were pairmatched such that pair members were geographically close together and as similar to each other as possible within each region. High and low stress wetlands were matched primarily based on similarity of their connectivity to Lake Huron and physical features found at each site (i.e., low stress drowned river mouth wetlands were matched with highly agriculturally-influenced drowned river mouth wetlands). To the extent possible, considerably stressed wetlands were matched with spatially adjacent low stress wetlands.

Based on the selection process described above, ten wetlands were selected and sampled between June and August 2017 - five within watersheds that contain significant amounts of agricultural activity in the contributing watershed (stress score >0.2, Table

2.1), and thus operationally defined as stressed wetlands; and five wetlands draining watersheds operationally classified as reference (stress scores <0.06; Table 2.1). One wetland, Stokes Bay Wetland 2, was designated as stressed despite having a very low agriculture stress score. It was believed that the score was not representative, based on examination of Google Earth® aerial images.

Table 2. 1 Lake Huron coastal wetland wet meadow sites sampled June-August, 2017. BP represents the Bruce Peninsula region, while NC represents the North Channel region of Lake Huron.

Site Number	Site Name	Sampling Days 2017	Lat	Long	Region	Regional Agriculture Score	Regional Euclidean Distance Score	Level of Stress
5704	Old Woman's River	20-21 August	44.96845	-81.34436	BP	0.62	0.63	H
5706	Fishing Island 5	24-25 August	44.71839	-81.27881	BP	0.02	0.14	L
5016	Baie du Dore 2	25-26 June	44.34172	-81.54984	BP	0.80	0.81	H
5727	Pike Bay 1	23-24 August	44.87549	-81.32893	BP	0.06	0.10	L
5953	Stokes Bay Wetland 2	18-19 August	44.99500	-81.37379	BP	0.01	0.07	H
5952	Stokes Bay 1	19-20 August	44.98502	-81.39395	BP	0.79	0.79	L
5013	Anderson Creek	04-05 August	46.33104	-83.97656	NC	0.15	0.16	H
5106	Blind River 1	07-08 August	46.23411	-83.04957	NC	0.01	0.10	L
5950	Stobie Creek 1	05-06 August	46.33137	-83.88453	NC	0.27	0.28	H
5137	Bullhead Bay	09-10 August	46.25600	-83.52496	NC	0.03	0.32	L

Figure 2. 1 Map of 10 wetlands sampled in August 2017.

Field Methods: Overall Wet Meadow Assessment of Environmental Variables

Transect Delineation

At each wetland site, three equidistant, parallel transects were delineated at intervals along the width of the wet meadow. Transects were oriented to extend from deeper to shallower water along the depth/dissolved oxygen gradient in order to cross the emergent wet meadow habitat zone of each wetland (Rose, 2006). The dissolved oxygen/depth gradient was identified by measuring the dissolved oxygen concentration at evenly spaced intervals, using a dissolved-oxygen meter (YSI Inc., Professional Plus).

The measurements were made at each site between 0845 and 1645 EDT, during which time DO was expected to be approaching or at peak saturation for the day. Transects extended from deeper water (up to 1 m deep) lakeward of the wet meadow (most exposed to the open water of the lake and therefore presumably having the highest DO) towards the water's edge (no shallower than 30 cm) to accommodate the DO data loggers without disturbing the sediment where DO concentrations were expected to be lowest. Transects were 15-30 m long, with sample locations being arranged equidistantly - at the two end points, and 1/3 and 2/3 of the distance along their length (3 transects with 4 sample points each, for a total of 12 sample points/wetland). A suite of environmental and biological measurements was collected at each sampling location.

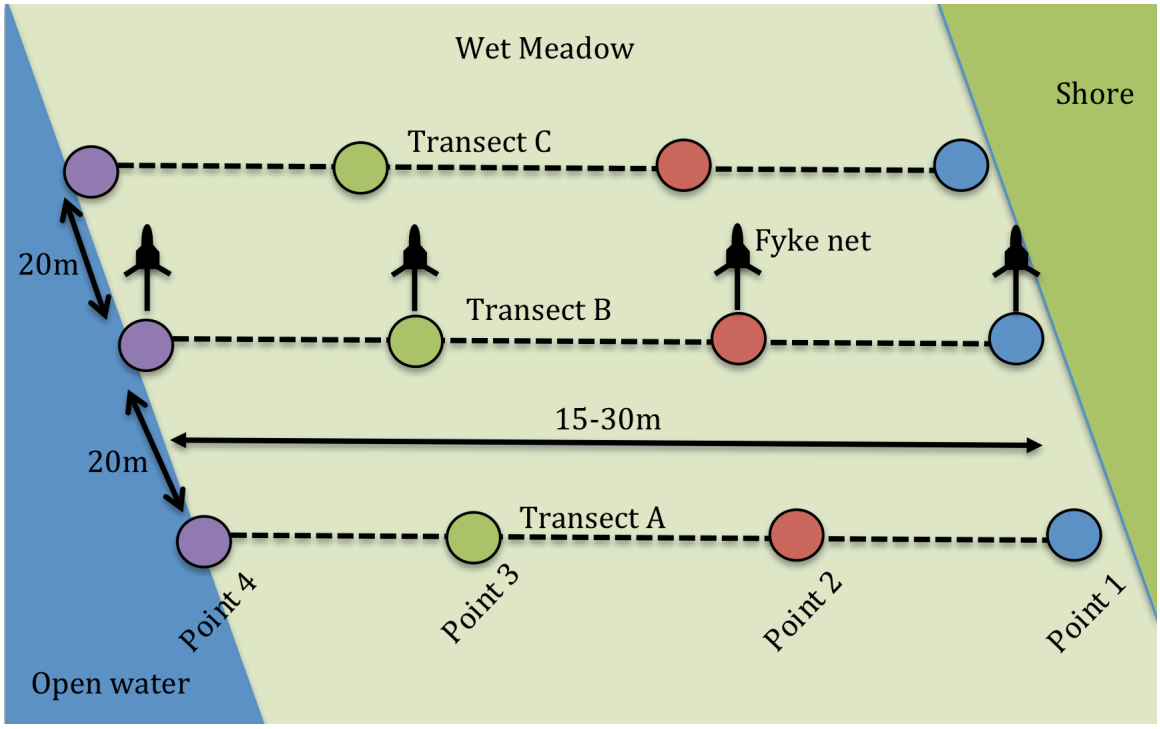

Figure 2. 2 Sampling set up at each wet-meadow.

Water quality

Water-quality data were collected at each sample point using the YSI water-quality meter referenced above. Temperature, dissolved oxygen concentration,

conductivity, pH and oxidation-reduction potential (ORP) were recorded at each sample point. A 6-L composite water sample was collected by combining aliquots taken from various locations at the study site. Composite water samples were stored on ice and shipped to the National Laboratory for Environmental Testing (NLET) in Burlington ON, for analysis of nutrients (phosphorus, nitrogen, etc.), chlorophyll *a*, and water clarity.

Diel Variation in Dissolved Oxygen

Dissolved oxygen (DO) loggers (Hobo U26-001Dissolved Oxygen Data Logger) were deployed at every sample point (12 loggers/site, Figure 2.2), to record the dissolved oxygen concentration every 15 min over a 25-h period. The DO loggers were placed so that the top of the logger was 15 cm below the surface of the water and were fastened to a stake. The DO loggers were oriented vertically with the sensor facing upwards. The DO loggers were deployed as early as possible in the day. Units were deployed in 4 wetlands (Bullhead Bay, Anderson's Creek, Pike Bay, and Stokes Bay 2) in the morning between 0845 and 1115 EDT, and were removed from the wetlands 45 min – one h after the deployment time the following day; thus, these 4 sites logged DO concentrations for a minimum of 24 h and 45 min, up to a maximum of 25 h. At the remaining 6 wetlands (Blind River, Stobie Creek, Fishing Island 7, Old Woman's River, Stokes Bay 1 and, Baie du Dore) the DO loggers were deployed in the afternoon (between 1145 and 1645 EDT), and they were removed from the wetland approximately 75 min after the deployment time the following day. The DO concentrations were recorded for at least 24 h, and for up to 25 h and 15 min. Dissolved oxygen records were available for a common 17-h period at all 10 wetlands - 0645 – 0930..

At each wetland, a DO logger was suspended within a 70-L Rubbermaid® plastic tub filled with tap water to serve as a reference 'blank'. The reference tub was placed near the center of the sample site, immersed in the wet meadow water and was secured in place with stakes. Unfortunately, tubs at all wetlands were flooded by wetland water due rising water levels, thus somewhat compromising these data.

Diel variation in Light and Temperature

Light and Temperature loggers (Hobo UA-002-64 Pendant® Temperature/Light 64K Data Logger) were placed at each sample point and recorded the light intensity and water temperature every 15 min over a 25-h period (Figure 2.2). These loggers (LT loggers) were fastened 15 cm below the water surface in a horizontal orientation.

Vegetation

Prior to sampling, it was expected that the distribution of wet meadow plant species might exhibit a zonation pattern reflecting the prevailing water depth since the tolerance response to inundation is unique to each plant species and thus controls zonation in deep water (Sorrell et al., 2012; Rose, 2006). Within each wet meadow, two factors were measured at each sample point; vegetation type (genus), and vegetation stem density. The two most dominant plants found at each sample site were documented. The species composition of the 10 wet meadows was uniform within wetlands and almost identical among wetlands. *Carex* was the most abundant genus at nearly all wet meadows and *Calamagrostis canadensis* was the second most dominant species at most of the wetland sampled (Table 5.3).

Vegetation density was measured using two methods and these were then compared to each other (Figures 5.1 and 5.2). Quadrat (50 cm x 50 cm) stem counts were

taken from a randomly selected transect for each wetland (4 quadrat stem counts/wet meadow). These quadrat stem counts were then compared to photographic images of vegetation obscuring a Robel Pole situated 4 m away from corresponding quadrat stem counting locations in anticipation of producing a calibration curve (Appendix 1; Figure 2.3). The recorded water depth was taken into account to determine how much of the pole was actually above water. A visual obstruction percentage was determined by calculating and how much of the Robel Pole (above water) was visible from a distance of 4 m.

Figure 2. 3 Robel Pole Photo example.

Laboratory Methods:

Data were downloaded from the DO and Pendant light/temperature loggers using a USB cable, upon the completion of sampling trips using HOBOware Pro 3.7.12 software (Onset Computer Corporation, 2017), and the information was saved as CVS files. Each file was then copied and pasted into Excel version 14.7.7 (Microsoft, 2011)

for further clean up and summary. Multiple sites' worth of data were captured on each sampling trip so the recorded date and time that loggers were deployed were used to identify and isolate each site's specific data.

Once the information pertaining to each wetland had been compiled into site-specific files, time of logging was standardized among sample point replicates based on when all loggers were recording information at the same time. For example, if the logger for transect 1 began recording at 1030 but the loggers for transects 2 and 3 began logging at 1045, then the 1030 logging information from transect 1 was excluded from the final set of data that would be used to summarize diel DO and temperature patterns. Time trend plots were visually examined to detect anomalous measurements (outliers). If a marked difference in the DO concentration was observed that did not correspond with the DO recordings 30 min before and after the recording of interest, the reading was operationally deemed to be an outlier and was excluded from further analysis (This occurred on one or two occasions when a DO reading of -888.88 was observed). Minor deviations from the pattern of DO recordings were compared to notes made about the weather conditions. Scattered showers and stream outflow explained the majority of these events, as was expected (Cornell, 2008). A handful of events resulted in odd DO recordings that are suspected to have been due to activities of muskrats or other wildlife near the DO loggers.

Once the replicates (Transects A, B and C, Figure 2.2) had been standardized within each wetland, simple summary information (mean, standard deviation (SD) and standard error (SE), n=3) was calculated for each relative sampling location (Point 1 – shallowest to Point 4 – deepest), as the distances between sampling points was not

exactly the same among sites. The summary table of means was used to determine the duration of mild (<4 mg/L) and moderate (< 2 mg/L) hypoxia at each point by summing the number of 15-min intervals during which a criterion was met.

To better compare sites to one another, further standardization was required. As mentioned before, DO loggers were deployed as early in the day as possible in each wetland. Although arrival times varied among wetlands all logger recorded data over the same 17-h period - between 1645 and 0930 EDT. The durations of mild and moderate hypoxia at each sampling point in each wetland were determined for this common 17-h period.

Each vegetation-and-Robel pole image was examined and the percentage of the above-water length of the Robel pole was subjectively estimated to the nearest 10%. The visual obstruction estimate for each photo was regressed against the corresponding quadrat stem counts to create an equation relating photo estimates to vegetation density (Appendix 1).

Statistical Analyses

Arithmetic means and standard deviations of environmental variables at each transect point along a depth gradient within each region were calculated using data collected from the 10 wetlands (Table 2.2). Principal Components Analysis (PCA; based on the correlation matrix, and using Varimax rotation) was used to summarize the variation among samples collected using the Factor Analysis module of Statistica 7.1 (Statsoft Inc.). Cluster analysis (Ward's method based on Euclidean distances) was performed to determine whether groups of samples having environmental characteristics were evident.

Results

Of the ten wetlands sampled, 7 (Blind River, Stokes Bay 1 and 2, Old Woman's River, Fishing Island 7, Baie du Dore and Pike Bay) displayed diel DO patterns that were expected of a coastal wet meadow zone. Dissolved oxygen concentrations were highest in the late afternoon, and the oxygen levels steadily declined through the night (Figures 2.4 – 2.10). Nocturnal anomalies were observed at 2 wetlands, Anderson's Creek and Stobie Creek (Figures 2.11 and 2.12). At Bullhead Bay an unexpected increase in DO concentration was observed in the middle of the night (Figure 2.13)

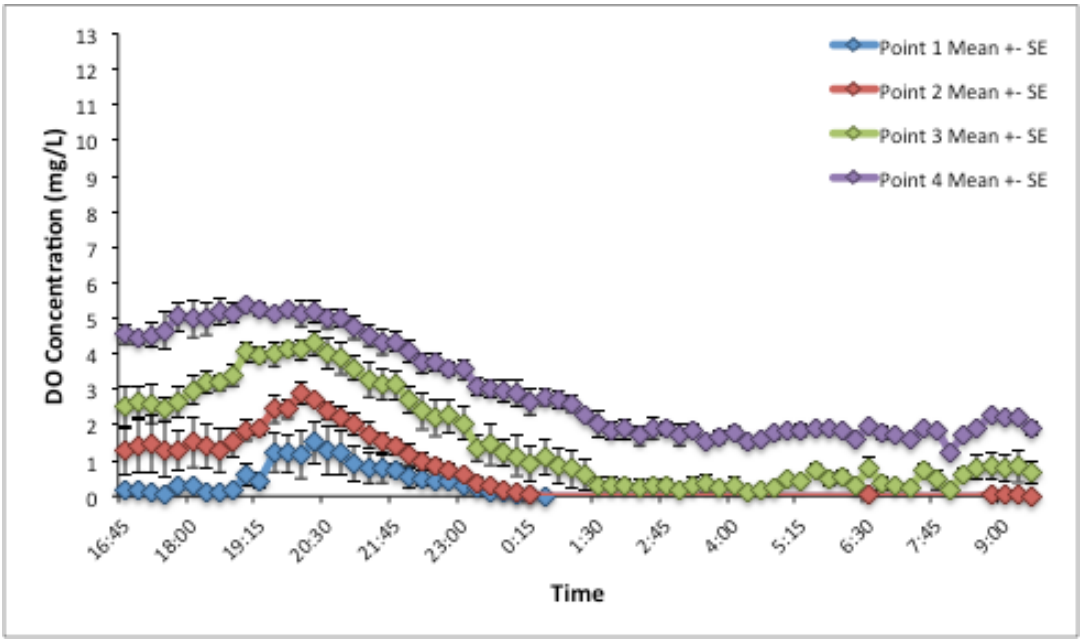

Figure 2. 4 The Blind River site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 7, 2017 and 0900 at August 8, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

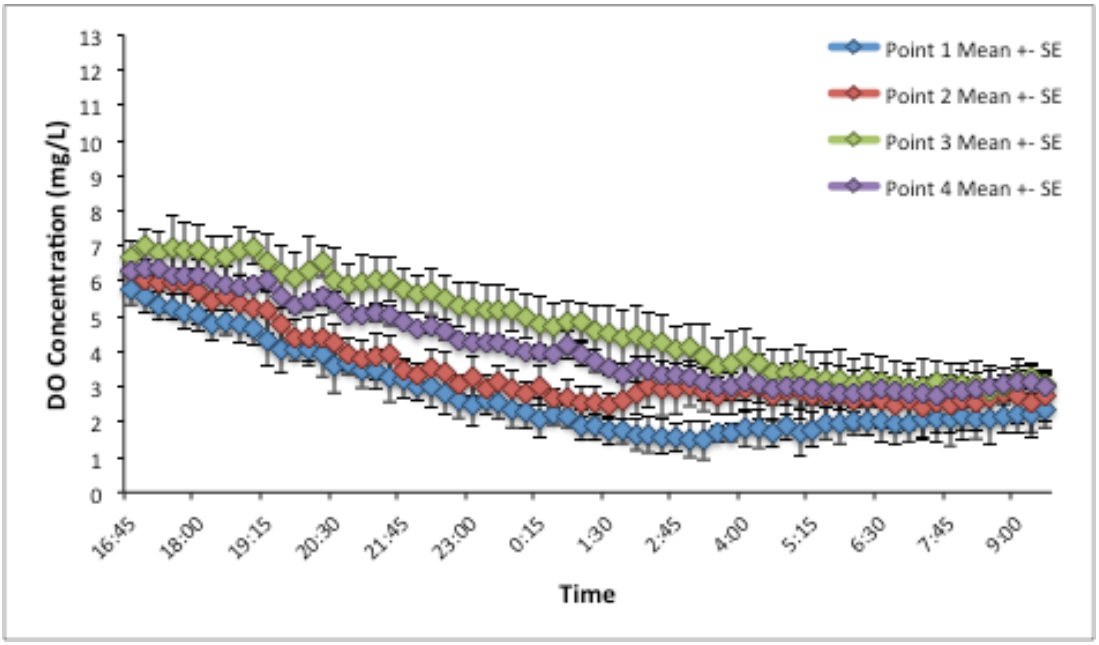

Figure 2. 5 The Stokes Bay 2 site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 18, 2017 and 0900 at August 19, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

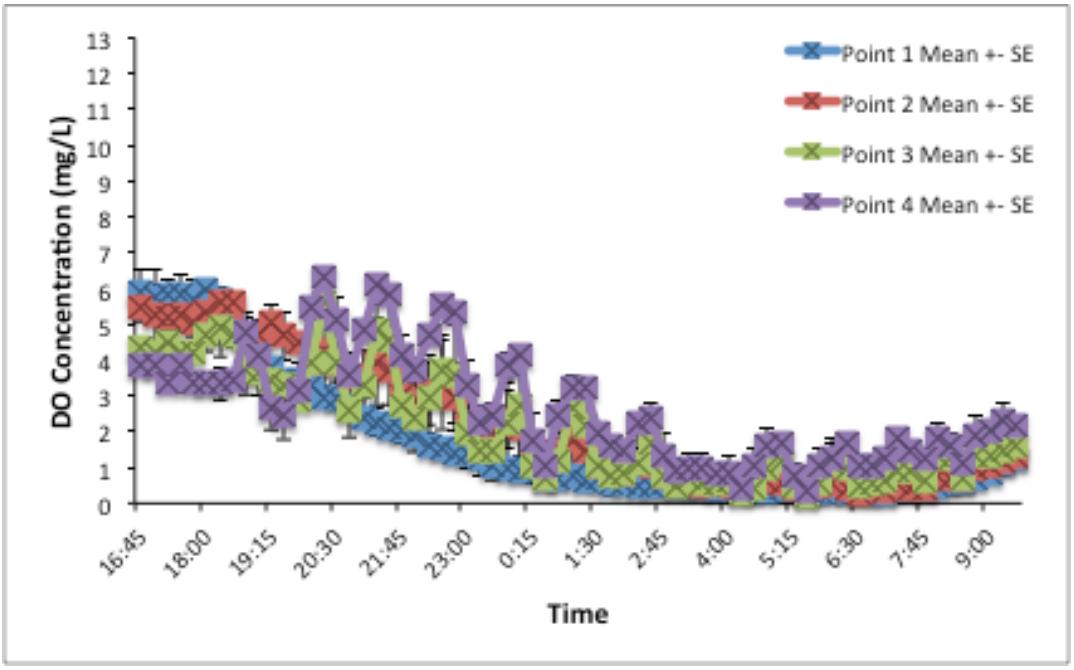

Figure 2. 6 The Stokes Bay 1 site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 19, 2017 and 0900 at August 20, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

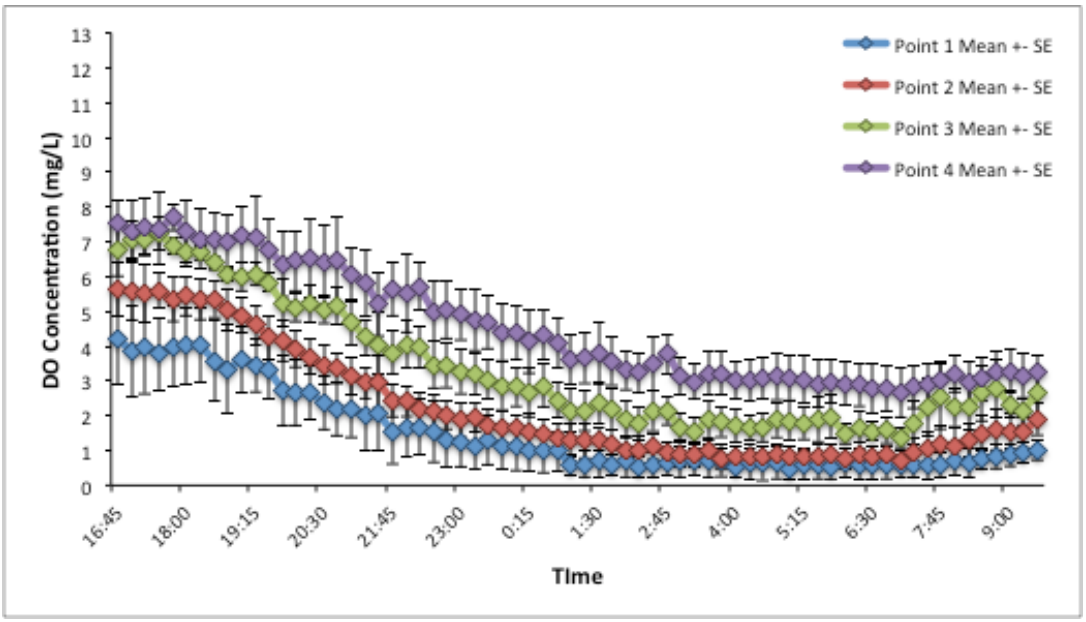

Figure 2. 7 The Old Woman's River site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 20, 2017 and 0900 at August 21, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

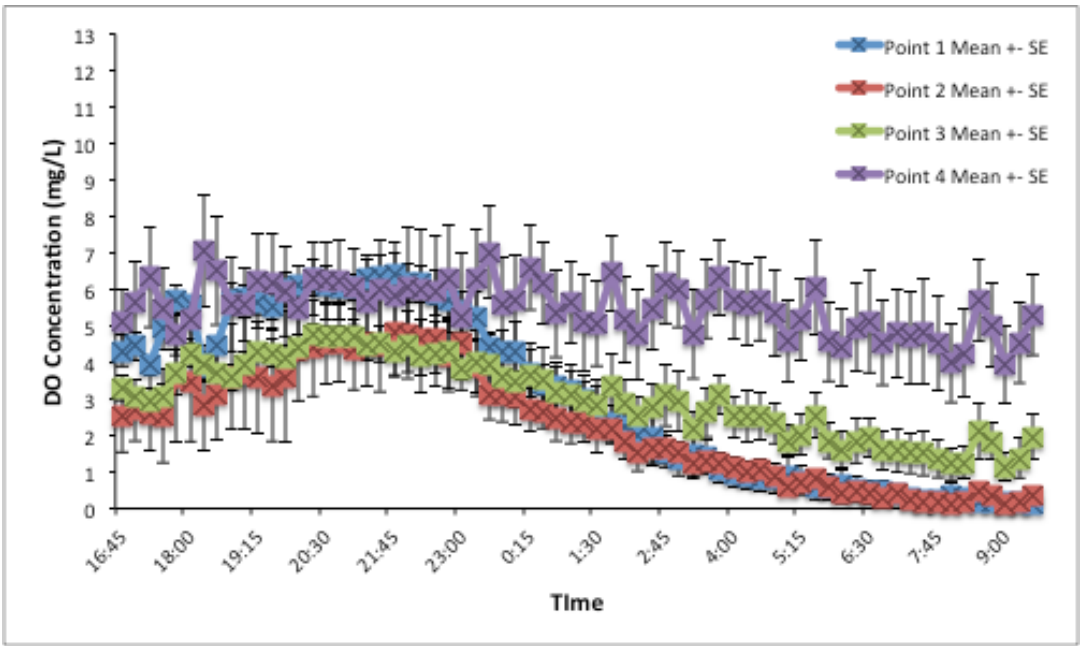

Figure 2. 8 The Fishing Island 7 site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 24, 2017 and 0900 at August 25, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

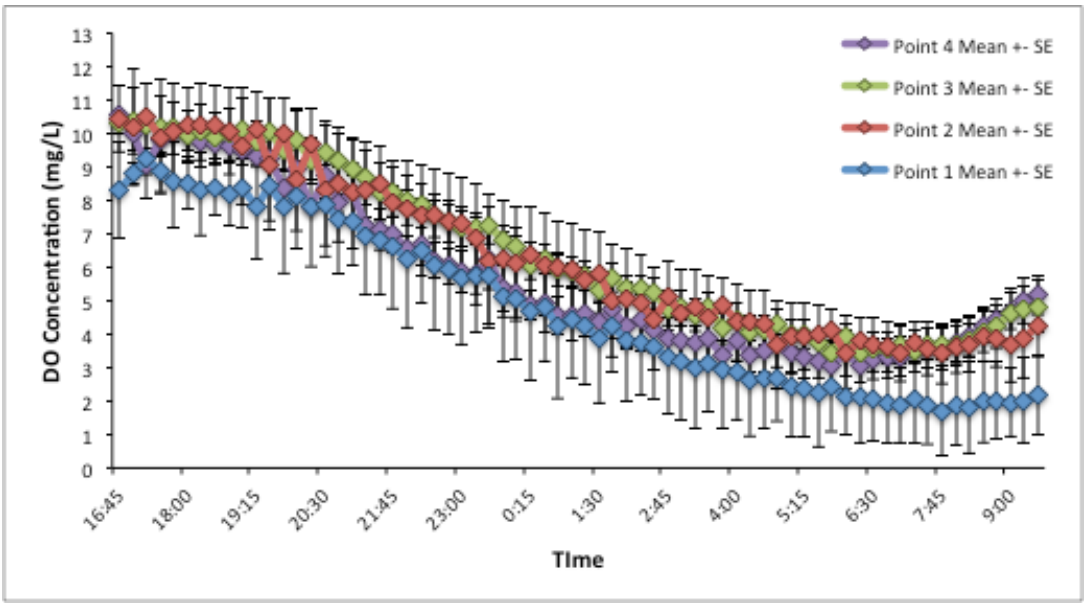

Figure 2. 9 The Baie du Dore site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on June 25, 2017 and 0900 at June 26, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

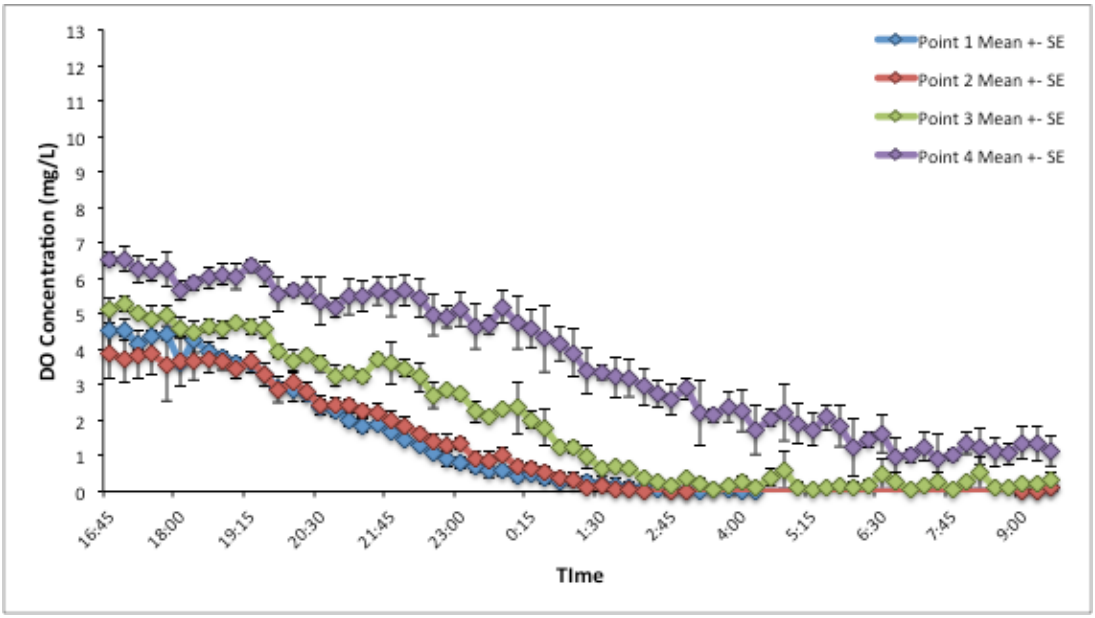

Figure 2. 10 The Pike Bay site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 23, 2017 and 0900 at August 24, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

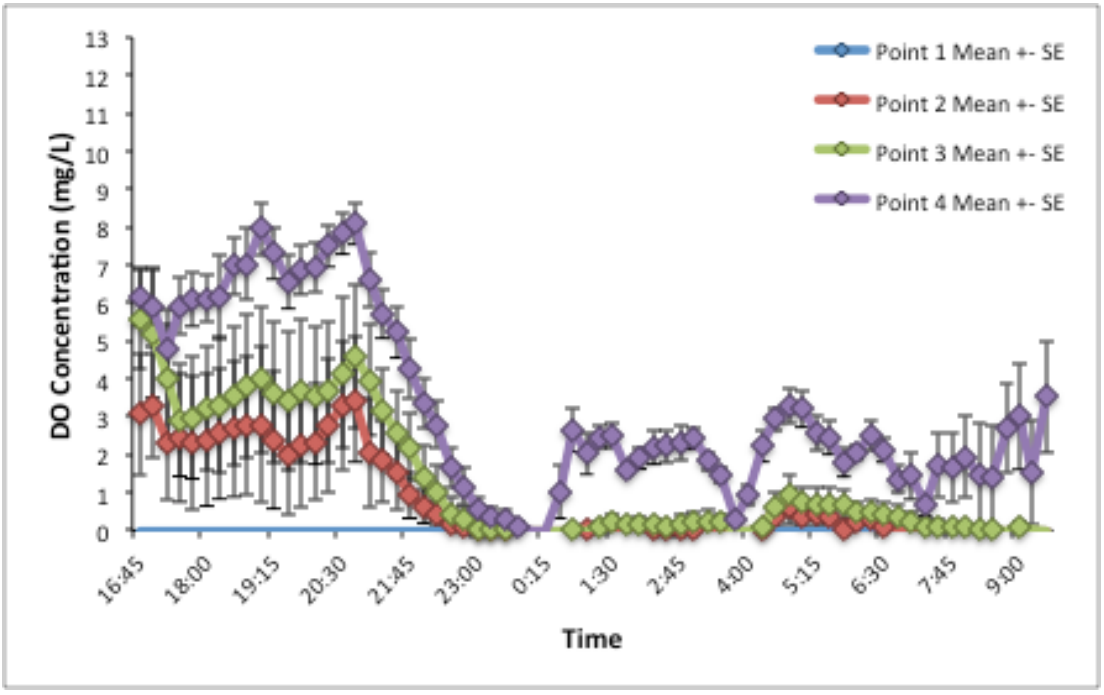

Figure 2. 11 The Stobie Creek site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 5, 2017 and 0900 at August 6, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

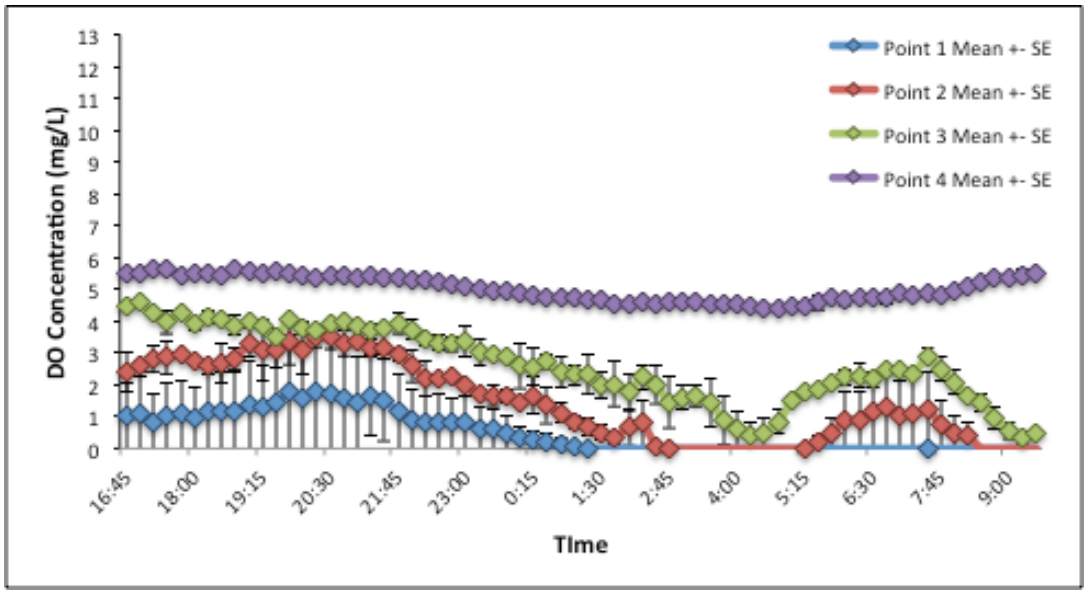

Figure 2. 12 The Anderson's Creek site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 4, 2017 and 0900 at August 5, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

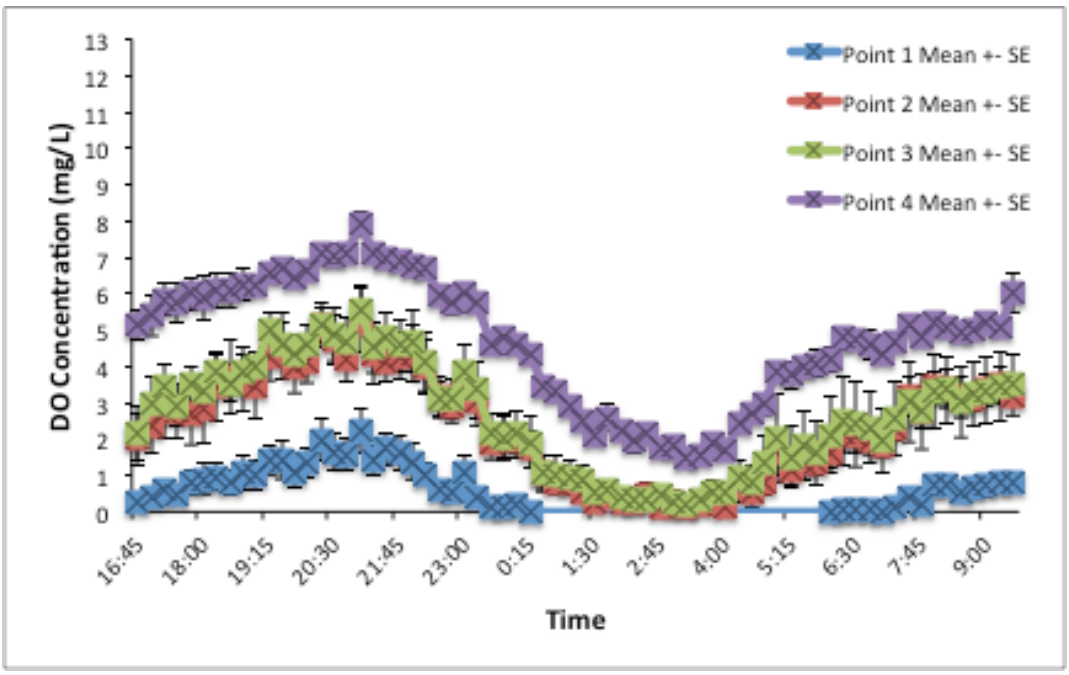

Figure 2. 13 The Bullhead Bay site DO concentrations (mean ± SE of three transects) recorded every 15 minutes between 1645 on August 9, 2017 and 0900 at August 10, 2017. Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

Reference tub estimates

Reference DO concentrations were only taken at 5 of the 10 sample sites, Fishing Island 7, Pike Bay, Old Woman's River, Stokes Bay 1 and Stokes Bay 2. The DO concentrations recorded by almost all of the wetland-based loggers were consistently lower than the records produced by the corresponding loggers in reference tubs for the Bruce Peninsula sites (Fig 2.14).

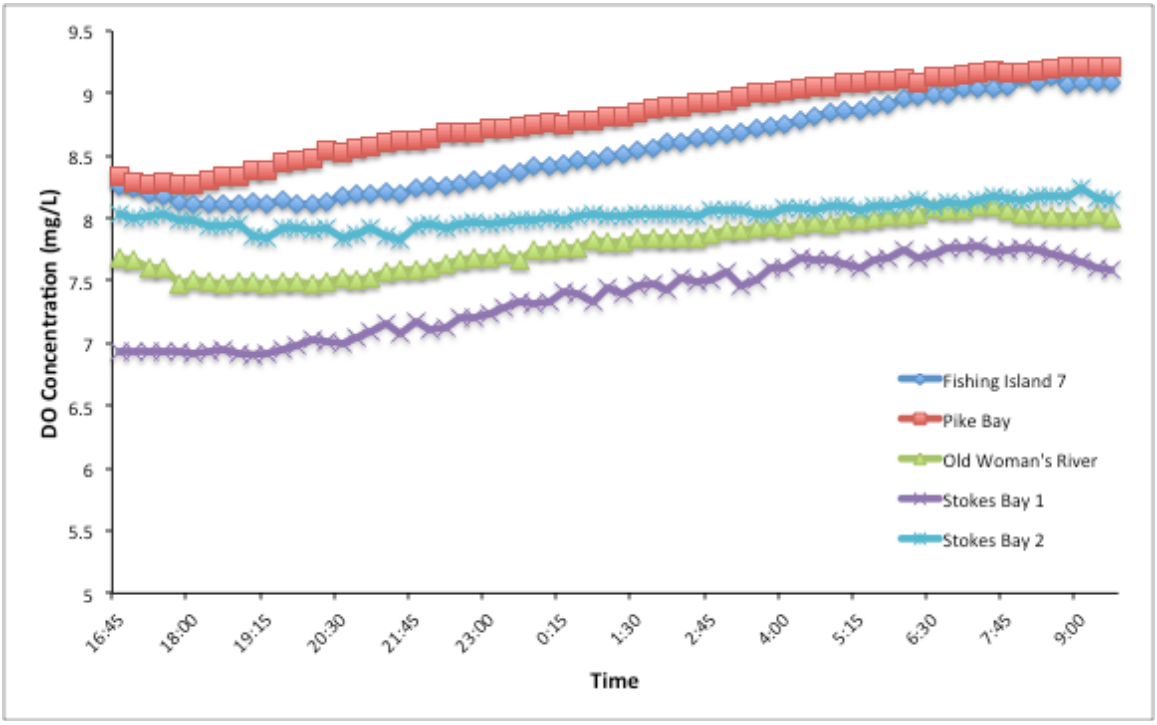

Figure 2. 14 The Reference Tote DO concentrations (mean ± SE of three transects) recorded every 15 minutes for 5 sites (Fishing Island 7, Pike Bay, Old Woman's River, Stokes Bay 1 and Stokes Bay 2). Point 1 represents the most protected and closest to shore, part of the wet-meadow transects, Point 4 represents the least protected part of the wet-meadow transects (Figure 2.3).

Environmental characteristics

Summary statistics for the 14 environmental characteristics measured at each sample location at every site are summarized in Table 2.2 (duration of mild hypoxia (<4 mg/L), duration of moderate hypoxia (<2 mg/L), DO maximum (mg/L), DO minimum (mg/L), DO range (mg/L), water temperature maximum (°C), water temperature minimum (°C), water temperature range (°C), specific conductance (uS/cm), pH, oxidation reduction potential or ORP (mV), water depth (cm), organic sediment depth (cm) and relative position within the wet-meadow (point)). These fourteen environmental characteristics were analyzed using a Principal Components Analysis (PCA) in Statistica (StatSoft Inc).

The North Channel region sites experienced a longer duration below 4 mg/L and 2 mg/L, or at hypoxia and anoxia, than the Bruce Peninsula sites. The Bruce Peninsula had greater DO concentration maxima recorded over the 25-hour monitoring period than the North Channel sites, which obtained the lowest DO concentrations between the two regions. The water temperatures were warmer at the Bruce Peninsula sites, and these sites also had the greatest temperature ranges over the diel sampling period. The North Channel sites were more acidic than the Bruce Peninsula sites due to the bedrock geology. Finally, the organic layer depths were much deeper in North Channel sites in comparison to the Bruce Peninsula sites.

Table 2. 2 Arithmetic mean ± SD of environmental variables for all sites based on region and transect point. Point 1 = shallowest, Point 4 = deepest.

		Hours <4mg/L	Hours <2mg/L	DO Max	DO Min	DO Range	Temp Max	Temp Min	Temp Range	Spec Cond	pH	ORP	Water Depth	Organic Depth
North	1 (n=4)	25.18 + 0.24	23.5 ± 2.54	2.13 ±1.63	0 ±0	2.13 ±1.63	20.42 ±1.8	18.18 ±1.14	2.25 ±0.77	119.8 ±14	6.84 ±0.13	-149.78 ±136	64.88 ±20.46	38.75 ± 24.5
	2 (n=4)	23±3.7	18.19±3.74	4.68±1.58	0±0	4.68±1.58	21.14±1.85	18.39±1.12	2.75±1	118.33±15.54	6.84±0.13	-120.65±107.12	61.88±20.54	38.75±24.5
	3 (n=4)	20.94±3.56	12.94±5.08	5.65±1.87	0.05±0.08	5.61±1.86	21.19±1.82	18.56±1.17	2.64±0.85	117.15±15	6.86±0.11	-103.2±50.54	66.63±13.6	39±24
	4 (n=4)	10.56±8.53	4.69±4	7.12±1.92	1.74±1.85	5.38±3.43	21.55±1.82	18.74±1.15	2.81±1.02	116.05±16.39	6.84±0.1	-64.63±24.36	76.88±8.4	38.5±25
South	1 (n=6)	19.38±3.2	11.79±5.13	6.64±1.94	0.65±0.98	5.99±1.06	24.49±4.21	19.23±1.61	5.26±4.44	281.33±84.84	7.19±0.52	59.25±135.1	43.33±20.02	6.33±7.71
	2 (n=6)	18.58±6.03	10.46±8.63	5.89±2.48	0.89±1.21	5±1.37	23.6±2.84	19.56±1.53	4.04±2.22	276.78±75.31	7.25±0.45	56.9±100.11	48.33±16.77	2.5±2.88
	3 (n=6)	13.29±5.61	4.58±4.77	6.99±1.45	1.27±0.95	5.72±0.98	23.52±2.57	19.73±1.61	3.79±1.76	268.68±68.83	7.35±0.45	5.9±113.39	51±12.39	2.5±3.83
	4 (n=6)	10.25±6.87	2.75±3.82	7.58±1.24	1.83±1.98	5.75±1.4	23.13±2.34	19.97±1.72	3.16±1.12	264.8±65.79	7.52±0.46	31.97±86.51	57.75±17.67	6.33±9.48

The PCA extracted 4 axes with eigenvalues greater than 1.0 (Figure 2.15), which explained approximately 83% of the variation (Table 2.3). The first two components had eigenvalues greater than 2 and together explained approximately 64% of the variation (Table 2.3). Temperature range, and mean depth were associated with PC1, with temperature range being positively correlated and mean depth being negatively correlated (Table 2.4). Thus, the shallowest sites also exhibited the greatest diel range in temperature, highest maximum temperature and higher pH. Duration of mild hypoxia (<4 mg/L), and duration of moderate hypoxia (< 2 mg/L) were negatively associated with PC2 (Table 2.4), whereas DO concentration minimum, maximum, Point and pH were positively associated with PC2 (Table 2.4). Samples from the Bruce Peninsula wetlands exhibited a broad range of PC1 values, whereas, samples from the North Channel wetlands ranged along PC2 (Figure 2.16). Among all wetlands, the relative location of sampling (Point 1,2,3 vs. 4) most markedly ordinated along the PC2 axis (Figure 2.17).

Temperature Minimum, Specific Conductivity and ORP were positively associated with PC3, while Organic depth was negatively associated with PC3. The DO Range and DO Maximum were positively associated with PC4 (Table 2.4). PC2 and PC4 accounted for all associations with DO concentration-related environmental variables. Together, they describe the relationship between the relative sampling location and DO concentration (Figure 2.18).

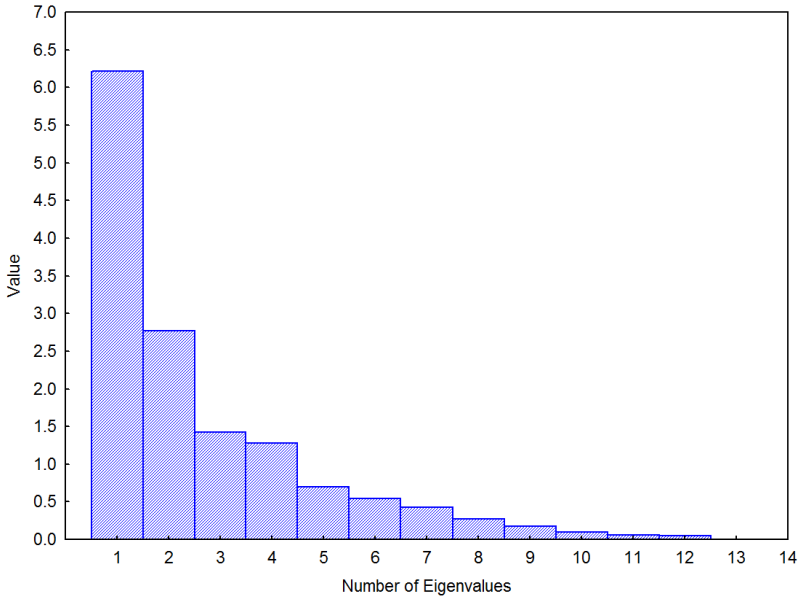

Figure 2. 15 Eigenvalues of environmental factors. The first four factors describe 84% of the variance (Table 2.3).

Table 2. 3 Percent of variance explained by each Factor.

Factor	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	6.22	44.40	6.22	44.40
2	2.77	19.75	8.98	64.15
3	1.42	10.14	10.40	74.29
4	1.28	9.14	11.68	83.43

Table 2. 4 Factor loadings of environmental variables. Bold-faced and red values indicate association with Factor axis (See Appendix for site loadings).

	PC1	PC2	PC3	PC4
Point	-0.50	**0.66**	0.16	0.28
Hours <4mg/L	-0.05	**-0.93**	-0.01	-0.22
Hours <2mg/L	-0.02	**-0.86**	-0.22	-0.31
DO Max	0.30	**0.63**	0.05	**0.69**
DO Min	0.29	**0.90**	-0.05	-0.17
DO Range	0.16	0.14	0.09	**0.95**
Temp Max	**0.65**	0.03	0.58	0.34
Temp Min	-0.02	-0.04	**0.94**	0.12
Temp Range	**0.84**	0.07	0.11	0.35
Spec Cond	0.64	0.24	**0.65**	0.13
pH	**0.66**	**0.57**	0.21	0.16
ORP	0.40	0.28	**0.42**	0.29
Depth Average	**-0.81**	-0.07	-0.09	-0.02
Organic Depth	-0.34	-0.21	**-0.72**	0.16

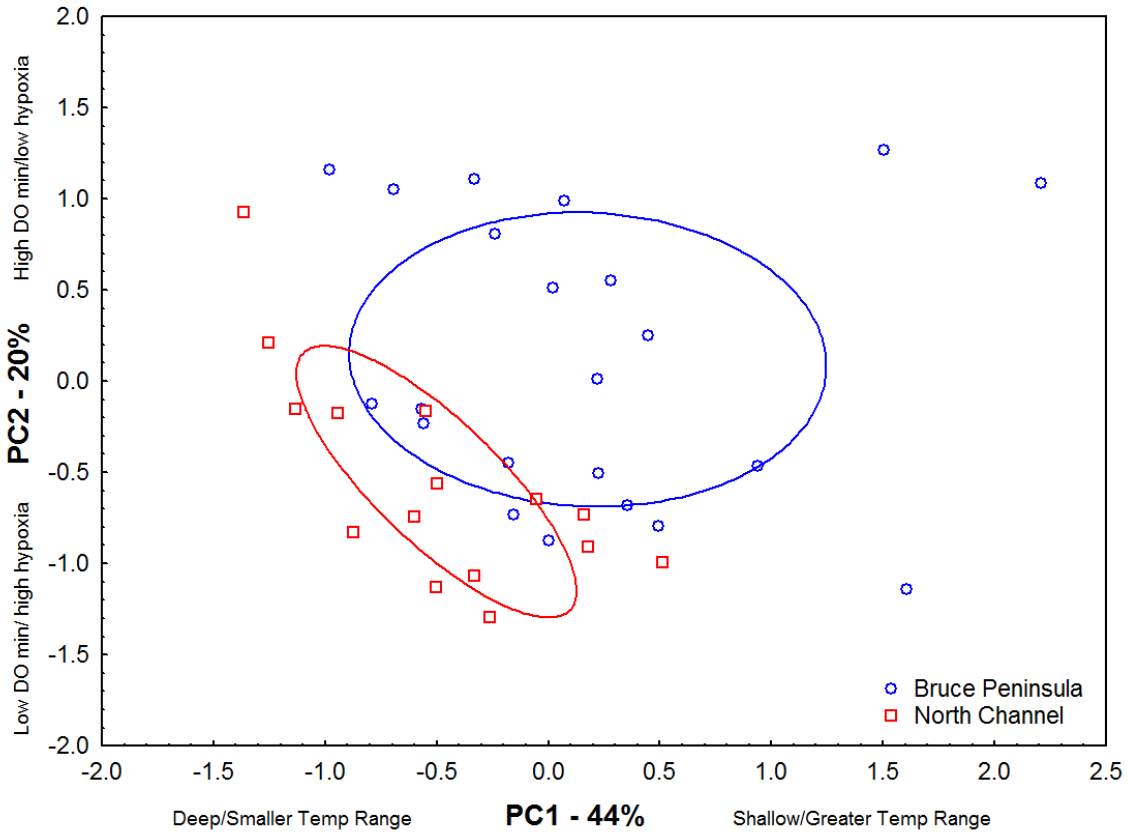

Figure 2. 16 Principal Component results of environmental sample loadings (n=37), excluding the three samples (Fishing Island 7's deepest transect point, Anderson's Creek deepest transect point, and Baie du Dore's shallowest point). Ellipse represents one standard deviation.

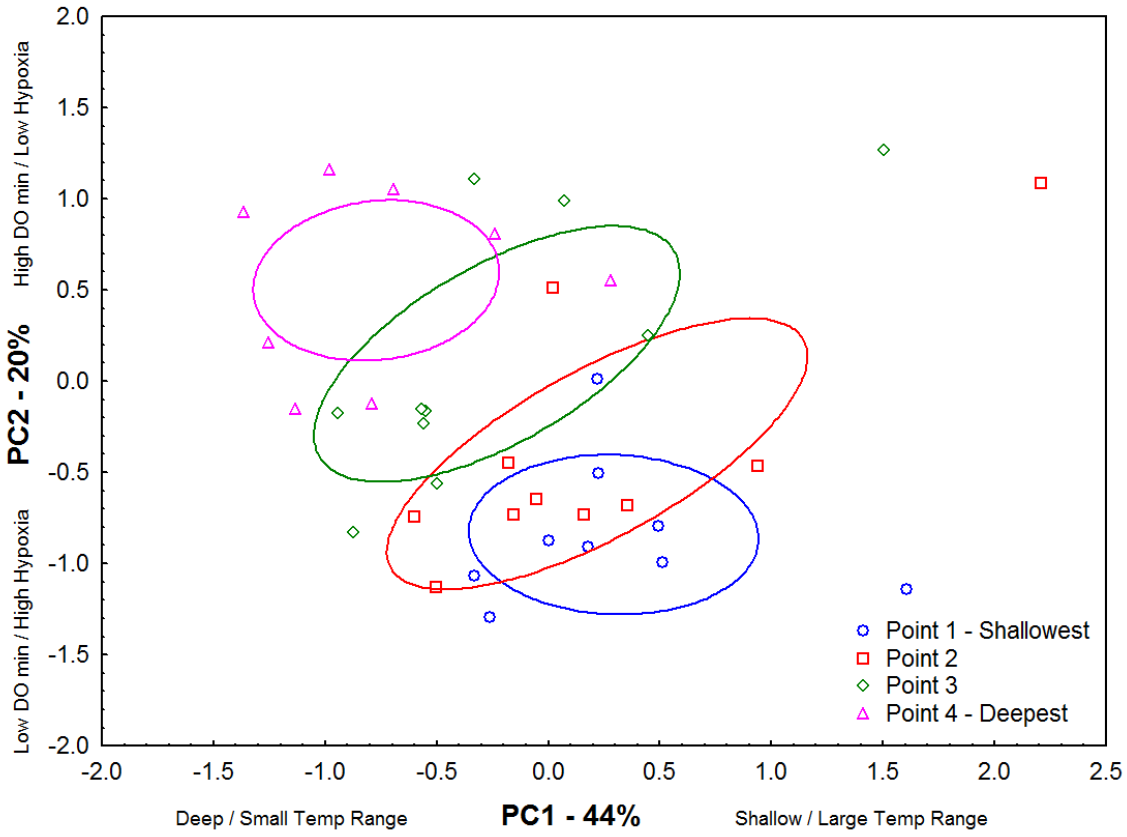

Figure 2. 17 Principal Component results of environmental sample loadings (n=37), excluding the three samples (Fishing Island 7's deepest transect point, Anderson's Creek deepest transect point and, Baie du Dore's shallowest point). Ellipses represent one standard deviation.

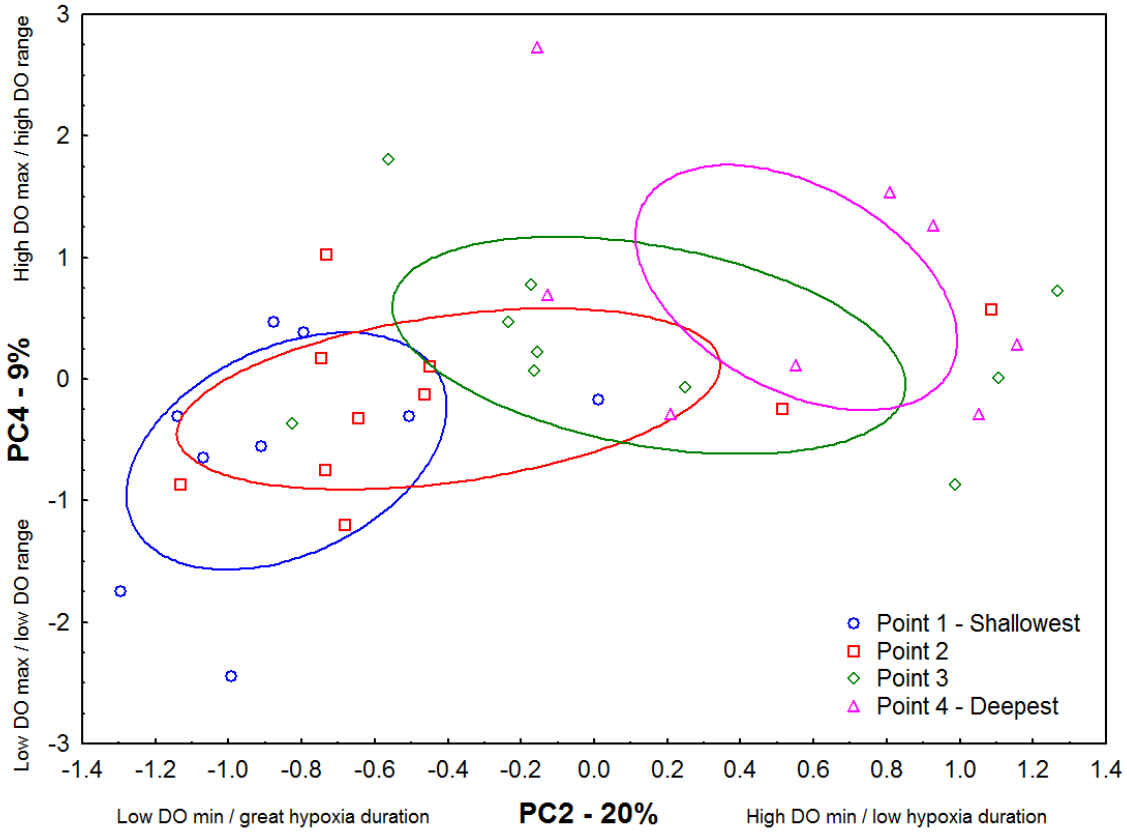

Figure 2. 18 Principal Component results of environmental sample loadings (n=37), excluding the three samples (Fishing Island 7's deepest transect point, Anderson's Creek deepest transect point and, Baie du Dore's shallowest point). Ellipses represent one standard deviation.

Cluster analysis of environmental factors identified 2 main groups of sample sites (1 and 2, Figure 2.19) each of which consisted of 2 subgroups (1A and 1B, and 2A and 2B). Group 1 samples (Bruce Peninsula) differed from Group 2 samples (North Channel) in that Group 1 had shallower mean water depth than Group 2. Groups 2A and 2B differed in that 2A samples had briefer periods of mild (<4 mg/L) and moderate (2 mg/L) hypoxia than samples in Group 2B, which exceeded the hypoxic thresholds for large portions of the day.

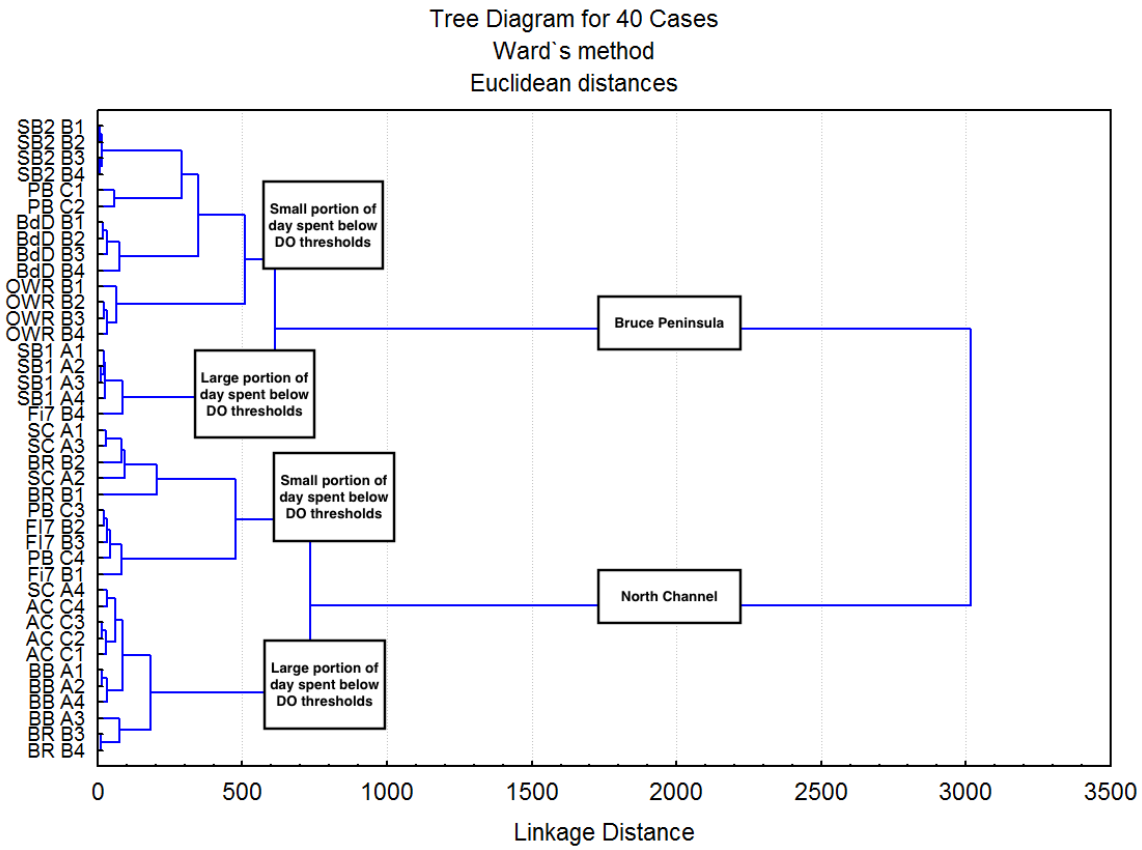

Figure 2. 19 Figure 2.19: Cluster analysis showing similarities among samples based on environmental variables collected at every sample point / site.

Discussion

The goal of this chapter was to identify the key environmental gradients within wet meadow zones of wetlands around Lake Huron, with a particular interest in documenting a DO concentration gradient. Due to the increase in Lake Huron water levels since 2014 (Gronewold, 2016), the water depth in the wet-meadow zone is much deeper than it had been in the previous 15 years when Lake Huron experienced relatively stable, low water conditions (Gronewold, 2016). The wet meadows of seven of the ten wetlands sampled (Bullhead Bay, Stobie Creek, Anderson's Creek, Old Woman's River, Stokes Bay 1 and Stokes Bay 2) were directly connected to open water. As mentioned in

the introduction, other vegetation zones often occur between the wet meadow and open water. The wet meadows of three of the ten wetlands (Baie du Dore, Fishing Island 7 and Blind River) were protected by other vegetation zones. The wet meadow at Baie du Dore was behind a very dense stand of *Phragmites*, while the wet meadows at Fishing Island 7 and Blind River were behind sparse bulrush vegetation (CWMP database). Water depth is one of many important factors that determine what types of vegetation are able to grow (Sorrell, 2012). The water beyond the lake ward side of the wet meadow was often too deep to support floating or emergent vegetation that might have been there prior to the rise in water levels. For example, Anderson's Creek, Bullhead Bay, Stobie Creek, Blind River, and Stokes Bay 1 were all sampled prior to the increase in water levels (2011-2012) and all 5 of these sites had vegetation zones that were no longer there at the time of sampling (Table 5.1).

Despite the changes that each wetland may have experienced since the rise in lake water level, the wet meadow vegetation was very similar among all of the wetlands with *Carex* being found at 42% of the 120 sample points (4 points x 3 transects x 10 wetlands) and *Calamagrostis canadensis* in 18% of the samples. Though inconclusive, the relationship between the percent visual obstruction that was calculated using the Robel pole method and traditional quadrat stem counts is promising (Figure 5.2). The relationship between these two methods of determining vegetation density appeared to be too weak to justify using the Robel pole in place of quadrat stem counts for this project. This relationship should be further investigated, as we did not have enough to time validate our methods. Moving from traditional quadrat stem counts to photo analysis would save a considerable amount of time when determining vegetation density!

Describing DO at each site:

The Reference tote that was deployed at the five sites in the Bruce Peninsula Region, consistently recorded diel DO trends that were the complement of the loggers recording directly in the wet meadows (Figure 2.14). Water in the reference tote was unaffected by biological processes and thus the variation in DO concentrations reflected only the effects of physical processes such as water temperature increasing and decreasing throughout the day. The DO concentrations in wet meadows however, reflect plant photosynthesis during the day and the continuous respiration of the suite of wetland organisms, ranging from sediment microbes, plants, macroinvertebrates and fishes. The daytime increases in DO concentration reflect net photosynthetic activity, and nighttime DO concentration decreases represent the effects of respiration in the absence of compensatory photosynthesis. These results are supported by reference tote comparisons done by Dings-Avery (2019). The reference tote DO concentration change for these 5 sites hardly differs by more than 0.5 mg/L. However, it is clear that the DO concentration in the reference totes follows the opposite trend to the DO concentration measured in the corresponding wet meadow (Figure 2.14).

Of the ten sites sampled, 7 (Blind River, Stokes Bay 1 and 2, Old Woman's River, Fishing Island 7, Baie du Dore and Pike Bay, Figures 2.4 - 2.10) displayed expected declines in DO concentration throughout the night, until photosynthesis resumed the following day.

Two of the ten sites, Stobie Creek and Anderson's Creek experienced sudden increases and decreases in the DO concentration. These were likely due to scattered showers. Just as Cornell (2008) found, precipitation disturbs the water surface, thus mixing air into the

water, disrupting the typical diel dissolved oxygen pattern (Figures 2.11 and 2.12). Scattered showers also occurred during the sampling of Stokes Bay 1, and the sharp changes in dissolved oxygen levels is quite apparent in the deeper (points 3 and 4) sample locations (Figure 2.7).

One site, Bullhead Bay is suspected to have experienced a storm surge or perhaps even a seiche event during sampling. This is suspected based on the gradual increase in dissolved oxygen, starting at about 0200 EDT, when respiration should be keeping the DO levels low (Figure 2.13). Trebitz (2006) found that oxygen depletion and temperature fluctuation were moderated with the increased mixing of water.

Almost all of the sample sites were hypoxic. Baie du Dore was the only site to not experience moderate hypoxia or anoxia. This was likely due to the flooded trail that cut across the wet meadow, giving the center of the wet meadow direct access to oxygenated lake water. Among all sites, point one, the shallowest and most protected part along thetransect, always had the longest duration of moderate hypoxia (<2 mg/L) and anoxia. Point 4, the deepest and least protected part of the transect, always had the shortest duration of moderate hypoxia and anoxia (Table 5.4 and 5.5).

Almost all of the sampling locations among all sites exhibited at least some level of mild hypoxia, including sample point 4, which was in the deepest and least protected area of the wet meadows. Anderson's Creek point 4 was the exception among the sites sampled (Table 5.4), and this is likely because the edge of the wet meadow interacted with a substantial stream that flowed through the wetland, and which would have prevented the drop of DO concentration at these sample points. These observations are consistent with other research findings, such as those of Losee and Wetzel (1993), who

also observed just how abruptly the flow of water, and thus the mixing of nutrients and gases can be reduced by a vegetation stand. In their case study, a bed of *Scirpus subterminalis* could reduce water flow, coming from the open lake towards shore, by 98% within the first 10-15 m of the vegetation stand (Losee and Wetzel, 1993).

Overall, all sample sites displayed diel dissolved oxygen concentration fluctuations that for the most part, reflected photosynthesis or lack thereof; and all portions of the wet meadows experienced these fluctuations at the same time and at about the same rate. Within each wet meadow however, I observed a distinct gradient in the duration of mild hypoxia, moderate hypoxia and anoxia, with the shallowest sample points always experiencing the longest durations of hypoxia and anoxia and the deepest sample points experiencing the shortest duration of hypoxia and anoxia. Within each wet meadow there is also a distinct gradient in DO concentration maximum and minimum recordings, with the shallowest sample points always having the lowest DO min/max in comparison to the deepest sample points always having the highest DO min/max.

I had predicted that sample locations would differ based on the duration of mild or moderate hypoxia. This was not entirely the case, though. The PCA indicated that samples were organized first by a "depth gradient" summarized by PC1 and by a "duration of hypoxia" gradient summarized by PC2 (Table 2.17). The two variables most highly correlated with PC1 were negatively correlated with each other - the daily water temperature range was negatively correlated with water depth at a point. Thus, samples that were high on PC1 are shallow and experience a large temperature range over a 24-h period, in comparison to samples that were low on PC1, being described as having deeper water and a smaller range in water temperatures over a 24 h. Many of the other variables

collected, correlate with depth (Sorrell, 2012). However, the reason that depth separates samples in the cluster analysis (Figure 2.19) is that all of the samples from the North Channel region were much deeper than samples from the Bruce Peninsula region (Table 5.6). The relative sampling location clusters are more strongly associated with PC2 than PC1 (Figure 2.18). Water temperature maximum was also positively associated with PC1 and it is important to consider the date at which samples were taken. North Channel wetlands were sampled earlier in the summer when water temperatures had not quite reached their seasonal peak (August 4-10, 2017). Samples from sites in the Bruce Peninsula region were collected in the late summer at which time daytime water temperatures were higher (August 18-25, 2017).

Geomorphology must also be considered, as pH was also positively correlated with the variables making up PC1. The North Channel wetlands were situated on the Canadian Shield granite, and Bruce Peninsula wetlands had carbonaceous bedrock. Thus, PC1 separates samples primarily based on region (Figure 2.16).

Overall, this chapter assessed the DO concentration because my thesis objective was to investigate the relationship between DO concentration and the community composition of aquatic macroinvertebrates and fishes (Chapter 2). The DO concentration variables measured were accounted for in PC2 and PC4 (Table 2.4). Figure 2.19 shows that within the first 15-30 m of a wet meadow there is a distinct DO gradient to beconsidered when sampling the wet meadow zone. Sample points closest to the open lake (point 4) experienced shorter duration of hypoxia and anoxia over the 24-hsampling period, whereas the most protected samples points (15-30 m into the wet meadow, nearest shore) experienced the much longer durations of hypoxia and anoxia.

Finally, the cluster analysis (Figure 2.19) shows that there are four distinct groups that are separated based on region first. These two regional groups are then subdivided based on the duration of hypoxia and anoxia. Within each subgroup, samples seem to cluster according to site.

Conclusions

This research shows that a diel dissolved oxygen gradient exists within the first 15-30 m of the lake-exposed side of coastal wet meadows and the rate of DO concentration change within a wetland similar regardless of the relative location of the sample. However, the diel range in DO concentration (maximum and minimum recordings) is smaller and the duration of hypoxia and anoxia increases the farther one samples into the wet meadow zone. All wet meadows exhibit a DO gradient along a 15-30 m transect extending from the lakeward edge of the emergent vegetation zone towards the wet meadow zone, with the duration of hypoxia increasing as a function of distance from the open water zone. However, among-wetland variation exceeds the differences observed among points along transects within wetlands. This is important, as knowledge of this short DO concentration gradient should guide researchers and conservation authorities in accounting for potential sampling biases when assessing the quality of wetlands' wet meadow zones, especially when difference are expected in macroinvertebrate and fish community composition along this short DO gradient.

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Chapter 3:
Variation in Invertebrate Community Composition along a Dissolved Oxygen Concentration gradient in Lake Huron coastal wet meadows.

Introduction:

In 2013 and 2014, lakes Superior, Michigan and Huron experienced a substantial water level increase over a 2-year period, ending a 15-year period of below average lake levels (Gronewold, 2016). The increase in water depth provided an opportunity to determine if dissolved oxygen (DO) levels influence the distributions of aquatic invertebrates and fishes within the wet meadow zone of Lake Huron coastal wetlands. I determined that there are defined DO boundaries within the wet meadow zone of wetland in the previous chapter, thereby potentially affecting the invertebrate community composition across a relatively short distance within the zone.

The shallow nature of wet meadows makes this habitat subject to large diel DO fluctuations, as described in Chapter 2 of this thesis. Because regions can become anoxic for several hours per day, aquatic organisms may require specific adaptations to reside in these areas. Kramer (1987) claims that in aquatic systems, oxygen should be considered a resource just as important as food, thereby modifying foraging and diet decisions when it comes to predicting fish habitat selection using the optimality approach. Fishes may use a variety of methods to deal with hypoxic stress: change in activity, air breathing, increasing their aquatic surface respiration and/or vertically or horizontally moving elsewhere in their habitat. Laboratory studies have shown that fishes will try to remain in a favoured spot by using the first three strategies mentioned above (Suthers & Gee, 1986). However, if oxygen is continuously lowered, thereby increasing the cost of respiration, the fish will ultimately move (Suthers & Gee, 1986). Tolerance to hypoxia is

species specific. However, areas where DO concentrations are less than 2 mg/L are typically avoided by fishes (Yimer, 2009). One species that is well known for having a much higher tolerance of hypoxia is the Central Mudminnow (*Umbra limi*), being found in habitats with DO concentrations considerably below 2 mg/L, due to their ability to breathe air (Klinger et al., 1982).

With regards to the influence of DO concentrations on aquatic invertebrates, Britt (1955), observed a massive decrease in *Hexagenia* populations in the western basin of Lake Erie, following a 2-day long anoxia event in the summer of 1953. Winter et al. (1996) found that even mild chronic hypoxia reduced growth rate and increased rates of mortality in laboratory-reared *Hexagenia* larvae. Wiley and Kohler (1980) also found that mayfly nymphs repositioned themselves in order to meet their respiratory requirements in streams. Thus, aquatic invertebrates are also vulnerable to the abiotic stress of hypoxia and anoxia, and the invertebrates meet the challenge of episodic hypoxia or anoxia with a variety of species specific physiological, morphological and behavioral adaptations (Harrison et al., 2018; Kolar, 1993; Nagell & Fagerstrom, 1978).

Aquatic invertebrates' adaptations to hypoxia and anoxia are numerous. These adaptations can range from some genera making use of atmospheric oxygen (e.g. snails, Penha-Lopes et al. 2010), or auxiliary respiratory appendages (e.g. Chironomidae pupae, Marziali et al. 2006). Some genera have an increased concentration of respiratory pigments (e.g. Chironomidae, Panis et al., 1996 and Oligochaeta, Van Horn, 1975), or use an alternative anaerobic respiratory metabolism pathway (Hamburger et al., 2000 , Harrison et al., 2018, Hoback and Stanley, 2001). Finally, some invertebrates will

physically move to areas with more oxygen or to at least to areas that experience a shorter duration of hypoxia throughout the day (e.g. Zygoptera, Teixeira et al., 2015).

Not only is the tolerance of hypoxia species-specific, but the risk or vulnerability to fish predation also varies among invertebrates at (Kolar, 1993). For example, Kolar (1993) found that stream-dwelling mayflies and amphipods were at a higher risk of being preyed upon in comparison to caddisflies and aquatic beetles under normal conditions. The risk of predation was so much greater for the former taxa that when fish were presented with all four prey-types in a tank, (mayflies, amphipods, caddisflies and beetles), only the mayflies and amphipods were eaten. Kolar (1993) ultimately found that invertebrates both sensitive to hypoxia and at high risk of fish predation would balance these conflicting stresses by spending more time in the hypoxic regions of their habitat when presented with predators. Eventually though, the hypoxia-sensitive invertebrates are forced into more normoxic regions and are ultimately preyed upon (Kolar 1993).

DO concentrations between 5 mg/L and 3 mg/L are stressful to most aquatic organisms (Scavia et al. 2006). DO concentrations below 2 mg/L are termed hypoxic for fishes, and typically do not support fish life (Yimer, 2009). Anoxia refers to conditions when DO concentrations are 0 mg/L (Scavia et al. 2006). In this study, I operationally define environmental hypoxia to be dissolved-oxygen concentrations between 1.0 and 3.9 mg/L. Environmental anoxia is defined as dissolved oxygen concentrations below 1.0 mg/L.

The objective of this chapter is to determine the relationship between the aquatic invertebrate community composition and the dissolved-oxygen concentration gradient in Lake Huron coastal wet meadows. I describe the variation in diversity and taxonomic

composition of aquatic macroinvertebrates with respect to locations along depth/distance transects extending from the land/water margin within each wetland's wet meadow zone. I report on the differences observed in the aquatic invertebrate taxa richness, Shannon diversity, and evenness, as well as the relative abundance of different respiratory functional groups and the relative abundance of groups differing in tolerance to thermal and oxygen stress along existing dissolved oxygen gradients within Lake Huron wet meadow zones. This chapter looks to understand the extent to which dissolved oxygen concentration structures the aquatic invertebrate communities in comparison to other environmental variables such as fish predation, within the coastal wet meadow zone.

Methods and Materials

Field Methods

Information regarding site selection, establishing transects, and details regarding the collection of various environmental variables are presented in Chapter 2.

Overall Assessment of Wet Meadow Biological Variables:

Aquatic Invertebrate Sampling

Zoobenthos at each transect point in wet-meadow areas of 10 wetlands were sampled with a D-frame dipnet (0.5-mm mesh). One-m benthic sweep samples were taken from an undisturbed area near each of the dissolved oxygen loggers (Chapter 2). Four consecutive sweeps were collected and combined into a single composite sample to represent the zoobenthos around each sampling point. The sample was emptied into a

labeled, heavy-duty polyethylene soil bag (25 x 48 cm) and preserved by adding equal parts of water from the sample site and concentrated, buffered formal-ethanol solution, a 2.5:1 ratio of 95% ethanol: formalin (38% formaldehyde, 15% methanol, 47% water), buffered with 3 g/L borax. The sample bags were transported to the lab, heat sealed, and stored until they could be processed.

Fish Sampling

Fishes were sampled at each point along one randomly selected transect in each wetland using fyke nets (Figure 3.1). Fyke nets were set parallel to shore, with the lead line of the fyke net staked near the DO logger position and the cod end of the fyke net being furthest away (Figure 3.1). The fyke nets were left to fish for approximately 24 h, after which fishes were taken out, identified to the species level, measured (total length, mm) and then released alive as per Great Lakes Coastal Wetland Monitoring Program Standard Operating Procedure (Brady et al. 2019). If more than 25 fish of a species were caught, only the first 25 individuals were measured. All others were counted but not measured. Fishes that were less than 20 mm long were not recorded at all.

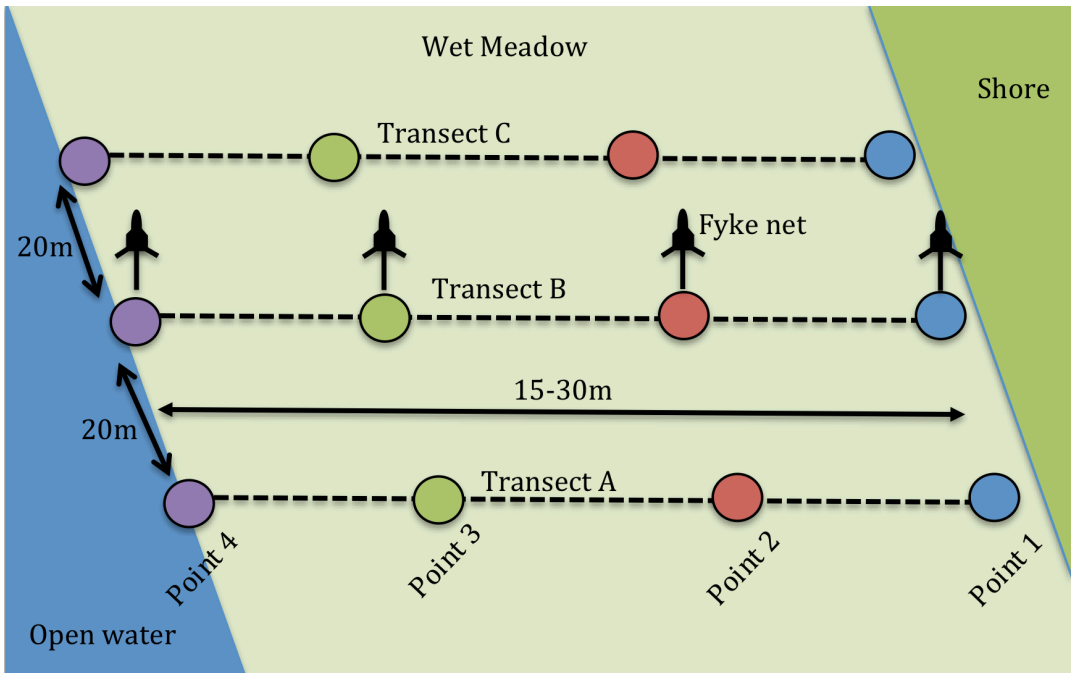

Figure 3. 1 Field sampling set up. Coloured circles represent the locations of DO loggers and invertebrate sampling. Point 4 (purple) sites were in deepest water; Point 1 sites (blue) were in shallowest water.

Lab sorting Methods

The samples collected along the transect in each wetland at which fyke nets were set were examined. The remaining samples were archived for later analysis

Material from each sweep sample was individually rinsed through a nested series of soil sieves (4.0 mm, 1.0 mm, 0.5 mm, and 0.25 mm) to facilitate separation of invertebrates from debris as well as to separate macroinvertebrates by size to facilitate efficient processing (Ciborowski, 1991).

Macroinvertebrates found in the 4.0, 1.0 and 0.5-mm size fractions were identified to the finest taxonomic resolution possible (usually genus) and stored in 70% ethanol. The materials retained in the 0.25 mm sieve were preserved in 70% ethanol and archived.

The debris remaining after invertebrates had been removed from each size fraction was placed on filter paper, dried at 60° C and weighed to the nearest mg (Ciborowski, 1991). When a size fraction was subsampled, the sorted and unsorted fractions were each dried and weighed, and the proportion of the sorted fraction inferred from the relative weights of the two fractions of detritus. The biomass of invertebrates remaining in the unsorted fraction was considered to be negligible

Counts of the invertebrates in each size fraction of a sweep sample were corrected for subsampling, summed, and expressed as total numbers per sample for each taxon identified. Rare taxa (those found in fewer than 10% of the samples) were pooled with other genera of the same family when possible; otherwise they were excluded from multivariate analyses.

Abundance = number enumerated / proportion of detrital mass sorted

Statistical Analyses:

The sample abundance (described above) was used to calculate the relative abundance of each invertebrate taxon within each sample. Relative abundances (percent) were then transformed into octaves ($Log_2(x+3)$, Gauch & Whittaker, 1972), with the constant of 0.001 being added to each value so that a value of zero before transformation was zero after the transformation. All transformed values were positive.

$$Relative\ Abundance\ i\ =\ \frac{Number\ of\ Individuals\ from\ Family\ x\ at\ Site\ i}{Total\ Number\ of\ Individuals\ at\ Site\ i}$$

Relative Abundance (Octave) = Log_2 (Relative Abundance (Percent) +3)

All community composition assessments were performed using the octave-transformed invertebrate values. Region-specific (North Channel vs. Bruce Peninsula), points-along-transect related, and region*point related differences in the abundance of individuals, taxa richness, Shannon's diversity and, Pielou's evenness, were evaluated by Analysis of Variance (ANOVA) using Statistica 7.1 software (StatSoft, 2005). The similarity of invertebrate community composition collected in each sample was assessed using hierarchical cluster analysis (Ward's method performed on Squared-Euclidean distances among samples based on relative abundances of taxa (octaves) within samples). Clusters were determined subjectively.

The functional group "air-breathing" represents aquatic invertebrates that respire directly with the atmosphere either through use of air bubbles, or with breathing tubes/siphons. Invertebrates in the "tracheal gills" respiratory functional group use the

outgrowths of their tracheal system as a gill (Batzer and Boix, 2016). These tracheal gills have a thin cutaneous layer that is permeable to both oxygen and carbon dioxide (Merritt et al., 2008). Insects with tracheal gills can fan their gills so that they may have constant contact with oxygenated water. Finally, some invertebrates are small enough to take advantage of direct diffusion through their body surface ("cutaneous respirers") due to their possession of a permeable, thin, cutaneous membrane as well as their high surface area to volume ratio, facilitating the transfer of oxygen and carbon dioxide (Batzer and Boix, 2016). The relative abundance of each respiratory group was calculated for each sample, and these relative abundances were transformed into octaves, using the same calculations described earlier.

Aquatic invertebrates can also be classified into tolerance categories. Tolerance scores were derived from Barbour et al. (1999), who compiled and summarized scores from the U.S. state organizations of Idaho, Ohio, North Carolina, Wisconsin and the Mid-Atlantic Coastal Streams workgroups. Ohio uses an in-house created IBI based upon the fish IBI created by Karr (1981) (Ohio EPA, 2015). Wisconsin (Weigel and Dimick, 2011), Idaho (Grafe, 2002), and North Carolina (NC Department of Environmental Quality, 2016) all incorporate or have modified the Hilsenhoff Biotic Index, which calculates taxa group tolerance to organic pollution. Hilsenhoff (1987) explained that increased nutrient and organic pollution resulted in lower dissolved oxygen levels, thus making it difficult for species of macroinvertebrates to survive in particular areas (Hilsenhoff, 1987).

Eighteen of the 33 taxa groups found in the study wetlands were assigned a tolerance score according to the classification created by Barbour et al. (1999), who used

a scoring system ranging from 1-10, with 1 representing the most sensitive taxa groups and 10 representing the taxa most tolerant to environmental degradation. Based upon the assigned tolerance scores, taxa groups were then pooled into 3 broader categories: Sensitive (scores ≤ 4), Mid-Tolerant (scores between 5 and 7) and, Tolerant (scores of 8 or more). The relative abundance of each tolerance group was calculated for each sample, and these relative abundances were transformed into octaves, as described earlier with invertebrate taxa groups themselves.

Redundancy analysis (RDA) was performed to assess the associations between the environmental variables (summarized in Chapter 2) and the invertebrate community composition observed at each sampling location.

Results

Overall Trends in Abundance, Richness and Diversity

In all, 21,225 invertebrates were captured from the 40 sweep samples analysed in this study (4 points along one transect within each of 10 wet meadows, situated on the Canadian coast of Lake Huron). The mean number of invertebrates captured at each wetland was calculated from the 4 samples collected at each wetland, and these means were averaged together to produce study-wide estimates of abundances and richness. The arithmetic mean±SE invertebrate abundance and taxa richness calculated across all transect locations and wetlands were: 531±315 individuals/sample and: 14±1 families/sample respectively (n=10).

Among all samples from all wet-meadows, the 8 most abundant invertebrates were (in descending order of abundance) Chironomidae midges, *Caenis* mayflies,

Oligochaeta (aquatic worms), *Caecidotea* isopods, *Gammarus* and *Hyalella* amphipods, Planorbidae snails, and Coenagrionidae damselflies (Table 3).

Arithmetic mean (±SE) taxa richness, Pielou's evenness and Shannon's Diversity were calculated independently for Bruce Peninsula (n=6) and North Channel sites (n=4). In other words, the mean number of families captured at each wetland was calculated and then the wetland means were averaged to create a region-specific mean. Mean (±SE) Bruce Peninsula site richness, evenness and diversity were 18±1, 0.58±0.02 and 1.64±0.07, respectively per wetland (n=6), whereas richness, evenness and diversity at North Channel wetlands averaged 17±20, 0.52±0.02 and 1.42±0.08, respectively (n=4). There was no significant difference in taxa richness (Table 3.1, F=2.701, p=0.109) between regions, but there was a significant difference in the Shannon Diversity score and the Pielou's evenness score between the two regions, with the Bruce Peninsula region having greater diversity and evenness than the North Channel region (Table 3.1; Analysis of Variance (Diversity, F=10.395, p=0.003) (Evenness; F=7.167, p=0.009).

Table 3. 1 Most-abundant taxa groups (Calculated totals over 1000). Mean represents mean / sample.

Order	Family	Genus/ Species	Total	Mean	SE
Diptera	Chironomidae		13015	325	48
Ephemeroptera	Caenidae	*Caenis*	12348	309	98
Oligochaeta			11467	287	67
Isopoda	Asellidae	*Caecidotea*	7583	190	102
Amphipoda	Gammaridae	*Gammarus*	3357	84	65
Gastropoda	Planorbidae		1768	44	19
Amphipoda	Hyalellidae	*Hyalella*	1529	38	15
Odonata	Coenagrionidae		1282	32	6

Table 3. 2 Analysis of Variance for Shannon Diversity, Pielou's Evenness and Taxa Richness among regions, sites and transect location (point).

		Degr. of Freedom	SS	MS	F	p
Diversity	**Region**	1	1.103	1.103	10.395	**0.003**
	Error	38	4.031	0.106		
	Total	39	5.134			
	Wetland	9	2.297	0.255	2.699	**0.020**
	Error	30	2.837	0.095		
	Total	39	5.134			
	Point	3	0.420	0.140	1.068	0.375
	Error	36	4.714	0.131		
	Total	39	5.134			
Evenness	**Region**	1	0.082	0.082	7.617	**0.009**
	Error	38	0.412	0.011		
	Total	39	0.494			
	Wetland	9	0.161	0.018	1.605	0.159
	Error	30	0.334	0.011		
	Total	39	0.494			
	Point	3	0.052	0.017	1.399	0.259
	Error	36	0.443	0.012		
	Total	39	0.494			
Richness	**Region**	1	49.504	49.504	2.701	0.109
	Error	38	696.396	18.326		
	Total	39	745.900			
	Wetland	9	219.400	24.378	1.389	0.237
	Error	30	526.500	17.550		
	Total	39	745.900			
	Point	3	11.700	3.900	0.191	0.902
	Error	36	734.200	20.394		
	Total	39	745.900			

Table 3. 3 Mean and standard error (SE) Invertebrate Taxa Richness, Pielou's Evenness Score and Shannon's Diversity Score, for Bruce Peninsula and North Channel sampling regions.

	Richness Mean	Richness SE	Pielou's Eveness Mean	Pielou's Eveness SE	Shannon's Diversity Mean	Shannon's Diversity SE
Bruce Peninsula	18.17	1.17	0.58	0.02	1.64	0.07
North Channel	16.81	1.84	0.52	0.02	1.42	0.08

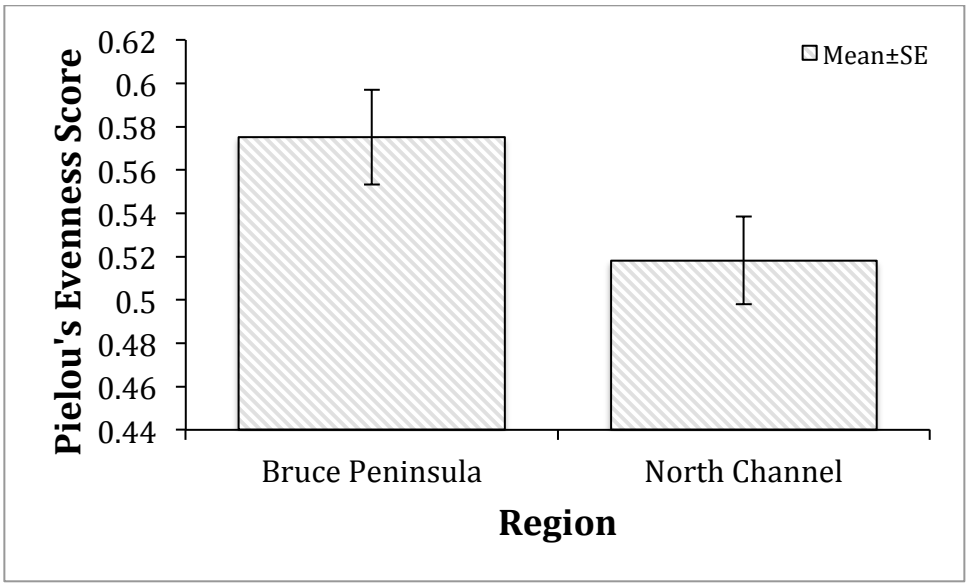

Figure 3. 2 Mean ± SE invertebrate Pielou evenness scores in the Bruce Peninsula (n=6) and North Channel (n=4) regions. The difference is significant (ANOVA, F= 7.617, $p<0.01$).

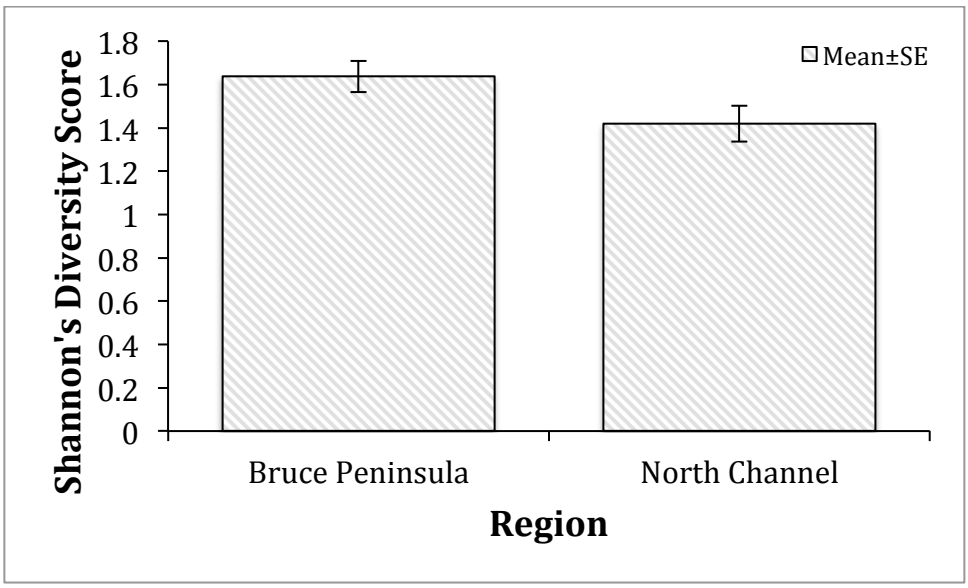

Figure 3. 3 Mean ± SE invertebrate Shannon's Diversity scores in the Bruce Peninsula (n=6) and North Channel (n=4) Regions. The difference is significant (ANOVA, $F= 10.395$, $p<0.01$).

Fish distribution and abundance

Fishes were commonly caught in this study. Central Mudminnows are known for their ability to endure hypoxic conditions (Klinger et al., 1982); thus, I postulated that their presence might influence invertebrate community composition. Fish captures varied greatly among wet-meadows. However, fishes were very rarely captured in fyke nets placed at point 1 - the most protected and closest to shore. In fact, fishes were caught in the shallowest zone in only two wetlands - Pike Bay and Blind River (Table 3.4).

Table 3. 4 Mean and standard deviation (SD) of number of fishes caught, and the proportion of those fishes being Central Mudminnows, along the dissolved oxygen transect. Point 1 is shallowest and closest to shore; Point 4 is the deepest and furthest from shore.

	All Fishes		Proportion Central Mudminnows	
	Mean	SD	Mean	SD
Point 1	2	4	0.05	0.16
Point 2	8	10	0.25	0.36
Point 3	6	9	0.27	0.41
Point 4	28	60	0.21	0.33

Table 3. 5 Mean, SD and standard error (SE) of all fishes caught within the two sampling regions.

	Mean	SD	SE
Bruce Peninsula (n=6)	13.63	38.96	15.91
North Channel (n=4)	6.94	14.68	7.34

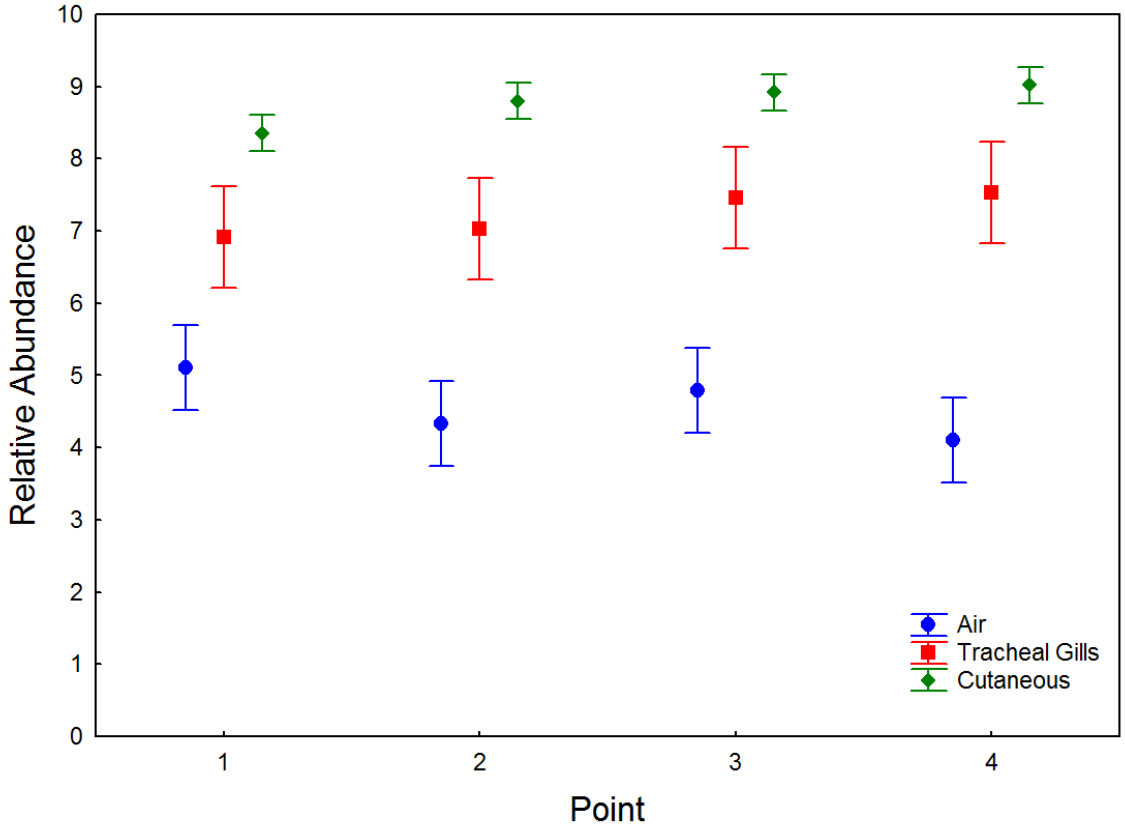

Figure 3. 4 Relative abundance (Octaves±SE; n=10) of the three different respiratory functional groups along a transect (Point 1 representing the shallowest point in the transect and closest to land, Point 4 representing the deepest point that is most exposed to open water interactions).

Table 3. 6 Relative abundance of different macroinvertebrate, respiratory functional groups (Octaves).

Respiratory Functional Group		Level of Factor	N	Mean	Std.Dev.	Std.Err	-68.00%	+68.00%
Cutaneous	Total		40	8.77	0.80	0.13	8.65	8.90
	Point	1	10	8.36	1.23	0.39	7.95	8.76
	Point	2	10	8.80	0.72	0.23	8.56	9.04
	Point	3	10	8.92	0.52	0.17	8.74	9.09
	Point	4	10	9.02	0.42	0.13	8.88	9.17
Tracheal	Total		40	7.23	2.14	0.34	6.89	7.57
	Point	1	10	6.91	3.02	0.95	5.91	7.92
	Point	2	10	7.03	2.68	0.85	6.14	7.92
	Point	3	10	7.47	1.32	0.42	7.03	7.90
	Point	4	10	7.53	1.21	0.38	7.13	7.93
Air	Total		40	4.59	1.82	0.29	4.29	4.88
	Point	1	10	5.11	2.23	0.70	4.37	5.85
	Point	2	10	4.33	2.04	0.64	3.65	5.01
	Point	3	10	4.79	1.79	0.57	4.19	5.39
	Point	4	10	4.11	1.19	0.38	3.71	4.50

Table 3. 7 ANOVA comparing the relative abundance (Octaves) of macroinvertebrate respiratory functional groups among sample points along a transect perpendicular to shore.

		Degr. of Freedom	SS	MS	F	p
Relative Abundance of Cutaneous Respiring Invertebrates	Point	3	2.58	0.86	1.39	0.26
	Error	36	22.27	0.62		
	Total	39	24.86			
Relative Abundance of Tracheal Gill Respiring Invertebrates	Point	3	2.86	0.95	0.20	0.90
	Error	36	175.24	4.87		
	Total	39	178.10			
Relative Abundance of Air Respiring Invertebrates	Point	3	6.11	2.04	0.59	0.62
	Error	36	123.68	3.44		
	Total	39	129.79			

Though the relative abundance of cutaneous respiring invertebrates was always greatest in comparison to the other respiratory functional groups, there was no significant trend that the abundance of any of these respiratory functional groups was affected by the relative location at which the sample was collected.

Table 3. 8 List of Invertebrates classified into three categories of tolerance to organic pollution and thus, decreased levels of dissolved oxygen. Classification is based on tolerance scores reported by Barbour et al. (1999).

Order	Family	Genus / Species	Very Tolerant	Moderately Tolerant	Intolerant
Amphipoda	Hyalellidae	*Hyalella*	x		
Decapoda	Cambaridae		x		
Ephemeroptera	Caenidae	*Caenis*	x		
Isopoda	Asellidae	*Lirceus*	x		
Isopoda	Asellidae	*Caecidotea*	x		
Odonata	Coenagrionidae	*Ischnura*	x		
Trichoptera	Hydroptilidae		x		
Diptera	Chironomidae			x	
Diptera	Ceratopogonidae			x	
Ephemeroptera	Baetidae			x	
Odonata	Libellulidae/ Corduliidae			x	
Odonata	Coenagrionidae			x	
Oligochaeta				x	
Trichoptera	Phryganeidae			x	
Amphipoda	Gammaridae	*Gammarus*			x
Odonata	Aeshnidae				x
Trichoptera	Leptoceridae				x
Tricladida					x

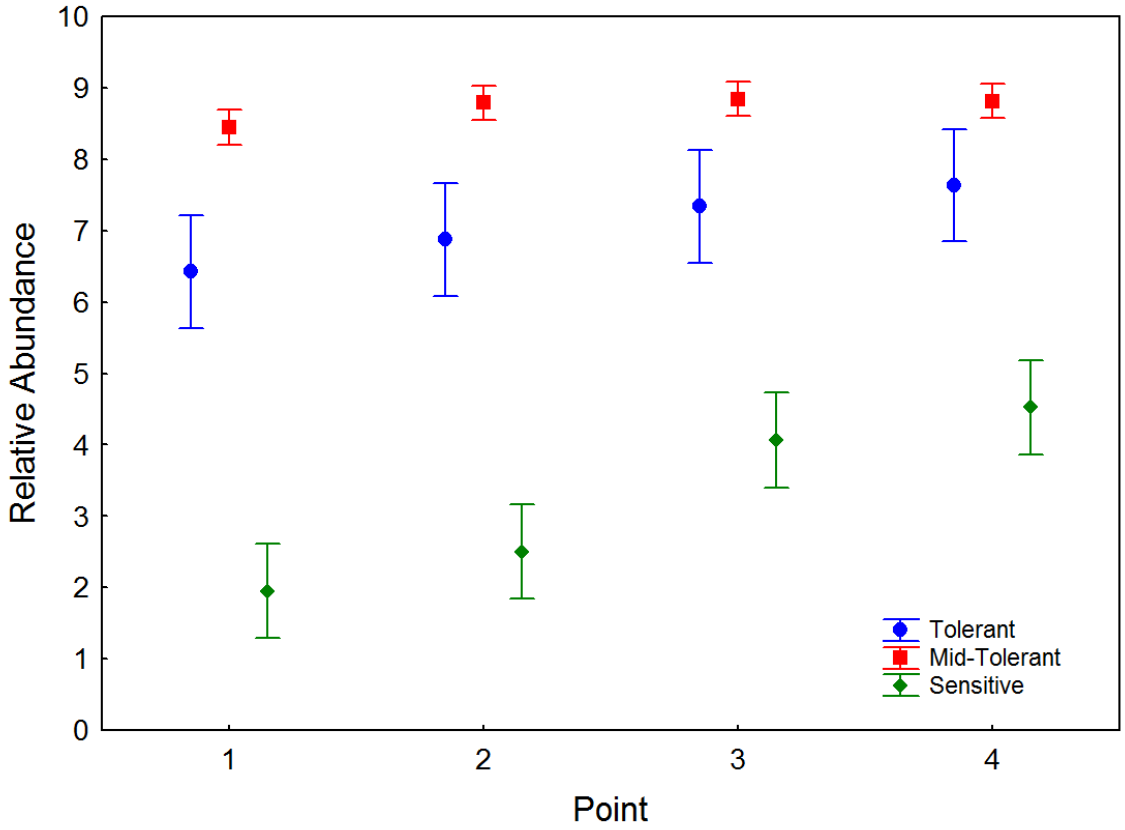

Figure 3. 5 Relative abundance (Octaves±SE; n=10) of invertebrates in 3 classes of tolerance to organic pollution/hypoxia at 4 points along wet meadow transects (Point 1 representing the shallowest point in the transect and closest to land, Point 4 representing the deepest point that is closest to open water).

Table 3. 9 Relative abundance (octaves) of different macroinvertebrate, environmental tolerance groups.

		Level of Factor	N	Mean	Std.Dev.	Std.Err	Mean1 SE	Mean+ ! SE
Sensitive	Total		40	3.26	2.27	0.36	2.90	3.62
	Point	1	10	1.95	1.77	0.56	1.36	2.54
	Point	2	10	2.50	2.26	0.71	1.75	3.25
	Point	3	10	4.06	2.25	0.71	3.32	4.81
	Point	4	10	4.52	2.01	0.64	3.85	5.19
Mid-Tolerant	Total		40	8.72	0.75	0.12	8.60	8.84
	Point	1	10	8.45	1.05	0.33	8.10	8.80
	Point	2	10	8.79	0.69	0.22	8.56	9.02
	Point	3	10	8.84	0.51	0.16	8.67	9.01
	Point	4	10	8.81	0.69	0.22	8.58	9.04
Tolerant	Total		40	7.07	2.42	0.38	6.68	7.45
	Point	1	10	6.43	3.63	1.15	5.22	7.63
	Point	2	10	6.88	2.88	0.91	5.92	7.84
	Point	3	10	7.34	1.40	0.44	6.87	7.80
	Point	4	10	7.64	1.00	0.32	7.30	7.97

Table 3. 10 Analysis of variances of the relative abundance (Octaves) of macroinvertebrates ,in 3 tolerance classes, among sample points along a transect perpendicular to shore.

		Degr. of Freedom	SS	MS	F	p
Relative Abundance of Sensitive Taxa	Point	3	45.24	15.08	3.48	**0.03**
	Error	36	155.81	4.33		
	Total	39	201.05			
Relative Abundance of Mid-Tolerant Taxa	Point	3	1.03	0.34	0.59	0.62
	Error	36	20.87	0.58		
	Total	39	21.90			
Relative Abundance of Tolerant Taxa	Point	3	8.45	2.82	0.46	0.71
	Error	36	220.28	6.12		
	Total	39	228.73			

There were no significant differences in the relative abundances of air, tracheal and cutaneous respiratory functional groups along the DO concentration gradient when all wet-meadows were considered (Table 3.7). However, a significant trend was found in the distribution of the sensitive environmental taxa group. The mean relative proportion of sensitive invertebrates, represented by *Gammarus,* Aeshnidae, Leptoceridae and Tricladida (Table 3.8), was significantly greater ($p= 0.03$) at transect points 3 and 4, the two points that are farthest from shore, than transect points 1 and 2, closest to shore (Table 3.9 and 3.10).

Trends in Community Composition among Wetlands and Among Sampling Points Within Wet Meadows.

Two redundancy analyses were conducted on the invertebrate community data. The first RDA used the four PCA axes that had eigenvalues greater than 1.0, derived from the analysis of 14 environmental variables (Chapter 2). These four principal components explained approximately 83% of the variation among sites. However, they explained only about 29% of the variation in the invertebrate community composition data (Table 3.). Principal Component 1, which was positively associated with temperature range, and negatively associated with water depth indicated that shallower areas had larger temperature ranges in comparison to the deeper portions. Scores of PC 2 were positively associated with DO concentration minima and negatively associated with the duration of hypoxia and anoxia. Thus, areas with low DO concentration minima exhibit longer periods of hypoxia than areas that recorded higher DO concentration minima. Scores of PC 3 were positively associated with water temperature minima and negatively

associated with the substrate organic layer thickness. Thus, areas where water temperature minima were high, had a thin organic substrate layer. Finally PC 4 scores were positively correlated with both the DO concentration range and the DO concentration maximum. Therefore, when the DO concentration maximum was relatively high, the DO range was also high (Table 2.4).

The second RDA used environmental PCA axes 2 and 4, because the dissolved oxygen concentration variables were associated with these factors. These two PCA axes explained approximately 16% of the variation in invertebrate community composition.

Table 3. 11 A list of each wetlands' region, number, name and, acronym used throughout this chapter in analysis.

	Site Number	Site Name	Site acronym
Bruce Peninsula	5704	Old Woman's River	OWR
	5706	Fishing Island 5	FI7
	5016	Baie du Dore 2	BdD
	5727	Pike Bay 1	PB
	5953	Stokes Bay Wetland 2	SB2
	5952	Stokes Bay 1	SB1
North Channel	5013	Anderson Creek	AC
	5106	Blind River 1	BR
	5950	Stobie Creek 1	SC
	5137	Bullhead Bay	BB

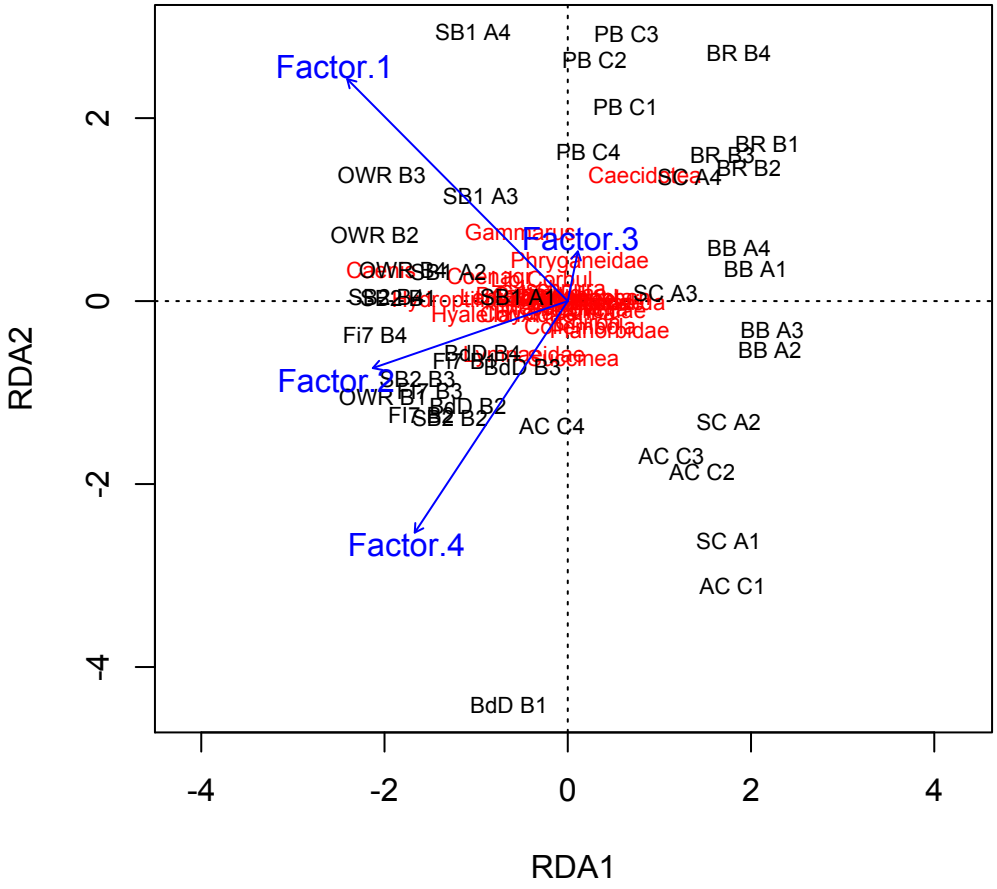

Figure 3. 6 RDA triplot involving all four main principal components calculated from the environmental variables PCA (PC factor 1 = water temperature range, PC factor 2 = duration of hypoxia, PC factor 3 = organic layer thickness, PC factor 4= DO concentration range), applied to sites and the invertebrate taxa data.

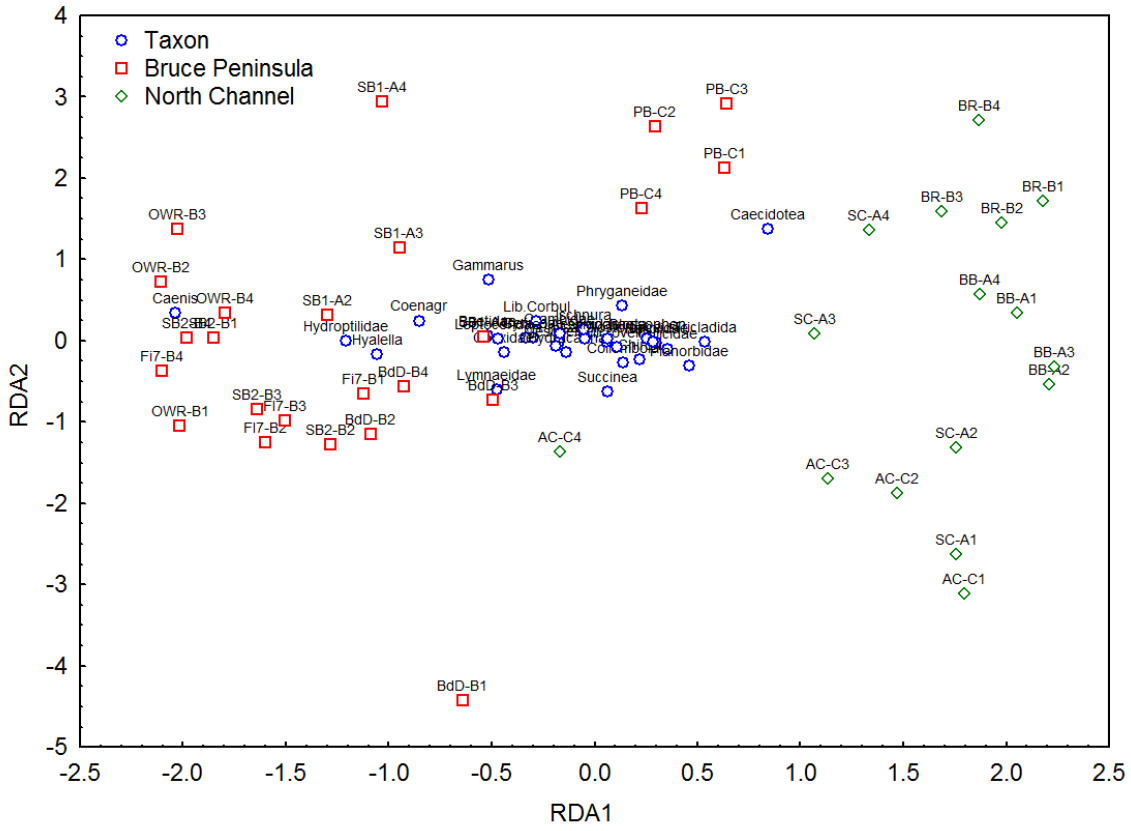

Figure 3. 7 RDA biplot involving all four main axis calculated in the environmental variables PCA (PCA 1 = water temperature range, PCA 2 = duration of hypoxia, PCA 3 = organic layer thickness, PCA 4= DO concentration range), applied to sites and the invertebrate taxa data. The green diamonds represent samples that were taken from wetlands in the North Channel Region, while the red squares represent samples that were taken from wetlands in the Bruce Peninsula Region.

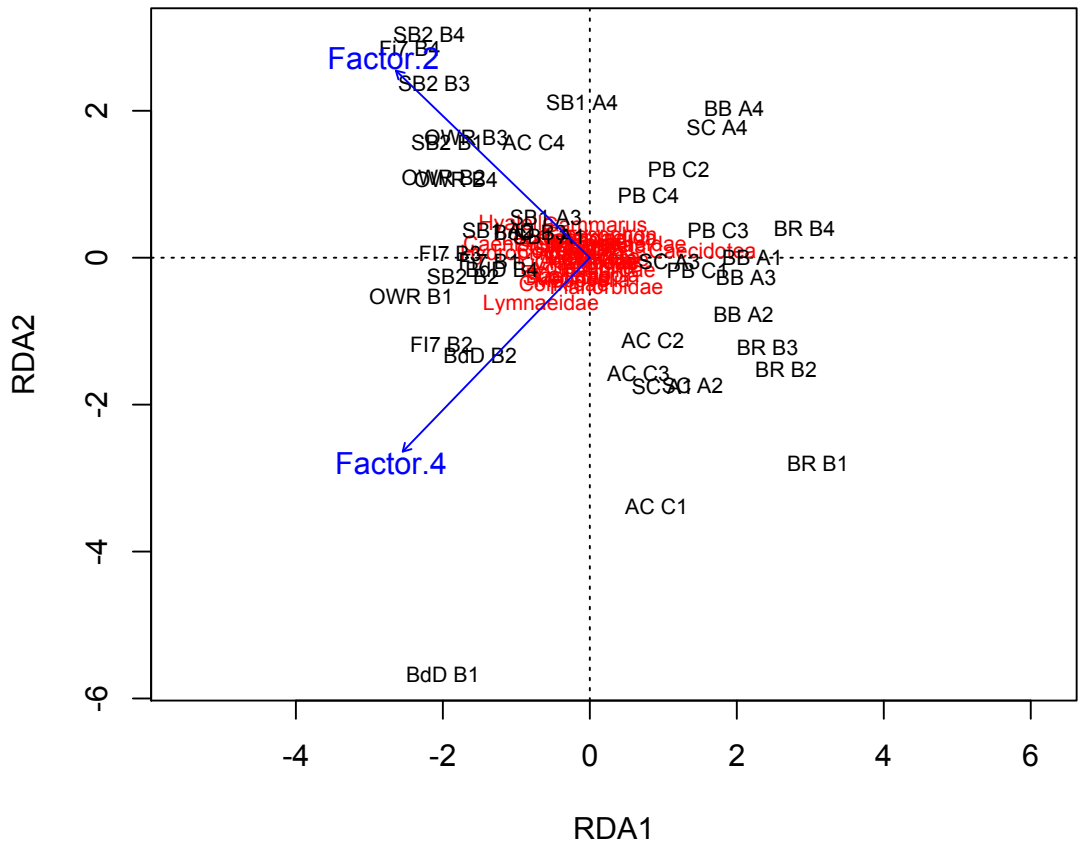

Figure 3. 8 RDA triplot involving only axis 2 (hypoxia duration) and 4 (DO concentration range) of the environmental PCA

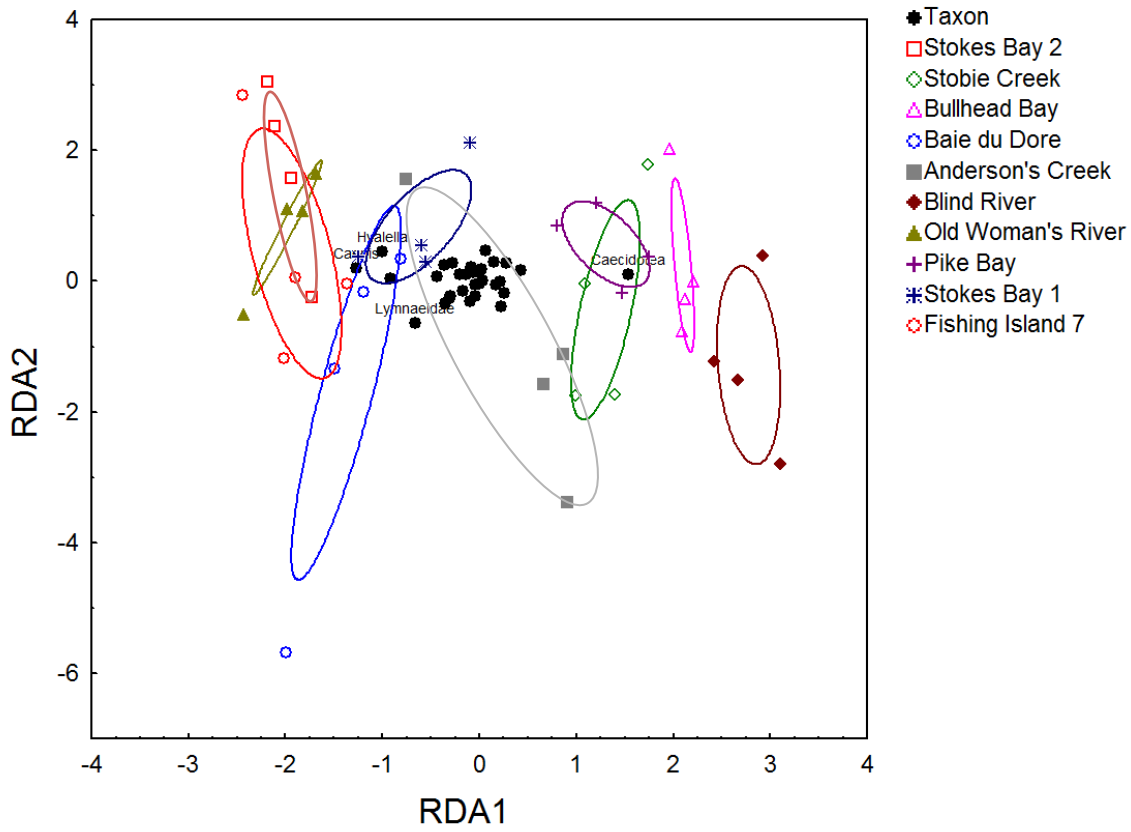

Figure 3. 9 RDA involving only axis 2 (hypoxia duration) and axis 4 (DO concentration range) of the environmental PCA. Ellipses represent 1 SE of the 4 sample points for each wetland (coloured symbols) and simplify visual interpretation of the patterns. Solid black points represent taxa. Only names of taxa at the periphery of the group of taxa points are shown. Loadings of taxa and sample points are listed in Appendix B.

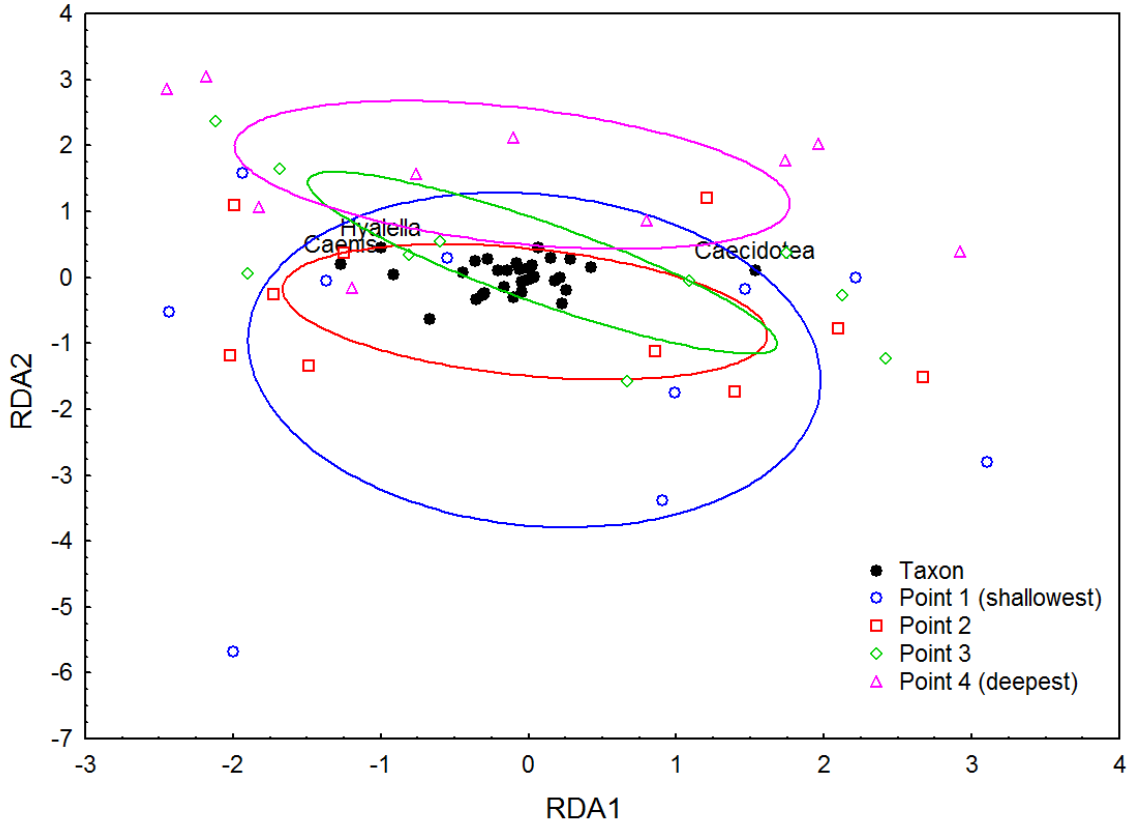

Figure 3. 10 RDA involving only axis 2 (hypoxia duration) and 4 (DO concentration range) of the environmental PCA. Ellipses represent 1 SE of the each of the 4 points sampled per wetland (coloured symbols) and simplify visual interpretation of the patterns. Solid black points represent taxa. Only names of taxa at the periphery of the group of taxa points are shown. Loadings of taxa and sample points are listed in Appendix B.

Table 3. 12

	RDA1	RDA2
Factor 2	-0.7196	0.6944
Factor 4	-0.6943	-0.7197

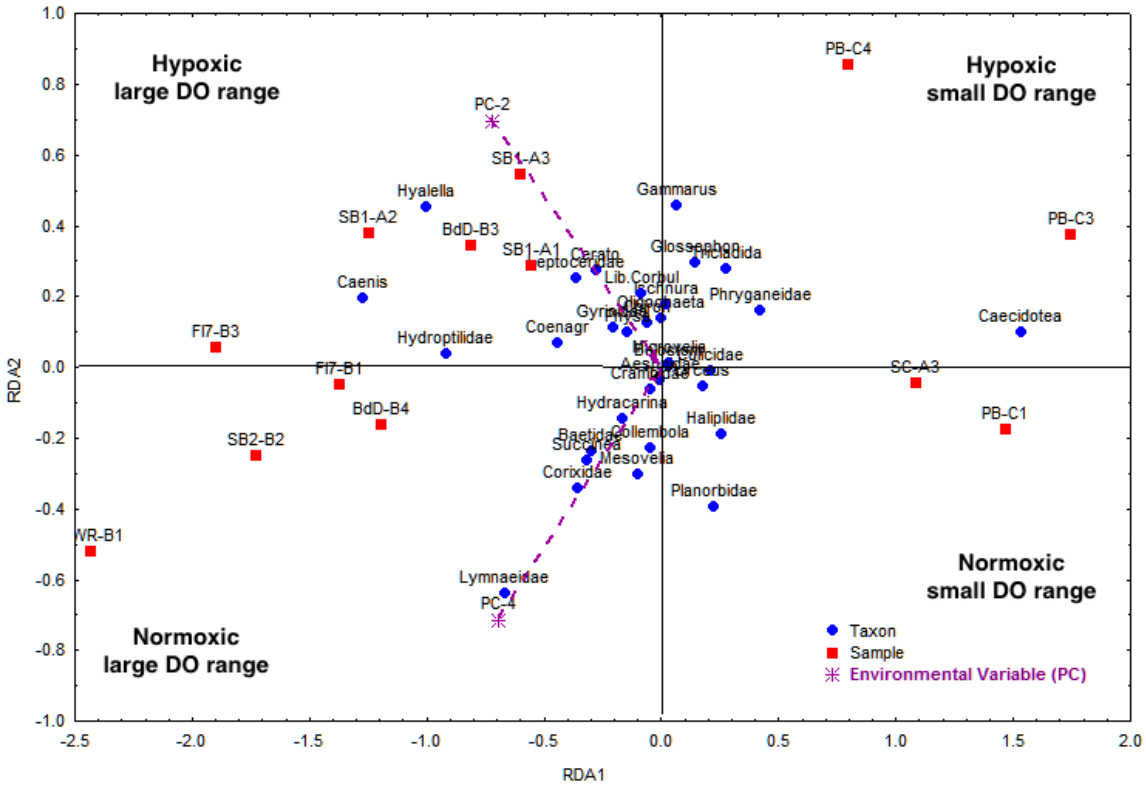

Figure 3. 11 Triplot representation of RDA calculations that used only axis 2 (hypoxia duration) and 4 (DO concentration range) of the environmental PCA data. Solid blue circles represent taxa. Solid red squares represent dissolved oxygen data collected at different sampling points within each wetland. The purple broken lines represent the correlation between the environmental variables with the RDA axis.

Examination of the ordination of samples according to the RDA model that used only the second and fourth environmental PC axis (duration of hypoxia and range of DO concentration over 24 h, respectively), indicated that samples were grouped based on wetland along the RDA 1 axis (corresponding to Figures 3.8 and 3.9). Thus, the invertebrate community composition in a sample was more similar to other samples from the same wetland regardless of their relative location along the DO gradient than to invertebrate communities being compared based on their relative location along a DO gradient among sites. The invertebrate community composition and dissolved oxygen patterns can somewhat be grouped together among wetlands based on the relative

location within the wet meadow where the samples were taken, along the RDA 2 axis (duration of hypoxia; Figure 3.10). The most notable difference is seen when comparing the deepest, least wave-sheltered locations to the shallowest, and most vegetation buffered sample locations.

As a refresher, PCA 2 represents hypoxia duration, while PCA 4 represents the DO concentration range. Both of these measurements were collected over the same 24-hour period. Table 3.11 shows that RDA1 has a negative correlation with both PCA2 and PCA4, while RDA2 has a positive correlation with PCA2 and a negative correlation with PCA4. This information is summarized by the names of the quadrants in Figure 3.11. Samples in the top left quadrant of Figure 3.11 were subject to a long duration of hypoxia as well as a large range of DO concentration over the 24 hour sample period. DO logger samples from Fishing Island 7(FI7), Baie du Dore(BdD), and Stokes Bay 1 (SB1) are found in this quadrant. Invertebrates such as *Caenis*, *Hyalella*, and Hydroptiliidae are also found in the top left quadrat, suggesting that there is strong correlation between these invertebrates and areas of a wetland that experience long durations of anoxia and a large range of DO concentrations throughout a 24 hour period.

Samples in the top right quadrant of Figure 3.11 experienced a long duration of hypoxia as well as a small range of DO concentration over the 24-hour period. DO logger samples from Pike Bay (PB) are found in this quadrat. Invertebrates such as *Caecidotea*, Phryganeidae, and Tricladida are also found in the top right quadrat, suggesting that there is strong correlation between these invertebrates and areas of a wetland that experience long durations of anoxia and a small range of DO concentrations throughout a 24 hour period.

Samples in the bottom right quadrant of Figure 3.11 experienced a short duration of hypoxia was recorded as well as a small range of DO concentration was over the 24 hour period. DO logger samples from Pike Bay (PB) and Stobie Creek (SC) are found in this quadrant. Invertebrates such as Planorbidae are also found in the bottom right quadrant, suggesting that there is strong correlation between these invertebrates and areas of a wetland that experience relatively normoxic conditions and a small range of DO concentrations throughout a 24 hour period.

Finally, samples in the bottom left quadrant of Figure 3.11 were subject to relatively normoxic conditions as well as a wide range of DO concentration over the 24 hour period. DO logger samples from Old Woman's River (OWR), Fishing Island 7 (FI7) and Stokes Bay 2 (SB2) are found in this quadrant. Invertebrates such as Lymnaeidae, Corixidae and Baetidae are also found in the bottom left quadrant, suggesting that there is strong correlation between these invertebrates and areas of a wetland that experience normoxic conditions and a large range of DO concentrations throughout a 24 hour period.

Cluster Analysis of Community Composition

Within the 40 invertebrate sweeps, 33 unique taxa groups were found. The dendrogram derived from cluster analysis of the relative abundances (octaves) of these

taxa revealed that samples fell into 4 groups (Figure 3.12) - 2 main groups of samples (A and B), each containing 2 subgroups (1A, 1B and, 2A, 2B). Group A samples were characterized as having a large relative abundance of *Caenis* mayflies, whereas Group B samples were dominated by *Caecidotea* isopod crustaceans (Table 3.11, 3.13). Groups A1 and A2 were separated based on the presence/absence of *Hyalella* amphipods (Group A1 = *Hyalella* present, Group A2 = *Hyalella* absent; Table 3.14-3.15). Groups B1 and B2 were separated based on the relative abundance of *Gammarus* amphipods (Group B1= low abundance of *Gammarus*, Group B2 = high abundance of *Gammarus*; Table 3.16-3.17).

The samples corresponding to each invertebrate community composition cluster class (A1, A2, B1 and B2) were colour- and shape-coded and ordinated against the Principal Component Factors that had been extracted from the PCA of environmental variables (Table 2.4).

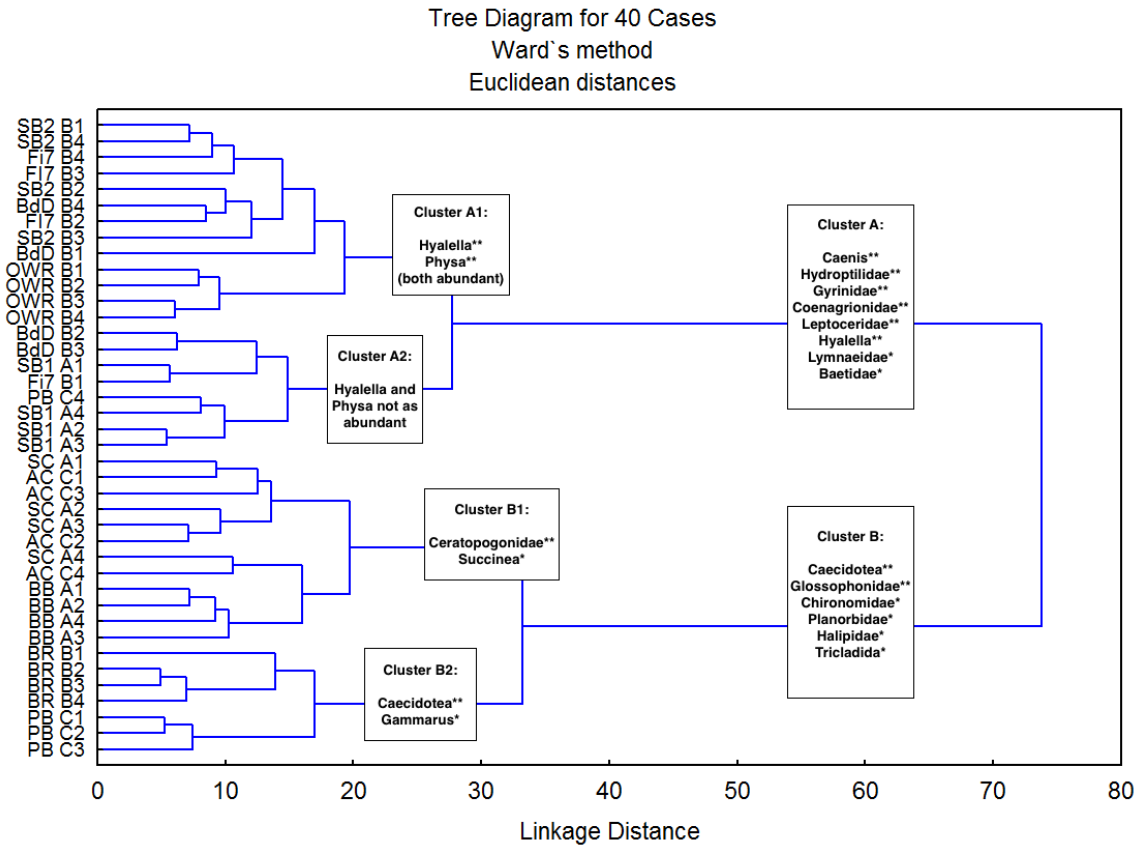

Figure 3. 12 Dendrogram showing similarities among samples (n=40) based on 33 taxa group relative abundances (octaves) captured in samples along the full length of a transect within each site. Taxa listed within boxes are significantly more relatively abundant in the cluster to which the box is connected than in the complementary cluster estimated from F-ratios of variance between clusters vs. variance among sites within clusters (Table 3.11). Asterisks indicate (**$p<0.001$, *$p<0.05$).

Table 3. 13 Significant taxa groups that separate samples into either clusters A and B, of the Cluster analysis (Figure 3.11).

		Degr. of Freedom	SS	MS	F	p
Caenis	Cluster Group	1	379.500	379.500	112.038	<0.001
	Error	38	128.715	3.387		
	Total	39	508.216			
Hydroptilidae	Cluster Group	1	124.675	124.675	75.198	<0.001
	Error	38	63.002	1.658		
	Total	39	187.677			
Caecidotea	Cluster Group	1	134.179	134.179	18.728	<0.001
	Error	38	272.260	7.165		
	Total	39	406.439			
Gyrinidae	Cluster Group	1	5.158	5.158	13.910	0.001
	Error	38	14.091	0.371		
	Total	39	19.249			
Coenagrionidae	Cluster Group	1	46.561	46.561	10.714	0.002
	Error	38	165.135	4.346		
	Total	39	211.696			
Glossophonidae	Cluster Group	1	11.473	11.473	10.512	0.002
	Error	38	41.476	1.091		
	Total	39	52.948			
Leptoceridae	Cluster Group	1	11.535	11.535	10.086	0.003
	Error	38	43.461	1.144		
	Total	39	54.997			
Hyalella	Cluster Group	1	56.122	56.122	9.059	0.005
	Error	38	235.408	6.195		
	Total	39	291.53			

			1			
Chironomidae	Cluster Group	1	6.756	6.756	7.132	0.011
	Error	38	35.996	0.947		
	Total	39	42.753			
Planorbidae	Cluster Group	1	31.914	31.914	6.951	0.012
	Error	38	174.478	4.592		
	Total	39	206.392			
Lymnaeidae	Cluster Group	1	12.418	12.418	6.667	0.014
	Error	38	70.784	1.863		
	Total	39	83.202			
Baetidae	Cluster Group	1	11.814	11.814	6.612	0.014
	Error	38	67.896	1.787		
	Total	39	79.710			
Halipidae	Cluster Group	1	5.272	5.272	5.864	0.020
	Error	38	34.163	0.899		
	Total	39	39.434			
Tricladida	Cluster Group	1	14.381	14.381	5.326	0.027
	Error	38	102.611	2.700		
	Total	39	116.992			
Crambidae	Cluster Group	1	1.541	1.541	5.059	0.030
	Error	38	11.578	0.305		
	Total	39	13.119			
Libellulidae and Corduliidae	Cluster Group	1	6.571	6.571	4.384	0.043
	Error	38	56.956	1.499		
	Total	39	63.527			

Table 3. 14 Relative abundance (octaves) of significant taxa groups in clusters A and B, of the Cluster analysis(Figure 3.11).

		Level of Factor	N	Mean	Std.Dev.	Std.Err	-95.00%	95.00%
Caenis	Total		40	4.587	3.610	0.571	3.433	5.742
	Cluster Group	A	21	7.667	1.476	0.330	6.976	8.358
	Cluster Group	B	19	1.507	2.144	0.479	0.504	2.510
Hydroptilidae	Total		40	1.946	2.194	0.347	1.244	2.647
	Cluster Group	A	21	3.711	1.633	0.365	2.947	4.475
	Cluster Group	B	19	0.180	0.806	0.180	-0.197	0.558
Caecidotea	Total		40	3.397	3.228	0.510	2.365	4.429
	Cluster Group	A	21	1.566	2.056	0.460	0.603	2.528
	Cluster Group	B	19	5.229	3.178	0.711	3.741	6.716
Gyrinidae	Total		40	0.383	0.703	0.111	0.158	0.607
	Cluster Group	A	21	0.742	0.858	0.192	0.340	1.143
	Cluster Group	B	19	0.023	0.076	0.017	-0.012	0.059
Coenagrionidae	Total		40	3.057	2.330	0.368	2.312	3.803
	Cluster Group	A	21	4.136	2.138	0.478	3.136	5.137
	Cluster Group	B	19	1.979	2.030	0.454	1.029	2.928
Glossophonidae	Total		40	0.639	1.165	0.184	0.266	1.011
	Cluster Group	A	21	0.103	0.288	0.064	-0.032	0.238
	Cluster Group	B	19	1.174	1.449	0.324	0.496	1.852
Leptoceridae	Total		40	0.595	1.188	0.188	0.215	0.975
	Cluster Group	A	21	1.132	1.490	0.333	0.435	1.829
	Cluster Group	B	19	0.058	0.259	0.058	-0.063	0.179
Hyalella	Total		40	2.386	2.734	0.432	1.512	3.260
	Cluster Group	A	21	3.570	2.743	0.613	2.287	4.854

	Cluster Group	B	19	1.201	2.206	0.493	0.169	2.234
Chironomidae	Total		40	7.586	1.047	0.166	7.252	7.921
	Cluster Group	A	21	7.175	0.874	0.195	6.767	7.584
	Cluster Group	B	19	7.997	1.064	0.238	7.500	8.495
Planorbidae	Total		40	2.593	2.300	0.364	1.857	3.329
	Cluster Group	A	21	1.700	1.961	0.438	0.782	2.618
	Cluster Group	B	19	3.486	2.310	0.517	2.405	4.567
Lymnaeidae	Total		40	0.745	1.461	0.231	0.278	1.213
	Cluster Group	A	21	1.303	1.870	0.418	0.428	2.178
	Cluster Group	B	19	0.188	0.479	0.107	-0.036	0.413
Baetidae	Total		40	0.665	1.430	0.226	0.208	1.123
	Cluster Group	A	21	1.209	1.851	0.414	0.342	2.075
	Cluster Group	B	19	0.122	0.382	0.085	-0.057	0.300
Halipidae	Total		40	0.457	1.006	0.159	0.136	0.779
	Cluster Group	A	21	0.094	0.335	0.075	-0.063	0.251
	Cluster Group	B	19	0.820	1.298	0.290	0.213	1.428
Tricladida	Total		40	0.752	1.732	0.274	0.198	1.306
	Cluster Group	A	21	0.152	0.471	0.105	-0.068	0.373
	Cluster Group	B	19	1.352	2.276	0.509	0.287	2.417
Crambidae	Total		40	0.274	0.580	0.092	0.089	0.460
	Cluster Group	A	21	0.471	0.731	0.164	0.128	0.813
	Cluster Group	B	19	0.078	0.273	0.061	-0.050	0.206
Libellulidae and Corduliidae	Total		40	1.371	1.276	0.202	0.963	1.779
	Cluster Group	A	21	1.776	1.398	0.313	1.122	2.430
	Cluster Group	B	19	0.966	1.022	0.228	0.487	1.444

Table 3. 15 Invertebrate taxa groups significantly distinguishing between Subgroups A1 and A2.

		Degr. of Freedom	SS	MS	F	p
Hyalella	Cluster Subgroup	1	122.5353	122.5353	108.0488	0.000000
	Error	18	20.4133	1.1341		
	Total	19	142.9486			
Physa	Cluster Subgroup	1	11.19069	11.19069	24.94487	0.000094
	Error	18	8.07511	0.44862		
	Total	19	19.26580			

Table 3. 16 Relative abundance (octaves) of significant taxa groups in subgroups A1 and A2 of Cluster A (Figure 3.11).

		Level of Factor	N	Mean	Std.Dev.	Std.Err	-95.00%	95.00%
Hyalella	Total		20	3.570	2.743	0.613	2.287	4.854
	Cluster Subgroup	A1	13	5.387	1.251	0.347	4.631	6.143
	Cluster Subgroup	A2	7	0.197	0.522	0.197	-0.285	0.680
Physa	Total		20	1.186	1.007	0.225	0.715	1.657
	Cluster Subgroup	A1	13	1.735	0.759	0.210	1.276	2.193
	Cluster Subgroup	A2	7	0.167	0.441	0.167	-0.241	0.574

Table 3. 17 Invertebrates distinguishing between Subgroups B1 and B2.

		Degr. of Freedom	SS	MS	F	p
Caecidotea	Cluster Subgroup	1	101.3953	101.3953	20.16264	0.000283
	Error	18	90.5196	5.0289		
	Total	19	191.9149			
Ceratopogonidae	Cluster Subgroup	1	15.50779	15.50779	15.45531	0.000979
	Error	18	18.06111	1.00340		
	Total	19	33.56890			
Gammarus	Cluster Subgroup	1	34.4383	34.43826	7.89350	0.011597
	Error	18	78.5316	4.36287		
	Total	19	112.9698			
Succinea	Cluster Subgroup	1	15.23164	15.23164	7.19612	0.015204
	Error	18	38.09964	2.11665		
	Total	19	53.33128			

Table 3. 18 Relative abundance (octaves) of significant taxa groups in subgroups B1 and B2 of Cluster B (Figure 3.11).

		Level of Factor	N	Mean	Std.Dev.	Std.Err	-95%	95%
Caecidotea	Total		20	5.229	3.178	0.711	3.741	6.716
	Cluster Subgroup	B1	9	2.739	2.981	0.994	0.448	5.031
	Cluster Subgroup	B2	11	7.265	1.393	0.420	6.329	8.201
Ceratopogonidae	Total		20	0.997	1.329	0.297	0.375	1.620
	Cluster Subgroup	B1	9	1.971	1.360	0.453	0.926	3.016
	Cluster Subgroup	B2	11	0.201	0.572	0.173	-0.183	0.585

Gammarus	Total		20	2.264	2.438	0.545	1.123	3.406
	Cluster Subgroup	B1	9	0.814	1.676	0.559	-0.474	2.102
	Cluster Subgroup	B2	11	3.451	2.368	0.714	1.860	5.042
Succinea	Total		20	1.032	1.675	0.375	0.248	1.816
	Cluster Subgroup	B1	9	1.997	2.039	0.680	0.430	3.564
	Cluster Subgroup	B2	11	0.243	0.697	0.210	-0.225	0.711

Discussion:

The purpose of this chapter was to determine how aquatic invertebrate community composition varies within the wet-meadow zone of Lake Huron wetlands, along the DO concentration gradient that exists from shore to open water. The analyses indicated that the invertebrate community composition varied greatly between the two geographic regions sampled and among individual wetlands. The spatial variation within wet meadow zones was relatively minor and inconsistent by comparison. In particular, there was a lack of consistent change in taxa richness, diversity and evenness of invertebrates along the DO concentration gradient. There was however, a significant trend found among the sensitive (hypoxia-intolerant) taxa ($p=0.03$), which were collected in greater relative abundance in the deeper and more open parts of the wet meadow. Other trends may be present as well, such as an increasing relative abundance of air breathing

invertebrates in transect points closer to shore, though this trend was not significant ($p=0.60$).

Evenness and diversity were significantly different between the two sampling regions, with the Bruce Peninsula having larger scores for both (Figures 3.2 and 3.3). The lower evenness value for the North Channel region is likely due to the complete lack of certain taxa groups, most notably the *Caenis* mayflies, but also of many other taxa groups, as the cluster analysis revealed (Figure 3.12). The North Channel wetlands - Anderson's Creek, Stobie Creek, Blind River and Bullhead Bay - were all sampled in the first week of August 2017. In contrast, the Bruce Peninsula wetland - Stokes Bay 1 and 2, Pike Bay, Baie du Dore, Old Woman's River and Fishing Island 7 - were all sampled during the last two weeks of August. Even though all of these samples were taken within the same month, the time between sampling events might have been enough to allow *Caenis* to be collected in the Bruce Peninsula sites prior to the populations' emergence because these sites were sampled at a later date. The family Caenidae are known to emerge as late as September in Idaho and Canada (Edmunds, 1976). In addition to the time difference between the two sampling events, differences in latitude and water temperature might also explain why the regions differ so greatly in the abundance *Caenis*.

The malacostracan crustaceans *Caecidotea* (Isopoda) and *Gammarus* (Amphipoda) were very abundant in the North Channel wet-meadows, particularly at the Blind River wet-meadow, in comparison to the Bruce Peninsula wet-meadow sites (Table 3.11). These crustaceans were also correlated with the sample locations that experienced long periods of hypoxia and a small range of DO concentrations (Figure 3.11). According to Barbour et al. (1999), *Caecidotea,* are considered to be a very tolerant taxa group to

changes in their environment (Table 3.8), thus, supports our results in finding these slow moving, benthic arthropods in areas that are consistently anoxic and experiences little change in DO concentration.

Hyalella amphipods are often used in as a toxicity test organism in laboratory studies to detect effects of trace metals (Borgmann et al., 2004), pharmaceuticals (Oviedo-Gómez, 2010) and pyrethroid insecticides (Weston, 2013). However, Weston et al., (2013) found that there can be large variation in the sensitivity among populations and that some populations of *Hyalella* have even become resistant to these commonly used pesticides. Though it would be circumstantial to suggest that that populations of *Hyalella* found within the Bruce Peninsula region may have developed pesticide resistance, it was noteworthy that *Hyalella* were only found in samples that came from sites experiencing high amounts of agricultural stress in the surrounding area (Figure 3.12). Barbour et al. (1999) classified *Hyalella* as pollution-tolerant relative to *Gammarus* (Table 3.8). As it turned out, both *Gammarus and Hyalella* correlated with sample locations that recorded long durations of hypoxia. However the range of DO concentration is what seems to separate *Gammarus and Hyalella. Gammarus* seem to be slightly more sensitive to environmental conditions in that they are correlated with sample location that have a more consistent range of DO concentration, in comparison to *Hyalella* which are correlated with sample locations that experienced a large range in DO concentrations (Figure 3.11).

Another likely reason why *Hyalella* were only found in the highly agriculture stressed sites would be due to the lower amount of fish predation. Fyke nets placed in wetlands surrounded by a lot of agriculture, captured fewer fishes than nets in wetlands

whose watersheds contained little agriculture (Figure B.7). Differences in fish abundance could also explain why the relative abundance of *Gammarus* seemed to separate the North Channel sites from the Bruce Peninsula wetlands (Andersson et al., 1986; Figure 3.12). In the case of the North Channel sites, fish abundance was greater in sites that were experiencing higher amount of surrounding agriculture pressure in comparison to sites experiencing less surrounding agriculture (Figure B.8).

There were no significant trends in the relative abundances of air, tracheal and cutaneous respiratory functional groups along the DO concentration gradient when all wetlands were considered (Table 3.7). Although we had not expected there to be a difference in abundance for the tracheal and cutaneous respiration functional groups, it was surprising to find that there was no trend for air-breathing (pneustonic) invertebrate taxa. We had predicted that there would be a greater relative abundance of air breathers at the shallower and closest to shore points along the transects (Points 1 and 2). Figure 3.4 shows that there might be a trend present; however, it was not at all significant ($p=0.60$). Perhaps we do not see a difference in the abundance of air breathers because there was abundant vegetation at all points that could provide cover to these organisms and protect them from predation in the water. Another point to consider is the time of day during which samples were collected. All samples were collected during the day, usually as the DO concentration was increasing in the morning. The DO concentration might have been low enough at the time to restrict fish access to the wet-meadow, but high enough to allow the invertebrate community to use all parts of the wet-meadow while it was predator free. Sampling at night when the DO concentration was at its lowest might have

yielded more significant trends between invertebrate respiratory functional groups and habitat selection.

There was one significant trend for the relative abundance of different tolerance groups, and that was for the sensitive (intolerant) group (Table 3.10). The group of intolerant taxa (consisting of Tricladida, Leptoceridae, Aeshnidae, and *Gammarus;* Table 3.8) was relatively more abundant in the deeper transect points closer to open water (Point 3 and 4) than at the shallowest points. The tolerance scores given to these four taxa groups by Barbour et al. (1999), were based upon a collection of 5 different of IBI protocols. Three of the five protocols were based upon or adapted from the Hilsenhoff Biotic Index, which rates aquatic invertebrates on their ability to tolerate organic and nutrient pollution, with the knowledge that DO concentration has a negative relationship with the increase in nutrients. The result of this investigation shows that the DO concentrations correlate with habitat selection when it comes to sensitive invertebrates, and that perhaps other tolerant groups of invertebrates use DO concentration to select suitable habitat too. Our results are consistent with those of Henry and Danielopol (1998) who found that *Gammarus* were dependent on DO concentration when it came selecting a habitat in a laboratory setting. In our study it seemed that the range of DO concentration was what influenced habitat choice within a wetland environment (Figure 3.11). Tricladida, have an incentive for seeking out normoxic conditions, as Larouche, et al. (2018) showed that planarians have greater success at regenerating lost tissue, following an amputation procedure, in normoxic conditions than in hypoxic conditions. In our wetlands, Tricladida were correlated with areas of relatively low, yet constant DO concentrations (Figure 3.11). Movement constraint and predation pressure from fish

might have influenced the Tricladida to remain the anoxic areas of the wetland. Ubhi and Matthews (2018) showed that Aeshnidae nymphs are also sensitive to hypoxia. These nymphs will seek out more normoxic waters once the frequency of ventilation due to hypoxic conditions, becomes too energetically expensive (Ubhi and Matthews, 2018). Unfortunately, Aeshnidae nymphs were not strongly correlated to anything (Figure 3.11) and this might be due to their ability to move quickly, perhaps allowing them to hunt in anoxic areas of a wetland for a time, then rest in normoxic areas of the wetland.

In the RDA model that used all four environmental PCA axis, samples were grouped by region along the RDA 1 axis (Figures 3.6 and 3.7), because they were segregated primarily based on water temperature ranges (Dissolved oxygen chapter). *Caenis*, as mentioned before, emerge in late summer in Canada (Merritt et al., 2011), and because sites in the Bruce Peninsula region were sampled at the end of the summer, these samples captured the *Caenis* likely right before an emergence event. The North Channel sites were sampled at the beginning of August or about mid-summer, when it seems it would be too early to in the season to capture large *Caenis* larvae.

Figure 3.8 and 3.9 indicated that the community composition of a sample was more similar to samples coming from the same wetland than to samples coming from different wetlands but sharing similar relative locations within each wetland. Figure 3.10 indicates that if the wetland was looked at individually, one could find a difference in the invertebrate community composition, based on the relative sampling location. Previous studies have found this along much longer transects that span multiple vegetation zones. Massri et al., (2019) have found that DO also plays a similar role in shaping community composition within streams. However, my study seems to document that community

composition differences can also be detected along a short transect within a single vegetation zone.

Overall, the relationship between invertebrate community composition and dissolved oxygen concentration should be further investigated, as many trends are likely being obscured in this study due to the site variability.

Conclusions

Ultimately, I have found that environmental factors greatly affect the community composition of benthic aquatic invertebrates, especially in diverse and dynamic regions such as freshwater wetlands. At a glance, the biological assessments conducted here suggest that variables associated with region (the granitic North Channel vs. the carbonaceous Bruce Peninsula) and varying among individual wetlands were more important determinants than features measured within the wet meadow zone of wetlands as a group (depth, duration of hypoxia, presence of fishes). However, biological responses to gradients, specifically the duration of hypoxia and the range of DO concentration a sample area experiences within wetlands were evident, and might be more apparent if wetlands were assessed and treated on an individual basis, as is commonly done in intensive studies. However, this chapter corroborates similar research done by Chapman et al. (2004), who found that the mean monthly DO concentration could be used to predict the abundance of certain respiratory groups in a swamp-river system. Diel periodicity of hypoxia and anoxia is widespread in Lake Huron wet meadows, and dissolved oxygen concentration does seem to be a consistently important

factor that structures aquatic invertebrate species within a system, as has been document in laboratory (Teixeira, 2015), marsh (Corkum, 1985), lake (Britt, 1955) and, stream (Wiley and Kohler, 1980) systems. These findings can aid future researchers and conservation authorities in designing appropriate protocols when studying these diverse and dynamic wetlands.

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Chapter 4
Thesis Discussion

Introduction

The goal of this project was to document the distribution and diel fluctuations of dissolved oxygen concentration (DO) in wet meadows of Lake Huron coastal wetlands and to determine if the aquatic invertebrate community composition within the wet meadow varies with respect to the DO patterns. In each wetland, Dissolved oxygen was measured for 24 h at 4 points along 3 transects that ran through the wet meadow from open water towards shore. Invertebrate samples and measurements of other water associated variables (temperature, depth, pH) were also taken at each point.

Within each wet meadow a DO gradient occurs from the most lakeward point of each transect extending approximately 15 – 30 meters towards shore. I found that the dissolved oxygen (DO) concentration gradient is defined primarily by the DO range, maximum and, minimum DO recordings over a 24-h period or best summarized as the duration of hypoxia experienced at a particular point over 24 h. Along the 15-30 m transect described above, sample points closest to open water (point 4) experienced the smallest DO range and had the greatest DO minimum recordings. In other words, transect points closest to open water endured hypoxic conditions for a relatively small portion of the day. Sample points that were furthest from open water (shallowest and nearest the shoreline) experienced a large day-night range of DO and lowest DO minimum values recorded over the same 24 h within the same wetland. This means that sample points furthest away from open water endured hypoxic or anoxic conditions for much longer

(Appendix A; Table 5.5 and 5.6). This indicates that potentially different assemblages of aquatic invertebrates may be encountered depending on where sampling efforts are concentrated, which may have contributed to an additional source of variation in this study. However, the among-wetland variation in invertebrate community composition was greater than the variation among points along transects within wetlands, meaning that sampling location within the wet meadow would likely not be a confounding factor in the analysis of either environmental or biological variables in multi-wetland studies. The invertebrate community composition was so different between each wetland's wet meadows that sampling location within the wet meadow is of little concern if researchers are comparing different wetlands to one another. Comparative studies are an important approach in wetland research, especially when trying to monitor the effectiveness of restoration and reclamation programs. This is a significant finding and important validation of the 'synoptic survey' study design commonly used for large sampling programs such as the Great Lakes Coastal Wetland Monitoring Program (CWMP; Uzarski et al., 2017), and Great Lakes Environmental Indicators study (GLEI; Niemi et al. 2007; Johnson et al. 2015) that integrate surveys conducted by many teams and individuals, who might at times interpret sampling protocols slightly differently (e.g. where in a vegetation zone to collect an invertebrate sample). Other vegetation zones should also be examined in the future though, as this project only focused on the wet meadow vegetation zone of wetlands.

In Chapter 3, I found that hypoxia-intolerant invertebrates represented by Tricladida, Leptoceridae, *Gammarus*, and Aeshnidae, did show a significant trend along the DO gradient. The relative abundance of sensitive invertebrates in comparison to all

other invertebrates was greater at wet meadow locations in deeper water farther from shore than at transect points nearer to shore . More tolerant types of invertebrates may also be constrained due to the DO, more specifically the duration of hypoxia and anoxia experienced in different locations of the wet meadow. Although I focused on dissolved oxygen in this thesis, a number of other factors also varied in parallel with DO gradients within wetlands. The biological component of the community is also very important, as seen with regards to organic sediment depth. The North Channel wetlands had a thicker layer of organic sediment in comparison to the wetlands sampled in the Bruce Peninsula and therefore likely created a greater biological oxygen demand, which was indicated in this study by the daytime maximum DO concentration being consistently lower than that of the Bruce Peninsula wetland sites (Chapter 2).

I found several trends indicating that the invertebrate community is constrained by dissolved oxygen, similar to the findings of Maasri et al., (2019), who observed that stream invertebrate communities were partitioned according to their functional and habit niche requirements. However, many of these trends found within the Lake Huron wet meadows seem to be masked by among-wetland differences in environmental variables and invertebrate community composition, which were greater than the differences observed within communities sampled along transects within wetlands.

My research was also designed to contrast regional differences in wetland characteristics, with 6 paired sites situated on the Bruce Peninsula and 4 sites in the North Channel. There were very clear differences in the composition of the invertebrate communities collected from the two regions, possibly relating to the marked differences in geomorphology and consequently to the types of stress relating to land use imposed by

those differences (Danz et al. 2007; Niemi et al. 2009). Nevertheless, I found that the invertebrate community composition was fairly distinct for each wetland sampled even within the two regions. A detailed comparison and contrast of the differential effects of stress on community composition within and between regions was beyond the scope of this thesis.

Limitations

Despite the trends observed, unmeasured wetland attributes may have contributed to the variation observed within and among wetlands. For example, the study of water depth variation during each survey could be improved with the use of water depth/pressure loggers, which could help researchers detect surges or wind shear effects on water levels, and provide a means of documenting diel and day-to-day depth changes. I measured and calculated water depth changes based on spot readings taken with a standard meter stick at the beginning and end of each survey. However, the heterogeneity of the sample sites made it difficult to replicate the exact spot a previous measurement was taken the day before due to the hummocky bottom.

Measuring environmental variables such as DO, depth and temperature over a longer time period, as in continuously monitoring these factors from the beginning of May to the end of August, at each of the sites could have allowed for a greater ability to determine days featuring abnormal conditions (Trebitz, 2006), thus affecting collection of fish and or invertebrates (Chapman et al., 2004). Different invertebrate taxa might depend on e different time frames of stable, favourable or unsuitable conditions to exploit, or seek refuge from predators in a different part of a wet meadow. Invertebrates such as the very tolerant Chironomidae might be able to endure lengthy and severe anoxic conditions

before seeking out a more favourable habitat (Van Hoven, 1975), in comparison to the very sensitive Aeshnidae. Invertebrates might also be better grouped together based on mobility groups. Aeshnidae nymphs for example would be able to swim much further and faster then Chironomidae (Merritt et al., 2008).

Revisiting sites could improve the representation of the aquatic invertebrate community over the course of the macrophyte growing season (perhaps once in June, July and August). The mayfly *Caenis*, in this study was found nearly exclusively at the Bruce Peninsula sites. This is inconsistent with the composition of samples collected at the same sites from previous years during which *Caenis* larvae were commonly encountered (CWM database). Because different invertebrates mature at different times and rates, sampling a site multiple times throughout the growing season, perhaps on three occasions, could have better represented the invertebrate community within each wet-meadow. Furthermore, collecting invertebrates throughout the summer might yield specimens large and mature enough to permit identification to finer taxonomic resolution, as this was a common limitation of this project. Identifying organisms more consistently to the genus and species levels would permit more accurate assessment of tolerance scores than was possible at the family level. For example, *Baetis flavistriga* is a sensitive stream-dwelling species, having a tolerance score of 4 in Barbour et al. (1999) tolerance scoring system, while sympatric *Baetis intercalaris* are more tolerant of eutrophic conditions, having a tolerance score of 6 (Hilsenhoff, 1988). Although these particular tolerant scores seem similar enough, these species would have fallen into different tolerance categories for some of the calculations performed in Chapter 3. *Baetis*

flavistriga would have been considered a sensitive species, while *Baetis intercalaris* would have been considered a mid-tolerant species.

The timing of invertebrate sampling during a day was constrained by the logistics of travel and scheduling for the field team. Consequently, samples were taken as early as 0830 and as late as 1630 in the day depending upon the wetland. Sampling each site at a consistent time of day, perhaps at about 0800, could have yielded a stronger relationship between the invertebrate community composition and DO concentration as the oxygen concentration is still very low in the morning, in comparison to later on in the day when oxygen levels rise considerably.

Implications and Future Studies

The data collected for my thesis further our understanding of aquatic organisms' distribution within wet meadows, highlighting the importance of when and where to collect invertebrate samples. Information from this thesis allows wetland researchers to make more informed decisions about how to capture the best representation of invertebrate community, as well as the important role that dissolved oxygen has in constraining at least the most sensitive aquatic invertebrates in habitat use. For example, if a research goal is to gather accurate and detailed data about a specific wetland, then samples from a variety of locations within a vegetation stand should be taken in order to effectively represent the variation in invertebrate community composition and abundance within that vegetation stand, especially when it comes to collecting the most oxygen-sensitive taxa. However, if researchers wish to compare multiple wetlands to one another, then their within-wetland sampling strategy need not need be as spatially intensive, as I found that the invertebrate communities are distinctive regardless of where one samples

within wet meadows . This research also highlights the need to for the continuous monitoring of as many wetlands as possible. Each wetland in this study was different enough to limit the statistical power to make comparisons among groups of wetlands. By sampling on multiple occasions over a longer period of time, possibly for a couple months, one might capture more of the invertebrate community biodiversity, as the different invertebrates mature over the growing season. This could result in greater similarity among 'replicate' wetlands. Corroborating my findings of similarities among wetlands - such as the trend of sensitive taxa groups being restricted to deeper and more oxygenated parts of the wet meadows within other vegetation zones would certainly strengthen our ability to predict changes to the invertebrate community at a basin wide level.

Overall, my research has documented important sources of environmental variability both within wet meadow zones (largely associated with spatial and day/night trends DO and associated parameters) and among wetlands (regional differences, land use differences, and, the type or amount of organic substrate) that otherwise appear to be homogeneous and comparable in terms of vegetation. Knowledge of these spatial patterns can help guide aquatic ecologists' study designs to minimize potential sampling biases, and to most accurately represent a specific wet meadow's health through the use of invertebrate indicators. This is essential in accommodating the difficulties and limitations of using aquatic invertebrates to compare the health of one wetland to another within enormous and complex ecosystems such as the Great Lakes.

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Appendix A:
Vegetation Density Analysis and Duration of Hypoxia and Anoxia according to surrounding Agricultural Impact

Vegetation density was sampled in the wet meadows of each wetland to allow me to quantify the relationship between measures and diel patterns of dissolved oxygen concentration and vegetation density, under the assumption that vegetation and its density attenuates waves and inhibits water mixing (Dalrymple et al. 1984; Cardinale, 1997), and therefore influences nutrients or gases dissolved in the water (Deng, 2018; Bachmann, 2000; Trebitz, 2006). A comparison between a traditional means of determining plant density - counting the number of stems per unit area within a quadrat was compared to that of the Robel pole method of quantifying plant density (Robel 1970) as described in detail in Chapter 2

Table 5. 1 Vegetation density comparison of Robel Pole visual obstruction (percent), derived from photos taken of the Robel pole from 4 m away, to quadrat stem count (number of stems observed within a 25 x 25 cm quadrat). Wet meadow Baie du Dore (BdD) data were not included.

Sample Point	Stem count (stems per 25 cm^2)	Visual obstruction %	Dominant Vegetation 1	Sub-ominant Vegetation
SB2 A1 2	178	10%	carex	Phalaris
SB2 A2 2	103	38%	carex	Cladium
SB2 A3 2	88	0%	Cladium	Schoenoplectus
SB2 A4 2	163	0%	Cladium	Schoenoplectus
SB1 B1 2	331	53%	carex	Calamagrostis
SB1 B2 2	96	9%	carex	Potentilla
SB1 B3 2	265	42%	carex	Calamagrostis
SB1 B4 2	246	64%	carex	Calamagrostis
SC B1 2	95	88%	carex	Phalaris
SC B2 2	88	69%	carex	Phalaris
SC B3 2	16	15%	carex	none
SC B4 2	65	40%	carex	Meadow Sweet
PB C1 2	122	42%	carex	Calamagrostis
PB C2 2	185	43%	carex	Calamagrostis
PB C3 2	74	23%	carex	Calamagrostis
PB C4 2	0	9%	carex	Calamagrostis
OWR B1 2	116	3%	carex	Iris
OWR B2 2	100	0%	Schoenoplectus	Juncus
OWR B3 2	103	0%	Schoenoplectus	Juncus
OWR B4 2	79	0%	Schoenoplectus	carex
FI7 C1 2	209	38%	carex	Calamagrostis
FI7 C2 2	302	31%	Calamogrostis	carex
FI7 C3 2	242	11%	Cladium	Phragmites
FI7 C4 2	68	0%	Cladium	Phragmites
BB B1 2	112	31%	carex	Calamagrostis
BB B2 2	168	13%	carex	Calamagrostis
BB B3 2	161	0%	carex	none
BB B4 2	23	89%	carex	Persicaria
BR B1 2	78	82%	carex	Utricularia
BR B2 2	155	100%	carex	Utricularia
BR B3 2	45	42%	carex	Utricularia
BR B4 2	87	0%	carex	Utricularia
AC C1 2	137	49%	carex	none
AC C2 2	168	46%	carex	none
AC C3 2	223	72%	carex	Calamagrostis
AC C4 2	343	6%	carex	Calamagrostis

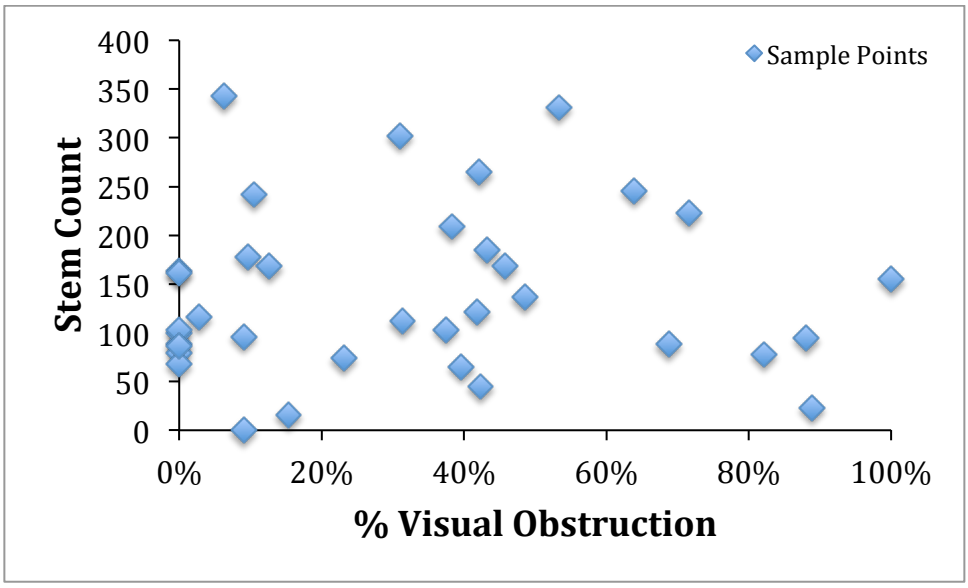

Figure 5. 1 Relationship between number of stems per 25 cm x 25c m quadrat at all sample points (4 transect points per wetland in 9 wetlands; n=36).

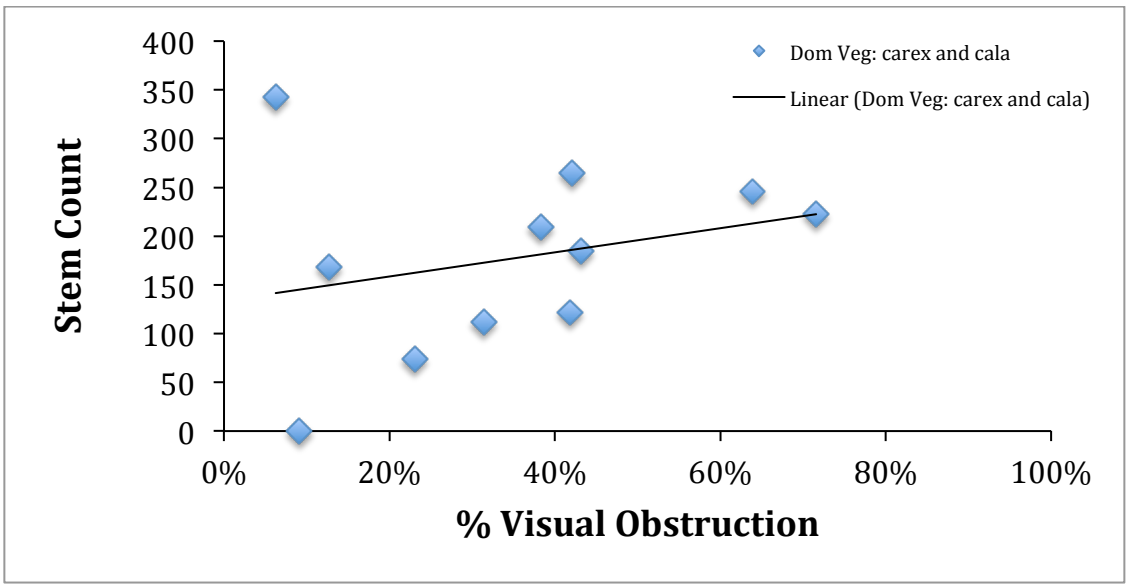

Figure 5. 2 Relationship between the Robel Pole visual obstruction method and Stem counts at quadrats dominated by the genera *Carex* or *Calamogrostis* (n=11).

The Coastal Wetland Monitoring Program has been monitoring and reporting on the the biota of Great Lakes coastal wetlands since 2011 (CWM Uzarski et al. 2017). The large number of wetlands that are found around the Great Lakes, limits return visits to individual wetlands by CWMP teams to once every 3-5 years. Table A1 lists the dominant and subdominant genera identified in the wet meadow zones of the study wetlands during previous surveys. Vegetation was surveyed by (Brady et al. 2019).

Table 5. 2 Vegetation zones sampled by Coastal Wetlands Monitoring teams prior to 2017. Sites not included (Old Woman's River, Stokes Bay 2 and Fishing Island 7) had not been sampled before 2017.

Site#	Site name	Previous Year Sampled	Zone 1	Zone 2
5016	Baie du Dore 2	2016	Phragmites	Wet Meadow
5013	Anderson's Creek	2012	Lily	Sparganium
5137	Bullhead Bay	2012	SAV	Typha
5950	Stobie Creek	2016	Typha	Wet Meadow
5106	Blind River	2013	SAV	
5727	Pike Bay	2015	Sparse Bullrush	Wet Meadow
5952	Stokes Bay 1	2015	Wet Meadow	

The mean (±SD) duration of mild and moderate hypoxia, and anoxia was calculated using the observations from the three transects set up at each wetland. For example, the duration of mild hypoxia (<4 mg/L) for point 1, is the mean of the duration of mild hypoxia among point 1 location of transects A, B and C.

Table 5. 3 Duration (hours) of mild hypoxia (DO <4.0 mg/L), moderate hypoxia (DO <2.0 mg/L) and anoxia (DO <1.0 mg/L) in each North Channel wetland.

Matched Pair 1	Low Agriculture	Site	Blind River			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	17	17	15.75	11.5
		Duration of Moderate Hypoxia (<2mg/L)	17	15.25	10.5	7.25
		Duration of Anoxia (<1mg/L)	15.5	11.5	9.25	0
	High Agriculture	Site	Anderson's Creek			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	17	17	15.25	0
		Duration of Moderate Hypoxia (<2mg/L)	17	10.5	5	0
		Duration of Anoxia (<1mg/L)	12.25	7.5	2.25	0
Matched Pair 2	Low Agriculture	Site	Bullhead Bay			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	17	14.25	13.75	5.25
		Duration of Moderate Hypoxia (<2mg/L)	16.75	7.25	5.75	1.75
		Duration of Anoxia (<1mg/L)	13.25	4.25	3.75	0
	High Agriculture	Site	Stobie Creek			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	17	17	15.75	11.75
		Duration of Moderate Hypoxia (<2mg/L)	17	12.5	11.75	6.25
		Duration of Anoxia (<1mg/L)	17	12	11.5	2.25

Table 5. 4 Duration (hours) of mild hypoxia (DO <4.0 mg/L), moderate hypoxia (DO <2.0 mg/L) and anoxia (DO <1.0 mg/L) in each Bruce Peninsula wetland

Matched Pair 3	Low Agriculture	Site	Pike Bay			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	15.5	17	14	8.75
		Duration of Moderate Hypoxia (<2mg/L)	12.5	11.75	9.25	4.75
		Duration of Anoxia (<1mg/L)	11	10.25	8.5	0.5
	High Agriculture	Site	Baie du Dore			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	5.75	3	4.5	8
		Duration of Moderate Hypoxia (<2mg/L)	0	0	0	2
		Duration of Anoxia (<1mg/L)	0	0	0	0
Matched Pair 4	Low Agriculture	Site	Fishing Island 7			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	10	13.75	12.75	0.5
		Duration of Moderate Hypoxia (<2mg/L)	7.25	7.75	4	0
		Duration of Anoxia (<1mg/L)	5.75	5	0	0
	High Agriculture	Site	Old Woman's River			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	16	13.75	11.75	8.75
		Duration of Moderate Hypoxia (<2mg/L)	12.25	10.75	5	0
		Duration of Anoxia (<1mg/L)	9.25	5	0	0
Matched Pair 5	Low Agriculture	Site	Stokes Bay 1			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	14.5	13.5	14.25	13.75
		Duration of Moderate Hypoxia (<2mg/L)	11.75	10	9.75	7.5
		Duration of Anoxia (<1mg/L)	9.5	6.5	5.25	2
	High Agriculture	Site	Stokes Bay 2			
		Relative sampling location	Point 1	Point 2	Point 3	Point 4
		Duration of Mild Hypoxia (<4mg/L)	13.5	13	6.5	9.25
		Duration of Moderate Hypoxia (<2mg/L)	5.5	0	0	0
		Duration of Anoxia (<1mg/L)	0	0	0	0

Appendix B:
Invertebrate and Fish Analysis and Summary Tables

Invertebrate taxa richness, evenness and diversity calculated for each wetland sampled for this project are summarized in the figures below. Means were calculated from the four invertebrate samples collected along a single transect within each wetland.

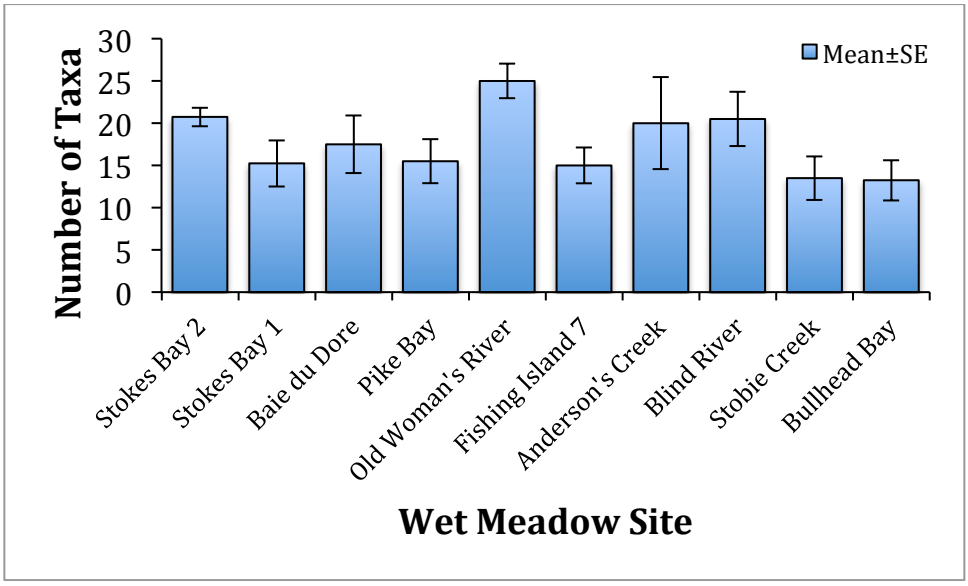

Figure 6. 1 (Mean (±SD)Taxa Richness (n=4) for each wet meadow site.

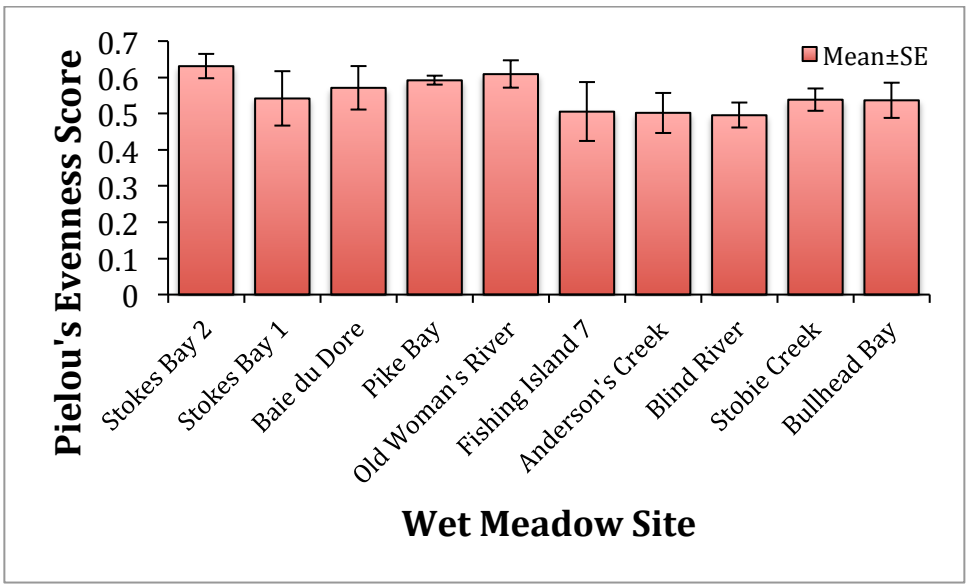

Figure 6. 2 Pielou's Evenness Score (mean±SD, n=4) for each wet meadow site.

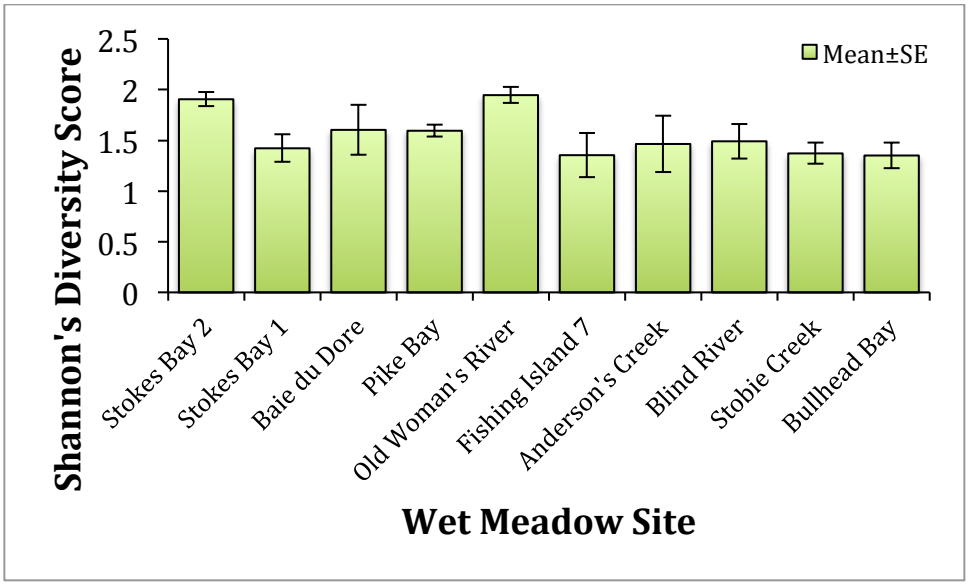

Figure 6. 3 Shannon's Diversity Score (mean±SD, n=4) for each wet meadow site.

Table 6. 1 Calculated mean and standard error (SE) of Taxa Richness, Pielou's Evenness Score and Shannon's Diversity Score, for all wet meadow sites.

Site	Richness Mean	Richness SD	Pielou's Evenness Mean	Pielou's Evenness SD	Shannon's Diversity Mean	Shannon's Diversity SD
Stokes Bay 2	20.75	1.11	0.63	0.03	1.91	0.07
Stokes Bay 1	15.25	2.75	0.54	0.08	1.42	0.14
Baie du Dore	17.50	3.43	0.57	0.06	1.60	0.25
Pike Bay	15.50	2.60	0.59	0.01	1.59	0.06
Old Woman's River	25.00	2.04	0.61	0.04	1.95	0.08
Fishing Island 7	15.00	2.12	0.51	0.08	1.35	0.22
Anderson's Creek	20.00	5.43	0.50	0.06	1.46	0.28
Blind River	20.50	3.20	0.50	0.03	1.49	0.17
Stobie Creek	13.50	2.60	0.54	0.03	1.37	0.10
Bullhead Bay	13.25	2.39	0.54	0.05	1.35	0.13

The comparison between Bruce Peninsula (BP; n=6) and North Channel (NC; n=4) wet meadow sites of the invertebrate taxa richness, evenness and diversity according to the relative sampling location within wet meadows (Point 1 being the shallowest and closest to shore, while Point 4 is deepest and open to lake effects) (Figure 6.4, 6.5 and 6.6).

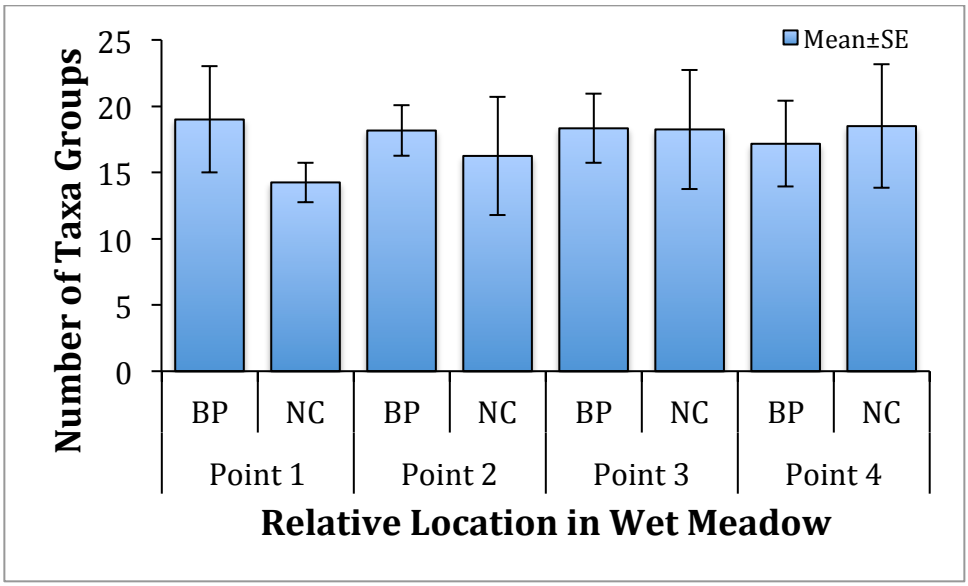

Figure 6. 4 Comparison of Taxa Richness among relative location along a sampling transect (Point 1 = shallowest and closest to shore, Point 4 = deepest and open to lake effects) within the two different sampling regions (BP = Bruce Peninsula, NC = North Channel).

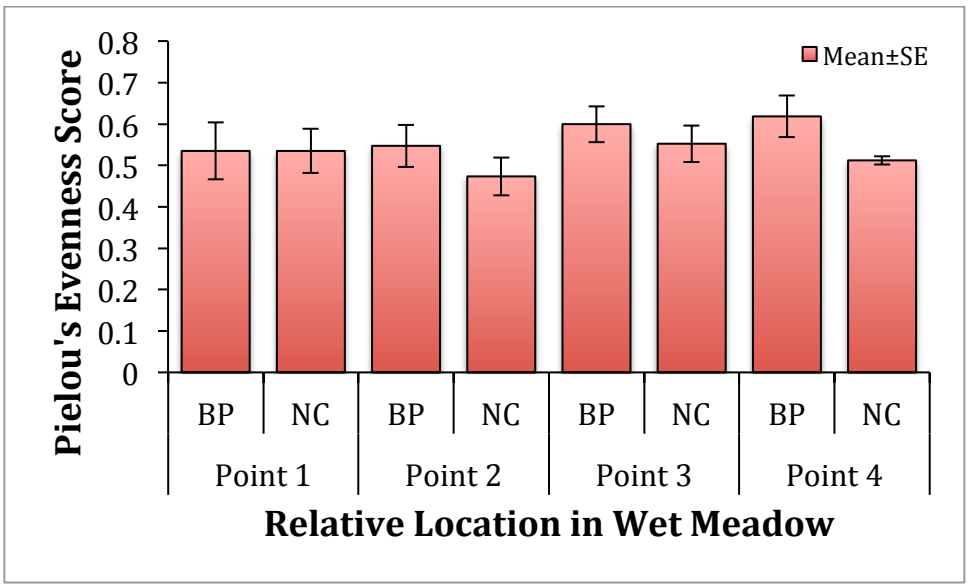

Figure 6. 5 Comparison of Pielou's Evenness Score among relative location along a sampling transect (Point 1 = shallowest and closest to shore, Point 4 = deepest and open to lake effects) within the two different sampling regions (BP = Bruce Peninsula, NC = North Channel).

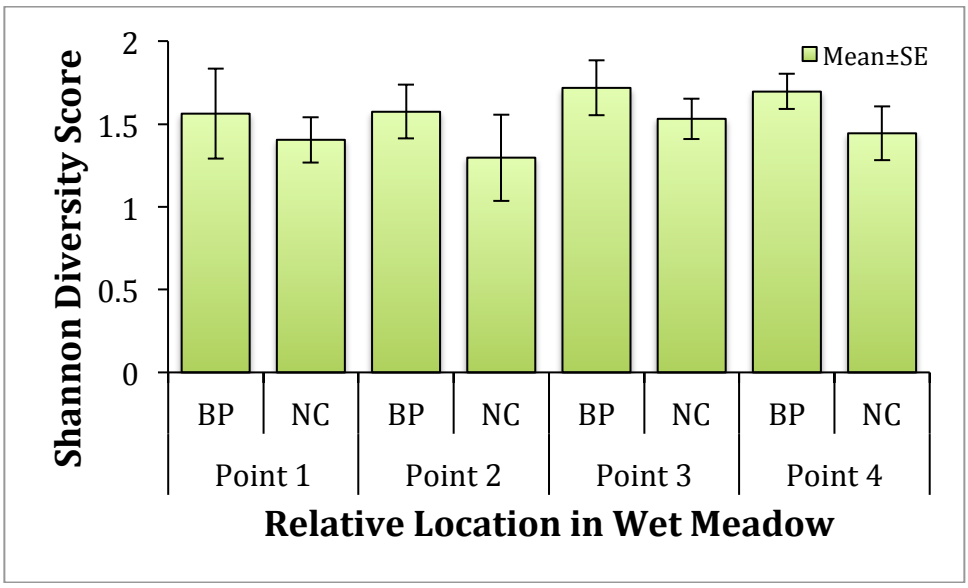

Figure 6. 6 Comparison of Shannon's Diversity Score among relative locations along a sampling transect (Point 1 = shallowest and closest to shore, Point 4 = deepest and open to lake effects) within the two different sampling regions (BP = Bruce Peninsula, NC = North Channel).

Table 6. 2 Mean (±SE, n=3) Taxa Richness, Pielou's Evenness Score and Shannon's Diversity Score, for each transect point(Point 1 = shallowest and closest to shore, Point 4 = deepest and open to lake effects) in two sampling regions (BP = Bruce Peninsula, NC = North Channel).

		Richness Mean	Richness SE	Pielou's Eveness Mean	Pielou's Eveness SE	Shannon's Diversity Mean	Shannon's Diversity SE
Point 1 (Shallowest)	Bruce Peninsula	19.00	4.01	0.53	0.07	1.56	0.27
	North Channel	14.25	1.49	0.53	0.05	1.40	0.14
Point 2	Bruce Peninsula	18.17	1.91	0.55	0.05	1.57	0.16
	North Channel	16.25	4.46	0.47	0.05	1.30	0.26
Point 3	Bruce Peninsula	18.33	2.60	0.60	0.04	1.72	0.16
	North Channel	18.25	4.50	0.55	0.04	1.53	0.12
Point 4 (Deepest)	Bruce Peninsula	17.17	3.23	0.62	0.05	1.70	0.11
	North Channel	18.50	4.66	0.51	0.01	1.44	0.16

Comparisons of fish caught in fyke nets between wetlands that experience high amounts of surrounding agriculture (High) and wetlands experiencing low amounts of surrounding

agriculture impact (Low) within each of the regions sampled with this study, Bruce Peninsula and North Channel respectively (Figure 6.7).

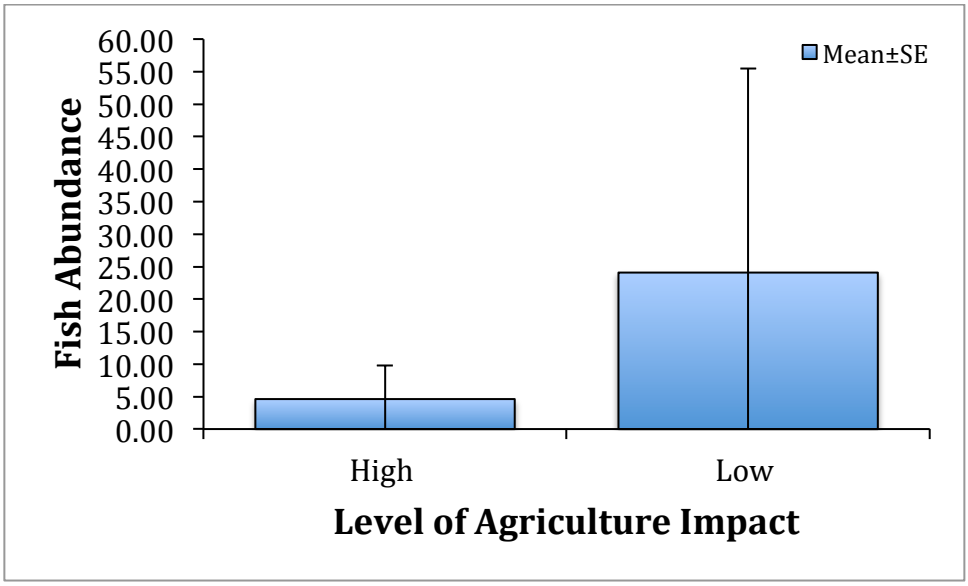

Figure 6. 7 Mean (±SE, n=3) number of fishes caught in fyke nets placed in wet meadows of wetlands categorized as experiencing high levels of surrounding agriculture impact (High) vs. low levels of surrounding agriculture impact (Low) in the Bruce Peninsula region.

Table 6. 3 Abundance of fish detected in Bruce Peninsula sites (n=3).

	High	**Low**
Mean	4.62	24.08
SD	9.03	54.46
SE	5.21	31.44

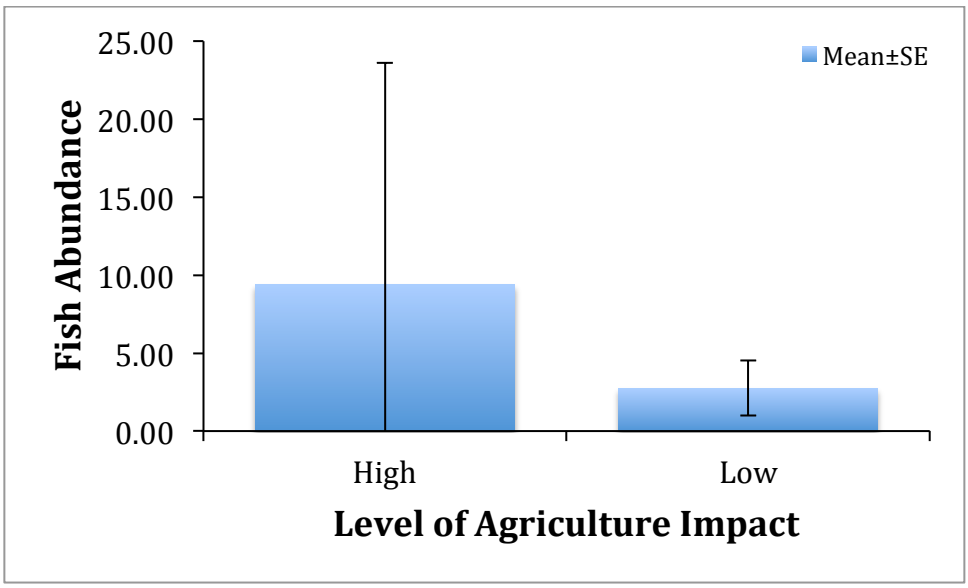

Figure 6. 8 Mean (±SE, n=2) number of fishes caught in fyke nets placed in wet meadows of wetlands categorized as experiencing high levels of surrounding agriculture impact (High) vs. low levels of surrounding agriculture impact (Low) in the North Channel region.

Table 6. 4 Abundance of fish detected in North Channel sites (n=2).

	High	**Low**
Mean	9.38	2.75
SD	20.14	2.50
SE	14.24	1.77

Scores of the taxa, and specific sample (wet meadow site, and relative sampling location) constrained by the environmental variables measured in Chapter 2 (Table 2.4) plotted in RDA space.

Table 6. 5 Summary table for (Chapter 3; Figure 3.7) of invertebrate community and samples constrained by the summarized environmental variables (PCA axes 1-4).

Name	RDA1	RDA2	RDA3	RDA4	PC1	PC2
Gammarus	-0.517	0.752	-0.129	-0.108	-0.787	0.510
Hyalella	-1.056	-0.165	-0.503	0.023	0.624	0.907
Gyrinidae	-0.300	0.039	-0.055	-0.082	0.149	0.171
Haliplidae	0.299	-0.029	0.215	-0.107	-0.153	-0.145
Collembola	0.137	-0.270	0.119	0.048	-0.196	-0.087
Ceratopogonidae	-0.337	0.033	-0.249	-0.060	0.190	-0.620
Culicidae	0.353	-0.098	0.000	-0.161	-0.053	-0.193
Chironomidae	0.221	-0.227	-0.325	0.188	-0.072	-0.213
Baetidae	-0.521	0.060	0.280	0.169	0.180	0.555
Caenis	-2.036	0.342	0.108	-0.032	1.761	-0.118
Lymnaeidae	-0.475	-0.599	0.398	0.358	-0.095	0.055
Physa	-0.174	-0.010	-0.062	-0.107	0.113	0.202
Planorbidae	0.458	-0.312	0.342	-0.164	-1.093	-0.798
Succinea	0.061	-0.622	0.103	-0.175	0.009	-0.145
Belostoma	0.057	-0.012	-0.010	-0.005	0.000	-0.083
Corixidae	-0.442	-0.146	0.337	0.093	-0.171	-0.315
Mesovelia	-0.186	-0.063	0.350	-0.040	0.069	0.128
Microvelia	0.109	-0.074	-0.019	-0.098	0.003	-0.092
Glossophonia	0.255	0.022	-0.299	-0.148	-0.314	0.233
Hydracarina	-0.136	-0.142	0.115	0.015	-0.120	0.194
Caecidotea	0.843	1.374	0.275	0.303	-1.510	1.068
Lirceus	0.285	-0.011	-0.063	0.268	-0.062	0.160
Crambidae	-0.169	0.085	0.121	0.004	0.142	0.028
Aeshnidae	-0.047	0.026	0.060	-0.002	0.004	0.007
Ischnura	-0.054	0.140	-0.130	-0.031	0.040	-0.386
Coenagrionidae	-0.854	0.247	0.136	-0.101	0.713	1.139
Libellulidae	-0.284	0.245	-0.116	0.013	0.216	-0.398
Oligochaeta	0.061	0.017	-0.221	0.180	0.175	-0.001
Hydroptilidae	-1.208	-0.003	-0.022	0.229	0.586	-0.118
Leptoceridae	-0.472	0.024	-0.166	-0.198	0.290	0.200
Phryganeidae	0.131	0.427	0.145	-0.452	-0.257	-0.329
Tricladida	0.537	-0.011	-0.451	0.234	-0.270	0.182
SB2-B1	-1.850	0.036	-0.515	-2.631	0.985	-0.269
SB2-B2	-1.285	-1.282	0.499	-3.581	0.739	-2.094
SB2-B3	-1.640	-0.847	-2.675	-2.048	0.730	-2.812
SB2-B4	-1.982	0.041	-2.613	-1.563	0.958	-0.384
SC-A1	1.756	-2.627	-0.404	-4.140	0.142	-1.277
SC-A2	1.757	-1.317	-0.147	0.934	0.241	0.185
SC-A3	1.069	0.084	-0.958	2.565	0.916	0.998

SC-A4	1.332	1.362	-2.125	1.774	-0.161	1.595
BB-A1	2.052	0.347	-1.049	1.248	-0.557	0.294
BB-A2	2.206	-0.535	-0.908	0.921	-0.237	-0.063
BB-A3	2.232	-0.316	-1.881	3.505	-0.894	0.543
BB-A4	1.870	0.578	-3.637	2.302	-0.941	0.722
BdD-B1	-0.643	-4.420	3.156	2.218	-1.610	0.169
BdD-B2	-1.089	-1.149	0.956	1.548	-1.164	-1.658
BdD-B3	-0.493	-0.726	-1.008	0.548	-1.192	-1.922
BdD-B4	-0.928	-0.564	-0.304	2.448	-0.358	-1.220
AC-C4	-0.169	-1.360	-3.025	-1.589	-0.262	1.232
AC-C3	1.131	-1.696	0.336	-2.703	0.322	-2.300
AC-C2	1.468	-1.873	-1.295	0.669	0.796	-0.512
AC-C1	1.798	-3.116	0.748	-1.535	0.333	-1.298
BR-B1	2.180	1.722	4.698	-2.444	-2.424	0.007
BR-B2	1.978	1.452	2.332	0.100	-2.152	-0.427
BR-B3	1.688	1.596	2.346	-0.400	-1.814	-0.272
BR-B4	1.867	2.711	1.224	-0.625	-2.945	0.250
OWR-B1	-2.015	-1.051	0.764	0.121	1.359	0.886
OWR-B2	-2.107	0.722	0.031	1.971	0.352	2.030
OWR-B3	-2.030	1.381	0.089	1.051	0.358	2.246
OWR-B4	-1.797	0.342	-0.556	2.420	0.591	1.918
PB-C1	0.631	2.120	2.091	-0.608	-0.447	0.177
PB-C2	0.292	2.635	0.745	0.406	-0.134	1.330
PB-C3	0.642	2.917	2.155	-0.710	-1.292	0.810
PB-C4	0.226	1.630	0.419	-0.423	-0.996	-0.555
SB1-A1	-0.541	0.043	0.008	-0.247	1.636	-0.814
SB1-A2	-1.300	0.322	0.397	0.715	1.671	-0.927
SB1-A3	-0.950	1.151	0.638	1.505	0.975	-0.320
SB1-A4	-1.031	2.942	0.411	1.202	0.778	0.996
Fi7-B1	-1.121	-0.654	0.312	-1.338	2.563	-0.052
FI7-B2	-1.599	-1.246	1.422	-0.926	2.197	0.053
FI7-B3	-1.505	-0.989	0.018	-1.148	0.531	0.807
Fi7-B4	-2.101	-0.366	-2.695	-1.513	0.408	1.927

Table 6. 6 Summary table for (Chapter 3; Figures 3.9 and 3.10 of invertebrate community and samples constrained by the summarized environmental variables of PC axes 2 and 4.

Name	RDA1	RDA2	PC1	PC2	PC3	PC4
Gammarus	0.068	0.458	-0.305	1.434	-0.654	0.113
Hyalella	-1.000	0.453	-0.729	-0.077	1.603	0.464
Gyrinidae	-0.205	0.111	-0.264	0.048	0.150	0.003
Haliplidae	0.257	-0.188	0.269	-0.142	0.067	0.150
Collembola	-0.046	-0.227	0.328	-0.071	0.100	0.147
Ceratopogonidae	-0.280	0.274	-0.057	-0.562	-0.077	0.469
Culicidae	0.210	-0.012	0.374	-0.340	0.067	-0.233
Chironomidae	-0.061	0.127	0.428	-0.259	-0.017	-0.328
Baetidae	-0.298	-0.237	-0.477	0.261	0.576	-0.133
Caenis	-1.272	0.194	-2.460	-0.288	-0.623	0.203
Lymnaeidae	-0.666	-0.641	-0.021	0.151	0.059	0.323
Physa	-0.143	0.097	-0.080	-0.115	0.530	0.062
Planorbidae	0.227	-0.396	1.143	-0.019	-0.324	1.137
Succinea	-0.314	-0.266	0.319	-0.310	-0.016	0.131
Belostoma	0.033	0.002	0.036	-0.059	-0.031	0.110
Corixidae	-0.353	-0.343	0.005	-0.104	0.097	0.654
Mesovelia	-0.099	-0.302	-0.199	0.037	0.200	0.291
Microvelia	0.036	0.012	0.124	-0.157	0.040	0.043
Glossophonia	0.147	0.296	0.390	0.132	0.380	0.096
Hydracarina	-0.166	-0.146	0.043	0.075	0.516	0.547
Caecidotea	1.534	0.098	0.433	1.927	0.186	0.060
Lirceus	0.175	-0.054	0.160	0.070	0.227	-0.008
Crambidae	-0.046	-0.064	-0.214	-0.059	0.136	0.091
Aeshnidae	-0.005	-0.039	-0.027	-0.016	0.078	0.035
Ischnura	0.022	0.178	-0.022	-0.243	-0.090	0.396
Coenagrionidae	-0.443	0.067	-1.310	0.579	0.555	0.068
Libellulidae	-0.082	0.208	-0.274	-0.283	-0.124	0.290
Oligochaeta	-0.003	0.139	0.014	-0.181	0.082	-0.337
Hydroptilidae	-0.917	0.039	-0.976	-0.015	-0.284	0.007
Leptoceridae	-0.360	0.251	-0.406	-0.009	0.158	-0.084
Phryganeidae	0.425	0.159	-0.030	0.087	-0.336	1.016
Tricladida	0.280	0.277	0.616	-0.048	0.557	-0.308
SB2-B1	-1.939	1.574	-1.492	-0.373	0.055	1.629
SB2-B2	-1.724	-0.251	-0.666	-1.824	-0.251	2.263
SB2-B3	-2.118	2.367	-0.572	-2.246	0.001	2.365
SB2-B4	-2.184	3.046	-1.372	-0.636	0.079	0.184
SC-A1	0.990	-1.755	1.471	-2.053	-0.782	-0.228

SC-A2	1.398	-1.732	1.247	-0.582	0.381	-0.679
SC-A3	1.087	-0.046	0.280	-0.181	1.016	-1.542
SC-A4	1.735	1.776	0.573	0.901	2.221	0.547
BB-A1	2.212	-0.001	1.042	-0.060	0.487	-0.823
BB-A2	2.093	-0.773	1.243	-0.840	1.063	-1.349
BB-A3	2.127	-0.275	1.673	-0.053	1.943	-0.507
BB-A4	1.965	2.027	1.581	0.534	0.752	-2.125
BdD-B1	-2.001	-5.679	1.937	0.691	0.706	1.173
BdD-B2	-1.490	-1.336	0.866	0.316	-2.082	-0.300
BdD-B3	-0.810	0.345	0.990	0.099	-2.469	-1.252
BdD-B4	-1.194	-0.163	-0.141	-0.358	0.374	0.278
AC-C4	-0.760	1.568	1.167	-0.476	2.283	1.055
AC-C3	0.665	-1.575	0.991	-2.018	-0.700	1.889
AC-C2	0.858	-1.123	0.849	-1.543	-0.317	-2.286
AC-C1	0.908	-3.381	1.462	-2.091	-0.121	-0.861
BR-B1	3.103	-2.798	1.194	1.411	-0.089	2.748
BR-B2	2.670	-1.518	1.067	1.221	-0.768	1.081
BR-B3	2.417	-1.226	0.700	1.124	-0.426	1.227
BR-B4	2.921	0.393	1.242	2.462	-1.149	0.298
OWR-B1	-2.430	-0.522	-1.790	-0.513	1.797	0.265
OWR-B2	-1.989	1.097	-1.681	1.279	1.755	0.040
OWR-B3	-1.681	1.644	-1.703	1.349	1.884	-0.447
OWR-B4	-1.821	1.062	-1.391	0.571	2.331	-1.105
PB-C1	1.468	-0.177	-0.389	1.183	-1.039	0.431
PB-C2	1.210	1.202	-0.874	1.405	-0.359	-0.845
PB-C3	1.747	0.373	0.013	2.095	-0.722	1.052
PB-C4	0.800	0.855	0.287	0.988	-1.754	0.064
SB1-A1	-0.552	0.289	-1.231	-1.046	-1.196	-1.339
SB1-A2	-1.247	0.377	-1.740	-0.822	-1.363	-0.696
SB1-A3	-0.598	0.545	-1.476	0.137	-1.258	-0.598
SB1-A4	-0.101	2.114	-1.789	1.525	-1.456	-1.054
FI7-B1	-1.370	-0.049	-1.533	-1.470	-0.413	-0.804
FI7-B2	-2.017	-1.178	-1.566	-1.200	-0.119	1.111
FI7-B3	-1.899	0.056	-0.453	0.384	-0.481	0.464
FI7-B4	-2.448	2.850	-0.013	0.710	0.184	-1.325

Vita Auctoris

Danielle Gunsch was born in Kitchener Ontario in 1994. She completed her high-school diploma at Huron Heights Secondary School in 2012. Danielle completed her BSc –Biological Sciences at the University of Windsor while competing with the Windsor Lancers Varsity Track and Field Team as a triple-jumper from 2012-2016. Danielle is currently a candidate for a Master's degree in the Biological Sciences program at the University of Windsor and hopes to graduate in October of 2020.