

INFLUENCES OF TAILINGS WATER, SEDIMENTS, MACROPHYTES AND  
DETRITUS ON ZOOBENTHIC COMMUNITY DEVELOPMENT IN  
CONSTRUCTED WETLANDS – RESULTS OF A RECIPROCAL TRANSPLANT  
STUDY

by  
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### **AUTHOR'S DECLARATION OF ORIGINALITY**

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## **Abstract**

Constructed wetlands using oil sand process materials are being used by the oil sands mining corporations to reclaim the post-mining landscape. A reciprocal sediment transplant study was conducted to measure effects of sediment, water, plant cover, detritus mass and year to year variation on zoobenthic richness, density and relative abundance. Density did not change between wetlands, but the oil sand process water-affected wetland had lower richness than the reference wetland. Zoobenthic relative abundance was influenced by water type, macrophyte density and amount of accumulated detritus in sediment. Zoobenthos density was significantly positively associated with amount of plant cover and detritus combined. Sediment did not directly influence zoobenthic abundance or richness. However, its inhibition of plant percent cover caused an indirect effect.

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# Chapter 1

## Overview of research and justification

### Background

The goal of this study is to contrast a constructed wetland with a reference wetland by using sediment reciprocal transplants to assess how water, sediment, plant cover and time influence zoobenthic abundance, richness and community composition. Benthic macroinvertebrate assemblages are a useful tool for examining characteristics of water and sediment quality such as salinity and contamination in various wetland habitats. Benthic invertebrates may serve as indicators of sediment quality because they are continually exposed to contaminants (Reynoldson 1987). The toxicity of sediment contaminants can also be measured directly via the invertebrate community condition (Kiffney and Clements 1994, Richardson and Kiffney 2000, Ciborowski et al. 1995). This study will contribute to the understanding of how constructed wetlands on reclaimed areas of the oilsands leases differ from naturally occurring wetlands in the area. Some benthic invertebrate taxa can accommodate conditions to which others are intolerant; these taxa can be expected to persist to the exclusion of others, when those conditions arise. For example, Aladin (1991) documented the decline in cladoceran species from 14 to 4 as the levels of salinity rose in their lake due to rerouted waterways.

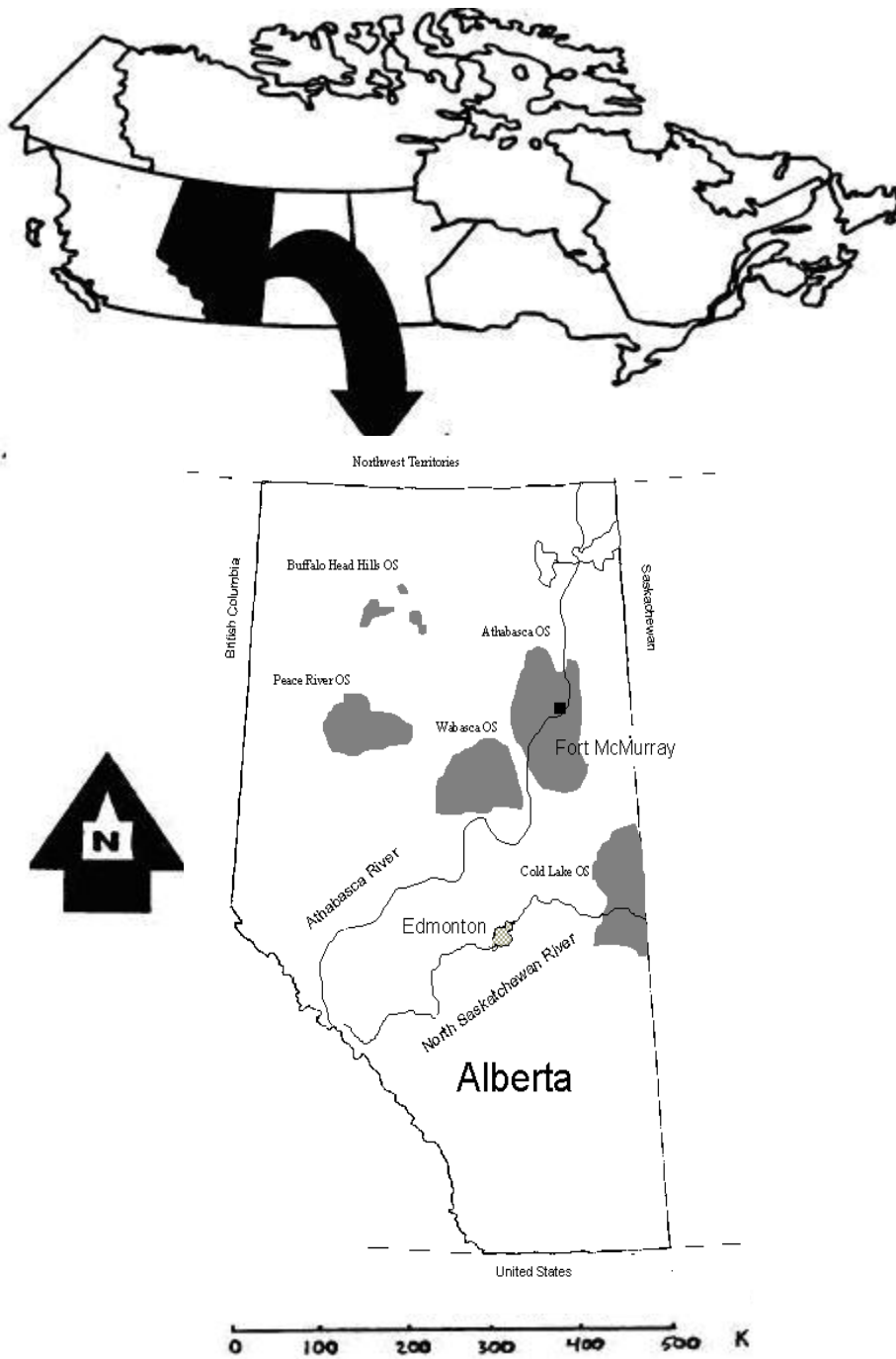
Wetlands are distinctive ecosystems, intermediate in characteristics between terrestrial and deeper aquatic habitats. A wetland is any land saturated with water long enough to promote wetland or aquatic processes indicated by poorly drained soils, hydrophytic vegetation, and various kinds of biological activity that are adapted to a wet environment (National Wetlands Working Group 1997, Leonhardt 2003). Changes in water chemistry can modify the habitat and influence the environment's capacity to support organisms found there. Overall, numbers of aquatic macroinvertebrates found in wetlands have been shown to correlate with pH levels (Friday 1987). Saline wetlands can be relatively high in biological production compared to similar freshwater wetlands (Batzer et al. 1999). These wetlands generally have lower richness of taxa but higher densities of taxa found (Whelly 1999). Differences in water chemistry, including

increased salinity, between reference/opportunistic wetlands and reclaimed experimental oilsand wetlands are expected to influence macroinvertebrate numbers found.

A close relationship exists between macrophyte and macroinvertebrate assemblages in wetlands. Macrophytes provide shelter and a substrate upon which macroinvertebrates can graze (Keast 1984, Balci and Kennedy 2000). The occurrence of macrophyte assemblages also enhances the quality of a wetland for consumers of macroinvertebrates by providing substrate for the growth of periphytic algae, a food source for many herbivorous invertebrates (Olson 1995, Dvorak and Best 1982). The study of colonization in newly created aquatic habitats increases understanding of the pattern and rates of macroinvertebrate assemblage development. Benoit et al. (1998) showed that macroinvertebrates can colonize new benthic habitats rapidly. They observed their artificial substrates colonized to half saturation within a mean of only 4 days. Invertebrates colonize new lakes and ponds at rates that reflect both their ability to disperse to new wetlands (Whelly 1999) and to persist under the prevailing conditions. Sediment characteristics such as particle size or simply the presence of aquatic macrophytes can facilitate or impede the establishment of various taxa in new wetland habitats.

### **Athabasca Oil Sands Mining**

In the Fort McMurray area of Alberta (Fig. 1), 21% of Alberta's provincial surface area is categorized as wetland. More than 90% of these wetlands are peatlands in the northern boreal forests of Alberta (Oil Sands Wetlands Working Group 2000). Here, open pit mining for oil sands has been taking place since the 1960s and is anticipated to affect an area of  $1.4 \times 10^3 \text{ km}^2$  by 2023 (Alberta Environmental Protection 1998). Open pit mining entails the removal of topsoil and mineral overburden followed by extraction of resource-bearing layer beneath. The topsoil can immediately be used in the reclamation of a mined site or it can be mixed with overburden and cached for later use (Foote and Cooper 2000). The terms of reference of the mining leases of Suncor Energy Inc. (Suncor) and Syncrude Canada Ltd. (Syncrude) require these companies to restore mined land to a condition of equivalent production capability of the land prior to disturbance.



**Fig 1.** Map of Province of Alberta depicting location of oil sands deposits.



The end land-use must not interrupt the continuity of the neighbouring landscape (Alberta Environment 1999).

Open pit mining removes the native surface ecosystem, and effective strategies for aquatic and terrestrial surface reclamation are being developed to return the land to productive levels post mining (FTFC 1995). Post-mining primary succession and assemblage development would occur more slowly without remediation efforts. Ecologists can learn what conditions will accelerate assemblage development by performing controlled experiments at small scales. The methodology for reclaiming terrestrial habitat has been relatively well developed (FTFC 1995). However, the reclamation of wetland areas poses different problems. Harris (2007) reports that reclamation of wetlands in the oil sands region differs from many of the situations documented in reclamation handbooks and published literature, in that it must be conducted in the context of larger-scale reclamation of whole landscapes or watersheds (Daly 2008).

### **Oil Sand Process Material (OSPM)**

Bitumen is extracted from oil sands using the Clark Hot Water Extraction Process (FTFC, 1995), which generates both tailings and wastewater that contain high concentrations of total dissolved solids, chlorides, sulfur, trace metals, polychlorinated aromatic hydrocarbons (PAHs) and naphthenic acids. Both tailings and mine process wastewater are slightly to moderately saline. The remaining oil sand process materials (OSPM) consist of oil sands process water (OSPW) and a slurry of sand, clays, gypsum and residual unextracted bitumen. The coarse sands quickly settle out of the slurry. The remaining mixture of clay and water is known as 'soft tails'. Oil sands process materials have elevated salt ion concentrations, and concentrations of soluble hydrocarbon compounds such as naphthenic acids and PAHs (polycyclic aromatic hydrocarbons) that are initially toxic (FTFC 1995, Matthews et al. 2002). The high water content of raw soft tails (80% or more) also poses reclamation difficulties because the clay particles settle very slowly. This material is referred to as 'mature fine tailings' (MFT). The addition of gypsum consolidates the clay particles in MFT tailings and hastens their settling out (Matthews et al 2002), producing a sediment variously referred to as 'consolidated

tailings' or 'composite tailings' (CT). When gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) is added to mature fine tails, calcium ions cause the particles to agglutinate into stronger floc structures that will dewater relatively rapidly with an applied stress. In this form, the tailings can be used more readily for load bearing surfaces in terrestrial applications (FTFC, 1995; Mathews et al. 2002). The use of CT in aquatic applications is a relatively new area of research, but because of its physical properties, it is expected to speed the rate of successional processes because it settles much more quickly than MFT.

Oil sands mining by-products are both plentiful and potentially toxic to organisms. In 2002 there were approximately 360 million cubic meters of tailings in holding areas on oilsand leases (Mathews et al. 2002). Both fresh tailings and fresh oil sands process water are toxic to vertebrates, including fish, amphibians and birds (FTFC 1995) in experimental wetlands. However, toxicity declines as wetlands age (FTFC 1995). My research evaluates the relative effects of oil sands process water and sediments on macroinvertebrate assemblage development in constructed wetlands in conjunction with indirect effects from aquatic plant cover. It will increase the knowledge base upon which reclamation decisions are made and will guide strategies that will permit accelerated assemblage development.

### **Sediment Types**

In 1999, Suncor Energy Inc. built a network of interconnected demonstration wetlands designed for treating wastewater released from their tailings ponds (Golder Associates Ltd. 2000; Daly and Ciborowski 2008). Constructed wetlands were built on a layer of composite/consolidated tailings (CT) and a layer of mature fine tailings (MFT). In this study, Suncor tailings were used.

### **Current Research**

At least four factors may be responsible for the marked differences in benthic macroinvertebrate assemblage composition between newly constructed wetlands and reference natural wetlands (Leonhardt 2003). These potential factors are altered water chemistry, altered sediment chemistry, quantity of organic matter, and age of wetland (Ciborowski and Liber 2002). A parallel research project is investigating the role of

organic matter as a factor in constructed wetlands (C. Wytrykush, Univ. of Windsor, in prep). Leonhardt (2003) investigated how macroinvertebrate assemblage composition varied as a function of age in 34 wetlands on oil sand leases. She found that zoobenthic richness was lower in OSPM-affected wetlands whereas overall density was not significantly lower than similar-aged reference wetlands. My research contrasts the differential effects of CT use as sediment, and oil sands process water in constructed wetlands on benthic macroinvertebrate assemblages by using a reciprocal transplant design between a natural wetland, a reference wetland (one that has formed from surface water collecting in a depression in the post-mining landscape), and a constructed experimental wetland. Differences in the density, richness and composition of benthic macroinvertebrate taxa are examined with respect to the water effects, the sediment effects, and the influence of macrophyte cover as well as variation between two consecutive years.

### **Thesis Overview**

In chapter 2, I examine the effects of four indicator variables on the benthic macroinvertebrate assemblages found in exchanged plots of a reference wetland (SW) and an experimental constructed wetland (4-m CT). The effects of oilsand process water, consolidated tailings sediment, plant percent cover and sample year are investigated using a reciprocal sediment transplant experiment. The data are analysed and interpreted with the aid of various multivariate statistical approaches, including principal components analysis (PCA), multiple regression, and structured equation modelling (SEM). Chapter 3 is a general summary discussion.

## **Chapter 2**

### **Zoobenthos assemblage diversity, density and relative abundance in OSPM constructed wetlands**

#### **Introduction**

The littoral zone of lentic habitats is the shallow region in which light can penetrate through the water column to reach the sediment. It is typically occupied by macrophytes - rooted vascular plants - and macroalgae. It generally supports a varied assemblage of aquatic invertebrates. Microhabitats include benthic and plant surfaces, the water column, and the surface film.

The purpose of this chapter is to investigate the effects of the sediment characteristics, water type and macrophyte cover in the littoral zone of a wetland constructed with composite tailings sediment, on benthic invertebrate abundance and community composition. The water of OSPM-affected wetlands has elevated salinity relative to reference wetlands in the region because ions such as sodium, sulphates and chlorides are concentrated during oil sand processing, and higher pH values, predominantly due to higher carbonate and bicarbonate ion concentrations (FTFC 1995, Ganshorn 2002).

Zoobenthic community level responses to sediment unsuitability may include a reduction in the number of organisms present, reduced taxonomic richness, the elimination of intolerant taxa or a change in the relative abundance of dominant taxa (Ciborowski et al. 1995).

Zoobenthos use macrophytes as substrates and/or may graze periphyton from their surface. Macrophytes generally have benthic invertebrate populations that include various functional feeding groups, including filter feeders such as *Rheotanytarsus* midges, which use mucous strands to trap food particles, periphyton grazers such as *Cricotopus* species, which shear material from the surface of submersed objects, and predators, such as members of the Tanypodinae, which pierce and engulf their prey (Armitage et al. 1997). Members of many benthic orders also use macrophytes as oviposition sites. Taxa like oligochaetes and deposit-feeding chironomids burrow in the surface sediment layer. Organic sediments are typical habitats for hunting odonates and some collector-filterer

chironomids. Surface dwelling taxa like gerrids and gyrids are regularly seen on the water's surface.

New reference wetlands in Athabasca oil sands lease areas form opportunistically in depressions of the reclaimed landscape (Harris 2007). Such areas initially have sodic inorganic sediments of sand or clays. As plants colonize the land-water interface, a layer of organic detrital material is built up. This facilitates development of an emergent zone, dominated by cattails, bulrushes and sedges. Submergent vegetation, largely *Chara*, or *Potamogeton* spp. develops at depths of 30- 50 cm. In contrast, OSPM affected wetlands are built with sediments of mature fine tailings (MFT) or consolidated tailings (CT), which have high clay content. The associated elevated concentrations of dissolved compounds such as ammonia or sulphate tend to bind phosphorus and other nutrients, which impedes plant establishment (FTFC 1995).

Land reclamation utilizing constructed wetlands post mining will undoubtedly produce wetlands that will be different from reference wetlands endemic to the region. The water and sediment and their effects on plants will likely produce wetlands with differing suitabilities to different aquatic invertebrates. The salinity of oil sands process water will prohibit species ill-equipped to manage their osmotic pressures. Given time, more acutely toxic organic compounds such as naphthenic acids, which are initially present in toxic concentrations, will decrease to negligible levels over a period of a few years (FTFC 1995). The fine nature of the clay sediment will affect the suitability of the wetlands to the sediment-dwelling fauna like oligochaetes that would typically be found in coarser, sandy sediment types. Fine clay is also expected to affect various plant species colonizing the wetlands and hence the associated epiphytic community. To investigate each of the effects of oil sands process water (OSPW), consolidated tailings (CT), and the subsequently developing plant cover, a reciprocal sediment transplant between a reference wetland and a constructed wetland was designed (Foote and Cooper 2000, Cooper 2004). Sediment transplants separated the effects of OSPW from effects of oil sands affected sediments.

The taxa richness, the overall density of invertebrates, and the community composition (relative abundances) of taxa were assessed. Constructed wetlands are expected to initially support high numbers of the relatively few taxa tolerant of the water

chemistry of a newly created wetland. Reference plots in the reference wetlands should have the greatest biodiversity and richness of taxa.

### **Wetland Descriptions**

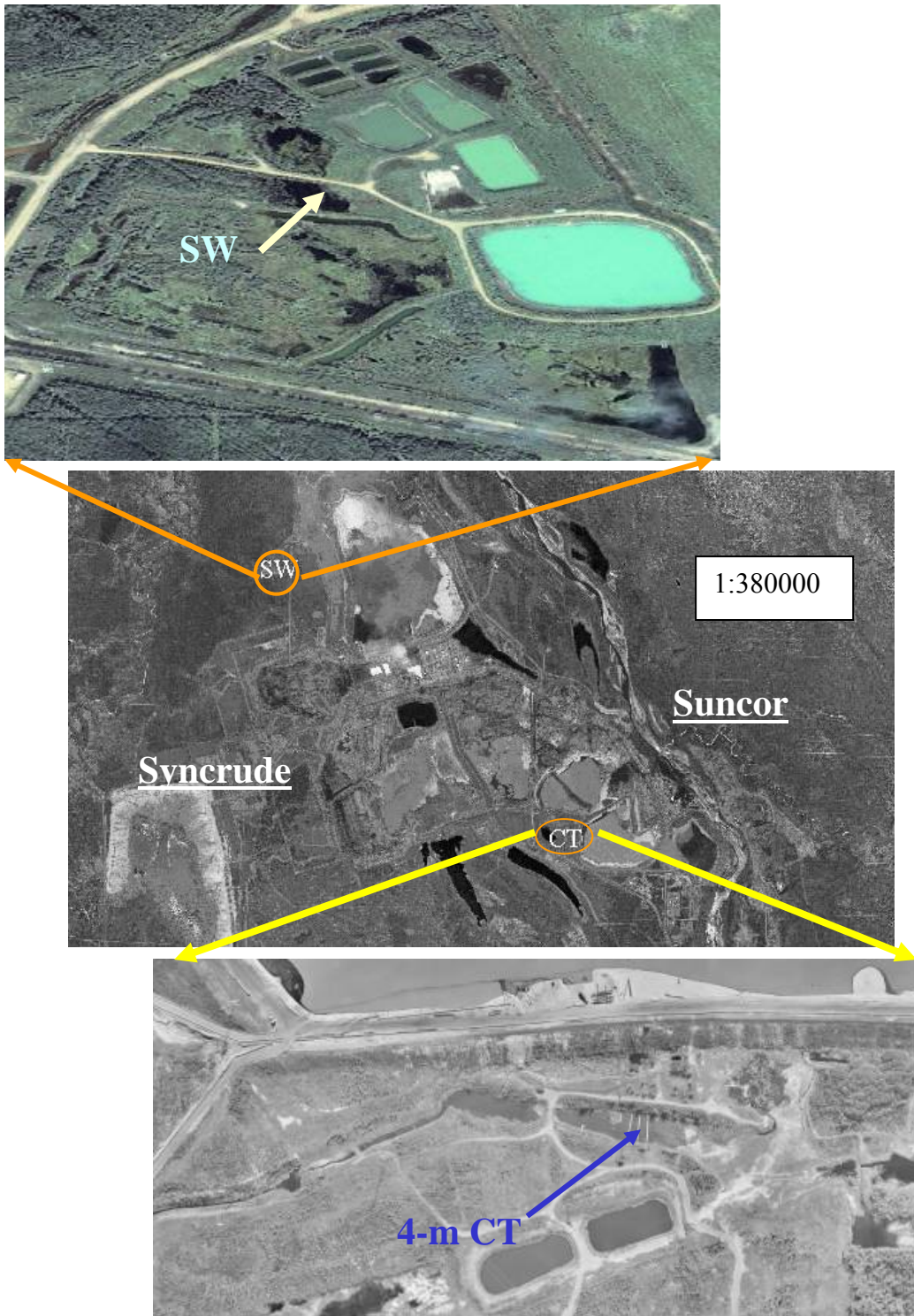
The reciprocal transplant study was originally designed as an exchange of sediments among three wetlands - a 'mature' wetland created by beaver activity (McLean Creek), a reference wetland constructed in 1992 (Shallow Wetland), and an oil sands process material affected wetland built in 2000 (4-m CT Wetland) (Foote and Cooper 2000, Cooper 2004).

#### **Shallow Wetland (SW)**

The Syncrude Shallow Wetland (SW) (57° 04.899'N 111° 41.427'W) is a constructed reference wetland in the test pond area west of the Northwest Interceptor Ditch appearing in 1993 on Syncrude's lease (Fig. 2). The wetland was initially filled with surface water from the nearby West Interceptor Ditch. Thereafter, water levels were maintained by snowmelt and precipitation. The substrate consists of tailings sand and sodic overburden. No additional amendments such as peat were applied.

#### **4-m Consolidated Tailings Demo Pond**

The 4-m CT Demo Pond (4-m CT) (56° 59.534'N 111° 31.914'W) was part of Suncor's network of interconnected demonstration wetlands designed for wastewater treatment. It was constructed in 1999 with a substrate consisting of 4-m depth of CT sediment (Daly and Ciborowski 2008). In some locations, the CT was covered with a 30-cm thick layer of 'muskeg' – the organic surface soil layer that overlies the mineral soil horizon. Muskeg is removed during the land-clearing phase and stored for later use in reclamation of the postmining landscape. The CT Research Wetland Complex receives a slow influx of process water, which is pumped from adjacent tailings ponds at a rate of 75 L per minute (Daly and Ciborowski 2008).



**Fig 2.** Aerial photo showing locations of the 4-m CT (CT) and Shallow Wetland (SW) wetlands.

## McLean Creek Wetland (McL)

The McLean Creek Wetland Complex (McL) (56° 53.275'N 111° 20.816W) was formed by beaver activity. It is located south of the Millennium mine on Suncor's lease area and is approx 30 years old. Unfortunately for this experiment, it became dry during the 2003 field season due to the failure of a beaver dam as a consequence of spring flooding. The complex subsequently became a terrestrial meadow. Zoobenthic samples related to McLean Creek wetland are not discussed further in this thesis.

## **Purpose**

This study had 4 objectives consisting of determining the influence of OSPW, the influence of CT, the difference from year to year, and the influence of the plant cover and detritus in the plots on zoobenthic characteristics.

### Objective 1: OSPW

To investigate the influence of OSPW on benthic macroinvertebrate density, richness, and relative composition.

### Postulate

If OSPW (Oil Sand Process Water) chemistry has an overall adverse effect on benthic macroinvertebrate assemblage condition, then aliquots of reference wetland sediment (collected from Syncrude Shallow Wetland) placed in the CT demo pond, will support fewer invertebrate taxa than equivalent aliquots of reference wetland sediment removed and replaced the reference wetland. If process water chemistry does not adversely affect zoobenthos, then benthic samples in the 4-m CT Pond containing reference wetland sediments will not have statistically significantly fewer animals or fewer types of invertebrates than samples comprised of reference wetland sediment in the reference wetlands.

### Assumptions

Sediment treatments in plots will not be affected by neighbouring sediment chemistry. All species of benthic macroinvertebrates have equal chances of colonizing



plots of both CT sediment and reference sediment. Benthic macroinvertebrate assemblage condition is only affected by the differences in water chemistry.

#### Expectations

Different taxa will be found in different abundances in each type of plot. The greater salt concentrations in 4-m CT water may result in higher overall zoobenthic density but fewer taxa for samples taken from the 4-m CT wetland than from Shallow Wetland (Whelly 1999).

#### Objective 2: Consolidated Tailings (CT sediment)

To investigate the influence of consolidated tailings sediment (CT) on benthic macroinvertebrate abundance, richness and relative composition.

#### Postulate

If CT sediments adversely affect suitability to the benthic macroinvertebrate assemblage condition, then plots containing a substrate of CT sediment will have statistically significantly fewer types and numbers of organisms than samples in the same wetland containing substrate of reference wetland sediment. If sediment type does not lower suitability for benthic macroinvertebrate assemblages, then plots comprised of CT sediment will not have different numbers and kinds of invertebrates than plots in the same wetland comprised of natural wetland sediment.

#### Assumptions

Sediment treatments in plots will not be affected by neighbouring sediment chemistry. All species of benthic macroinvertebrates have equal chances of colonizing plots of CT sediment and reference sediment. Benthic macroinvertebrate assemblage condition is only affected by the differences in sediment chemistry.

#### Expectations

Through time, the toxic compounds originally present in the consolidated tailings (ammonia, naphthenic acids, residual hydrocarbons) will be reduced to non-toxic levels

in areas where benthic invertebrates will be sampled, and consequently sediment toxicity will not have adverse effects on benthic macroinvertebrate assemblage condition. Physical characteristics of the CT mineral sediment (fine particle size and lack of organic content) may adversely affect sediment-dwelling invertebrates and macrophyte development. This may also indirectly and adversely affect the benthic community associated with plant cover. The differences, if any, between plots with CT sediment and those with natural wetland sediment will be related to macrophyte development, which may be influenced by CT, rather than by the direct effects of CT on zoobenthos.

#### Objective 3: Year

To assess whether there are any year to year differences of taxa density and diversity.

#### Postulate

Increases or decreases in overall number and number of taxa through time will be investigated by using sampling dates. If there is increased diversity in the 4-m CT wetland sediments and benthos as time progresses, then it will imply an increased suitability for invertebrates.

#### Assumptions

Sediment treatments in plots will not be affected by neighbouring sediment chemistry. All species of benthic macroinvertebrates have equal chances of colonizing plots of CT sediment and reference sediment. Benthic macroinvertebrate assemblage condition is only affected by the differences in water chemistry.

#### Expectations

The abundance and diversity of benthic invertebrates will increase as the constructed wetland ages, possibly due to decreases in toxicity of oilsands-associated compounds and/or increases in macrophyte coverage through time. If there is no change in SW but an increase in CT, then this would produce a time x wetland interaction.

#### Objective 4: Percent Cover and Detritus mass

To investigate the influence of macrophytes (expressed as percent cover developing in experimental plots) on the density, richness and relative abundance of zoobenthos.

#### Postulate

Within any of the study wetlands, diversity and abundance of benthic invertebrates collected should be directly correlated with the amount of plant cover and detritus mass found within plots.

#### Assumptions

Sediment treatments in plots will not be affected by neighbouring sediment chemistry. All species of benthic macroinvertebrates have equal chances of colonizing plots of CT sediment and reference sediment.

#### Expectations

The abundance and diversity of benthic invertebrates will increase as the amount of plant percent cover increases and as the amount of detritus mass increases, possibly due to increased surface area for biofilm for nutrition and physical hiding spaces for diversity of taxa.

#### Study Sites

This study builds upon a reciprocal transplant design created by Foote and Cooper (2000) to investigate the effect of sediments transferred among wetlands on macrophyte assemblage development (Cooper 2004). Experimental sites were established in three wetlands on oil sand lease areas in northern Alberta. Two wetlands were located on the Suncor Energy Inc. lease area and one was located on the Syncrude Canada Ltd. Lease area (Fig. 2).

## Methods

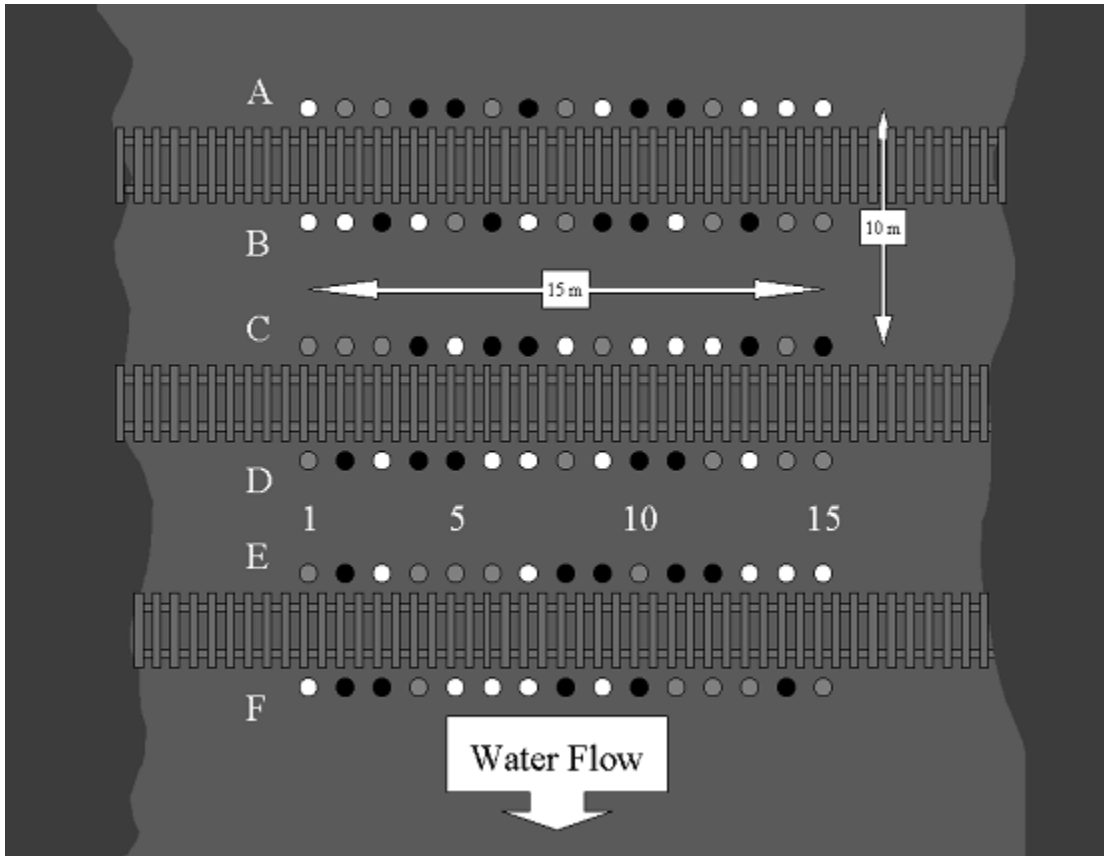
*Terminology* Plot: one transplant location (A1:F15) Ninety plot locations per wetland  
Transect: one boardwalk (AB, CD, EF). Three transects per wetland  
Block: Each side of boardwalk (A:F). Six blocks per wetland  
Replicate: Five sediment transplant replicates per block  
Sample: a collection of benthic macroinvertebrates and detritus taken from a plot with coring tube or a dip-net

### Experimental Design

#### Field Sampling Methods:

Plot Design: Study areas were laid out in each wetland in June 2002 by N. Cooper, University of Alberta as 3 blocked pairs of 15-m long transects, one block on each side of three wooden boardwalks (Fig. 3). Boardwalks were constructed, as necessary, into each wetland to permit safe access. Each transect had room for 15 potential sample unit sites spaced 1-m apart (Fig. 4). The blocked pairs of transects were spaced at 10-m intervals across the wetlands. Two hundred and ten sampling units were laid out in total (2 reference wetlands, each with 10 sample units per transect, 2 transects per block, and 3 blocks = 120; one constructed wetland, with 15 sample units per transect, 2 transects per block, and 3 blocks = 90). Reference wetlands each contained 60 sample units opposed to the 90 in the experimental wetland because reference wetlands were not reciprocally transplanted between each other. Ten sample unit sites were randomly selected from among the units available along the 15-m long transects in the reference wetlands.

Sampling Units: Sample units consisted of 10-L, sediment-filled buckets (30 cm in diameter x 30 cm deep), which kept the experimental sediments from washing into other plots or out into the wetlands. A series of 10-mm diameter holes had been drilled into the bottom of each bucket to permit ion, nutrient, and water exchange between the



**Fig. 3** Experimental plot layout of the CT Wetland.

sediment inside and outside the buckets. Four columns of holes were drilled through the sides and one hole was drilled through the bottom. Fine mesh fabric (commercial landscape material) was secured around the outside of the buckets to minimize loss of sediment through the holes. Each bucket was dug into the sediment so that the lip was flush with the sediment surface. Water depth ranged from approximately 30 cm to 50 cm at the time of placement. Buckets were filled with 10cm of native substrate (CT at 4-m CT, SW sediment at SW, and McLean Creek sediment at McL). Enough donor soil was then added, unmixed, to fill the remaining 20 cm of bucket depth (Fig. 5).

#### Zoobenthic Sampling:

Invertebrates were sampled twice annually over 3 summers (2002-2004). Sampling occurred in late spring (June) and at the end of the summer (August). Samples sorted were from August 2002 and August 2003. Two types of samples were collected to ensure representative assessment of the fauna in each bucket. Sweep sampling with a small fine mesh brine dipnet was used to collect relatively large and rarer epibenthic, epiphytic, and pelagic macroinvertebrates that would otherwise not be sampled adequately by coring (Leonhardt 2003). Coring tubes were used to collect organisms living on and in the sediment. Samples were preserved in the field and sorted, enumerated, and identified in the laboratory.

#### Sweep samples:

Sweep samples were collected using a 10 x 8- cm brine shrimp dip-net. Mesh size was approximately 0.25 mm. Prior to sweep sampling a plot, a 20-L bucket, with the bottom removed, was fitted around the inner rim of the sample bucket to isolate the water column above the sampling area from that outside the plot. The sediment surface layer and water were then swept for 30 transits of the bucket. Care was taken to gently agitate any macrophytes within the buckets to dislodge invertebrates without damaging the plants. The sample contents were emptied by rinsing the inverted dip-net in a shallow pan partly filled with wetland water using the water tension to remove the solid material from the net. The sample was then poured through a 0.25-mm mesh sieve bucket or sieve bag, and the material retained was preserved in a labelled plastic bag containing

approximately 250 mL of formalin-ethanol solution (5:2:7 v/v/v 95% ethanol : 100% formalin : water).

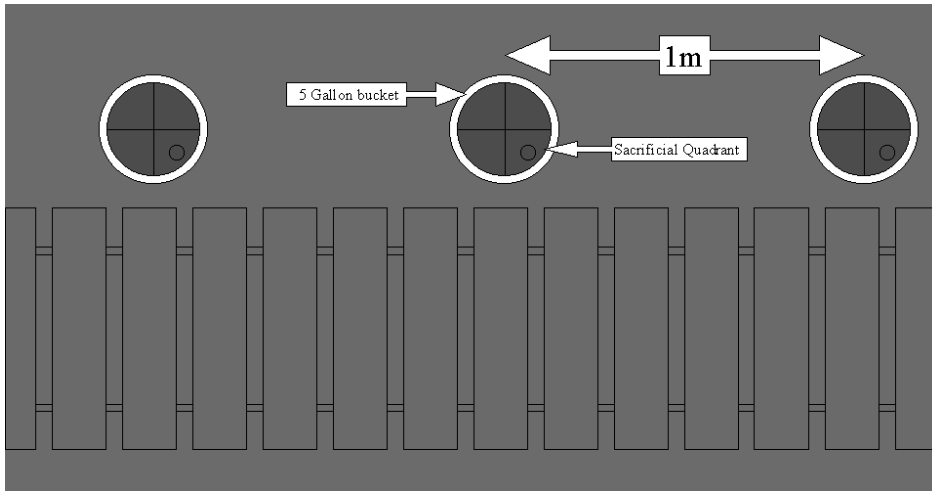
#### Core samples:

On each sampling date, a five-cm diameter x 15-cm deep sediment core was taken from one ('sacrificial') quadrant of each bucket (Fig. 4). The coring device was a polyvinyl chloride (PVC) tube twisted into the substrate until 15 cm of the coring tube was inserted (approximately 20 cm<sup>2</sup> surface area; 295 cm<sup>3</sup> sediment volume). A rubber stopper was then placed into the top of the coring tube, and the tube and enclosed sediment were removed by hand. The removed sediment and overlying water were emptied into a 0.25-mm mesh bag and washed to remove fine materials. The sample was subsequently transferred into a labelled plastic bag and preserved with approximately 250 mL of formal-ethanol solution.

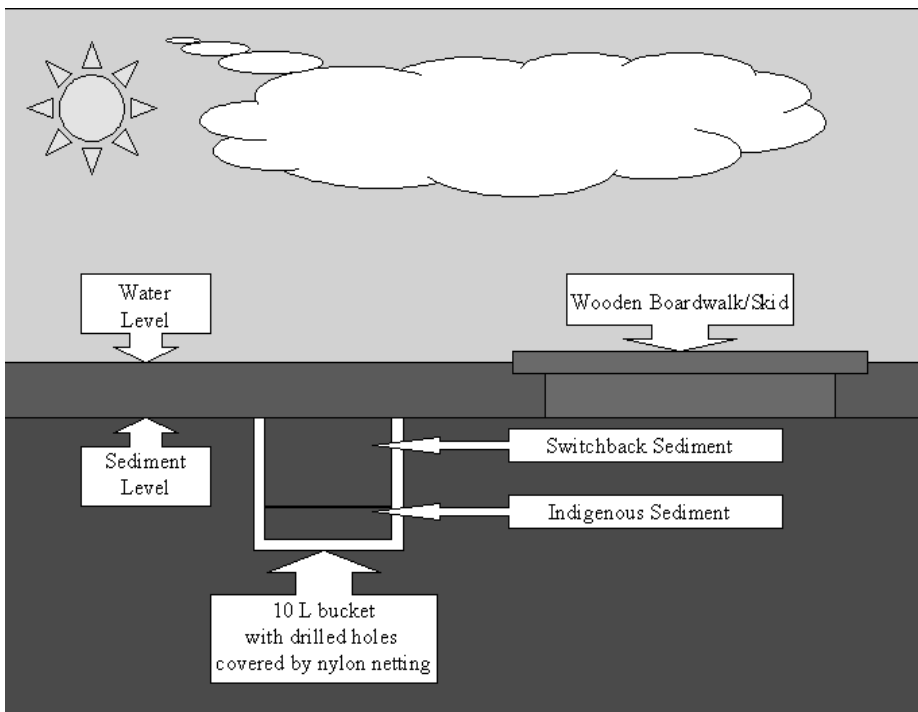
Macrophyte cover values were acquired from Natalie Cooper (Univ. Alberta) taken during sampling dates in June and August. Water chemistry was determined at each wetland by taking 3-5 measurements during sampling in August 2003 of salinity, conductivity and temperature at 3-5 locations with a YSI Model 33 multi-parameter meter. pH was measured with an Orion QuiKcheck model 106 pocket meter.

#### Laboratory Methods

Early on in the processing stage it was decided that only a subset of samples could be analysed due to time constraints in sorting. Approximately 15 core samples and 15 sweep samples of each combination of sediment and water type were randomly selected and sorted. In addition, groups of 20 sweep samples were chosen at random and their chironomids specimens were all mounted and identified to genus. This was done for samples taken from the SW and the 4-m CT in both 2002 and 2003. In all, a total of 60 core samples and 80 sweep samples were sorted and enumerated.



**Fig. 4** Overhead view of sacrificial quadrant location and spatial distances between sample buckets.



**Fig. 5** Cross-section diagram showing 10 L bucket with sediment orientation.



### Sample Processing:

Samples were processed in the laboratory following the methods of Ciborowski (1991) and Leonhardt (2003). Organic materials were separated into size fractions by rinsing the sample material through a nested series of brass sieves with mesh sizes of 4-mm, 1-mm, 0.5-mm and 0.25-mm to facilitate sorting. The preservative was rinsed out of the sample material prior to separation in a 180- $\mu$ m sieve. Samples were rinsed until a generally consistent and uniform particle size fraction was obtained in each sieve. A sieve fraction was emptied into an enamelled tray flooded with water and stirred to separate clumps of debris, and then the lighter, organic materials were poured back into the sieve, leaving behind the denser, inorganic material. When large amounts of organic material were found in a size fraction, that fraction was further separated into less dense materials (plus invertebrates) and denser materials using Ludox<sup>®</sup> (Dupont) solution, a colloidal silica polymer with a specific gravity of 1.15 g/cm<sup>3</sup> (Leonhardt 2003).

Each size fraction of organic material was examined in grid-marked petri dishes beneath a dissecting microscope. The material was repeatedly scanned until no additional invertebrates could be found. The 4 and 1-mm size fractions were entirely sorted. One-quarter subsamples of the 0.50-mm and 0.25-mm size fractions were sorted, if they contained large amounts of organic material or animals. Detritus was dried for at least 48 h and weighed.

### Identification of Zoobenthos

The macroinvertebrates were enumerated and identified to the lowest practicable level using keys of Clifford (1991), Merritt and Cummins (1996) and Oliver and Roussell (1983). All taxa were identified at least to family level. Most families in these wetlands are represented by a single genus (Leonhardt 2003).

Chironomidae from samples collected in 2002 and 2003 were identified to genus using the keys of Oliver and Roussell (1983) and Ferrington and Coffman (1996). Organisms identified were preserved in ethanol and archived in the University of Windsor reference collection.

Chironomidae were slide-mounted for taxonomic identification to the genus level (Epler 1999) using CMC-9AF aqueous mounting medium (Master's Chemical Company,

Des Plaines, Illinois). Chironomid larvae of similar size were mounted on the same slide with up to 10 larvae/slide. A glass cover slip was positioned over the larvae and gently compressed to expand the mouthparts. After 24-48 h, excess CMC-9AF was trimmed from the slide and the coverslip was ringed and sealed with opaque nail polish to prevent evaporation of the mounting medium. The slide was set aside to clear for at least 72 h. Chironomids were examined beneath a compound light microscope at 100x – 400x magnification.

### Statistical Analyses

All summary data, regressions and principal components analyses were performed using Statistica<sup>®</sup> software release 6.0 (Statsoft Inc., 2001). AMOS<sup>®</sup> software release version 17 (SPSS 2009) was used to estimate the structural equation model.

Invertebrates collected by each sampling method (core and sweep net) were enumerated separately. Data for each sample were recorded in raw form (count tabulated per sieve size fraction per sample, corrected for subsampling where applicable). These values were then summed to yield the total numbers per sample. These values were then converted to densities (No./m<sup>2</sup>) prior to further analysis (Appendix 9).

### Measures of Invertebrate Community Condition

Three measures of the invertebrate community were analysed.

Richness: Number of taxa per sample.

Overall Density: Total number of invertebrates per sample divided by the surface area of the sample.

Community Composition: Relative abundance of each taxon was octave-transformed ( $\text{Log}_2(\text{percent}+0.125)$ ) (Gauch et al. 1984). A constant (3.0) was added so that all values would be positive. A few dominant species control the results of multivariate analyses because the biological processes controlling abundance of species are exponential in nature (Gauch 1984). Consequently, logarithmic transformation of taxonomic data gives more weight to rarer taxa (Gauch 1984).

Rarely collected taxa were excluded from multivariate analyses of community composition. To be included, a taxon had to occur in at least 5% of the samples and comprise at least 2% of invertebrate count within any one wetland. We operationally termed the taxa retained for further analyses as “common” (i.e, commonly encountered) to distinguish them from the excluded taxa.

### Multivariate Summary of Zoobenthic Community Composition

Principal components analysis (PCA) performed on the correlation matrix of zoobenthic relative abundances using Varimax rotation identified taxonomic principal components with eigenvalues greater than 1.00. Principal components analysis expresses multivariate data as a smaller number of statistically independent, normally distributed indices (principal components). The original variables are each correlated with the principal components to a greater or lesser extent. Suites of intercorrelated variables can thus be expressed in terms of the principal component with which they are most highly correlated. When applied to the relative abundances of aquatic invertebrates in individual samples, the PCA thus identifies ‘assemblages’ of co-occurring taxa, each independent of all others. Typically, a relatively small number of statistically independent principal components can account for a large proportion of the among-sample variation in the original variables. Accordingly, the principal component scores for a sample can serve as surrogate dependent variables for the original univariate data. Because the scores are normally distributed and statistically independent, the principal components meet the assumptions required for parametric statistical tests.

Eighty samples were included in each of the two principal components analyses (one analysis for core samples; one for sweep samples - 20 from each treatment in each of SW and 4-m CT). The principal component scores for each sample were then used as the dependent variable in analyses to evaluate the effects of sediment type, water type, and environmental covariates on community composition.

Multiple linear regression was used to determine the effect of detritus (g dry mass per core sample or sweep sample), macrophyte cover (percent), water type (SW or 4-m CT), sediment source (SW or 4-m CT), year of sampling (2002 vs. 2003) and their interactions on each principal component grouping of taxa. In each of several analyses,

the dependent variable was the principal component score representing relative abundance of an assemblage of aquatic invertebrates. One-tailed tests of significance were applied to tests of the slopes because specific expectations were defined *a priori*.

### Contrasts and Expectations

Samples from plots at which SW (reference) sediment were transferred into the 4-m CT wetland were compared to samples from plots of SW sediment in the SW. If the samples in 4-m CT wetland have statistically significantly lower abundance and richness, then oil sands process water (OSPW) will be judged to have negative effects on benthic macroinvertebrate abundance and richness, independently of any negative effect of oil sands mine-derived sediments (CT).

The effect of water type (a categorical variable with two classes – ‘Reference’ and ‘OSPW’) on principal component was tested using multiple linear regression. Plant % cover and detritus mass were included as additional covariate independent variables to assess their effect on invertebrate taxa.

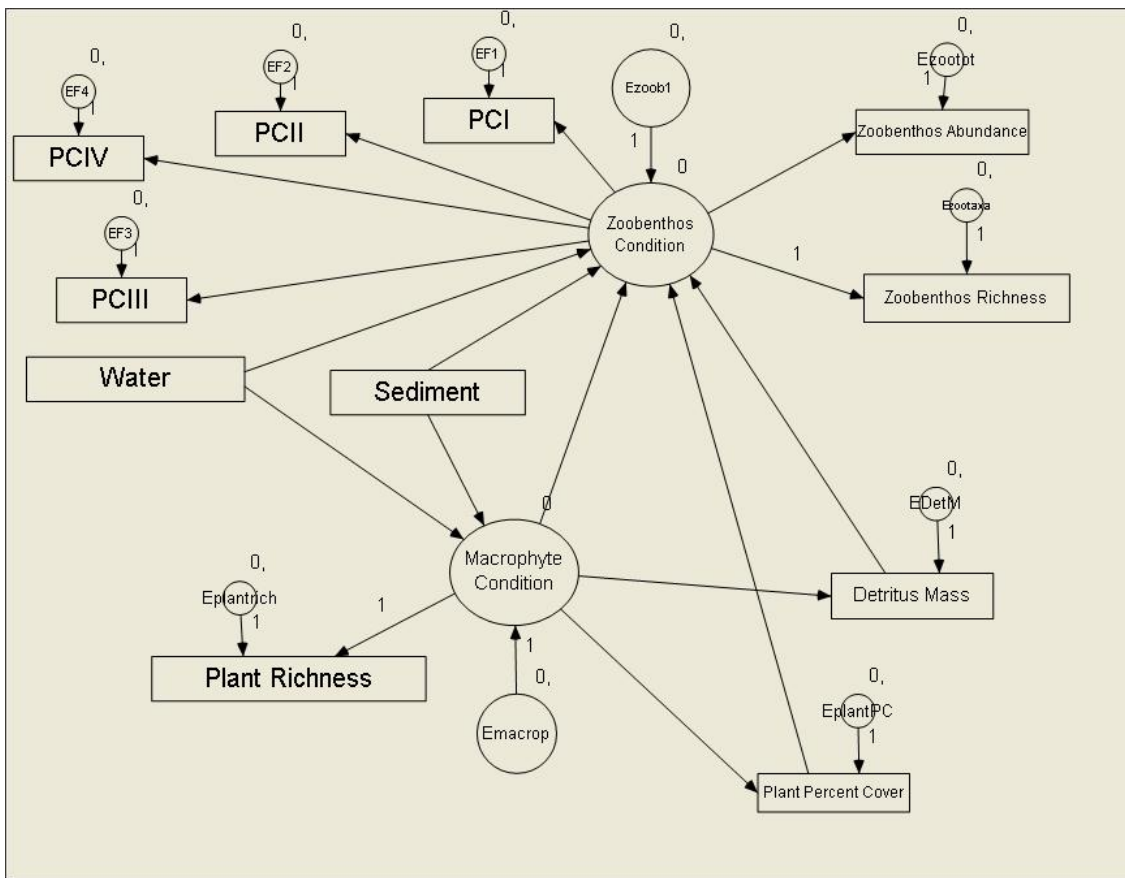
CT sediment was taken from the 4-m CT and placed into sample sites in SW (reference). If benthic invertebrate samples collected from CT sediments placed in SW have statistically significantly fewer individuals and lower richness than samples collected from reference sediment plots in SW, then CT sediment will be judged to be more unsuitable than natural wetland sediment for benthic macroinvertebrates. Multiple linear regression was used to relate the PC scores to sediment type, plant cover, detritus mass and their interactions as outlined above.

### Structural Equation Modelling (SEM)

Structural Equation Modelling is a method that measures multifaceted hypotheses linking multiple causal pathways among variables (McCune and Grace 2002). It enables researchers to estimate unobserved latent variables from specific measured indicator variables and the strength of the direct and indirect pathways between variables. Grace and Pugasek (1997) used structural equation modelling to examine the importance of disturbance, community biomass and abiotic conditions on plant species richness. This enabled them to model density and abiotic effects at the same time.

Two latent variables were created, zoobenthos condition and macrophyte condition. Water and sediment were linked to both zoobenthos and macrophyte condition. Macrophyte condition was estimated by plant species richness, plant percent cover, and detritus mass. Plant percent cover and detritus mass both linked to zoobenthos condition. Zoobenthos condition linked to PCI, PCII, PCIII, PCIV, zoobenthos abundance and zoobenthos diversity (Fig 6.).

Data were  $\log_{10}$  transformed when appropriate to meet assumptions of normality. The model was laid out in Amos Graphics (SPSS 2009), and baseline values of 1.00 were set for the loading effect from macrophyte condition to plant richness and from zoobenthos condition to zoobenthos diversity in order to meet the requirements of an identified model (Kline 2005).



**Fig 6.** Structural Equation Model representation of inferred Zoobenthos – Macrophyte interactions and influences.

## Results

### Wetland Observations

#### Environmental Characteristics

The 4-m CT wetland had the highest salinity, followed by SW and then McL (Table 1). Dissolved oxygen concentration was near or exceeded saturation in all three wetlands. Shallow wetland had the lowest concentrations of dissolved oxygen (DO), the 4-m CT wetland had slightly higher DO, and DO was highest in McL water; however this was likely due to the shallowness of the water at the time of sampling. The temperature at time of sampling was also highest for McLean creek water (following loss of the beaver dam). The 4-m CT was the coolest (Table 1). Sediments were damp or dry values in McL but water depth was approximately 25-35 cm in SW and 4-m CT.

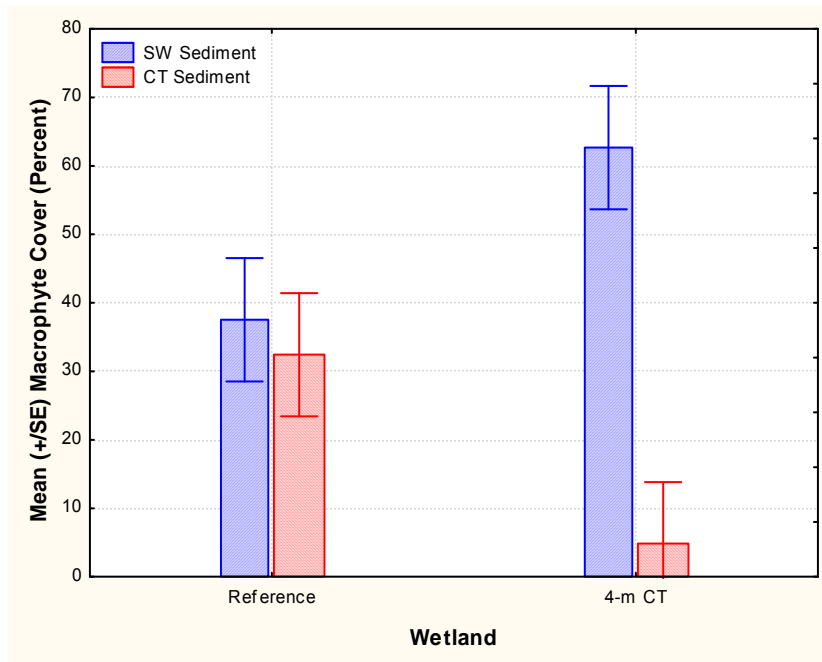
In terms of qualitative observations, the wind blowing across the surface of the SW was unobstructed by physical structures and was not very sheltered from the surrounding terrestrial landscape. In contrast, the 4-m CT was located the base of a large berm and was surrounded by 2-m tall conifers. McLean creek wetland also received some shelter from trees surrounding the wetland.

#### Macrophyte Cover:

Mean percent cover varied. Analysis of variance ANOVA (details in Appendix 5.) of the data showed that CT sediment plots in the CT wetland were significantly less in plant percent cover than reference sediment plots in the CT wetland. CT plots in the CT wetland were significantly lower in plant cover than CT plots in the reference wetland. (Fig 7.).

#### Detritus:

There was no significant difference in mean detritus mass in any of the plots (Fig 8 Appendix 6)



**Fig 7.** Mean macrophyte percent cover in Reference and CT tailings sediment within reference (Shallow Wetland) and OSPW-affected (4-m CT Wetland) wetlands (n=30).

#### Sample quantities

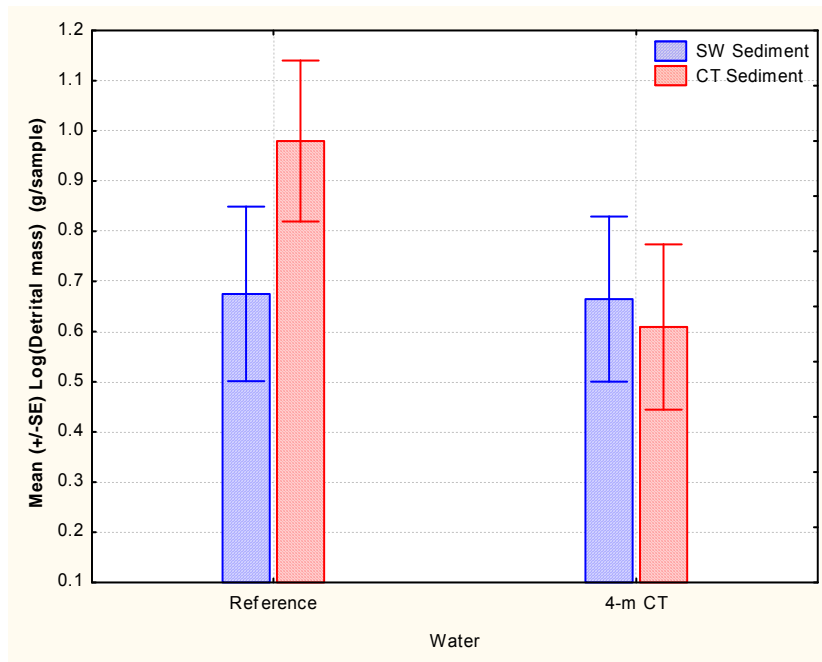
#### Overall Abundance (Density)

A total of 1,888 invertebrates were identified from 40 sweep samples taken in 2002 in the 4-m CT wetland and 2,070 were identified from 40 samples taken 2003. Shallow wetland samples contained 526 individuals in 2002 and 1,812 in 2003. The increase in density between years was largely due to a larger number of oligochaetes being collected in 2003 (412 individuals). Mean density of Chironomidae was significantly greater in 4-m CT than in the SW (Fig. 9, Appendix 7)

Mean density of chironomid taxa indicated increased density in OSPW in reference sediment plots (Fig 9. Appendix 7).

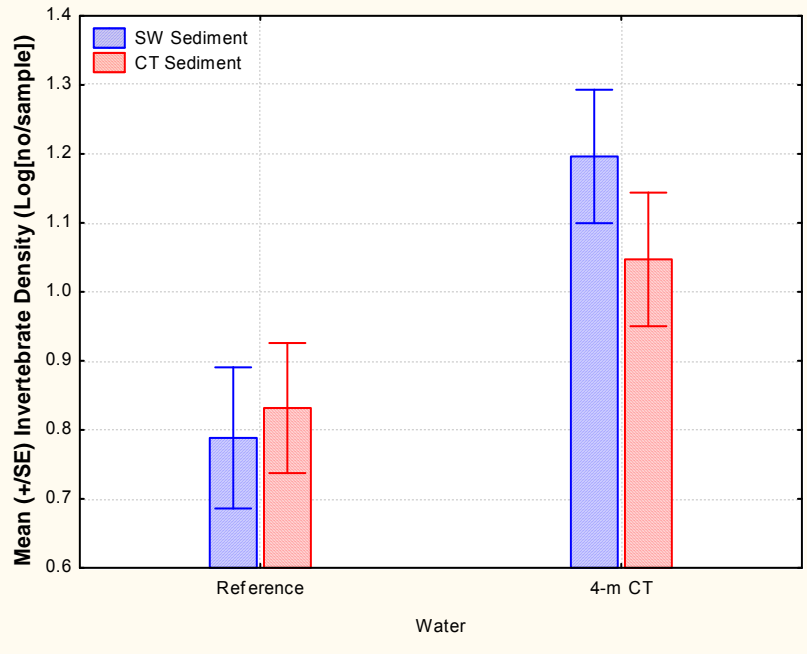
**Table 1.** Ranges of water chemistry values of the study wetlands. Values are ranges of 3-5 measurements taken in August 2003.

	4-m CT Demo Pond	Shallow Wetland	McLean Creek Wetland Complex
pH	7.7 - 7.8	7.8	7.9 - 8.1
Salinity (parts per thousand)	1.18 - 1.21	0.19 - 0.20	0.05
Conductivity ( $\mu$ S)	1888 - 1902	414 - 418	108.6 - 108.9
Dissolved Oxygen (mg/L)	9.9 - 10.1	8.5 - 8.6	12.1 - 12.2
Temperature ( $^{\circ}$ C)	14.4 - 14.5	16.4	22.3 - 22.4

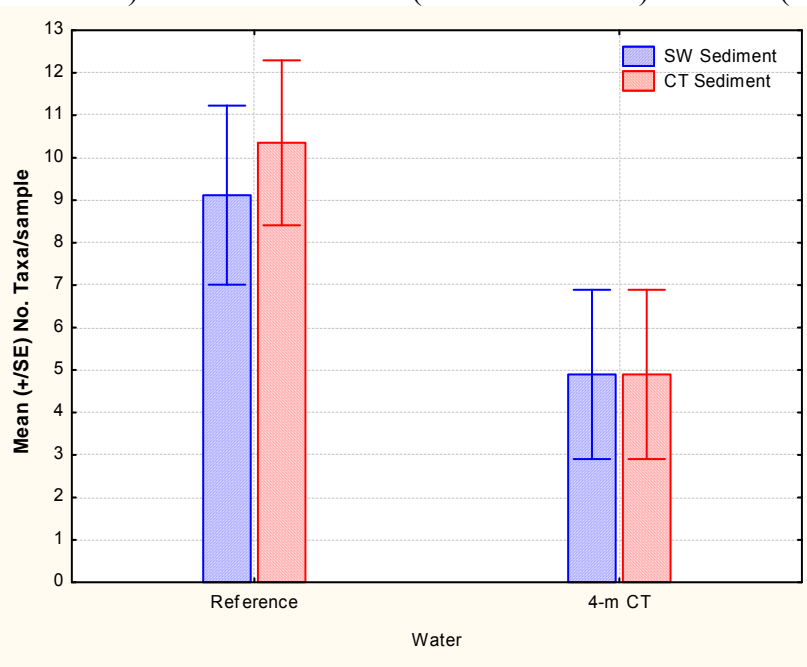


**Fig 8.** Mean detritus mass in grams/sweep in Reference and CT tailings sediment within reference (Shallow Wetland) and OSPW-affected (4-m CT Wetland) wetlands (n=20).





**Fig 9.** Mean invertebrate density in Reference and CT tailings sediment within reference (Shallow Wetland) and OSPW-affected (4-m CT Wetland) wetlands (n=20).



**Fig 10.** Mean richness (Taxa/572cm<sup>2</sup>) in Reference and CT tailings sediment within reference (Shallow Wetland) and OSPW-affected (4-m CT Wetland) wetlands (n=20).

### Zoobenthic Taxa Richness

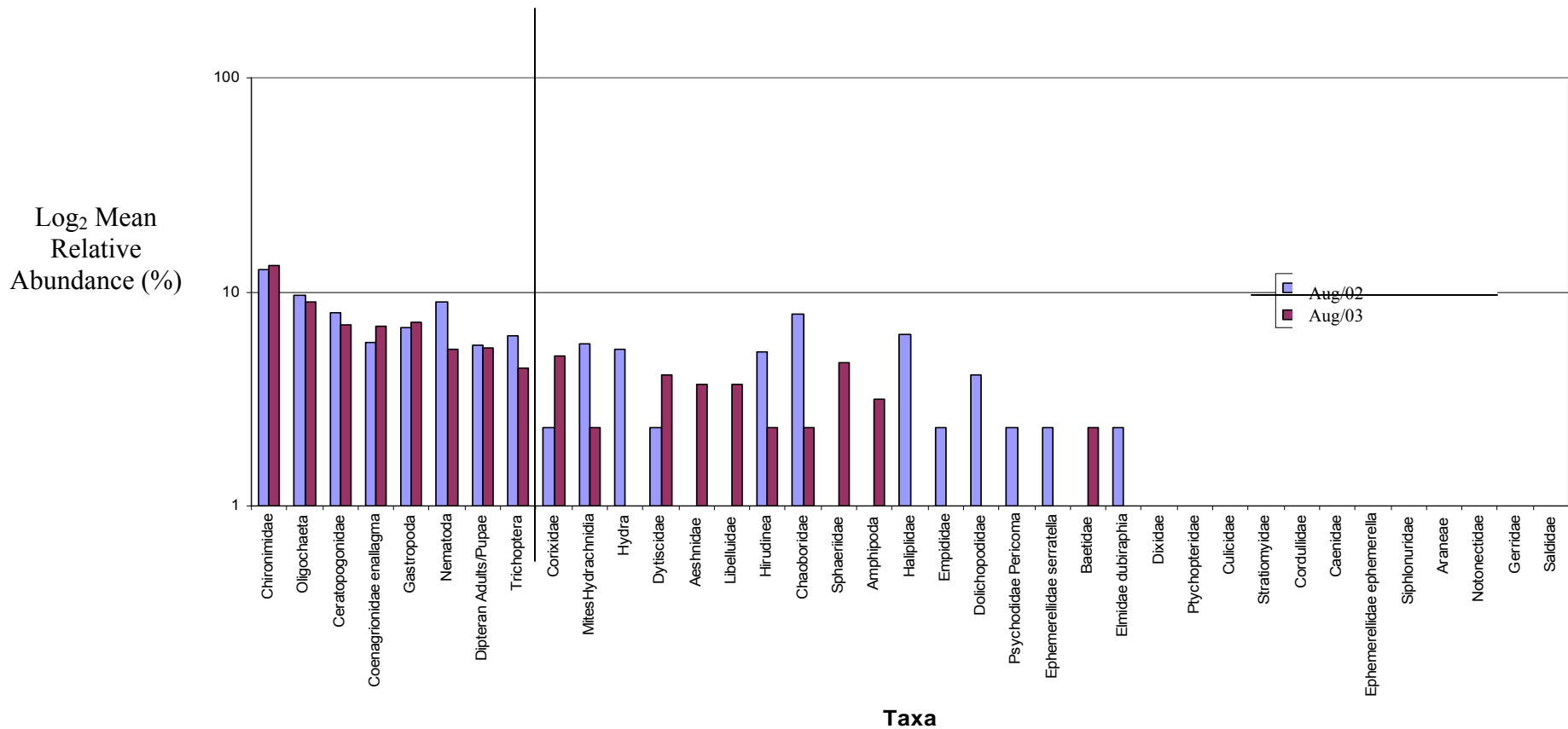
The analysis of taxa richness indicated that there were more taxa found in reference water samples when taxa occurring 1 time only were excluded (Fig 10. Appendix 8). When all taxa were considered, thirty-six taxa were identified from sweep samples and thirty-seven taxa were identified in core samples (Fig 11 and Fig 12).

### Zoobenthic Relative Abundance

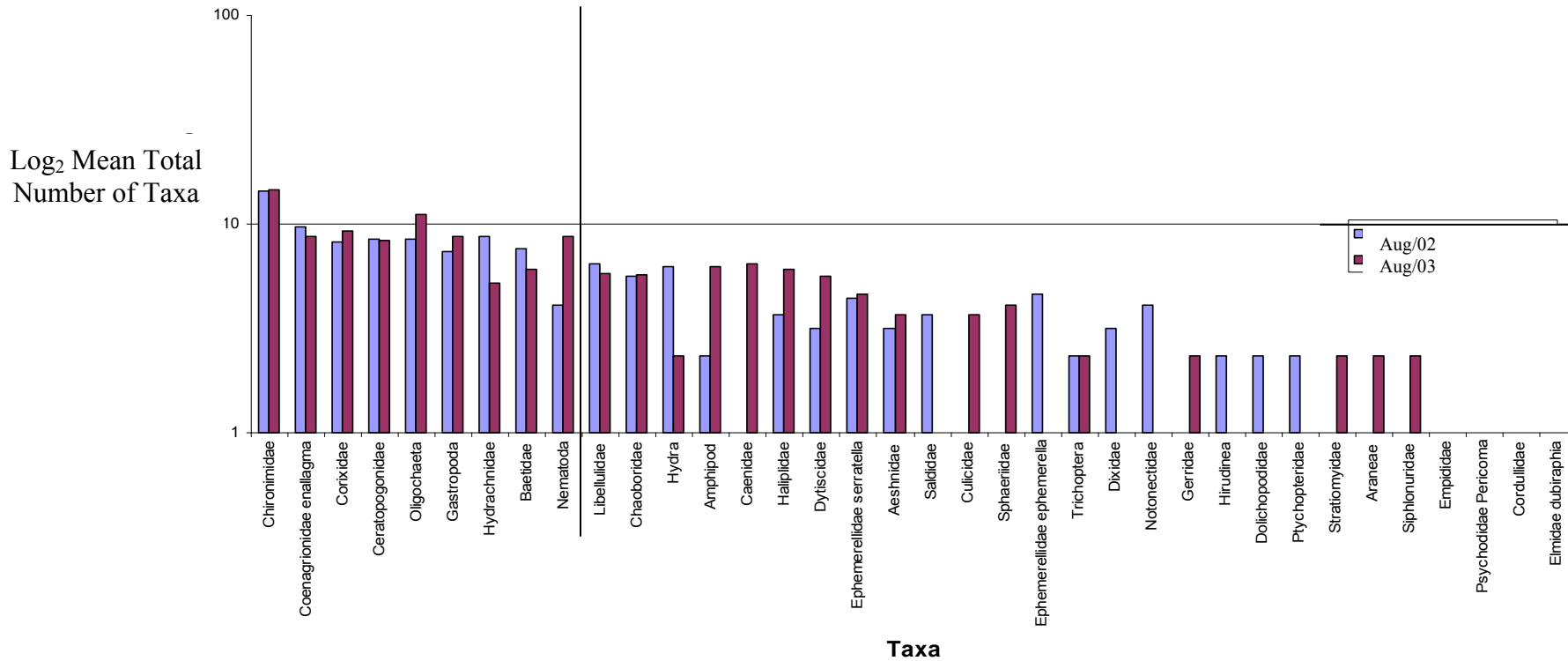
Prior to mounting and identifying the chironomids, preliminary core and sweep sample data were analysed using principal components analysis followed by multiple regression analysis of the components (Table 2, Fig. 11, Fig. 12 and Table 3).

The most common taxa found in core samples were oligochaetes, Ceratopogonidae, *Enallagma* damselflies, Gastropoda and Nematoda. Similarly in sweep samples the most common taxa included Oligochaeta, Gastropoda, Ceratopogonidae as well as *Enallagma* (damselflies) and Corixidae (water boatmen). In all, 8 taxa from core samples and 9 taxa from sweep samples met the criteria for inclusion in principal component analyses (see below) (Figures 11 and 12).

Twenty-six chironomid genera were found to occur in at least 5% of all sweep samples while representing at least 2% of the invertebrates in those samples when chironomids had been identified to the genus level (Fig 12). Taxa richness differed between the two wetlands. The Shallow Wetland had all 26 taxa whereas 18 taxa were identified from the 4-m CT Demo Pond samples in 2002 and 2003. In 2002, sweep samples from the 4-m CT wetland had 13 taxa, whereas 16 taxa were collected in 2003. In 2002, the SW sweep samples contained 22 taxa; 25 taxa were collected in 2003. *Psectrocladius* and *Cladotanytarsus* chironomids were the most abundant zoobenthos in the 4-m CT wetland, whereas oligochaetes and *Monopelopia* chironomids were the most numerous invertebrates in the Shallow Wetland. (Fig 10).

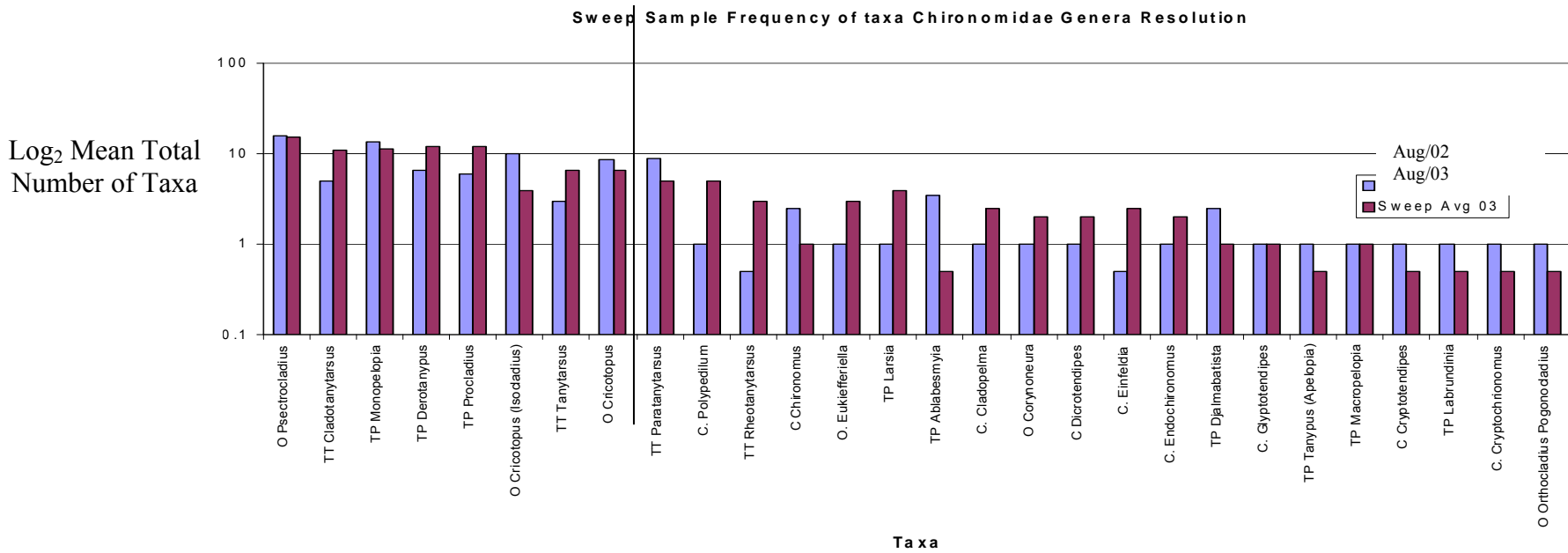


**Fig. 11** Frequency distribution of relative abundances of all taxa identified in core samples (n = 60; 5,837 invertebrates). Dividing line demarcates ‘more common’ taxa, which occurred in at least 5% of samples and represented an average of 2% of the invertebrates/sample from ‘rarer’ taxa. Only the more common taxa were used in multivariate analyses. Data were Log<sub>2</sub> transformed and a constant of 3 was added.



**F**

**ig. 12.** Frequency distribution of relative abundances of all taxa identified in sweep samples (n = 60; 6,756 invertebrates). Dividing line demarcates ‘common’ taxa, which occurred in at least 5% of samples and represented an average of 2% of the invertebrates/sample from ‘rarer’ taxa. Only common taxa were used in multivariate analyses. Data were Log<sub>2</sub> transformed and a constant of 3 was added.



**Fig. 13.** Frequency distribution of relative abundances of chironomid genera identified in sweep samples (n = 80; 1,509 invertebrates). Dividing line demarcates ‘common’ taxa, which occurred in at least 5% of samples and represented an average of 2% of the invertebrates/sample from ‘rarer’ taxa. Only common taxa were used in multivariate analyses. Data were log<sub>2</sub> transformed and a constant of 3 was added.

**Table 2.** Principal component (PC) factor loadings of relative abundances of taxa collected from core samples and sweep samples in reference (SW) and Oil sands process water (OSPW) wetlands (4-m CT) wetlands.

Cores	PC I	PC II	PC III	PC IV
Chironomidae	<b>-0.813</b>	0.030	0.329	0.003
Oligochaeta	<b>0.705</b>	0.005	0.378	-0.094
Nematoda	<b>0.654</b>	-0.099	-0.016	0.338
Trichoptera	<b>0.348</b>	0.118	0.121	0.257
Anisoptera	-0.204	<b>0.831</b>	0.148	0.130
Gastropoda	0.329	<b>0.664</b>	-0.197	-0.365
Ceratopogonidae	0.062	-0.037	<b>-0.914</b>	0.094
<i>Enallagma</i>	-0.042	0.000	0.115	<b>-0.902</b>
Variance Explained	1.862	1.157	1.175	1.162
Prop. Total	0.233	0.145	0.147	0.145
Cumulative Prop. Total	0.233	0.378	0.525	0.670
Sweeps	PC I	PC II	PC III	PC IV
Chironomidae	<b>0.802</b>	0.253	-0.070	-0.116
Baetidae	<b>-0.718</b>	-0.037	0.067	0.174
Hydrachnidae	<b>-0.792</b>	0.059	-0.095	0.189
Gastropoda	<b>-0.608</b>	-0.487	0.178	-0.107
Oligochaeta	-0.362	<b>-0.763</b>	0.104	-0.045
Nematoda	0.314	<b>-0.698</b>	0.200	0.263
Corixidae	0.270	<b>0.582</b>	0.494	-0.042
<i>Enallagma</i>	0.102	0.142	<b>-0.861</b>	0.005
Ceratopogonidae	0.153	0.049	0.012	<b>-0.938</b>
Variance Explained	2.491	1.738	1.086	1.044
Prop. Total	0.277	0.193	0.121	0.116
Cumulative Prop Total	0.277	0.470	0.591	0.707

## Principal Components Analysis

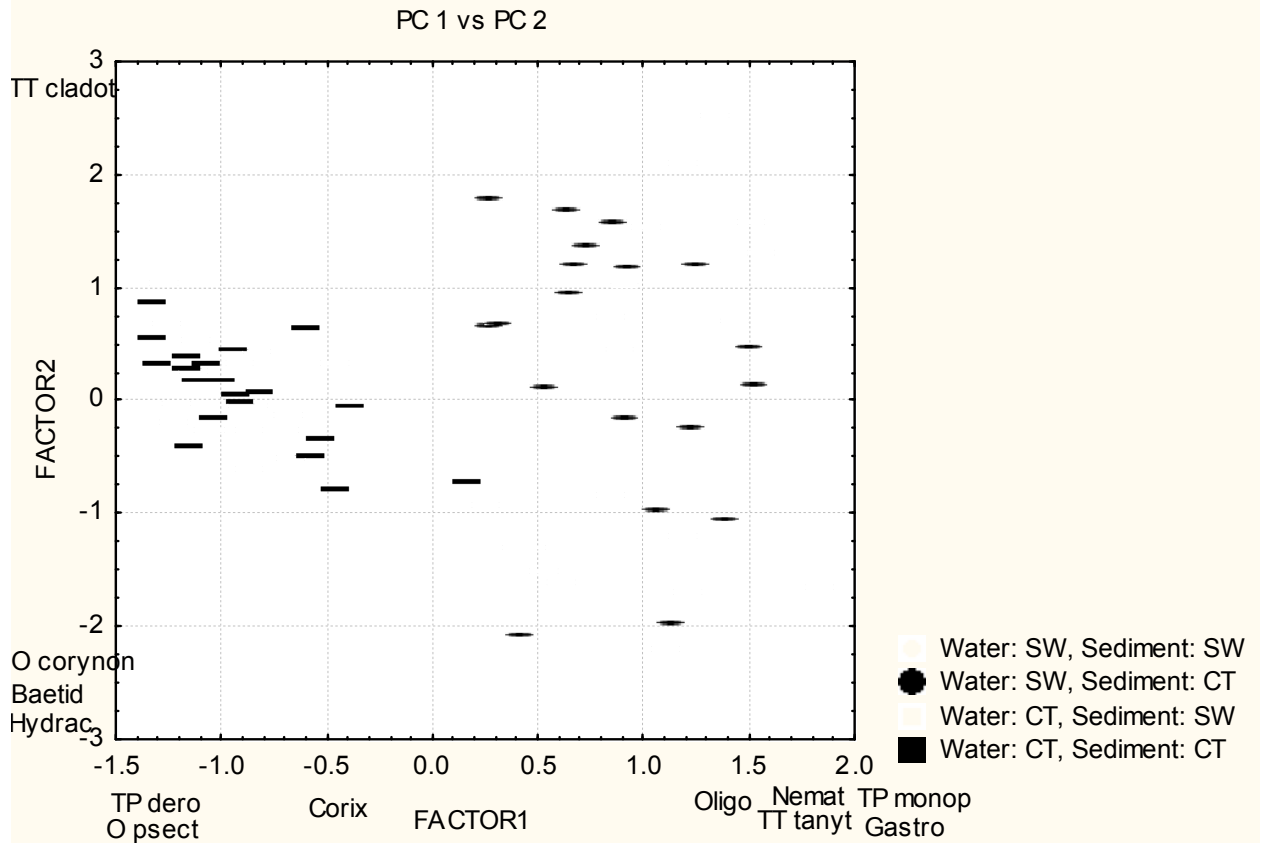
When principal components analysis was performed on the sweep samples resolved to the level of chironomid genera, 9 components representing 67.8% of the original variance were detected (Table 4 and Fig 15). Taxa whose relative abundance was positively associated with values of PCI, were Oligochaeta, *Tanytarsus*, Gastropoda, *Monopelopia* and Nematoda. Negatively associated taxa were Corixidae, *Psectrocladius* and *Derotanypus*. The relative abundance of only *Cladotanytarsus* was positively associated with values of PCII (Fig 14). Negatively associated taxa were *Corynoneura*, Baetidae and Hydrachnidae. For PCIII there were no positively associated taxa. Negatively associated taxa were *Rheotanytarsus* and *Larsia*. For PCIV positively associated taxa were *Polypedilum* and *Cladopelma* chironomids. There were no negatively associated taxa with PCIV. For PCV *Dicrotendipes* and *Chironomus* were positively associated taxa. Ceratopogonidae relative abundance was negatively associated with PCV. For PCVI, the only positively associated taxon was *Ablabesmyia*, and the only, strongly negatively associated taxon was *Cricotopus*. For PCVII positively associated taxa were *Enallagma*, *Cricotopus (Isocladius)* and *Procladius*. For PCVIII the positively associated taxon was *Eukiefferiella*. There were no negatively associated taxa. For PCIX positively associated taxon was *Paratanytarsus*. There were no negatively associated taxa.

## Taxa in the literature

The predominant taxa sampled have previously been categorized with respect to their affinity for salinity/conductivity, and plant cover (Leonhardt 2003). The invertebrates composing the principal component groupings can be contrasted with the literature (Table 5).

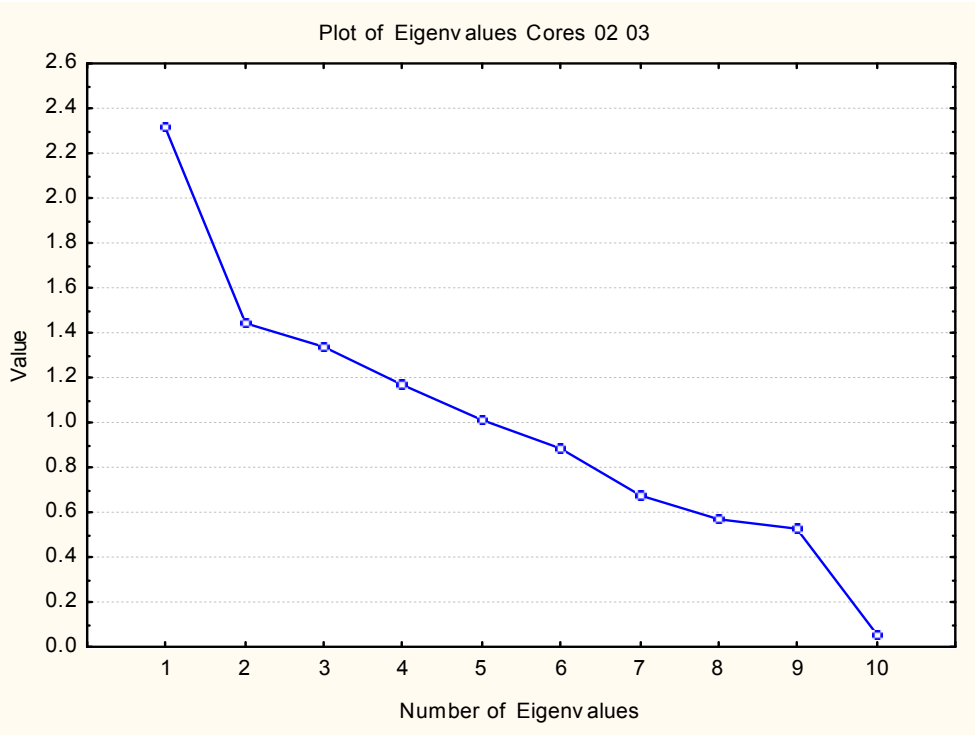
### PC I Positive

Oligochaeta: In terms of sensitive taxa, oligochaetes are rarer at sites with high conductivity, with relative abundances ranging from 0-19%, compared to reference sites with values of 20% or more (Whelley 1999).

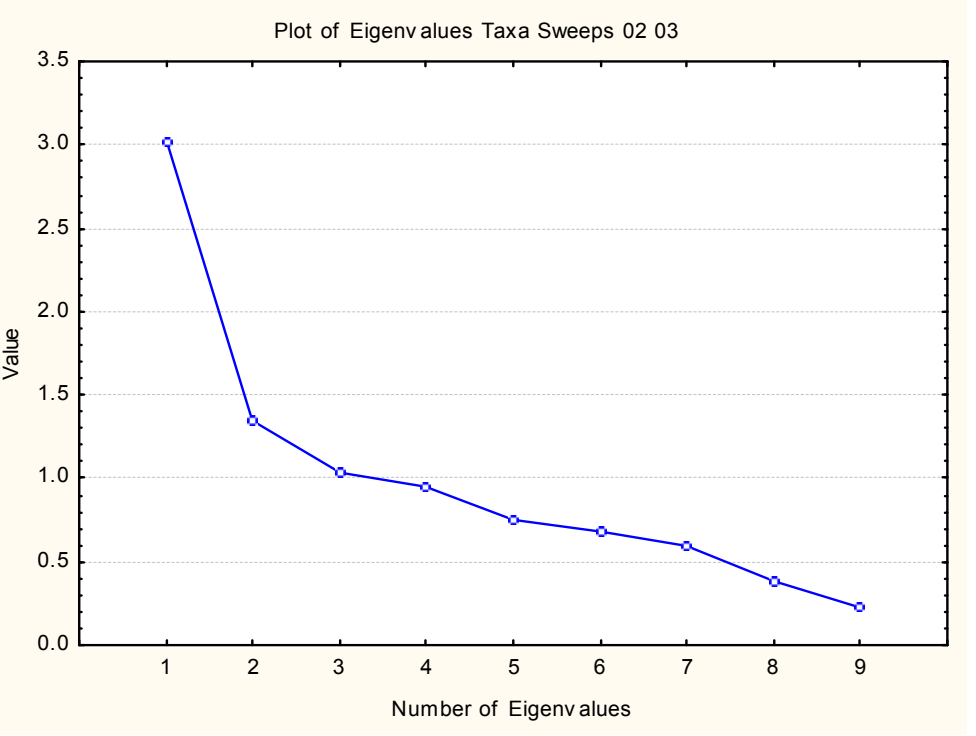


**Fig 14.** Scatterplot contrasting principal component scores for sweep samples of zoobenthos. Each point represents a sample. Taxa whose relative abundances are associated with each compound are listed on the axes. TT cladot: *Cladotanytarsus*, O corynon: *Corynoneura*, Baetid: Baetidae, Hydrac: Hydrachnidae, TP dero: *Derotanypus*, O psect: *Psectrocladius*, Corix: Corixidae, Oligo: oligochaeta, Nemat: nematoda, TT tanyt: *Tanytarsus*, Gastro: gastropoda, TP monop: *Monopelopia*





**Fig 15.** Plot of Eigenvalues for PCA of core samples; resolved only to Chironomidae family.



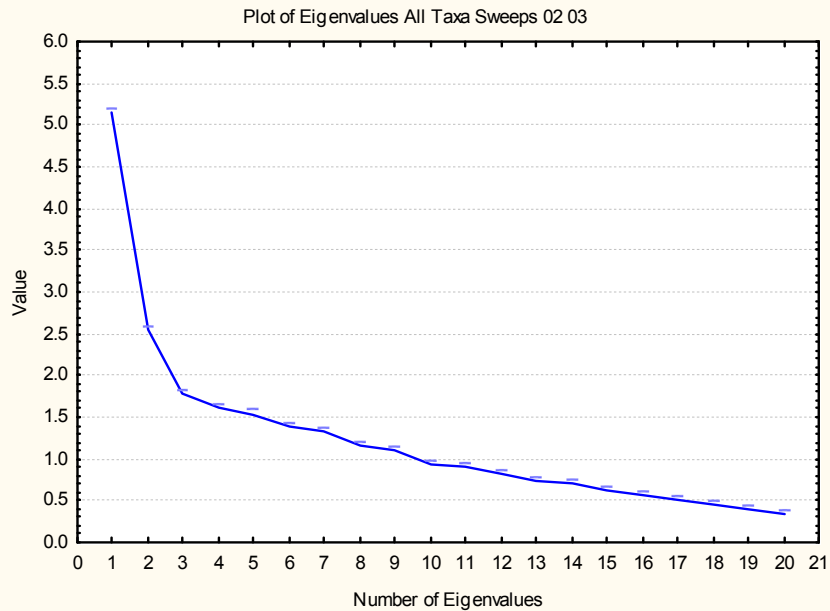
**Fig 16.** Plot of Eigenvalues for PCA of sweep samples; all taxa, resolved only to Chironomidae family.

**Table 3.** Multiple Regression models of the relationships between environmental variables and values of each of 4 principal component summaries of zoobenthic relative abundance.

<b>CORES (n= 60)</b>	PC I	R <sup>2</sup>	PC II	R <sup>2</sup>	PC III	R <sup>2</sup>	PC IV	R <sup>2</sup>
Intercept	0.74 ±0.71	-	-0.33 ±0.95	-	1.07 ±0.89	-	0.26 ±0.95	-
% Cover x Water Depth	-	-	-	-	5.19x10 <sup>-4</sup> ±2.32x10 <sup>-4</sup> *	0.043	-	-
Wetland (Water)	-1.65±0.26***	0.426	-	-	-	-	-	-
Wetland (Water) x Year	1.26±0.43**	0.144	-	-	-	-	-	-
Total R <sup>2</sup>		0.54		0.17		0.28		0.18
Associated Species +ve	Oligochaeta Nematoda Trichoptera		Anisoptera Gastropoda					
Associated Species -ve	Chironomidae				Ceratopogonidae		<i>Enallagma</i>	
<b>SWEEPS (n= 60)</b>	PC I	R <sup>2</sup>	PC II	R <sup>2</sup>	PC III	R <sup>2</sup>	PC IV	R <sup>2</sup>
Intercept	1.83±0.30***	-	-0.35 ±0.48	-	1.22±0.53*	-	0.45±0.57	-
% Cover	-	-	-	-	0.02±0.01*	0.042	-	-
% Cover x Water Depth	-	-	-	-	-0.00±0.00*	0.032	-	-
Wetland (Water)	-1.98±0.13***	0.649	-	-	0.66±0.23**	0.063	-	-
Year	-	-	1.81±0.64 **	0.059	-	-	-	-
Wetland (Water) x Year	0.51±0.19*	0.054	-1.51±0.30***	0.164	-1.54±0.33***	0.144	-0.91±0.36*	0.048
Total R <sup>2</sup>		0.76		0.39		0.26		0.13
Associated Species +ve	Chironomidae		Corixidae					
Associated Species -ve	Baetidae Hydrachnidae Gastropoda		Oligochaeta Nematoda		<i>Enallagma</i>		Ceratopogonidae	

**Table 4.** Principal component (PC) factor loadings of relative abundances of taxa collected from sweep samples in reference and oil sands process water (OSPW) wetlands.

Variable	PC I	PC II	PC III	PC IV	PC V	PC VI	PC VII	PC VIII	PC IX
<i>Oligochaeta</i>	<b>0.802</b>	-0.142	-0.056	0.142	0.014	0.095	-0.069	0.113	0.164
<i>Tanytarsus</i>	<b>0.784</b>	0.040	0.041	0.125	0.166	-0.032	-0.211	-0.057	-0.231
Gastropoda	<b>0.722</b>	-0.286	-0.039	0.081	-0.070	0.178	0.008	0.252	0.126
<i>Monopelopia</i>	<b>0.529</b>	-0.286	-0.242	0.296	0.307	0.025	0.024	0.009	-0.089
Nematoda	<b>0.507</b>	0.361	0.233	-0.206	0.132	-0.081	0.048	-0.494	-0.124
Corixidae	<b>-0.434</b>	0.350	-0.352	-0.181	-0.275	-0.031	-0.282	-0.013	-0.163
<i>Psectrocladius</i>	<b>-0.473</b>	0.247	0.152	-0.128	-0.190	-0.392	0.020	0.049	-0.371
<i>Derotanypus</i>	<b>-0.585</b>	0.383	0.218	0.067	0.092	0.243	-0.235	0.072	-0.263
<i>Cladotanytarsus</i>	-0.095	<b>0.650</b>	0.057	-0.371	-0.078	0.115	0.129	0.082	-0.085
<i>Corynoneura</i>	0.283	<b>-0.538</b>	0.163	-0.198	-0.381	0.026	-0.206	-0.095	0.098
Baetidae	0.110	<b>-0.635</b>	-0.210	-0.056	0.145	0.134	0.241	-0.197	0.161
Hydrachnida	0.162	<b>-0.820</b>	0.122	-0.178	0.052	0.060	0.123	0.029	0.035
<i>Rheotanytarsus</i>	0.310	0.222	<b>-0.442</b>	0.152	0.239	0.172	-0.042	-0.103	0.263
<i>Larsia</i>	0.039	-0.004	<b>-0.843</b>	-0.054	-0.038	-0.076	-0.040	-0.017	-0.169
<i>Polypedilum</i>	0.195	0.098	-0.010	<b>0.724</b>	0.271	-0.127	-0.021	-0.104	0.117
<i>Cladopelma</i>	0.175	0.085	0.098	<b>0.716</b>	-0.281	0.214	0.101	-0.036	0.021
<i>Dicrotendipes</i>	0.099	-0.040	0.062	0.129	<b>0.647</b>	0.076	-0.112	-0.003	0.241
<i>Chironomus</i>	0.415	0.277	0.063	-0.091	<b>0.522</b>	0.022	0.062	0.115	0.272
Ceratopogonidae	-0.061	0.346	-0.004	0.121	<b>-0.640</b>	0.102	-0.013	0.140	0.116
<i>Ablabesmyia</i>	0.103	-0.099	0.062	0.070	-0.096	<b>0.792</b>	0.096	-0.076	0.026
<i>Cricotopus</i>	0.376	-0.146	-0.211	0.115	-0.235	<b>-0.475</b>	-0.012	-0.128	0.329
<i>Enallagma</i>	-0.146	0.004	0.077	0.027	0.037	0.106	<b>0.813</b>	0.186	0.007
<i>Cricotopus (isocladius)</i>	-0.164	-0.183	0.226	0.299	-0.172	-0.417	<b>0.514</b>	-0.016	-0.061
<i>Procladius</i>	-0.226	0.218	0.267	0.139	0.067	-0.056	<b>-0.536</b>	0.393	-0.123
<i>Eukiefferiella</i>	0.218	0.132	0.059	-0.157	-0.004	-0.060	0.129	<b>0.823</b>	-0.025
<i>Paratanytarsus</i>	0.023	-0.115	0.159	0.065	0.074	-0.003	0.027	0.001	<b>0.856</b>
Variance Explained	3.848	2.841	1.532	1.697	1.856	1.456	1.589	1.332	1.515
Prop. Total	0.148	0.109	0.059	0.065	0.071	0.056	0.061	0.051	0.058
Cumulative Prop. Total	0.148	0.257	0.316	0.381	0.452	0.508	0.569	0.620	0.678



**Fig 17.** Plot of Eigenvalues for sweep samples for all taxa and with chironomid genus resolution.

*Tanytarsus*: Species of this genus are indicators of clean-water conditions in still water (Oliver and Roussel 1983). They are relatively common taxa (Pardalis 1997). Leonhardt (2003) reported that Tanytarsini were typical of young (less than 7 y old) wetlands in the region. They are regarded as a generally sensitive taxon (Pontasch and Cairns 1991).

**Gastropoda**: found more commonly in “older” wetlands (operationally defined as wetlands more than 7 y old.) (Leonhardt 2003).

*Monopelopia*: typically inhabit warm, shallow, organically rich still water (Oliver and Roussel 1983). They can be found in waterbodies with low pH (EPA 2005).

**Nematoda**: Nematodes as a group are a prevailing component of the meiofauna of aquatic sediments. Species found in brackish or estuarine waters are distinct from the Nematoda that are normally found in freshwater habitats (Thorp and Covich 2001 p. 264). Salt-tolerant species of nematodes could/would be able to colonize and survive in saline wetlands.

**Table 5.** Summary of literature-reported distribution/tolerance of zoobenthic taxa with respect to their salinity and wetland age.

Typical of old wetlands (>7y old) or Salinity intolerant		Typical of younger wetlands (<7y old) or Salinity tolerant	
Oligochaeta	Rarer in wetlands of higher conductivity	<i>Derotanypus</i>	Predator prevalent in OSPM-affected wetlands
Nematoda	Species-specific salinity tolerance <sup>1</sup>	<i>Psectrocladius</i>	OSPM chemistry can increase abundance <sup>2</sup>
Gastropoda	Typical of older wetlands <sup>4</sup>	<i>Cladotanytarsus</i>	Typical of younger wetlands <sup>4</sup>
<i>Tanytarsus</i>	Indicator of good conditions <sup>2</sup>	<i>Rheotanytarsus</i>	Typical of younger wetlands <sup>4</sup>
<i>Monopelopia</i>	Prefer organically rich water <sup>4</sup>	<i>Larsia</i>	Typical of younger wetlands and tolerant to diverse conditions <sup>4</sup>
<i>Corynoneura</i>	Characteristic of still water with plants, typical of older wetlands <sup>4</sup>	Corixidae	Mobile taxa, colonizer of younger wetlands tolerant to OSPW salinity <sup>2</sup>
Baetidae	OSPM chemistry can reduce Ephemeroptera; typical of older wetlands <sup>4</sup>		
Hydrachnida	Not very tolerant to salinity <sup>3</sup>		
<i>Polypedilum</i>	Typical of older wetlands <sup>4</sup>	<i>Polypedilum</i>	Tolerant of salinity <sup>2</sup>
<i>Cladopelma</i>	Typical of older wetlands <sup>4</sup>		

<sup>1</sup>Thorp & Covich (2001), <sup>2</sup>Whelley (1998), <sup>3</sup>Oliver & Roussel (1983), <sup>4</sup>Leonhardt (2003)

### PC I Negative

*Corixidae*: Frequently invade temporary wetlands. In northern latitudes, adults of lentic species fly to larger water bodies to overwinter. Adults disperse widely, invading temporary ponds (Thorp and Covich 2001 p. 684). In addition, young wetlands also have relatively high numbers of corixids. These are highly mobile (adults are strong fliers) taxa and are often colonizers of new areas (Merritt and Cummins 1996, Leonhardt 2003)

*Psectrocladius*: resilient to creosote toxicity concentration of up to 5 ppb; they are relatively common taxa (Pardalis 1997). A mixture of chlorides, ammonia, organics and metals was related to reduced mayflies, and to an increase in Orthoclaudiini (Pontasch and Cairns 1991).

*Derotanypus*: Whelley (1999) found *Derotanypus* to be the main predatory chironomid in saline wetlands.

### PC II Positive

*Cladotanytarsus*: Typical of young wetlands (Leonhardt 2003).

### PC II Negative

*Corynoneura*: predominate in standing water on submerged or floating aquatic plants (Oliver and Roussell 1983); typical of older wetlands (Leonhardt 2003).

Baetidae: typical of older wetlands (Leonhardt 2003); generally sensitive to changes in wetland condition (Pontasch and Cairns 1991)

Hydrachnida: found in fresh water; intolerant of salinity (Thorp and Covich 2001 p. 568).

### PC III Negative

*Rheotanytarsus*: typical of young wetlands (Leonhardt 2003).

*Larsia*: typical of young wetlands (Leonhardt 2003); live in a wide variety of habitats (Oliver and Roussell 1983).

#### PC IV Positive

*Polypedilum*: Chironomini typical of mature wetlands (Leonhardt 2003); can inhabit brackish water (Oliver and Roussell 1983).

*Cladopelma*: Chironomini typical of mature wetlands (Leonhardt 2003).

#### Multiple Regression Analysis of the Principal Components

Multiple regression analysis using the PCA with chironomid genera resolved yielded significant relationships with a number of variables (Table 6.).

#### Objective 1: OSPW

For sweep samples, water type significantly influenced three principal component groups. Values of principal component I were highly significantly influenced ( $p < 0.001$ ), those of principal component II were mildly ( $p < 0.05$ ) significantly influenced and scores of principal component III were significantly influenced ( $p < 0.01$ ) (Table 6).

Relative abundances of taxa associated with PC-I (Oligochaeta, *Tanytarsus*, Gastropoda, *Monopelopia* and Nematoda were reduced in OSPW affected wetland (4-m CT) and taxa such as Corixidae, *Psectrocladius* and *Derotanypus*, which were negatively associated with PC-I were more abundant in OSPW. Relative abundances of taxa associated with PC-II *Cladotanytarsus*, were greater in OSPW, and taxa such as *Corynoneura*, Baetidae and Hydrachnidae were all reduced in the OSPW wetland. Relative abundances of taxa negatively associated with PC-III *Rheotanytarsus* and *Larsia* were greater in OSPW (Table 6).

**Table 6.** Multiple Regression table of 9 principal components from sweep samples representing 68% of overall variability.

	PC I	R <sup>2</sup>	PC II	R <sup>2</sup>	PC III	R <sup>2</sup>	PC IV	R <sup>2</sup>		
Intercept	1.27±0.37**	-	-0.05±0.66		1.06±1.00		-			
Detritus Mass x % Cover	0.01±0.00**	0.122	-		-		-			
Wetland (Water)	-1.62±0.13***	0.707	0.49±0.23*	0.066	-0.95±0.36**	0.103	-			
Percent Cover x Year	-		-		-		0.03±0.01*	0.066		
Wetland (Water) x Year	-		-1.61±0.35***	0.261	1.93±0.53***	0.179	-1.05±0.52*	0.063		
Total		0.87		0.37		0.20		0.16		
Associated Species +ve	Oligochaeta <i>Tanytarsus</i> Gastropoda <i>Monopelopia</i> Nematoda		<i>Cladotanytarsus</i>				<i>Polypedilum</i> <i>Cladopelma</i>			
Associated Species -ve	Corixidae <i>Psectrocladius</i> <i>Derotanypus</i>		<i>Corynoneura</i> Baetidae Hydrachnidae		<i>Rheotanytarsus</i> <i>Larsia</i>					
	PC V	R <sup>2</sup>	PC VI	R <sup>2</sup>	PC VII	R <sup>2</sup>	PC VIII	R <sup>2</sup>	PC IX	R <sup>2</sup>
Intercept	-		-		-		-		-	
Detritus Mass x % Cover	-	-	-	-	-	-	-	-	-	-
Wetland (Water)	-	-	-	-	-	-	-	-	-	-
Percent Cover x Year	-	-	-	-	-	-	-	-	-	-
Wetland (Water) x Year	-	-	-	-	-	-	-	-	-	-
Total		0.07		0.16		0.04		0.06		0.16
Associated Species +ve	<i>Dicrotendipes</i> <i>Chironomus</i>		<i>Ablabesmyia</i>		<i>Enallagma</i> <i>Cricotopus</i> ( <i>Isocladius</i> )		<i>Eukiefferiella</i>		<i>Paratanytarsus</i>	
Associated Species -ve	Ceratopogonidae		<i>Cricotopus</i>		<i>Procladius</i>					



## Objective 2: Consolidated Tailings

Sediment type within wetlands did not significantly influence abundance or diversity of zoobenthic taxa directly. However, there was a significant indirect effect of sediment on plant cover, which (as Objective 4 will indicate) significantly affected relative abundances of principal component I taxa (Table 6.).

## Objective 3: Year

The relative abundances of taxa summarized by principal components II and III varied highly significantly between years ( $p < 0.001$ ; Table 6). There were marginally statistically significant year x wetland and year x plant cover interaction effects on taxa whose relative abundances were represented by PC-IV.

Relative abundances of *Corynoneura* chironomids, baetid mayflies and hydrachnid mites were all greater in 2003.

There was a highly significant year x wetland interaction effect on relative abundances. Taxa associated with scores of PC-II and PC-III became rarer in the CT wetland but not in the SW wetland in 2003 ( $p < 0.001$ ; Table 6) compared to 2002.

Relative abundances of *Polypedilum* and *Cladopelma* chironomids increased in 2003 as a function of increasing plant cover, but there was no relationship between relative abundance and plant cover in 2002 (significant year x percent cover interaction,  $p < 0.05$ , Table 6).

## Objective 4: Plant Cover and Detritus Mass

The interaction of macrophyte cover and detritus mass had a significant effect on PC-I scores. Oligochaetes, *Tanytarsus*, Gastropoda, *Monopelopia* and Nematoda relative abundances were all positively associated with the detritus mass x % cover interaction. Corixidae, *Psectrocladius* and *Derotanypus* relative abundances were all negatively correlated with the detritus mass x % cover interaction. There was no specific taxon that dominated plots of greater plant cover and detritus. There was an indirect effect from sediment on zoobenthos condition acting through macrophyte condition.

## Structural Equation Model

The structural equation model was designed as a confirmatory model whose linkages were based on the results of the multiple regression analyses. It was designed to attempt to separate the direct effects of OSPW and CT sediment from indirect effects mediated through influences of these variables on plant growth and detrital deposition.

In this section, the magnitudes of the effects between indicator and latent variables are called loadings similarly to the term used to describe the correlations of individual taxa with principal component scores.

The  $\chi^2$  test value for this model provided by AMOS (SPSS 2009) was over 100, indicating a highly significant lack of fit ( $p < 0.001$ ). There were 76 data samples included in the model and while the literature indicates this as adequate, it may be the reason for the large  $\chi^2$  value.

## Macrophyte Condition

Macrophyte condition loading from water was -0.07. Plant species richness from macrophyte condition was 1.00. Plant percent cover loading from macrophyte condition was 9.17. Detritus mass loading from macrophyte condition was 0.43. Values of PC I, II, III and IV loadings from detritus mass (inferred from the path through the zoobenthos condition latent variable) were zero. This suggests that detritus biomass had no direct effect on relative abundances of the various zoobenthic taxa. PC I, II, III and IV loadings from macrophyte condition were -4.77, 2.64, -0.09 and -0.73 respectively. PC I, II, III and IV loadings from percent cover were 9.18, -2.32, 0.08 and 0.64 respectively.

## Zoobenthos Condition

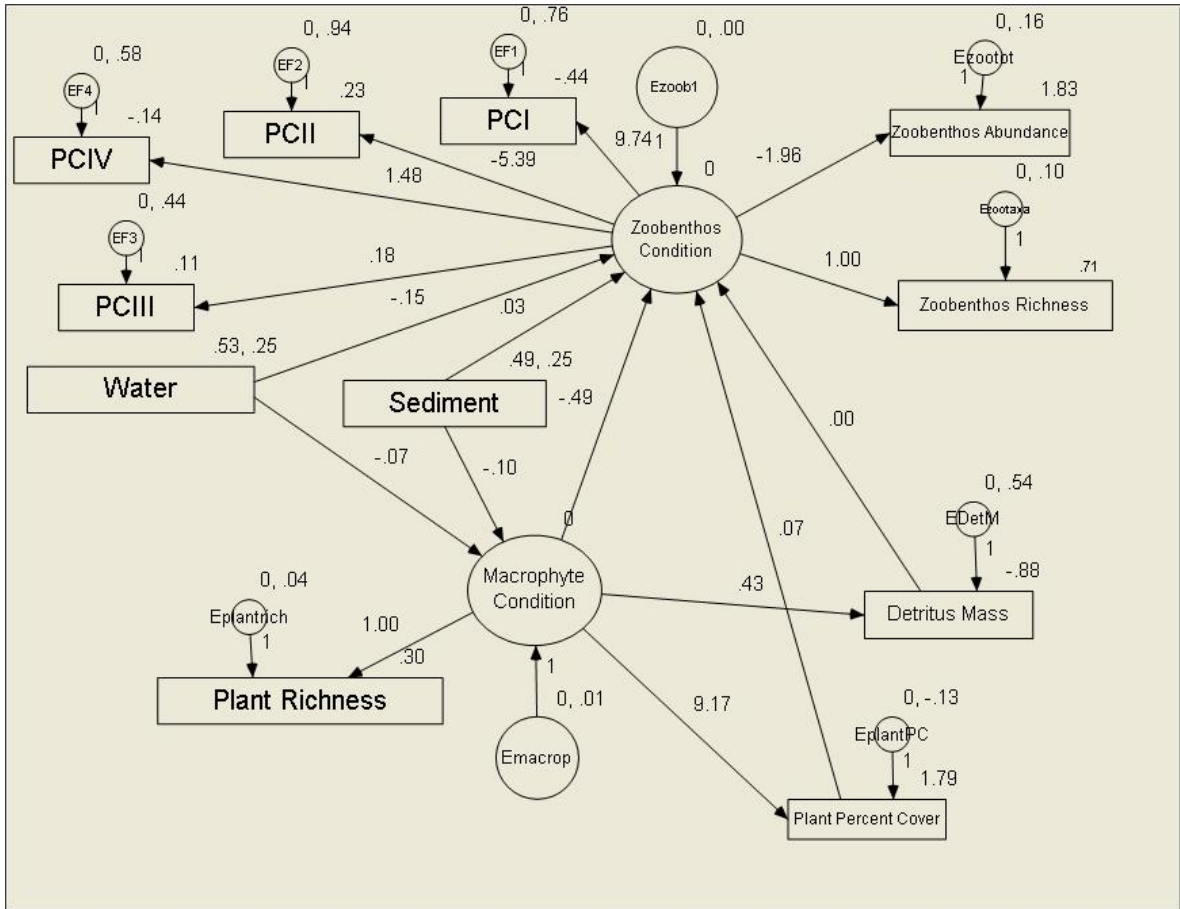
Zoobenthic condition richness was assigned unit loading from 20 Principal component I, II, III, IV loadings from zoobenthos condition were 9.74, -5.39, 0.18 and 1.48 respectively, implying that the taxa associated with PCI were those whose relative abundances were most representative of the 'zoobenthic condition' latent variable. Zoobenthic density was somewhat negatively related to zoobenthos condition (Fig 18). Nevertheless, the majority of variation in zoobenthic condition was unaccounted for (Error term loading was 9.71), indicating that other, unmeasured features of the system affected the distribution of zoobenthos among plots.

Zoobenthos abundance loading from zoobenthos condition was -1.96. This means that abundance of zoobenthos was negatively proportional to the zoobenthos condition latent variable. In contrast, the zoobenthos richness loading from zoobenthos condition was 1.00, which is half the magnitude but directly proportional to the zoobenthos condition.

## Overall Effects on Benthos:

Overall effects loadings were calculated by multiplying loadings effects values for routes through the macrophyte condition latent variable. Direct effects loadings on PC I, II, III and IV from sediment were 0.29, -0.16, 0.01 and 0.04 respectively. Indirect effects loadings on PCI, II, III and IV from sediment were -0.15, 0.08, -0.00 and -0.02 respectively. Overall effects loadings from sediment on PCI, II, III and IV were 0.19, -0.08, 0.01 and -0.02 respectively (Table 7.) These linkages are relatively low indicating that there may not be a relationship.

Direct effects loadings on PC I, II, III and IV from water (through zoobenthos condition) were -1.46, 0.81, -0.03, and -0.22 respectively. Indirect effects loadings on PCI, II, III and IV from water were -0.10, 0.06, -0.00 and -0.02 respectively. Overall effects loadings from water on PCI, II, III and IV were -1.56, 0.87, -0.03 and -0.24 respectively (Table 7.). These values are relatively high indicating that water effects are more important than the effects from sediment.



**Fig 18.** Estimated structural equation model with latent variables, indicator variables and loadings.

**Table 7** Structural Equation Model loadings and associated taxa for principal component I, II, III and IV

	Principal Component 1	Principal Component 2	Principal Component 3	Principal Component 4	Absolute Mean
Zoobenthos Condition	9.74	-5.39	0.18	1.48	
Detritus Mass	-	-	-	-	
Macrophyte Condition	-4.77	2.64	-0.09	-0.73	
% Cover	9.18	-2.32	0.08	0.64	
Sediment Direct	0.29	-0.16	0.01	0.04	
Sediment Indirect	-0.15	0.08	-0.00	-0.02	
Total Sediment Loading	<b>0.19</b>	<b>-0.08</b>	<b>0.01</b>	<b>-0.02</b>	<b>0.08</b>
Water Direct	-1.46	0.81	-0.03	-0.22	
Water Indirect	-0.10	0.06	-0.00	-0.02	
Total Water Loading	<b>-1.56</b>	<b>0.87</b>	<b>-0.03</b>	<b>-0.24</b>	<b>0.68</b>
Associated Species Positive	Oligochaeta <i>Tanytarsus</i> Gastropoda <i>Monopelopia</i> Nematoda	<i>Cladotanytarsus</i>		<i>Polypedilum</i> <i>Cladopelma</i>	
Associated Species Negative	Corixidae <i>Psectrocladius</i> <i>Derotanypus</i>	<i>Corynonneura</i> Baetidae Hydrachnidae	<i>Rheotanytarsus</i> <i>Larsia</i>		

Zoobenthos condition loading from water was -0.15, (which is a relatively small magnitude) and negative, which indicates that water was negatively proportional to zoobenthos condition. Zoobenthos condition loading from sediment was 0.03. This is a small magnitude indicating that direct effects from water are approximately 5 times larger than sediment.

Zoobenthos condition loading from macrophyte condition was -0.49. This is a larger effect than either sediment or water, and the negative loading indicates that it is negatively proportional to zoobenthos condition. Zoobenthos condition loading from plant percent cover was 0.07, which indicates a relatively small positive effect on the zoobenthos condition. Zoobenthos condition loading from detritus mass was 0.00 indicating that detritus mass was not important to zoobenthos condition. Macrophyte condition loading from sediment was -0.10.

## **Discussion**

In this chapter, I identified groupings of co-occurring benthic macroinvertebrate taxa and investigated the variation in their abundance and richness in a constructed OSPM-affected wetland and a reference wetland with regard to water, plant cover, sediment and year. In addition to assessing overall trends in abundance and richness, I determined how relative abundances of particular groups of taxa varied as a function of the independent variables.

OSPM-affected wetlands have higher salinity and pH, and lower levels of dissolved oxygen in the surface waters than reference wetlands in the area (Ganshorn 2002). The richness of chironomid genera declines as OSPM concentration increases in wetlands on oil sand leases (Whelly 1999). Whelly (1999) and Leonhardt (2003) also established that benthic macroinvertebrate assemblage composition, especially of chironomid genera of reference wetlands differed from the composition of environmentally comparable OSPM-affected wetlands. Salinization of freshwater habitats often reduces invertebrate community species richness. Aladin (1991) documented that 10 of 14 cladoceran species were extirpated from a lake as it underwent an 18% increase in salinity over 30 years. Aquatic insects need to maintain a proper internal salt and water balance. The energy requirement for invertebrates to osmoregulate the homeostasis is

quite high. Normant (2005) exposed the brackish water amphipod *Gammarus oceanicus* to salinity levels below ideal and noted that there was an increase in energy expenditure predominantly attributed to the high energy cost of osmoregulation.

Saline wetlands in Saskatchewan are dominated by Chironomini (*Chironomus*, *Cryptochironomus*) Tanytarsini (*Tanytarsus*) and Tanypodinae (*Procladius*) similar to saline wetlands of inland British Columbia (Cannings and Scudder 1978). These taxa were also commonly collected from the OSPW-affected 4-m CT wetland. In addition to predominant chironomid taxa in reference and OSPW-affected wetlands Whelley (1999) found that *Chironomus tentans* was negatively influenced when larvae were artificially reared in high concentrations of OSPW.

Wetland/water and year were both highly significant for principal component 1 (PC1). PC1 is the most important component, explaining about 21% of the variance in zoobenthic taxonomic composition. By comparison, PC II (the next most important component) explained only 9%.

Clearly, changes in water chemistry can modify the habitat and influence the environment's capacity to support organisms found there. Differences in water chemistry between the reference and OSPW-affected wetland were expected to influence macroinvertebrate abundance. Indeed, water type affected overall zoobenthic abundance, and richness directly, and influenced macrophyte cover. In terms of effects on zoobenthic community composition, water type was a highly significant variable influencing principal component 1. The OSPW wetland had lower relative abundance of oligochaete and nematode worms, *Tanytarsus*, *Corynoneura* and *Monopelopia* chironomids, gastropods, baetid mayflies and hydrachnid mites. Oligochaete worms and *Tanytarsus* chironomids are both relatively sensitive taxa to OSPM-affected wetland condition, and their reduced number would be anticipated in the altered chemistry found there. Nematode worms are distinctly saline-tolerant or saline-intolerant (Thorp and Covich 2001) and consequently the indigenous species would be expected to be eliminated.

The OSPW (4-m CT) samples contained greater proportions of *Psectrocladius*, *Derotanypus*, *Cladotanytarsus*, *Rheotanytarsus* and *Larsia* chironomids and water boatmen (Corixidae) than samples in the reference wetland, regardless of whether a plot contained CT sediment or reference wetland sediment. This again indicates that water

type exerts a greater influence on zoobenthic composition than sediment type, even among sediment-dwelling (as opposed to epiphytic) taxa.

High Orthocladiinae larval abundance has been reported as especially noticeable in younger wetlands (Leonhardt 2003). Some genera of these chironomids were noted to tolerate conductivity levels of 488-741 $\mu$ S/cm in saline lakes in central British Columbia (Cannings and Scudder 1978). The conductivity measured in the OSPM –affected wetland is roughly 2-3 times this level. Consequently, the osmoregulating stresses may play more of a factor than in these previous studies. However, the observation of *Psectrocladius* chironomids in OSPM-affected wetlands is consistent with expectations derived from known habitat requirements. The Tanypodinae *Derotanypus* has previously been shown to be predominant in OSPW (Whelly 1999, Leonhardt 2003). Its prevalence in the OSPM-affected wetland is not unexpected.

Members of the hemipteran family Corixidae, are able to fly to temporary wetlands and are generally one of the first invertebrates colonizing habitable wetlands. Their apparent tolerance to OSPM-affected wetland conditions results in their being one of the most prevalent taxa found.

Overall, water type exerted a major influence on benthic macroinvertebrate assemblage abundance and this finding is consistent with previous studies.

Sediment did not directly affect zoobenthos richness in either the reference or the OSPW-affected wetland. However the influence of CT sediment is not limited by direct effects on zoobenthos (Table 7). As previously indicated, Cooper (2003) found significantly lower levels, of vascular plant cover in CT plots than in reference sediment plots in reference wetlands. This influence of sediment type on the habitat structure important to zoobenthic taxa indicates an indirect influence on the benthic macroinvertebrate assemblage sampled. Plants increase the surface area available for development of the benthic microbial community, which may be an important source of nutrition for grazing taxa. Results from the structural equation model indicated that plant percent cover effects were more important and directly proportional to PCI, PCIII and PCIV and negative for PC II than overall sediment effects, which followed the same proportionality except PCIV, which was negative (Table 7.)



Interaction terms involving the year of sampling were highly significant in influencing principal component II and III, and marginally significant in influencing principal component IV. Year was marginally significant in influencing principal component IV when interacting with percent cover. *Corynoneura* chironomids, baetid mayflies and hydrachnid mites all increased in relative abundance in 2003. In contrast, relative abundances of *Cladotanytarsus*, *Rheotanytarsus*, and *Larsia* were lower in the second year. The highly significant year x wetland interaction (Table 6.) indicated that the change was more pronounced in the CT wetland than in the SW. The taxa that increased in relative abundance are typical of maturing wetlands or those taxa characteristic of stable, suitable conditions. Conversely, the taxa that exhibited reduced relative abundance in the 4-m CT wetland are those typical of younger wetlands (Leonhardt 2003). Leonhardt (2003) indicated that OSPM-affected and reference wetlands older than 7 years old had the same family richness. Consequently, she referred to wetlands age 7 or older as 'mature' from a macroinvertebrate community richness perspective. However, differences in community composition among groups were still evident after 13-15 years. The patterns observed in this study may simply reflect the maturation of the reference wetland.

Of special interest, *Polypedilum* and *Cladopelma* chironomids, which were grouped as PC IV, showed inconsistent patterns - two differing interactions with year. Decreasing in relative abundance in 4-m CT in 2003 and increasing in plots of increased plant cover in 2003.

Relative abundances of *Polypedilum* and *Cladopelma* chironomids were greater in 2003 as a function of plant cover, but there was no relationship between relative abundance and plant cover in 2002 (significant year x percent cover interaction,  $p < 0.05$ , Table 5). Species of *Polypedilum* are able to survive in brackish conditions while the literature does not indicate the same for *Cladopelma*.

In general, the trends of the invertebrates over the summers of August 2002 and August 2003 seem to reflect the transition from a younger wetland to those of a more mature wetland (<7years).

Although the main questions of this study were to distinguish between the direct effects of OSPM (sediment) from OSPW (water) on macroinvertebrates, this led to an

additional investigation of the indirect effect from consolidated tailings on macrophytes to the macroinvertebrates. The macrophyte assemblages in plots from the 4-m CT wetland were species poor compared to plots from Shallow Wetland (SW) and McLean Creek Wetland (McL), the two reference wetlands (Cooper 2003). The differences were also evident in the relative amounts of accumulated detritus and degree of plant cover among wetlands. Both detritus and percent cover significantly influenced the relative abundances of several taxa - oligochaete and nematode worms, gastropods and *Tanytarsus* and *Monopelopia* chironomids increased in relative abundance as detritus mass and percent cover increased. Although herbivory by zoobenthos has not been recognized as a major source of nutrition, there is now considerable evidence to suggest that herbivory on living macrophytes may be much more important than previously suspected (Lodge 1991; Newman 1991). Newman (1991) found that herbivores from primarily aquatic groups of invertebrates, were generalists and also detritivores in addition to consuming living macrophyte.

*Corixidae*, and *Psectrocladius* (detritivores) and *Derotanypus* (predators) relative abundances decreased with increased detritus mass and percent cover. These are taxa associated with prevalence in younger, less stable wetlands (Leonhardt 2003). Their negative relationship contrasts with the relative increased abundance of macrophyte-preferring taxa such as oligochaete (particularly Naididae) and nematode worms, gastropods, *Tanytarsus*, and *Monopelopia* chironomids, which are more characteristic of mature, stable wetlands and can be sensitive to environmental stresses.

### Structured Equation Modelling

#### PCI

Total effects loadings from sediment were positive while total effects of loadings for water were negative. Total water effects loadings were approximately 8 times larger in magnitude than total sediment effects loadings, indicating that water has a greater overall influence on PCI-associated taxa than sediment.

## PCII

Total effects loadings from sediment were negative while total effects loadings for water were positive. Total water effects loadings were approximately 10 times larger than total sediment effect loadings in magnitude, indicating that water has a greater total influence on PCII-associated taxa than sediment.

## PC III

Total effects loadings from sediment were positive while total effects loadings for water were negative. Total water effects loadings were 3 times larger in magnitude than

The magnitudes of both sediment and water (0.01 and -0.03 respectively), were small, which indicates that neither was very important in influencing PCIII taxa

## PC IV

Total effects loadings from sediment and water were both negative. Total effects loadings of water were approximately 12 times greater than sediment.

## Structural Equation Modelling Summary

Water has a much stronger direct effect on zoobenthic community composition than sediment. The absolute average value of total effects for sediment loadings is 0.08 while the absolute average value of total effects for water is 0.68 (Table 6.).

## Conclusions

Overall, OSPW exerted significant direct negative effects on the zoobenthic condition compared to reference wetland water. Consolidated tailings sediment layered in the constructed wetland had an indirect effect on the zoobenthos condition by negatively influencing the percentage plant cover. The effects of water were seen on the overall density, taxa richness and relative abundance of the taxa. Year to year variation also had some significant interactions which indicate wetland maturation.

The use of oil sand mining by-products in wetland reclamation procedures that had neutral or positive effects on resident assemblages would achieve two purposes: disposal of mining by-products, and the regeneration of productive landscape. The

development of suitable strategies for reclaiming oil sand mining areas is increasingly important as the area of mined land increases and the quantities of stored by-products increase. The ability to effectively transform cleared landscapes, such as oil sand mining areas and wetlands, into ecologically productive areas, is based on many factors. My results suggest that the use of OSPW and CT sediment in the construction of wetlands results in direct effects on the zoobenthos relative abundance from OSPW, and indirect effects through inhibited plant development. Amelioration strategies, such as using peat substrates or direct planting of macrophytes capable of surviving in the wetlands may mitigate the indirect effects of CT sediment on zoobenthos in constructed wetlands. In terms of restoring land to original levels of productivity, the use of OSPW and OSPM may be suitable in constructing wetlands. However, the zoobenthos assemblages found in these wetlands would be expected to differ from those of wetlands pre-mining. These factors need further study in order to increase the knowledge base from which decisions on the procedure to prepare the most ideal conditions for constructed wetlands are made.

## **Chapter 3**

### **Discussion, Summary and Future Research**

#### **Discussion**

The purpose of this research was to investigate the effects of the sediment, water and macrophyte cover in a wetland constructed with oil sands process water and composite tailings sediment, on benthic invertebrate abundance and community composition in littoral habitats. The surface waters of OSPM-affected wetlands generally have higher salinity, pH and lower levels of dissolved oxygen than reference wetlands (Ganshorn 2002).

In general, zoobenthic community level responses to sediment contamination may include a reduction in the number of organisms present, a reduction in taxonomic richness, the elimination of intolerant populations and/or a change in the relative abundance of dominant taxa (Ciborowski et al. 1995). The littoral zone of lentic habitats is typically occupied by submergent macrophytes, rooted emergent vascular plants and macroalgae. It generally supports a varied group of insects with members from most aquatic orders. Habitats include benthic and plant surfaces, the water column, and the surface film. Zoobenthos can use macrophytes as substrates or may graze periphyton from their surface. Macrophytes generally harbour populations of filter feeders, periphyton grazers and predators. Members of some benthic orders also use macrophytes as oviposition sites. Several researchers have demonstrated the direct effect of oil sands process water on zoobenthos in laboratory toxicity tests (e.g. Whelly 1999, Sabo and Ciborowski 2005). Similarly, field studies have shown that aquatic plant colonization and production are inhibited in both oil sands process water and sediments (Crowe et al. 2002). One goal of the current project was to determine the direct and combined effects of OSPW and CT on zoobenthic community composition. The appearance of submergent macrophytes and deposition of detritus in study plots (Cooper 2004) provided an opportunity to measure the effects of OSPW and CT mediated through zoobenthic-macrophyte interactions.

The acute toxicity of OSPM (oil sands process materials) decreases quickly over its first year when exposed to natural conditions and free from the input of fresh OSPM

(FTFC 1995). Survival of trout and *Daphnia magna* in exposure studies involving tailings water that had aged for 10 months increased from 0% to 60-80% vs. unaged tailings water as a result of hydrocarbon breakdown (FTFC 1995). The detoxification of OSPM in wetlands seems to be an aerobic process that is likely phosphorus limited (FTFC 1995). Laboratory studies have shown that the addition of phosphate to surface water from Suncor ponds resulted in a detoxification period between 7-10 weeks and at field-scale there was also an enhanced ability to detoxify Suncor dyke drainage water (FTFC 1995).

This study used multivariate analysis to identify groupings of benthic macro-invertebrate taxa and investigated the variation in their abundance and diversity caused by components of a constructed OSPM-affected wetland and a reference wetland with regard to water type, sediment type, plant cover, detritus mass and year to year effects.

The richness of chironomid genera declines as OSPM concentration increases in wetlands on oil sand leases (Whelly 1999). Whelly (1999) also established that benthic macroinvertebrate assemblage composition, especially genera of chironomid larvae of reference wetlands, differed from the composition of environmentally comparable OSPM-affected wetlands. Zoobenthic relative abundances are influenced by the amounts of macrophyte development and accumulated detritus (Leonhardt 2003). Aged consolidated tailings did not seem to exert a direct negative effect on zoobenthic community composition. However, macrophyte development tended to be inhibited in these sediments relative to reference sediments, producing an indirect alteration of zoobenthic community composition. The magnitude of this response varied between study years, likely reflecting the increasing amount of macrophyte development occurring in study plots as a function of time since the start of the study. If these trends persist over periods longer than the duration of this study, then ultimately the effects of water quality (elevated salinity and other oil sands process water constituents) are likely to be of more concern than sediment quality in wetlands constructed with oil sands process materials.

Elevated salinity of freshwater habitats reduced invertebrate community species richness by extirpating salt-intolerant species. This is a global phenomenon. Aladin (1991) documented the local extinction of 10 out of 14 indigenous cladoceran species

from a lake as it underwent an 18% increase in salinity over 30 y. Aquatic insects need to maintain a proper internal salt and water balance. Energy is needed to osmoregulate cellular homeostasis. Normant (2005) exposed the brackish water amphipod *Gammarus oceanicus* to salinity levels below ideal and observed increased energy expenses mostly attributed to the high energy cost of osmoregulation. Differences between the fauna of reference sediments and CT sediments may decrease through time as organic material becomes deposited (Leonhardt 2003); however elevated salinity likely will remain a permanent characteristic of the chemistry of OSPW-affected constructed wetlands, and the associated zoobenthic community may never become similar to that of low-salinity reference constructed wetlands.

Whelly found that chironomid larva were less dominant in reference wetlands than in OSPM-affected wetlands in the area. Saline wetlands in Saskatchewan are dominated by Chironomini (*Chironomus*, *Cryptochironomus*) Tanytarsini (*Tanytarsus*) and Tanypodinae (*Procladius*) similar to saline wetlands of inland British Columbia (Cannings and Scudder 1978), and also similar to what was seen in the OSPM-affected wetland in this study. In addition to predominant chironomid taxa in reference and OSPW-affected wetlands, Whelly (1999) found that *Chironomus tentans* was negatively influenced when larvae were laboratory-reared in high concentrations of OSPW. Although Leonhardt (2003) found that OSPM-affected and reference wetlands older than 7 years old had the same family richness, differences in community composition among groups, including chironomid genera, were still evident after 13-15 years.

Changes in water chemistry can modify the habitat and influence the capacity to support organisms. Overall numbers of aquatic macroinvertebrates found in wetlands have been shown to be maximum when water is at near neutral pH levels (Friday 1987). In addition, wetlands with increased concentrations of salt have higher densities of invertebrates but generally lower richness of taxa than wetlands with low dissolved salt concentrations (Whelly 1999). Changes in water chemistry in reclaimed oil sands wetlands were expected to influence macroinvertebrate diversity. Water type was a highly significant variable influencing principal component 1, which accounted for about 21% of the variation in zoobenthic relative abundances among samples. The OSPW wetland had lower relative abundance of oligochaete and nematode worms, *Tanytarsus*,

*Corynoneura* and *Monopelopia* chironomids, gastropods, baetid mayflies and hydrachnid mites (the taxa whose relative abundance correlated highly with values of PC1). Oligochaete worms and *Tanytarsus* chironomids were both relatively sensitive to the OSPM-affected wetland condition and their decrease in number would be anticipated in the adjusted chemistry found there. All plots in the OSPW wetland supported larger numbers of *Psectrocladius*, *Derotanypus*, *Cladotanytarsus*, *Rheotanytarsus* and *Larsia* chironomids and water boatmen (Corixidae) than plots in the reference wetland, regardless of the sediment type. Larval abundance of Orthocladiinae is relatively high in younger wetlands (Leonhardt 2003). Some species are able to tolerate relatively high conductivity (up to around 7000  $\mu\text{S}/\text{cm}$  in saline lakes in central British Columbia; Cannings and Scudder 1978). The conductivity measured in the OSPM-affected wetland is less than 2000  $\mu\text{S}/\text{cm}$ . Consequently the osmoregulating stresses wouldn't be expected to be an over-riding factor in eliminating these taxa from constructed wetlands. The chironomids *Psectrocladius* and *Derotanypus* are Tanytopodinae frequently found in naturally saline waterbodies. Their occurrence in the OSPM-affected wetlands is consistent with what is known of their biology (Oliver and Rousell 1983). Corixidae (water boatmen) are able to fly to temporary wetlands and are generally among the first invertebrates to colonize habitable wetlands. Their apparent tolerance to OSPM-affected wetland conditions has enabled them to be one of the most prevalent taxa found.

Overall, the differences in zoobenthic relative abundances observed as a function of exposure to water type (the low conductivity Shallow Wetland vs. the OSPM-affected 4-m CT Wetland), were consistent with the findings of previous studies that contrast reference wetlands with OSPM-affected wetlands. Therefore, zoobenthic taxa are governed by the influence of water regardless of the source of sediment from which they were collected.

There was no significant direct influence of sediment type on the relative abundance of benthic taxa collected from either sweep net or core samples. Sediment type (Shallow Wetland sediment vs. 4-m CT sediment) did not influence zoobenthic overall density or the relative abundance of any of the assemblages summarized by the Principal Component Analysis factor scores, in either of the wetland individually or across the two wetlands combined. However, the influence of CT



sediment is not limited to direct effects on zoobenthos. As indicated previously, Cooper (2004) found significantly lower biomass and vascular plant cover in CT sediment plots in reference wetlands than in reference sediment plots. Macrophytes also provide substrate for the benthic microbial community to develop, which supports the nutrition for grazing. In addition some taxa are herbivorous and depend directly on macrophyte tissues rather than on epiphytic materials for food (Thorp and Covich 1991). Consequently, epiphyte-associated zoobenthos dependent on macrophytes are affected indirectly by CT sediment.

Among-year interaction terms significantly influenced taxa whose relative abundances were correlated with principal components II, III and IV. A year by plant cover interaction significantly influenced scores of principal component IV. This indicates that the effects of water, sediment and plant cover on zoobenthic relative abundance varied between the two years for which data were analyzed.

*Corynoneura* chironomids, baetid mayflies and hydrachnid mites all increased in relative abundance in 4-m CT wetland in 2003, a full year after the plots had been established. In contrast, relative abundances of *Cladotanytarsus*, *Rheotanytarsus*, and *Larsia* decreased in that wetland but not in SW (the reference wetland) in the second year. The taxa that differentially increased in abundance are those that are typical of maturing wetlands or those taxa indicating stable suitable conditions. In contrast, the taxa whose relative abundance declined between years are those more typical of younger wetlands. This may be seen as a sign of change toward mature reference wetlands. The patterns are in agreement with other research (Leonhardt 2003). Taxa whose relative abundances were positively associated with scores of PC-II and PC-III became rarer in the CT wetland but not in the SW wetland in 2003 compared to 2002. Relative abundances of *Polypedilum* and *Cladopelma* chironomids increased in the CT wetland 2003 as a function of increasing plant cover, but there was no relationship between relative abundance and plant cover in 2002.

The main questions of this study were to assess and contrast the direct effects of oil sands process water vs. sediments on macroinvertebrates. However, because OSPM also affected the plant communities, this permitted an evaluation of the indirect effect of CT sediment on the zoobenthos, mediated through plant development. Cooper's (2004)

investigation showed that macrophyte assemblages in the Suncor Energy Inc. 4-m CT consolidated tailings wetland were species poor compared to the two reference wetlands (Shallow Wetland (SW) and McLean Creek Wetland (McL)). OSPW is approximately 6-24 times more saline than the naturally occurring waters of the reference wetlands.

The interaction between detritus and percent cover was statistically significant in its influence on taxa from PC1. The relative abundances of oligochaete and nematode worms, gastropods and *Tanytarsus* and *Monopelopia* chironomids increased as detritus mass and amount of plant cover increased. The impact of herbivorous insects on many living plants has previously been considered to be low. However, the nitrogen content of macrophytes is similar to that of terrestrial plants and there is now evidence to suggest that herbivory on living macrophytes may be much more important than previously suspected (Lodge 1991; Newman 1991). Newman (1991) found that herbivores from primarily aquatic groups of invertebrates were generalists and also detritivores.

The relative abundance of Corixidae and *Psectrocladius* and *Derotanypus* chironomids, which occupy benthic sediments rather than plants, decreased with increased detritus mass and percent cover. These are taxa associated with prevalence in younger, less stable wetlands (Leonhardt 2003). Their negative relationship complements the relative increased abundance of macrophyte-preferring taxa such as oligochaete and nematode worms, gastropods, *Tanytarsus*, and *Monopelopia* chironomids, which characterize mature, stable wetlands and can be sensitive to environmental stresses.

## **Summary**

Water effects, sediment effects, inter-year effects and the effects of macrophyte percent cover on the benthic macroinvertebrate assemblages of a reference wetland and a constructed experimental wetland were investigated using a reciprocal sediment transplant. The reciprocal sediment transfer designed for this experiment enabled the effects of OSPW to be assessed independently of those of reference sediments in terms of the zoobenthos condition. OSPW was the most important single factor in influencing zoobenthic condition, while CT sediment effects were indirect, acting through plant cover. Multiple regression analysis of principal components identified groupings of taxa that tended to co-occur. There was higher chironomid density in OSPW samples, but

more taxonomic diversity in reference water samples. There was higher plant percent cover in plots containing reference (SW) sediment. Principal component analysis identified groupings of taxa that co-occurred. *Corixidae*, *Psectrocladius*, *Derotanytus*, *Rheotanytus*, *Cladotanytus* and *Larsia* chironomids were seen to group together in OSPW conditions while *Oligochaeta*, *Tanytus*, *Gastropoda Monopelopia*, *Nematoda*, *Corynoneura*, *Baetidae* and *Hydrachnidae* were generally grouped in reference conditions. Structural equation modelling fit the overall data patterns poorly (as indicated by a high chi-square value) but indicated that overall water effects were more important than overall sediment effects to the principal component groupings. It also indicated importance of plant percent cover to the principal component groupings.

### **Future Research**

This project assessed results of reciprocal transplant effects in a relatively young OSPW wetland (5 years old at the end of the study) with an older reference wetland.

Zoobenthos are more strongly affected by OSPW than by oil sands process sediments (consolidated tailings). However, this study could not separate the influence of elevated salinity from those of NA or other residual compounds from oil sands processing. Reciprocal transplant studies that contrasted sodic wetlands lacking NA with OSPW-affected wetlands might be able to separate these effects. Wetlands and lakes on oil sand leases generally have increased salinity in conjunction with residual hydrocarbons. Salt will likely be more important to the zoobenthos condition in reclaimed wetlands than residual NA or other hydrocarbons, which would be expected to break down fairly rapidly through time. In general however, salinity levels are not likely to change rapidly through time unless dilution and elimination from the watershed occurs.

Indirect effects of oil-sands derived water and sediment on invertebrates, mediated through detrital and macrophyte accrual may become reduced as wetlands age. As the constructed wetland acquires more detritus and builds up an organic sediment layer, the sediment should become more similar to that of a reference wetland and the indirect effect of the CT sediment on zoobenthos should be reduced. Another reciprocal transplant study between older constructed wetlands with increased organic sediment and

macrophyte populations or simply a transplant between parts of a wetland with and without dense submerged macrophyte cover could be useful in investigating macrophyte effects. However, the single greatest need is likely to be continued monitoring of constructed wetlands as they age in order to track the relative influences from oil sands tailings water and sediment on the development of the macrophytes and the associated zoobenthic community.

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## Appendices

**Appendix 1.** Summary of multiple regression analysis of principal component scores summarizing relative abundances of zoobenthic taxa in all sweep samples (n=80).

	PC I	R <sup>2</sup>	PC II	R <sup>2</sup>	PC III	R <sup>2</sup>	PC IV	R <sup>2</sup>
Intercept	1.27±0.37**		-0.05±0.66		1.06±1.00		-0.49±0.98	
Detritus Mass	0.00±0.14	0.000	-0.12±0.25	0.004	0.24±0.39	0.006	-0.08±0.38	0.001
% Cover	0.00±0.01	0.003	-0.02±0.02	0.017	-0.04±0.02	0.044	0.02±0.02	0.008
Detritus Mass x % Cover	0.01±0.00**	0.122	-0.01±0.01	0.012	0.00±0.01	0.001	0.00±0.01	0.002
Water Depth	-0.021±0.01	0.029	-0.02±0.02	0.022	-0.03±0.03	0.011	0.01±0.03	0.003
% Cover x Water Depth	-0.00±0.00	0.007	0.00±0.00	0.004	0.00±0.00	0.030	0.00±0.00	0.004
Wetland (Water)	-1.62±0.13***	0.707	0.49±0.23*	0.066	-0.95±0.36**	0.103	0.66±0.35	0.056
Sediment Type	0.06±0.19	0.002	0.14±0.33	0.003	0.50±0.50	0.016	-0.19±0.49	0.002
Year	0.05±0.46	0.000	1.57±0.81	0.058	-1.16±1.24	0.014	0.17±1.21	0.003
Detritus Mass x Year	-0.32±0.29	0.020	0.01±0.52	0.000	0.56±0.79	0.008	-0.61±0.77	0.010
Percent Cover x Year	-0.00±0.00	0.014	0.01±0.01	0.014	0.02±0.01	0.025	0.03±0.01*	0.066
Water Depth x Year	0.01±0.02	0.006	0.02±0.03	0.009	-0.01±0.04	0.000	-0.03±0.04	0.008
Wetland (Water) x Year	-0.42±0.20	0.069	-1.61±0.35***	0.261	1.93±0.53***	0.179	-1.05±0.52*	0.063
Sediment Type x Year	-0.35±0.23	0.036	-0.23±0.41	0.005	-0.56±0.63	0.013	1.14±0.61	0.053
<b>Total</b>		<b>0.87</b>		<b>0.37</b>		<b>0.20</b>		<b>0.16</b>
Associated Species +ve	Oligochaeta <i>Tanytarsus</i> Gastropoda <i>Monopelopia</i> Nematoda		<i>Cladotanytarsus</i>				<i>Polypedilum</i> <i>Cladopelma</i>	
Associated Species -ve	Corixidae <i>Psectrocladius</i> <i>Derotanypus</i>		<i>Corynoneura</i> Baetidae Hydrachnidae		<i>Rheotanytarsus</i> <i>Larsia</i>			

**Appendix 2.** Summary of multiple regression analysis of principal component scores summarizing relative abundances of zoobenthic taxa in all sweep samples (n=80).

	PC V	R <sup>2</sup>	PC VI	R <sup>2</sup>	PC VII	R <sup>2</sup>	PC VIII	R <sup>2</sup>	PC IX	R <sup>2</sup>
Intercept	-0.07±1.11		-0.60±1.04		0.54±1.17		-0.17±1.14		0.26±1.10	
Detritus Mass	-0.02±0.43	0.000	0.29±0.40	0.008	-0.08±0.45	0.001	0.04±0.44	0.000	-0.03±0.42	0.000
% Cover	-0.01±0.03	0.002	0.00±0.02	0.000	-0.02±0.03	0.012	0.00±0.03	0.000	-0.01±0.03	0.003
Detritus Mass x % Cover	-0.00±0.01	0.000	-0.01±0.01	0.012	0.01±0.01	0.022	-0.01±0.01	0.005	0.02±0.01	0.030
Water Depth	0.01±0.04	0.003	0.00±0.03	0.000	-0.03±0.04	0.010	0.03±0.04	0.015	-0.03±0.03	0.014
% Cover x Water Depth	0.00±0.00	0.001	0.00±0.00	0.000	0.00±0.00	0.014	-0.00±0.00	0.004	0.00±0.00	0.007
Wetland (Water)	-0.23±0.40	0.006	0.32±0.37	0.012	0.18±0.42	0.003	-0.36±0.41	0.013	0.46±0.39	0.022
Sediment Type	-0.57±0.55	0.017	0.45±0.52	0.012	0.15±0.58	0.001	-0.46±0.57	0.011	0.34±0.55	0.006
Year	0.88±1.37	0.007	2.03±1.29	0.039	-0.05±1.44	0.000	0.31±1.41	0.000	1.52±1.36	0.020
Detritus Mass x Year	0.91±0.67	0.018	-1.61±0.82	0.060	-1.17±0.92	0.026	0.93±0.89	0.018	-0.73±0.86	0.012
Percent Cover x Year	-0.01±0.01	0.008	-0.01±0.01	0.007	0.01±0.01	0.007	0.01±0.01	0.005	-0.02±0.01	0.031
Water Depth x Year	-0.02±0.05	0.003	-0.03±0.05	0.005	-0.01±0.05	0.000	-0.06±0.05	0.024	-0.01±0.05	0.000
Wetland (Water) x Year	0.16±0.58	0.001	-0.99±0.55	0.050	-0.29±0.62	0.004	0.77±0.60	0.027	-0.60±0.58	0.017
Sediment Type x Year	-0.04±-0.70	0.000	-1.20±0.60	0.052	0.19±0.73	0.001	0.23±0.71	0.002	-1.23±0.69	0.050
Total		0.07		0.16		0.04		0.06		0.16
Associated Species +ve	<i>Dicrotendipes</i> <i>Chironomus</i>		<i>Ablabesmyia</i>		<i>Enallagma</i> <i>Cricotopus (Isocladius)</i>		<i>Eukiefferiella</i>		<i>Paratanytarsus</i>	
Associated Species -ve	<i>Ceratopogonidae</i>		<i>Cricotopus</i>		<i>Procladius</i>					

**Appendix 3.** Summary of multiple regression analysis of principal component scores summarizing relative abundances of zoobenthic taxa in all core samples (n=60).

<u>CORES</u>	PC I	R <sup>2</sup>	PC II	R <sup>2</sup>	PC III	R <sup>2</sup>	PC IV	R <sup>2</sup>
Intercept	0.74±0.71		-0.33±0.95		1.07±0.89		0.26±0.95	
Detritus Mass	0.10±0.07	0.033	0.04±0.10	0.004	-0.09±0.09	0.021	-0.07±0.10	0.007
% Cover	0.01±0.01	0.020	0.01±0.01	0.002	-0.01±0.01	0.017	-0.01±0.01	0.006
Detritus Mass x % Cover	-0.00±0.00	0.010	-0.00±0.00	0.003	0.00±0.00	0.006	0.00±0.00	0.002
Water Depth	-0.00±0.02	0.002	0.00±0.03	0.000	-0.05±0.03	0.049	0.03±0.03	0.008
% Cover x Water Depth	0.00±0.00	0.004	0.00±0.00	0.000	0.00±0.00*	0.043	-0.00±0.00	0.000
Wetland (Water)	-1.65±0.26***	0.426	-0.32±0.35	0.013	-0.18±0.33	0.004	-0.19±0.35	0.004
Sediment Type	0.02±0.40	0.000	-0.01±0.54	0.000	0.03±0.50	0.000	-0.61±0.54	0.021
Year	-1.11±0.89	0.022	-0.46±1.19	0.004	-0.70±1.11	0.010	0.48±1.19	0.002
Detritus Mass x Year	-0.12±0.10	0.023	0.20±0.13	0.037	0.00±0.12	0.000	0.12±0.13	0.012
Percent Cover x Year	-0.01±0.01	0.015	-0.00±0.01	0.000	0.01±0.01	0.019	0.00±0.01	0.001
Water Depth x Year	0.02±0.03	0.008	0.04±0.04	0.008	0.03±0.04	0.004	-0.08±0.04	0.059
Wetland (Water) x Year	1.26±0.43**	0.144	-0.19±0.58	0.002	-0.93±0.54	0.053	0.99±0.58	0.048
Sediment Type x Year	-0.27±0.48	0.006	0.58±0.65	0.015	-0.17±0.60	0.001	0.42±0.64	0.007
Total		0.54		0.17		0.28		0.18
Associated Species +ve	Oligochaeta Nematoda Trichoptera		Anisoptera Gastropoda					
Associated Species -ve	Chironomidae				Ceratopogonidae		<i>Enallagma</i>	

**Appendix 4.** Summary of multiple regression analysis of principal component scores summarizing relative abundances of zoobenthic taxa in initial subset of sweep samples (n=60)

<u>SWEEPS</u>	PC I	R <sup>2</sup>	PC II	R <sup>2</sup>	PC III	R <sup>2</sup>	PC IV	R <sup>2</sup>
Intercept	1.83±0.30***		-0.35±0.48		1.22±0.53*		0.45±0.57	
Detritus Mass	-0.06±0.19	0.000	-0.01±0.30	0.000	0.14±0.33	0.002	-0.19±0.36	0.002
% Cover	-0.00±0.00	0.003	-0.00±0.01	0.002	0.02±0.01*	0.042	-0.01±0.01	0.010
Detritus Mass x % Cover	0.01±0.00	0.023	-0.00±0.00	0.000	-0.01±0.01	0.014	0.01±0.01	0.008
Water Depth	-0.01±0.01	0.012	-0.01±0.02	0.001	0.04±0.02	0.026	-0.02±0.02	0.006
% Cover x Water Depth	-0.00±0.00	0.000	-0.00±0.00	0.000	-0.00±0.00*	0.032	0.00±0.00	0.005
Wetland (Water)	-1.98±0.13***	0.649	0.37±0.21	0.025	0.66±0.23**	0.063	0.39±0.24	0.020
Sediment Type	-0.24±0.17	0.017	0.26±0.26	0.008	0.27±0.29	0.007	-0.24±0.31	0.005
Year	-0.77±0.41	0.028	1.81±0.64 **	0.059	-0.47±0.71	0.003	0.25±0.77	0.000
Detritus Mass x Year	0.09±0.19	0.002	0.02±0.30	0.000	-0.18±0.33	0.002	0.16±0.36	0.002
Percent Cover x Year	0.00±0.00	0.000	-0.00±0.01	0.000	0.00±0.01	0.002	0.01±0.01	0.015
Water Depth x Year	0.00±0.02	0.000	0.00±0.03	0.000	0.01±0.03	0.001	-0.02±0.03	0.005
Wetland (Water) x Year	0.51±0.19*	0.054	-1.51±0.30***	0.164	-1.54±0.33***	0.144	-0.91±0.36*	0.048
Sediment Type x Year	-0.11±0.23	0.002	-0.54±0.37	0.017	-0.15±0.41	0.001	0.43±0.44	0.008
Total		0.76		0.39		0.26		0.13
Associated Species +ve	Chironomidae		Corixidae					
Associated Species -ve	Baetidae Hydrachnidae Gastropoda		Oligochaeta Nematoda		<i>Enallagma</i>		<i>Ceratopogonidae</i>	



**Appendix 5.** ANOVA of plant percent cover for CT and reference sediment plots in the 4-m CT and the SW

	df	SS	MS	F	p
Intercept	1	166.15	166.15	693.651	0.000
Wetland	1	3.17	3.17	13.226	0.000
Sed	1	15.28	15.28	63.801	0.000
Wetland *Sed	1	10.12	10.12	42.246	0.000
Error	114	27.31	0.24		
Total	117	55.03			

**Appendix 6.** ANOVA of detritus mass for CT and reference sediment plots in the 4-m CT and the SW.

	df	SS	MS	F	p
Intercept	1	40.07	40.07	84.799	0.000
Wetland	1	0.29	0.29	0.612	0.436
Sed	1	0.68	0.68	1.435	0.234
Wetland *Sed	1	0.61	0.61	1.283	0.261
Error	71	33.55	0.47		
Total	74	35.20			

**Appendix 7.** ANOVA of Invertebrate density for CT and reference sediment plots in the 4-m CT and the SW.

	Df	SS	MS	F	p
Intercept	1	49.68	49.68	269.496	0.000
Wetland	1	0.37	0.37	2.021	0.160
Sed	1	0.04	0.04	0.222	0.639
Wetland *Sed	1	0.02	0.02	0.117	0.734
Error	71	13.09	0.18		
Total	74	13.62			

**Appendix 8.** ANOVA of number of taxa (excluding taxa occurring once) for CT and reference sediment plots in the 4-m CT and the SW.

	df	SS	MS	F	p
Intercept	1	3998.28	3998.28	210.297	0.000
Wetland	1	7.09	7.09	0.373	0.543
Sed	1	437.52	437.52	23.012	0.000
Wetland *Sed	1	7.09	7.09	0.373	0.543
Error	71	1349.89	19.01		
Total	74	1811.95			

**Appendix 9.** Raw data from sweep samples taken August 2002 and August 2003 in SW and 4-m CT.

PLOT NUMBER	Chironimidae	Coenagrionidae <i>enallagma</i>	Corixidae	Ceratopogonidae	Oligochaeta	Gastropoda	MitesHydrachnidia	Baetidae	Adults/Pupae	Libellulidae	Chaoboridae	Nematoda	Hydra
<b>CTDemoPond</b>													
<b>49 samples AUG</b>													
<b>02</b>	49	35	27	27	5	1	5	2	6	2	10	1	0
A1 CT	30	0	2	1	0	0	0	0	0	0	0	0	0
A9 CT	52	1	0	0	0	0	0	0	0	0	0	0	0
A13 CT	45	0	10	6	0	0	0	0	0	0	0	0	0
A2 SW	149	3	1	0	0	0	0	0	0	0	0	0	0
A6 SW	74	0	0	2	0	0	0	1	0	0	0	0	0
A12 SW	132	4	0	0	0	0	0	0	0	0	0	0	0
A4 MC	80	4	0	3	0	0	0	0	0	0	0	0	0
A5 MC	80	3	5	0	0	0	0	0	0	0	0	0	0
A11 MC	25	2	1	0	1	0	0	0	0	0	0	0	0
B5 SW	97	0	0	0	1	0	1	0	0	0	1	0	0
B12 SW	123	7	3	0	0	0	0	0	4	0	0	0	0
B15 SW	265	9	1	0	0	0	1	0	1	0	1	0	0
B13 MC	200	6	0	2	0	0	0	0	1	0	0	0	0
C5 CT	86	1	3	4	0	0	1	0	0	0	1	0	0
C8 CT	43	0	4	0	0	0	0	0	0	0	1	0	0
C10 CT	22	1	2	0	0	0	0	0	2	0	0	0	0
C12 CT	47	0	1	1	0	0	0	0	0	0	0	0	0
C1 SW	178	10	2	1	0	0	0	0	0	0	0	0	0
C2 SW	327	19	1	6	0	0	0	0	0	0	0	0	0
C9 SW	74	1	6	1	0	0	0	0	0	0	2	0	0
C14 SW	70	10	0	0	0	0	0	0	0	0	0	0	0
C4 MC	256	6	1	3	0	0	0	0	1	0	0	0	0
C6 MC	117	3	1	1	0	0	0	0	0	0	2	0	0
C15 MC	60	0	2	4	0	0	0	0	0	0	0	0	0
D6 CT	74	5	3	7	0	0	0	0	0	0	1	0	0
D1 SW	569	15	2	10	0	0	0	0	0	0	0	0	0
D8 SW	66	13	2	5	0	0	0	0	0	1	0	0	0
D12 SW	451	21	1	0	0	0	0	0	0	0	1	0	0
D2 MC	154	4	0	0	1	0	0	0	0	0	0	0	0
D4 MC	111	2	0	0	0	0	2	0	0	0	0	0	0
E7 CT	8	1	0	1	0	0	0	0	0	0	0	0	0
E13 CT	13	0	0	0	0	0	0	0	0	0	1	0	0
E14 CT	17	0	0	1	1	0	0	0	0	0	0	0	0
E15 CT	18	0	0	0	0	0	0	0	0	0	0	0	0
E1 SW	16	2	0	0	0	0	0	0	0	0	0	0	0

PLOT NUMBER	Chironimidae	Coenagrionidae <i>enallagma</i>	Corixidae	Ceratopogonidae	Oligochaeta	Gastropoda	MitesHydrachnidia	Baetidae	Adults/Pupae	Libellulidae	Chaoboridae	Nematoda	Hydra
<b>AUG 02</b>													
E5 SW	66	4	1	3	0	0	0	0	0	0	0	0	0
E6 SW	8	1	0	0	0	0	0	0	0	0	0	0	0
E10 SW	103	1	0	0	0	0	0	0	0	0	0	0	0
E2 MC	34	3	0	1	0	0	0	0	0	0	0	0	0
E8 MC	22	1	0	7	0	0	0	0	0	0	0	0	0
E9 MC	10	0	2	1	0	0	0	0	0	0	0	0	0
E11 MC	26	1	1	3	0	0	0	0	0	0	0	0	0
F1 CT	8	0	0	1	0	7	0	8	1	2	1	0	0
F5 CT	23	0	0	3	0	0	1	0	0	0	0	0	0
F11 SW	65	4	1	1	0	0	0	0	0	0	0	0	0
F13 SW	39	5	1	0	0	0	0	0	0	0	0	0	0
F2 MC	46	3	0	0	0	0	0	0	0	0	0	0	0
F3 MC	29	2	2	0	4	0	0	0	0	0	0	0	0
F10 MC	37	0	0	4	0	0	0	0	0	0	0	1	0
<b>SUM</b>	4645	178	62	83	8	7	6	9	10	3	12	1	0

PLOT NUMBER	Amphipod	Caenidae	Halipidae	Dytiscidae	Ephemereilidae serratella	Aeshnidae	Saldidae	Culicidae	Sphaeriidae	Ephemereilidae ephemerella	Tricoptera total	Dixidae	Notonectidae
<b>AUG 02</b>													
<b>CTDemoPond</b>													
<b>49 samples</b>	0	0	1	2	0	1	1	0	0	0	1	1	1
A1 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A9 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A13 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A2 SW	0	0	0	1	0	0	0	0	0	0	0	0	0
A6 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
A12 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
A4 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
A5 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
A11 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
B5 SW	0	0	0	0	0	0	0	0	0	0	0	0	3
B12 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
B15 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
B13 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
C5 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C8 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C10 CT	0	0	0	1	0	0	0	0	0	0	0	0	0
C12 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C1 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C2 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C9 SW	0	0	0	0	0	1	0	0	0	0	0	0	0
C14 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C4 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
C6 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
C15 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
D6 CT	0	0	0	0	0	0	0	0	0	0	1	1	0
D1 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D8 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D12 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D2 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
D4 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
E7 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
E13 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
E14 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
E15 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
E1 SW	0	0	0	0	0	0	0	0	0	0	0	0	0

PLOT NUMBER	Amphipod	Caenidae	Halipidae	Dytiscidae	Ephemereillidae serratella	Aeshnidae	Salidae	Culicidae	Sphaeriidae	Ephemereillidae ephemerella	Tricoptera total	Dixidae	Notonectidae
<b>AUG 02</b>													
E5 SW	0	0	1	0	0	0	0	0	0	0	0	0	0
E6 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
E10 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
E2 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
E8 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
E9 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
E11 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
F1 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
F5 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
F11 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
F13 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
F2 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
F3 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
F10 MC	0	0	0	0	0	0	1	0	0	0	0	0	0
<b>SUM</b>	0	0	1	2	0	1	1	0	0	0	1	1	3

PLOT NUMBER	Gerridae	Hirudinea	Dolichopodidae	Ptychopteridae	Stratiomyidae	Spider	Siphonuridae	Empididae	Psychodidae <i>Pericoma</i>	Cordulidae	Elmidae <i>dubraphia</i>	Total numbers in sample
<b>AUG 02</b>	0	0	0	0	0	0	0	0	0	0	0	
<b>CTDemoPond 49</b>	0	0	0	0	0	0	0	0	0	0	0	
<b>samples</b>	0	0	0	0	0	0	0	0	0	0	0	
A1 CT	0	0	0	0	0	0	0	0	0	0	0	33
A9 CT	0	0	0	0	0	0	0	0	0	0	0	53
A13 CT	0	0	0	0	0	0	0	0	0	0	0	61
A2 SW	0	0	0	0	0	0	0	0	0	0	0	154
A6 SW	0	0	0	0	0	0	0	0	0	0	0	77
A12 SW	0	0	0	0	0	0	0	0	0	0	0	136
A4 MC	0	0	0	0	0	0	0	0	0	0	0	87
A5 MC	0	0	0	0	0	0	0	0	0	0	0	88
A11 MC	0	0	0	0	0	0	0	0	0	0	0	29
B5 SW	0	0	0	0	0	0	0	0	0	0	0	103
B12 SW	0	0	0	0	0	0	0	0	0	0	0	137
B15 SW	0	0	0	0	0	0	0	0	0	0	0	278
B13 MC	0	0	0	0	0	0	0	0	0	0	0	209
C5 CT	0	0	0	0	0	0	0	0	0	0	0	96
C8 CT	0	0	0	0	0	0	0	0	0	0	0	48
C10 CT	0	0	0	0	0	0	0	0	0	0	0	28
C12 CT	0	0	0	0	0	0	0	0	0	0	0	49
C1 SW	0	0	0	0	0	0	0	0	0	0	0	191
C2 SW	0	0	0	0	0	0	0	0	0	0	0	353
C9 SW	0	0	0	0	0	0	0	0	0	0	0	85
C14 SW	0	0	0	0	0	0	0	0	0	0	0	80
C4 MC	0	0	0	0	0	0	0	0	0	0	0	267
C6 MC	0	0	0	0	0	0	0	0	0	0	0	124
C15 MC	0	0	0	0	0	0	0	0	0	0	0	66
D6 CT	0	0	0	0	0	0	0	0	0	0	0	92
D1 SW	0	0	0	0	0	0	0	0	0	0	0	596
D8 SW	0	0	0	0	0	0	0	0	0	0	0	87
D12 SW	0	0	0	0	0	0	0	0	0	0	0	474
D2 MC	0	0	0	0	0	0	0	0	0	0	0	159
D4 MC	0	0	0	0	0	0	0	0	0	0	0	115
E7 CT	0	0	0	0	0	0	0	0	0	0	0	10
E13 CT	0	0	0	0	0	0	0	0	0	0	0	14
E14 CT	0	0	0	0	0	0	0	0	0	0	0	19
E15 CT	0	0	0	0	0	0	0	0	0	0	0	18
E1 SW	0	0	0	0	0	0	0	0	0	0	0	18

PLOT NUMBER	Gerridae	Hirudinea	Dolichopodidae	Ptychopteridae	Stratiomyidae	Spider	Siphonuridae	Empididae	Psychodidae <i>Pericoma</i>	Cordulidae	Elmidae <i>dubraphia</i>	Total numbers in sample
<b>AUG 02</b>												
E5 SW	0	0	0	0	0	0	0	0	0	0	0	75
E6 SW	0	0	0	0	0	0	0	0	0	0	0	9
E10 SW	0	0	0	0	0	0	0	0	0	0	0	104
E2 MC	0	0	0	0	0	0	0	0	0	0	0	38
E8 MC	0	0	0	0	0	0	0	0	0	0	0	30
E9 MC	0	0	0	0	0	0	0	0	0	0	0	13
E11 MC	0	0	0	0	0	0	0	0	0	0	0	31
F1 CT	0	0	0	0	0	0	0	0	0	0	0	28
F5 CT	0	0	0	0	0	0	0	0	0	0	0	27
F11 SW	0	0	0	0	0	0	0	0	0	0	0	71
F13 SW	0	0	0	0	0	0	0	0	0	0	0	45
F2 MC	0	0	0	0	0	0	0	0	0	0	0	49
F3 MC	0	0	0	0	0	0	0	0	0	0	0	37
F10 MC	0	0	0	0	0	0	0	0	0	0	0	43
<b>SUM</b>	0	0	0	0	0	0	0	0	0	0	0	5034

PLOT NUMBER	Chironimidae	Coenagrionidae enallagma	Corixidae	Ceratopogonidae	Oligochaeta	Gastropoda	MitesHydrachnidia	Baetidae	Adults/Pupae	Libellulidae	Chaoboridae	Nematoda	Hydra
<b>AUG 02</b>													
<b>Shallow Wetland 30</b>													
<b>samples</b>	30	8	7	5	19	15	21	18	6	11	0	2	10
A2 CT	2	2	0	0	1	1	6	1	0	0	0	0	0
B5 CT	51	4	0	0	12	2	2	5	0	0	0	2	2
B2 SW	4	1	0	0	1	2	9	1	0	0	0	0	1
B4 SW	45	0	2	0	24	2	6	3	0	0	0	0	1
C1 CT	3	0	0	0	1	1	3	0	0	0	0	0	0
C3 CT	12	0	0	1	1	2	9	0	0	0	0	0	0
C14 CT	4	0	0	0	0	0	1	0	0	0	0	0	0
C8 SW	4	0	0	0	0	0	4	3	0	0	0	0	0
C11 SW	18	1	0	0	2	0	3	2	0	1	0	0	0
C12 SW	23	2	0	0	0	1	3	2	1	0	0	0	0
D8 CT	8	1	0	0	0	0	5	2	0	1	0	0	0
D15 CT	23	0	0	1	5	0	0	0	0	0	0	1	0
D3 SW	4	0	1	0	0	1	7	1	0	1	0	0	0
D6 SW	6	0	0	0	0	0	3	0	0	0	0	0	1
D7 SW	11	0	0	0	2	5	4	2	2	1	0	0	3
D9 SW	10	1	0	0	0	0	1	1	0	0	0	0	0
D13 SW	67	0	0	0	4	7	13	2	1	0	0	0	5
E5 CT	9	0	1	0	0	2	0	0	0	0	0	0	0
E6 CT	23	0	0	0	8	3	0	4	0	0	0	0	0
E10 CT	8	0	2	0	2	0	0	1	0	3	0	0	0
E3 SW	9	0	0	0	1	0	0	3	0	0	0	0	1
E7 SW	37	0	0	1	1	0	3	2	2	1	0	0	2
E13 SW	33	24	0	1	0	0	6	0	2	3	0	0	2
E14 SW	24	0	1	0	2	0	7	0	1	2	0	0	0
F4 CT	15	0	1	0	0	0	0	4	0	2	0	0	0
F12 CT	10	0	0	0	8	0	0	0	0	2	0	0	0
F15 CT	18	0	1	1	3	1	0	0	0	0	0	0	0
F5 SW	46	0	0	0	1	1	0	2	0	2	0	0	0
F6 SW	5	0	0	0	0	0	4	0	0	0	0	0	0
F9 SW	20	0	0	0	1	4	1	0	0	0	0	0	1
<b>SUM</b>	552	36	9	5	80	35	100	41	9	19	0	3	19



PLOT NUMBER AUG 02 Shallow Wetland 30 samples	Amphipod	Caenidae	Halipidae	Dytiscidae	Ephemereilidae serratella	Aeshnidae	Saldidae	Culicidae	Sphaeriidae	Ephemereilidae ephemerella	Tricoptera total	Dixidae	Notonectidae
A2 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
B5 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
B2 SW	1	0	0	0	0	0	0	0	0	0	0	0	0
B4 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C1 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C3 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C14 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C8 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C11 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C12 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D8 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
D15 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
D3 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D6 SW	0	0	0	0	0	0	0	0	0	0	0	1	1
D7 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D9 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D13 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
E5 CT	0	0	1	0	0	0	0	0	0	4	0	0	0
E6 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
E10 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
E3 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
E7 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
E13 SW	0	0	0	0	0	0	1	0	0	0	0	0	0
E14 SW	0	0	0	0	0	1	1	0	0	0	0	0	0
F4 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
F12 CT	0	0	0	0	3	0	0	0	0	0	0	0	0
F15 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
F5 SW	0	0	1	0	0	0	0	0	0	0	0	0	0
F6 SW	0	0	0	0	2	0	0	0	0	2	0	0	0
F9 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>SUM</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>6</b>	<b>0</b>	<b>1</b>	<b>1</b>

PLOT NUMBER	Gerridae	Hirudinea	Dolichopodidae	Ptychopteridae	Stratiomyidae	Spider	Siphonuridae	Empididae	Psychodidae <i>Pericoma</i>	Cordulidae	Elmidae <i>dubraphia</i>	Total numbers in sample
<b>AUG 02</b>												
<b>Shallow Wetland 30</b>												
<b>samples</b>	0	1	1	1	0	0	0	0	0	0	0	
A2 CT	0	0	0	0	0	0	0	0	0	0	0	13
B5 CT	0	0	0	0	0	0	0	0	0	0	0	80
B2 SW	0	0	0	0	0	0	0	0	0	0	0	20
B4 SW	0	0	0	0	0	0	0	0	0	0	0	83
C1 CT	0	0	0	1	0	0	0	0	0	0	0	9
C3 CT	0	0	0	0	0	0	0	0	0	0	0	25
C14 CT	0	0	0	0	0	0	0	0	0	0	0	5
C8 SW	0	0	0	0	0	0	0	0	0	0	0	11
C11 SW	0	0	0	0	0	0	0	0	0	0	0	27
C12 SW	0	0	0	0	0	0	0	0	0	0	0	32
D8 CT	0	0	0	0	0	0	0	0	0	0	0	17
D15 CT	0	0	0	0	0	0	0	0	0	0	0	30
D3 SW	0	0	0	0	0	0	0	0	0	0	0	15
D6 SW	0	0	0	0	0	0	0	0	0	0	0	12
D7 SW	0	0	1	0	0	0	0	0	0	0	0	31
D9 SW	0	1	0	0	0	0	0	0	0	0	0	14
D13 SW	0	0	0	0	0	0	0	0	0	0	0	99
E5 CT	0	0	0	0	0	0	0	0	0	0	0	17
E6 CT	0	0	0	0	0	0	0	0	0	0	0	38
E10 CT	0	0	0	0	0	0	0	0	0	0	0	16
E3 SW	0	0	0	0	0	0	0	0	0	0	0	14
E7 SW	0	0	0	0	0	0	0	0	0	0	0	49
E13 SW	0	0	0	0	0	0	0	0	0	0	0	72
E14 SW	0	0	0	0	0	0	0	0	0	0	0	39
F4 CT	0	0	0	0	0	0	0	0	0	0	0	22
F12 CT	0	0	0	0	0	0	0	0	0	0	0	23
F15 CT	0	0	0	0	0	0	0	0	0	0	0	24
F5 SW	0	0	0	0	0	0	0	0	0	0	0	53
F6 SW	0	0	0	0	0	0	0	0	0	0	0	13
F9 SW	0	0	0	0	0	0	0	0	0	0	0	27
<b>SUM</b>	0	1	1	1	0	0	0	0	0	0	0	

PLOT NUMBER	Chironimidae	Coenagrionidae <i>enallagma</i>	Corixidae	Ceratopogonidae	Oligochaeta	Gastropoda	MitesHydrachnidia	Baetidae	Adults/Pupae	Libellulidae	Chaoboridae	Nematoda	Hydra
<b>CTDemoPond 33</b>													
<b>samples AUG 03</b>	33	19	29	18	2	1	0	0	9	2	0	3	0
A1 CT	93	0	5	3	0	0	0	0	0	0	0	0	0
A9 CT	35	0	3	0	0	0	0	0	0	0	0	0	0
A13 CT	11	2	2	1	0	0	0	0	1	0	0	0	0
A14 CT	58	0	11	0	0	0	0	0	8	0	0	0	0
A15 CT	40	0	3	1	0	0	0	0	0	0	0	0	0
A2 SW	157	0	3	0	0	0	0	0	0	0	0	0	0
A3 SW	136	2	1	1	0	0	0	0	0	0	0	0	0
A6 SW	112	0	2	8	1	0	0	0	1	2	0	0	0
A8 SW	58	1	1	1	0	0	0	0	0	0	0	0	0
A12 SW	75	0	1	3	0	0	0	0	1	0	0	0	0
A10 MC	212	1	0	1	0	0	0	0	4	0	0	0	0
B1 CT	148	1	4	3	0	0	0	0	0	0	0	0	0
B2 CT	184	0	1	1	0	0	0	0	0	0	0	0	0
B4 CT	35	2	9	1	0	0	0	0	0	0	0	0	0
B7 CT	87	1	7	0	0	0	0	0	2	0	0	0	0
B11 CT	223	1	13	8	0	0	0	0	0	0	0	0	0
B5 SW	36	4	2	0	0	0	0	0	2	0	0	0	0
B8 SW	16	0	7	0	0	0	0	0	0	0	0	1	0
B12 SW	319	5	9	5	0	0	0	0	0	0	0	0	0
B14 SW	131	9	1	0	0	0	0	0	0	0	0	5	0
B15 SW	228	0	8	4	0	0	0	0	1	0	0	0	0
C5 CT	25	0	1	0	0	0	0	0	0	0	0	0	0
C8 CT	51	0	5	0	0	1	0	0	0	0	0	0	0
C10 CT	62	2	6	0	0	0	0	0	0	0	0	0	0
C11 CT	121	0	18	2	0	0	0	0	0	0	0	0	0
C12 CT	93	3	5	0	0	0	0	0	0	0	0	0	0
C1 SW	27	0	0	0	0	0	0	0	0	0	0	0	0
C2 SW	75	1	7	1	1	0	0	0	0	0	0	0	0
C3 SW	125	6	5	0	0	0	0	0	0	0	0	0	0
C9 SW	52	1	10	2	0	0	0	0	2	0	0	1	0
C14 SW	354	5	0	4	0	0	0	0	0	0	0	0	0
D1 SW	299	10	2	0	0	0	0	0	0	1	0	0	0
D14 SW	165	12	0	0	0	0	0	0	0	0	0	0	0
<b>SUM</b>	3843	69	152	50	2	1	0	0	22	3	0	7	0

PLOT NUMBER	Amphipoda	Caenidae	Halipidae	Dytiscidae	Ephemereilidae serratella	Aeshnidae	Saldidae	Culicidae	Sphaeriidae	Ephemereilidae ephemerella	Tricoptera total	Dixidae	Notonectidae
<b>CTDemoPond 33</b>	0	0	0	3	0	0	0	3	0	0	1	0	0
<b>samples AUG 03</b>	0	0	0	0	0	0	0	0	0	0	0	0	0
A1 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A9 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A13 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A14 CT	0	0	0	0	0	0	0	1	0	0	0	0	0
A15 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A2 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
A3 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
A6 SW	0	0	0	1	0	0	0	0	0	0	0	0	0
A8 SW	0	0	0	0	0	0	0	1	0	0	0	0	0
A12 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
A10 MC	0	0	0	0	0	0	0	0	0	0	0	0	0
B1 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
B2 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
B4 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
B7 CT	0	0	0	0	0	0	0	0	0	0	1	0	0
B11 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
B5 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
B8 SW	0	0	0	0	0	0	0	1	0	0	0	0	0
B12 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
B14 SW	0	0	0	1	0	0	0	0	0	0	0	0	0
B15 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C5 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C8 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C10 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C11 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C12 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
C1 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C2 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C3 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C9 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C14 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D1 SW	0	0	0	1	0	0	0	0	0	0	0	0	0
D14 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>SUM</b>	0	0	0	3	0	0	0	3	0	0	1	0	0

PLOT NUMBER	Gerridae	Hirudinea	Dolichopodidae	Ptychopteridae	Stratiomyidae	Spider	Siphonuridae	Empididae	Psychodidae <i>Pericoma</i>	Corduliidae	Elmidae <i>dubraphia</i>	total number in sample
<b>CTDemoPond 33</b>	0	0	0	0	0	0	0	0	0	0	0	
<b>samples AUG 03</b>	0	0	0	0	0	0	0	0	0	0	0	
A1 CT	0	0	0	0	0	0	0	0	0	0	0	101
A9 CT	0	0	0	0	0	0	0	0	0	0	0	38
A13 CT	0	0	0	0	0	0	0	0	0	0	0	17
A14 CT	0	0	0	0	0	0	0	0	0	0	0	78
A15 CT	0	0	0	0	0	0	0	0	0	0	0	44
A2 SW	0	0	0	0	0	0	0	0	0	0	0	160
A3 SW	0	0	0	0	0	0	0	0	0	0	0	140
A6 SW	0	0	0	0	0	0	0	0	0	0	0	127
A8 SW	0	0	0	0	0	0	0	0	0	0	0	62
A12 SW	0	0	0	0	0	0	0	0	0	0	0	80
A10 MC	0	0	0	0	0	0	0	0	0	0	0	218
B1 CT	0	0	0	0	0	0	0	0	0	0	0	156
B2 CT	0	0	0	0	0	0	0	0	0	0	0	186
B4 CT	0	0	0	0	0	0	0	0	0	0	0	47
B7 CT	0	0	0	0	0	0	0	0	0	0	0	98
B11 CT	0	0	0	0	0	0	0	0	0	0	0	245
B5 SW	0	0	0	0	0	0	0	0	0	0	0	44
B8 SW	0	0	0	0	0	0	0	0	0	0	0	25
B12 SW	0	0	0	0	0	0	0	0	0	0	0	338
B14 SW	0	0	0	0	0	0	0	0	0	0	0	147
B15 SW	0	0	0	0	0	0	0	0	0	0	0	241
C5 CT	0	0	0	0	0	0	0	0	0	0	0	26
C8 CT	0	0	0	0	0	0	0	0	0	0	0	57
C10 CT	0	0	0	0	0	0	0	0	0	0	0	70
C11 CT	0	0	0	0	0	0	0	0	0	0	0	141
C12 CT	0	0	0	0	0	0	0	0	0	0	0	101
C1 SW	0	0	0	0	0	0	0	0	0	0	0	27
C2 SW	0	0	0	0	0	0	0	0	0	0	0	85
C3 SW	0	0	0	0	0	0	0	0	0	0	0	136
C9 SW	0	0	0	0	0	0	0	0	0	0	0	68
C14 SW	0	0	0	0	0	0	0	0	0	0	0	363
D1 SW	0	0	0	0	0	0	0	0	0	0	0	313
D14 SW	0	0	0	0	0	0	0	0	0	0	0	177
<b>SUM</b>	0	0	0	0	0	0	0	0	0	0	0	

PLOT NUMBER	Chironimidae	Coenagrionidae <i>enallagma</i>	Corixidae	Ceratopagonidae	Oligochaeta	Gastropoda	MitesHydrachnidia	Baetidae	Adults/Pupae	Libellulidae	Chaoboridae	Nematoda	Hydra
<b>Shallow Wetland 29</b>													
<b>samplesAUG 03</b>													
29	15	3	13	26	20	4	10	3	6	9	10	1	
A3 CT	30	0	0	0	6	0	0	0	0	0	0	2	0
A6 CT	11	0	0	0	3	1	0	0	0	0	0	0	0
A8 CT	13	0	2	0	0	4	0	1	0	0	0	0	0
A1 SW	116	0	0	0	39	4	0	0	0	0	1	4	0
B8 CT	61	0	1	1	20	4	0	1	0	0	1	0	0
B12 CT	133	1	0	2	24	7	0	0	0	2	0	0	0
B15 CT	130	0	0	0	13	3	0	0	0	5	0	72	0
B1 SW	19	0	0	0	0	0	0	0	0	0	1	1	0
B4 SW	40	0	0	2	13	2	3	0	0	0	0	0	0
B7 SW	22	2	0	0	10	4	0	0	0	1	0	0	0
B11 SW	113	2	1	0	10	3	0	1	1	0	0	5	0
C3 CT	127	1	0	0	75	0	0	1	2	0	3	0	0
C14 CT	38	2	0	3	2	0	0	0	0	0	0	0	0
C5 SW	47	0	0	1	8	1	0	0	0	0	0	1	0
C8 SW	24	1	0	0	16	10	1	0	0	0	2	0	0
C10 SW	69	0	0	0	12	0	0	1	0	0	0	0	0
C11 SW	32	0	0	0	3	0	0	0	0	0	0	0	0
C12 SW	47	4	0	2	4	2	0	2	0	0	2	0	0
D1 CT	168	1	0	7	110	6	0	0	0	0	0	2	0
D8 CT	170	3	0	3	7	1	0	2	0	0	0	0	0
D12 CT	38	0	0	0	0	0	0	0	0	0	1	0	0
D14 CT	52	2	0	1	5	4	0	0	0	0	0	0	0
D15 CT	72	4	0	1	6	8	0	0	1	0	0	0	0
D3 SW	63	2	0	0	54	5	4	0	0	0	0	0	0
D7 SW	40	1	0	0	60	0	0	0	0	0	0	5	1
D13 SW	38	2	0	1	13	0	1	2	0	1	0	0	0
E13 SW	57	5	0	0	14	19	0	4	0	1	1	1	0
F15 CT	107	0	0	3	31	11	0	0	0	1	1	1	0
F7 SW	122	0	0	3	4	4	0	2	0	0	0	0	0
<b>SUM</b>	1999	33	4	30	562	103	9	17	4	11	13	94	1

PLOT NUMBER	Amphipoda	Caenidae	Halipidae	Dytiscidae	Ephemereilidae serratella	Aeshnidae	Salidae	Culicidae	Sphaeriidae	Ephemereilidae ephemerella	Tricoptera total	Dixidae	Notonectidae
<b>Shallow Wetland 29</b>													
<b>samples AUG 03</b>	9	9	6	1	2	2	0	0	3	0	0	0	0
A3 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A6 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A8 CT	0	0	0	0	0	0	0	0	0	0	0	0	0
A1 SW	0	0	0	0	0	0	0	0	2	0	0	0	0
B8 CT	0	0	0	0	0	2	0	0	0	0	0	0	0
B12 CT	0	0	0	0	0	0	0	0	1	0	0	0	0
B15 CT	0	0	7	9	0	0	0	0	0	0	0	0	0
B1 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
B4 SW	1	1	2	0	0	0	0	0	0	0	0	0	0
B7 SW	0	1	3	0	2	0	0	0	0	0	0	0	0
B11 SW	1	4	2	0	0	0	0	0	0	0	0	0	0
C3 CT	1	0	0	0	0	0	0	0	0	0	0	0	0
C14 CT	1	0	0	0	0	0	0	0	0	0	0	0	0
C5 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C8 SW	1	0	1	0	0	0	0	0	0	0	0	0	0
C10 SW	0	0	0	0	4	0	0	0	0	0	0	0	0
C11 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
C12 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D1 CT	0	4	0	0	0	0	0	0	0	0	0	0	0
D8 CT	0	1	0	0	0	0	0	0	0	0	0	0	0
D12 CT	0	1	0	0	0	0	0	0	0	0	0	0	0
D14 CT	2	3	0	0	0	0	0	0	0	0	0	0	0
D15 CT	0	4	0	0	0	0	0	0	0	0	0	0	0
D3 SW	0	0	0	0	0	0	0	0	0	0	0	0	0
D7 SW	6	0	0	0	0	1	0	0	0	0	0	0	0
D13 SW	5	0	2	0	0	0	0	0	0	0	0	0	0
E13 SW	0	2	0	0	0	0	0	0	0	0	0	0	0
F15 CT	0	0	0	0	0	0	0	0	1	0	0	0	0
F7 SW	1	0	0	0	0	0	0	0	0	0	0	0	0
<b>SUM</b>	19	21	17	9	6	3	0	0	4	0	0	0	0

PLOT NUMBER	Gerridae	Hirudinea	Dolichopodidae	Ptychopteridae	Stratiomyidae	Spider	Siphonuridae	Empididae	Psychodidae <i>Pericoma</i>	Cordulidae	Elmidae <i>dubraphia</i>	total number in sample
<b>Shallow Wetland 29</b>												
<b>samples AUG 03</b>	1	0	0	0	1	1	1	0	0	0	0	
A3 CT	0	0	0	0	0	0	0	0	0	0	0	38
A6 CT	0	0	0	0	0	0	0	0	0	0	0	15
A8 CT	0	0	0	0	0	0	0	0	0	0	0	20
A1 SW	0	0	0	0	0	0	0	0	0	0	0	166
B8 CT	0	0	0	0	0	0	0	0	0	0	0	91
B12 CT	0	0	0	0	0	0	0	0	0	0	0	170
B15 CT	0	0	0	0	0	0	0	0	0	0	0	239
B1 SW	0	0	0	0	0	0	0	0	0	0	0	21
B4 SW	0	0	0	0	0	0	0	0	0	0	0	64
B7 SW	0	0	0	0	1	1	0	0	0	0	0	47
B11 SW	0	0	0	0	0	0	0	0	0	0	0	143
C3 CT	0	0	0	0	0	0	0	0	0	0	0	210
C14 CT	0	0	0	0	0	0	0	0	0	0	0	46
C5 SW	0	0	0	0	0	0	0	0	0	0	0	58
C8 SW	0	0	0	0	0	0	0	0	0	0	0	56
C10 SW	0	0	0	0	0	0	0	0	0	0	0	86
C11 SW	0	0	0	0	0	0	0	0	0	0	0	35
C12 SW	0	0	0	0	0	0	0	0	0	0	0	63
D1 CT	0	0	0	0	0	0	0	0	0	0	0	298
D8 CT	0	0	0	0	0	0	0	0	0	0	0	187
D12 CT	0	0	0	0	0	0	0	0	0	0	0	40
D14 CT	0	0	0	0	0	0	0	0	0	0	0	69
D15 CT	0	0	0	0	0	0	0	0	0	0	0	96
D3 SW	0	0	0	0	0	0	0	0	0	0	0	128
D7 SW	0	0	0	0	0	0	0	0	0	0	0	114
D13 SW	0	0	0	0	0	0	0	0	0	0	0	65
E13 SW	0	0	0	0	0	0	0	0	0	0	0	104
F15 CT	1	0	0	0	0	0	1	0	0	0	0	158
F7 SW	0	0	0	0	0	0	0	0	0	0	0	136
<b>SUM</b>	1	0	0	0	1	1	1	0	0	0	0	



### Vita Auctoris

Lyndon Barr was born in 1979 in St. Thomas, Ontario. He graduated from East Elgin Secondary School in 1996. From there he went to the University of Western Ontario where he obtained an H. B. Sc. in ecology and evolution. In 2006 he went to Queen's University where he obtained a B. Ed. He is currently a candidate for the Master's degree in Biology at the University of Windsor.