# ZOOBENTHIC SUCCESSION IN CONSTRUCTED WETLANDS OF THE FORT McMURRAY OIL SANDS REGION: DEVELOPING A MEASURE OF ZOOBENTHIC RECOVERY

by

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A Thesis
Submitted to the Faculty of Graduate Studies and Research
through the Department of Biological Sciences
in Partial Fulfillment of the Requirements for the
Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

2003

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## ABSTRACT

This study investigated the effect of oil sands process material (OSPM) on the zoobenthic community of constructed wetlands in the Fort McMurray oil sands region. The unique characteristics of OSPM-affected wetlands may modify the successional trajectory of invertebrate communities compared to that of high or low-conductivity reference wetlands.

The zoobenthic community of 31 wetlands, aged 0 to 30 years, was simultaneously sampled, allowing inference into the chronological sequence of change that results with wetland succession. Wetlands were categorised *a priori* into one of three classes: low conductivity (<700 μS/cm) or high conductivity (700-2,500 μS/cm) reference wetlands or OSPM-affected wetlands (700-4,000 μS/cm) containing tailings and/or water from bitumen extraction. Invertebrate communities in each wetland were assessed using core, artificial substrate, and sweep net sampling methods.

Zoobenthic richness and abundance reached asymptotes in reference wetlands aged 5 years and older. Richness, but not abundance, was significantly lower in young OSPM-affected wetlands than in equally young reference wetlands. Water pH, naphthenic acid concentration, detrital abundance, conductivity, salinity and sediment ORP were all significantly associated with taxa richness. Zoobenthic abundance was correlated with extent of macrophyte development, water conductivity, and detrital abundance. Water toxicity, sediment characteristics, and development of macrophytes may initially limit the zoobenthic community development in OSPM-affected wetlands.

Principal components analysis and discriminant function analysis were used to classify each OSPM-affected wetland as being "equivalent to young" or "equivalent to

mature" reference wetlands. Tanypodinae and Orthocladiinae midges and dytiscid beetles characterised young wetlands. Gastropods, Chironomini midges, caenid and baetid mayflies, and amphipods characterised mature reference wetlands.

Metrics developed for artificial substrate and sweep samples best separated young from mature reference wetlands. The sweep sample metric provided the most robust benthic community description. However, metrics must be verified against other saline systems in western Canada.

Restoration of mined areas to pre-mining conditions of diversity and abundance of habitat types, using wetlands as a component of a reclamation strategy, is a viable option. Older "one-time-addition" OSPM-affected wetlands must be re-sampled to determine if they will develop as diverse a zoobenthic community as local reference wetlands.

## ACKNOWLEDGEMENTS

There are a great number of people I'd like to thank for their assistance with this project. Dr. Jan Ciborowski for his thoughtful advising, dedication to research, statistical knowledge at all hours and days of the week, and his drive in the field. My committee members, Dr. Lynda Corkum (Department of Biological Sciences) and Dr. Stan Reitsma (Department of Civil and Environmental Engineering) provided guidance and insight. Many people assisted me both in the lab and in the field including Kevin Ganshorn, Paige Short, Elisabeth Sabo, Jesse Ballairgeon, Kathryn Kuntz, Laura St. John, Erin Copeland, Sepal Bonni, Stella Chan, and Manjit Shah. The great staff at the Syncrude Environmental Complex provided much needed logistical and field support. Many thanks to Neil Rutley, Terry Van Meer, Clara Qualizza, and Joanne Hogg for their assistance and information provided on wetlands in the region. The equally great staff at Suncor Mine Engineering and Reclamation also provided much needed logistical support on-site (including several mud-induced vehicle extractions), thank you Leo Paquin, Wayne Tedder and Bruce Anderson, and summer staff. Dr. Michael MacKinnon and the staff at Syncrude Canada Research Analytical Group provided water chemistry analysis. Dr. MacKinnon provided advice on sampling locations and graciously provided editorial comments for this thesis.

I wish to thank the great people associated with the Department of Entomology at the University of Alberta (J.A. Heibert, J.R. Spence, D.A. Craig, K.M. Fry, K.A. Justus, and S. Bjornson) for first stimulating my interest in invertebrates and research, and for all of their encouragement while working on my thesis. And of course, my lab mates, and fellow graduate students for sharing the stress and beer so necessary for graduate work.

I would like to extend a great big thank you to all of my family and friends for their support. Lastly, I would like to thank André Bachteram for helping me keep things in perspective, and for his support in more ways than I can count. He tolerated my long absences with good humour and the stressful times with even greater humour.

This study was supported by research grants received by Jan Ciborowski from Syncrude Canada Limited, the Environmental Science and Technology Alliance of Canada (ESTAC), and the Natural Sciences and Engineering Research Council of Canada (NSERC). In kind support and logistic assistance from Syncrude Canada Limited, Suncor Energy Incorporated, and Golder Associates Limited are also gratefully acknowledged.

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# Chapter 1

# General Introduction

## Introduction

Wetland reclamation has become an important area of scientific study in the past 30 years. Since arrival of Europeans to North America over 50% of wetlands have been lost in the contiguous United States (Bildstein *et al.*, 1991 in Murkin 1998), and 85% loss of wetlands has occurred in southern Ontario (Murkin 1998). Wetlands are an integral part of the landscape. They act as a buffer between upland areas and aquatic areas (Hook 1993; Gopal 1999). They are the kidneys of the land and the home to many plants and animals. The development of wetlands is affected by many factors. Hydrology, underlying geology and topography are key geophysical factors that determine the effectiveness of a constructed wetland (Brown *et al.* 1997; Sistani *et al.* 1999; Stolt *et al.* 2000). The development of the vegetation is also influenced by hydroperiod. The plant community, along with hydroperiod and microtopography, then influences the benthic invertebrate and vertebrate community development.

The Fine Tailings Fundamentals Consortium (FTFC) was a group of provincial and federal government agencies (Environment Canada, National Research Council, Alberta Energy, and Alberta Research Council), oil sands companies and agencies (Syncrude Canada Ltd., Suncor Energy Inc., OLSO (Other Six Lease Owners), AOSTRA (Alberta Oil Sands Technology and Research Authority), and CANMET (Canada Centre for Mineral and Energy Technology)) and university researchers whose mandate was to develop methods to eliminate or reduce the rate of accumulation of fine tailings resulting from oil sands mining and to enhance reclamation potential through the modification of tailings properties (FineTailingsFundamentalsConsortium 1995a). The work initiated by the FTFC

is now continued through the Canadian Oil Sands Network for Research and Development (CONRAD).

CONRAD has defined constructed wetlands as: "a designed and human-made complex of saturated substrates, emergent and submergent vegetation, animal life, and water that simulates a natural wetland for the treatment of wastewater" (Hammer and Bastien 1989 cited in Oil Sands Wetlands Working Group (2000)). Wetlands have been identified as a reclamation design option for oil sands companies operating in northern Alberta. However, the development time and the composition of benthic invertebrate communities that comprise these wetlands are not well known. The goal of this research is to characterize and ordinate benthic community composition in constructed wetlands with respect to time since reclamation, and to produce a scale against which progress towards achievement of a "natural state" can be measured. The wetlands in this study are the result of human-created depressions that fill with water. These wetlands have all been formed by disturbance and differ in the length of time since the disturbance occurred (0- 30 + years).

Wetlands are "land that is saturated with water long enough to promote wetland or aquatic processes as indicated by poorly drained soils, hydrophytic vegetation, and various kinds of biological activity which are adapted to a wet environment" (National Wetlands Working Group, 1997). Wetlands generally form in low-lying areas with poor drainage that collect and retain water. I define a constructed wetland as a water-saturated expanse forming due to human-related alteration of drainage patterns that creates a pooling of water.

Wetlands that form on existing natural substrate in the Fort McMurray region accumulate surface runoff. These wetlands tend to have conductivity and nutrient concentrations typical of undisturbed northern Alberta water-bodies (Fine Tailings Fundamentals Consortium, 1995a). For example, ambient conductivity in the Athabasca River was 292 µS/cm, and 360 µS/cm in Ruth Lake (Fine Tailings Fundamentals Consortium 1995b). Such areas will be referred to as 'low conductivity reference wetlands'.

Wetlands that form in depressions of oil sands reclamation material in northern Alberta (areas from which overburden has been removed and replaced with saline and/or sodic parent material, or where peat has been stockpiled) are saline. These wetlands have higher conductivity, and water chemistry that may be analogous to saline wetlands in other parts of North America such as waterbodies in southern Saskatchewan (Rawson and Moore 1944; Hammer 1978a; Hammer and Haynes 1978b), the British Columbia interior (Cannings 1973; Topping and Scudder 1977; Cannings and Scudder 1978), and Wyoming (Lovvorn *et al.* 1999; Hart and Lovvorn 2000). I will refer to these wetlands as 'high conductivity reference wetlands' in my study area.

The other wetlands in this study have been affected by oil sands waste products, typically, as a one-time addition. These wetlands received or are built into waste materials (extraction tails, overburden) and/or their release or seepage water and are referred to as oil sands process material (OSPM)-affected wetlands. Some of these designated wetlands may receive process-affected water on a continuous or intermittent basis.

Successional rate is defined as the change in species composition over time (the length of time for the total number of resident species to reach an asymptote) while the successional trajectory is the direction of species change over time, either increasing or decreasing the total number of species. Successional rates and trajectories of zoobenthic communities may be limited by invertebrate dispersal characteristics (Gore 1982; Barnes 1983; Kovats *et al.* 1996), toxicity (Lovvorn *et al.* 1999), water chemistry (Euliss *et al.* 1999), habitat suitability (Cyr and Downing 1988; Oertli and Lachavanne 1995; de Szalay and Resh 2000) and diversity of the substrates (Paterson and Fernando 1970; Cantrell and Lachlan 1977; McLachlan 1985; Francis and Kane 1995; Oertli and Lachavanne 1995) or development of an appropriate food base (Angerilli and Beirne 1980; Lovvorn *et al.* 1999). The unique characteristics of OSPM-affected wetlands may modify the successional trajectory of invertebrate communities compared to that of high or low- conductivity reference wetlands.

I studied approximately 10 constructed wetlands of each class, ranging in age from 0-30 years old. Physico-chemical characteristics were measured to define the similarities and differences in wetlands among classes. Zoobenthic samples were collected by three methods (cores, artificial substrates, and sweep netting) and were used to assess the infaunal and epibenthic communities. Changes in taxon richness and benthic abundance of the two classes of reference communities were evaluated with respect to wetland age, amount of macrophyte development, and sediment organic content. I used discriminant function analysis to determine which zoobenthic taxa best distinguished the youngest reference wetlands from older reference wetlands. The resulting function was then used to classify each OSPM-affected wetland as being " equivalent to young" or "equivalent to mature". Lastly, I used various statistical techniques to develop an "index of wetland recovery" whereby the zoobenthic community of a particular OSPM-affected wetland could be assessed with respect to attainment of a natural state (equivalent to a mature reference wetland).

This thesis is organised into four chapters. The first chapter consists of a general introduction to the topic and the rationale behind the project. The second chapter examines the physical and biological variables that delineate these wetlands. The third chapter examines the development and use of an index of wetland recovery. The fourth chapter provides a general discussion and conclusion to my thesis.

## Background

### Wetlands

A wetland is any land saturated with water long enough to promote wetland or aquatic processes as indicated by poorly drained soils, hydrophytic vegetation, and various kinds of biological activity that are adapted to a wet environment (National Wetlands Working Group 1997).

## Wetlands in the Boreal Forest of Northern Alberta

Wetlands can be divided into two types, peat forming (with > 40 cm of accumulated organic material) and non-peat forming (with < 40 cm of accumulated organic material). Non-peat forming wetlands are classified as shallow open-water wetlands, marshes, and swamps. Peat-forming wetland (peatlands) are subdivided into fens and bogs (Vitt 1996; Oil Sands Wetlands Working Group 2000).

In the oil sands mining region bogs, fens, and marshes occur throughout the area, with bog and fen peatlands being characteristic wetland types in the region (Oil Sands Wetlands Working Group 2000). Vitt et al. (1996) estimated that 18% of Alberta's landbase is comprised of wetlands. Most of these areas are peatlands (90.4%), occurring within the boreal forest region of northern Alberta (Oil Sands Wetlands Working Group 2000).

## Wetland Function

Wetlands are foci of biodiversity, and are valued for their ability to act as filters, sinks and transformers for sediments, nutrients and pollutants. Wetlands act as buffer systems between other aquatic systems and human activities on upland areas (Hook 1993). Examples of anthropogenic uses include storage of municipal sewage sludge for detoxification (Sawhill and Ferguson 1998), retention of water and filtration using reed beds in the U.K. (Chaloner and Wotton 1996), and use of wetlands to remediate nutrient loads from aquaculture wastewater (Shuskey and Baca 2001).

## Importance of Wetlands to the Boreal Ecosystem

Wetlands in the boreal zone are very important for wildlife. Westworth (1993) identified at least 236 bird species and 43 mammal species in the Boreal Forest Region of

Alberta (Oil Sands Wetlands Working Group 2000). The Athabasca region provides important habitat for waterfowl with 66 species identified in the region, 33 of which were seen in suitable nesting or breeding habitat (J. Fraser, pers.comm. Wood Buffalo Wild Bird Club spring survey, May 2000). Many mammals depend on wetlands for habitat and food, including beaver, deer, moose, mink, caribou and muskrat. Sustained populations of these animals are important for local First Nations people (Oil Sands Wetlands Working Group 2000).

**Athabasca Oil Sands Region**

The Athabasca oil sands deposit is located in northern Alberta, approximately 500 km north of Edmonton (Figure 1.1). The deposit is composed of the Athabasca and McMurray geological formation, which is an oil saturated sand that is the remnant of an ancient sea with fluvial, estuarine, and marine depositional environments (Wightman *et al.* 1997). Oil sand deposits in Canada are the largest globally. The Athabasca deposit is the largest in the province, covering 40,000 $km^2$ and containing an estimated 300 billion barrels of recoverable oil. By 2025 the National Energy Board of Canada expects that the Alberta oil sands will provide 70% of national oil production (Laird 2001).

In 1776, Peter Pond was the first European to document the occurrence of oil sand in the Athabasca region. Since this time, various companies and consortia have sought economical means of extraction. The Athabasca oil sands are currently being surface-mined by several companies, the two largest in the area being Suncor Energy Inc. and Syncrude Canada Ltd. Intensive oil sands mining has occurred since the 1970's and is expected to continue for the next 100 years (Syncrude Canada Ltd., pers. comm.). The purpose of this activity is the extraction of bitumen from the oil sands and production of saleable crude oil.

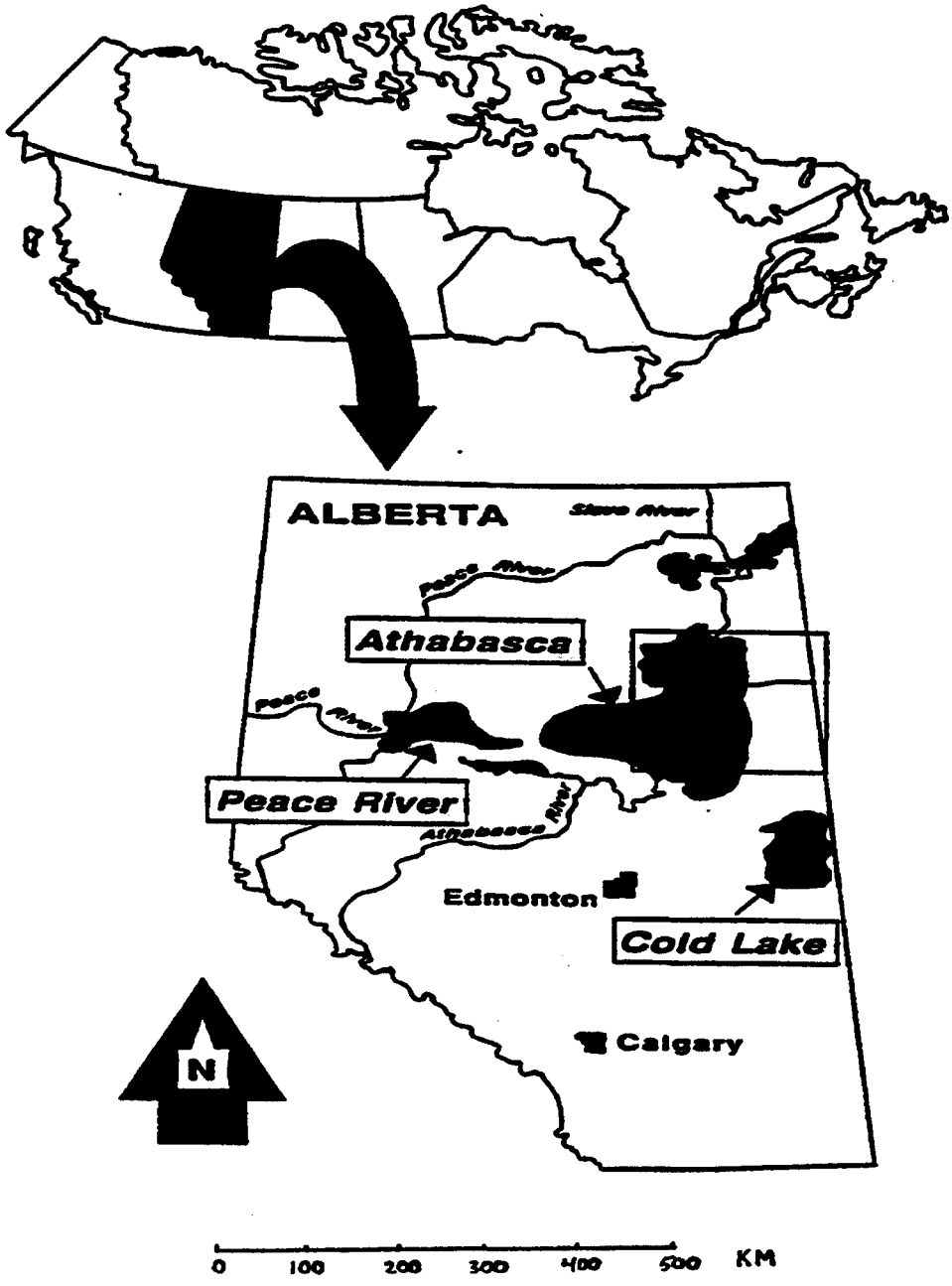



Figure 1.1. Map of Alberta indicating the location of the Athabasca Oil Sands in the province. Also indicated on this map are the other major oil sands deposits in the province. (Source: van den Heuvel, M.R., *et al.* 1999. Can. J. Fish. Aquat.Sci. **56**:1213-1225)

## The Mining Process

Oil sand mining entails stripping standing vegetation and removing several metres (<100 m) of overburden (vegetation, topsoil, and clays) to expose the oil-rich sands. The overburden is stockpiled for later use in reclamation. The oil sand is digested and conditioned in large tumblers with hot water, caustic soda (NaOH), and steam (Fine Tailings Fundamentals Consortium 1995a). The end products of this process are unrefined bitumen (oil) and tailings. Unrefined bitumen is transported to Edmonton via pipeline for refining.

## Tailings and Effluent Storage and Disposal

The tailings slurry is pumped into large settling basins or tailings ponds. Tailings are comprised of a coarse fraction (sand), a fine fraction (clay and silt), un-recovered bitumen, and water (Fine Tailings Fundamentals Consortium 1995). Pore water in the tailings is extruded as the tailings settle ("densify"). This resulting, low suspended solids content water is re-used in the extraction process. As the extraction tailings segregate, coarser solids (sand) form beaches while most of the finer fraction (silts and clays) and the tailings waters enter the settling ponds. Fine clays and sands form a slowly settling aqueous suspension, known as fine tails, in the tailings ponds, below the release water zones (MacKinnon *et al.* 2000).

## Tailings Toxicity

Fresh tailings and oil sand process water (OSPM) are toxic to laboratory test animals, including *Daphnia magna*, rainbow trout (*Onchorhynchus mykiss)* and bacteria (See Table 1.1 for specific test results) (Fine Tailings Fundamentals Consortium, 1995a). For this reason mining companies are not permitted to discharge tailings or tailings water into the surrounding area (Oil Sands Wetlands Working Group 2000).

Recent improvements in extraction and refinement processes have permitted increased production for both companies. The result has been a significant increase in the rate and volume of fluid waste generated and the pressing problem of what to do with it. No single reclamation option can handle the projected volumes of tailings waste in a technically, environmentally, and economically acceptable way (Fine Tailings Fundamentals Consortium 1995b). Thus, an integrated approach includes the use of "wet" and "dry" landscape techniques. "Dry" landscape reclamation uses de-watered ("consolidated" or "mature") fine tails mixed with overburden to form a soil matrix, on which vegetation can be re-planted or if in low-lying areas, where wetlands can form (Fine Tailings Fundamentals Consortium 1995b). "Wet" landscape reclamation involves using fine tailings sludge as the sediment base in a lake, and capping the lake with fresh water (Fine Tailings Fundamentals Consortium 1995b).

**Tailings Constituents**

Constituents contained within the extraction tailings include silts and clays (quartz and layered silicates such as kaolin, illite, muscovite), dissolved materials (salts, dissolved organics), and un-recovered hydrocarbons. The major hydrocarbon components are unrecovered bitumen and naphtha, with some polycyclic aromatic hydrocarbons (PAHs) and hetercyclic aromatics. In the fine tails zone, hydrocarbons comprise up to 2% by volume. Humic acids are composed of aromatic hydrocarbons, and strongly bound organic matter is present (Fine Tailings Fundamentals Consortium 1995a). Naphthenic acids (groups of acyclic and cyclic aliphatic carboxylic acids) account for most of the dissolved organic matter in process-affected waters and have been shown to be a major factor contributing to toxicity due to their surfactant properties (Madill *et al.* 2001). Tailings waters are saline, with the dominant salts including NaCl, $Na_2SO_4$, and $NaHCO_3$, with only minor levels of the divalent ion ($Ca^{+2}$, $Mg^{+2}$) salts being seen. Trace metal concentrations

**Table 1.1.** Summary of acute toxicity test results.

| Water Source | Test | | | |
|---|---|---|---|---|
| | Microtox ($IC_{50}$) | *Daphnia magna* ($LC_{50}$) | *Hyallela azteca* (% survival) | Rainbow trout (*O. mykiss*) ($LC_{50}$) |
| Tailings pond water | | | | |
| Syncrude | 22 - 43 % [1,2,3,4,5] | 76 % [1] | - | 12.5 - 13 % [1,3] |
| Suncor | 16 - 50 % [1,2,3,5,6] | 98 - 100 % [1,6] | - | 3.2 - 28 % [4,7,8] |
| Fine tailings porewater | | | | |
| Syncrude | 20 - 40 % [1,2] | - | - | - |
| Suncor | 30 - 80 % [1,2,6] | 100 % [6] | 10 % [6] | - |

Table reproduced from: Fine Tailings Fundamentals Consortium. 1995b. Fine Tails and Process Water Reclamation. *Advances in Oil Sands Tailings Research.* Edmonton, AB, Alberta Department of Energy Oil Sands and Research Division. **II:** page 16.


1 Mackay, W.C. and A.G. Verbeek. 1993. AOSTRA # 8878M (Sludge 681)

2 Nelson, L.R., M. MacKinnon, and J.R. Gulley. 1993. Proceedings of Oil Sands - Our Petroleum Future Conference. Fine Tailings Fundamentals Consortium. April 4-7, 1993. Edmonton.

3 Zenon Environmental Inc., 1986. Prep. for Environment Canada

4 MacKinnon, M.D. and J.T. Retallack. 1982. Proc. of the 4th Annual Meeting of the International Society of Petroleum Industry Biologists. Denver, CO. pp 185-210.

5 Enviro-Test Laboratories and HydroQual Laboratories. 1994. Prep. for Fine Tails Fundamentals Consortium. 58 pp.

6 EVS. 1992. Prep. for Suncor Inc., Oil Sands Group.

7 Nix, P.G. and R.W. Martin. 1992. Environmental Toxicology and Water Quality 7: 171-188.

8 Nix, P.G. and E.A. Power. 1989. 1989 Summary Report. Prep. for Suncor Inc. Oil Sands Division.

are low and no evidence of trace metal build-up in these tailings waters have been indicated (M. MacKinnon, pers. comm.). The relative concentration of these ions varies due to the quality of the ore at each lease and the use of caustic soda (Fine Tailings Fundamentals Consortium 1995b).

**Wetland Role in Reclamation**

A "fully functioning wetland community" is one of the goals of reclamation on the oil sands leases. Wetlands are expected to act as passive buffer systems between the reclamation area and receiving area. Wetlands are also bioreactors for the degradation of toxic tailings constituents (Fine Tailings Fundamentals Consortium 1995b). The constituents remaining in the process water may affect constructed wetlands in a manner causing the zoobenthic community to differ from reference natural areas with respect to richness or abundance. Companies operating on these leases are expected to return the mined area to pre-mining conditions of diversity and abundance of wildlife habitat types and qualities (Oil Sands Wetlands Working Group 2000).

The Oil Sands Wetlands Working Group (2000) has delineated the end uses (endpoints) for reclaimed wetlands on oil sands leases. Five wetland types have been identified as existing on the reclaimed landscape: altered wetlands, opportunistic wetlands, constructed wetlands (flood control, water treatment, habitat), vegetated watercourses, and littoral zones of lakes (Oil Sands Wetlands Working Group 2000). The wetlands in this study fall into altered wetlands, opportunistic wetlands, constructed wetlands for water treatment and for habitat. The importance of hydrology, and vegetation in the formation and maintenance of wetlands, as well as traditional subsistence use and habitat for fish and waterfowl have also been assessed (Oil Sands Wetlands Working Group 2000). Thus far, there has been no clear delineation of what comprises a "mature" constructed wetland in this area, nor of the invertebrate community that would comprise it.

Studies of benthic invertebrate communities, with emphasis on the Chironomidae, have found that OSPM-affected wetlands have reduced taxa richness and abundances compared to saline reference communities, and that OSPM-affected wetlands tend to be dominated by the Chironomidae (Whelly 1999; Bendell-Young 2000). McDonald (1998) reported that chironomid production rates are lower at OSPM-affected wetlands than at environmentally similar matched-pairs. Whelly (1999) found no difference in the incidences of mentum deformities in the mouthparts of chironomid larvae collected from reference and OSPM-affected wetlands.

**Reclaiming Peatlands in the Oil Sands Region**

Removal of overburden destroys naturally occurring peatlands in the mining area, re-routes entire drainage systems, and significantly alters the hydrology of the landscape (Oil Sands Wetlands Working Group 2000). Replacement of naturally peat-dominated wetlands takes many years, as the accumulation of organic matter is determined by the interrelated combination of hydrologic, chemical and biotic factors that result in a decrease in organic matter decomposition relative to plant production (Oil Sands Wetlands Working Group 2000).

Destroyed or altered peatlands will be replaced with wetlands of soils composed mainly of clays and sands unless the wetland is constructed to contain > 40 cm of peat. Constructed wetlands containing OSPM and/or tailings will also be saline. Naturally saline water-bodies are not common in northern Alberta, with the exception of a few salt meadows or saline lakes in local discharge areas (Oil Sands Wetlands Working Group 2000). Thus, reclamation wetlands on the oil sands leases will be unlike any wetlands in the region.

**Wetland Colonisation and Succession**

Numerous factors influence the colonisation potential and successional processes of wetlands. They include distance from other 'source' wetlands (Barnes 1983), upland vegetation (Lovvorn *et al.* 1999), water chemistry (Cannings 1973; Cannings and Scudder 1978; Barnes 1983; Bervoets 1996; Euliss *et al.* 1999; Lovvorn *et al.* 1999), and substrate (Francis and Kane 1995). Other factors include the presence of competing species (McLachlan 1985), availability of prey items for predators (Osborn 1996), and time of arrival (Barnes 1983). Sheldon (1984) described colonisation as 'the sequence of events that leads to the establishment of individuals, populations, species, or higher taxa in places from which they were, however temporarily, absent'.

Colonisation of new areas is largely accomplished by the adult stage of many aquatic taxa. The benthic larvae are often capable of limited dispersal, perhaps depending on aid from a passing duck or muskrat. The flying, adult stage of these insects allows for dispersal across larger ranges. The ephemerid mayfly, *Hexagenia*, can fly several kilometres inland (Kovats *et al.* 1996). As well, Trichoptera (caddisflies) and chironomid (midge) adults disperse widely (see (Kovats *et al.* 1996). Dragonfly species richness is greatest in shallow, well-vegetated ponds with clear, oxygenated water (Osborn 1996). The determinant of species assemblages in dragonflies is influenced by variables that represent different successional stages, such as water quality, vegetation type and microsite diversity (Osborn 1996).

Colonisation of wetlands by less mobile taxa, such as oligochaetes, molluscs and micro-crustacea is thought to be through passive dispersal of reproductive propagules such as eggs or resting stages (Holland and Jenkins 1998; Jenkins and Underwood 1998; Caceres and Soluk 2002). Recent studies have examined the relative importance of passive dispersal mechanisms for zooplankton and have found that dispersal is infrequent and

limited to few species (Jenkins and Underwood 1998). However, older aquatic systems with greater interconnections will be more likely to have regional species disperse to a site (Jenkins and Underwood 1998). There is also the possibility of dried pond sediments (as may be found in stockpiled overburden destined for reclamation uses) acting as "seed-banks" for microinvertebrates (Jenkins and Underwood 1998).

Talling (1951) discussed the element of chance in succession and colonisation (Cited in (Jefferies 1989)). He stated that not every animal or plant seeks out every available pond in an area to colonise. Generally, the area they land in is random, and the suitability of that habitat determines whether they survive or not. Succession is the pattern of changes in the species composition of a community after a disturbance or after opening of a new patch (Horn 1974), i.e., the change in species composition of a community over time. Succession can occur after a radical event such as mining or fire, or gradually as animals and plants alter the habitat to better ensure their survival. This process may then change the suitability of the habitat for other species, resulting in exclusion or elimination of some individuals (Cantrell and Lachlan 1977; McLachlan 1985). This may in turn open a new patch in the environment and the cycle continues.

**Studying Succession**

There are several ways by which one can measure the change in a community over time. An opening created in an area can be followed from time zero to a given age (Craft 1999). Another method of documenting succession is to simultaneously measure many areas of differing ages and infer a time sequence of change. This method has been used by several researchers to examine long term changes that occur in a wetland after a disturbance (Barnes 1983; Galatowitsch and derValk 1996; Noon 1996), or after an area is allowed to return to a previous state, e.g., a salt marsh that is no longer impounded (Craft 1999).

Noon (1996) proposed that there are two phases of succession for wetland plants. The first phase is arrival and establishment of taxa, in which assembly rules apply and where the highest diversity initially occurs. The second phase, autogenic dominance, is where competition between taxa occurs and diversity decreases somewhat. The diversity of a wetland plant community correlates with a wetland's ability to rebound from a perturbation. Increased soil organic matter (which provides nutrient storage & nutrient cycling potential) was highest in older sites, implying that older wetlands are more stable and more capable of rebound after a perturbation (Noon 1996).

**Biological Monitoring and Restoration in Wetlands of the Oil Sands Area**

Benthic invertebrates form an integral link between the terrestrial and aquatic food ecosystems and provide an indication of the ecological status of aquatic systems (Ciborowski *et al.* 1995). Biological monitoring, or biomonitoring, is the 'systematic use of biological responses to evaluate changes in the environment'. In this broad sense, evaluating the establishment of the benthic invertebrate community of constructed wetlands and the community-level changes that may occur is biomonitoring.

Various factors affect the types of climax communities including microtopography, drainage characteristics, nutrient loading (Worrall *et al.* 1997) and disturbance history (Knott 1997). Whelly *et al.* (1998) and Ciborowski (1997) have documented and ordinated environmental variables that characterize natural and OSPM-affected wetlands in the Athabasca oil sands region, providing reference variables against which to document the convergence of the invertebrate community toward natural conditions.

Other research in the oil sands area has assessed the response of various organisms to OSPM at the organismal, population, and community levels. Microcosm experiments with natural indigenous phytoplankton communities have been used to assess the effects of

naphthenates and salt constituents (Leung 2001). OSPM mesocosms (test ponds) from age 0 - 8 yr. old were used, with naphthenate concentration greater than 20 mg/L. Significant community composition differences were found, but no overall difference in total phytoplankton biomass occurred. Nine taxa were identified as tolerant to elevated naphthenic acid concentration.

van den Heuvel *et al.* (1999) studied fish survival in mesocosms (test ponds) containing substrates of either oil sands fine tailings or clay and lean oil sands. They stocked adult yellow perch (abundant in many boreal lakes, with large geographic distribution) immediately post-spawning and sampled fish 5 and 11 months later. Using the gonadosomatic index of fish condition (length, weight, liver weight, gonad weight, gender and sexual maturity) they found no negative effect on somatic indices in ponds containing OSPM. Energy storage and utilisation were equivalent to or slightly greater than was observed in fish in natural lakes in the surrounding area. However, high liver weight (and size) could be due to increased exposure to petroleum-related chemical challenge. Gould (2000) found that adult yellow perch in a constructed OSPM-affected small lake had similar condition factors to off-lease reference areas (survived, grew and reproduced successfully). However, OSPM affected perch showed signs of chronic stress, with altered liver enzyme activation, altered gill structure and suppression of immune system (See van den Heuvel *et al.*, 1999).

Gould (2000) assessed invertebrates in a small constructed lake system (Demo Pond) and compared the benthic community to that of three natural lakes in the area (off-lease site). She evaluated invertebrates in terms of the suitability as a forage base in the constructed pond for supporting a population of yellow perch. She compared benthic invertebrate abundance with a diet analysis of yellow perch guts. Benthos were significantly less abundant in the OSPM-affected pond (Demo Pond) than in reference lakes (Gould 2000).

## Developing Metrics and Multivariate Techniques

### Importance of Reference Sites

Biotic integrity is the ability of a system to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organisation comparable to that of natural habitat of the region (Karr and Dudley 1981 in Karr *et al.* (1986)). A major challenge for oil sands companies is the remediation or reclamation of areas that have been adversely affected by mining activity. Remediation targets require precise definition to establish when sufficient action has been taken (Reynoldson and Metcalfe-Smith 1992).

The best method to measure whether an anthropogenically disturbed area has returned, or is returning, to pre-disturbance conditions is to compare it to other local areas that are relatively undisturbed (reference sites). The reference site, or best available condition, is representative of a minimally disturbed site (or sites) organised by selected physical, chemical and biological characteristics (Reynoldson *et al.* 1997). Comparing reference sites to impacted or disturbed sites is common in field biological studies (see Somers 1998; Galatowitsch *et al.* 1999; Barbour and Yoder 2000; Resh *et al.* 2000, Ferraro and Cole 1990; James *et al.* 1995; Bowman 1997; Reynoldson and Wright 2000; Bailey *et al.* 2001; King and Richardson 2002). Various attributes of the biological community can then be used to determine the degree to which a site has been affected by human activity. To establish when the benthic community of OSPM-affected wetlands is comparable to reference wetlands, it will be necessary to develop an index or measure. Multimetric and multivariate approaches that use benthic invertebrates are similar in data collection methods, but differ in the way reference sites are selected, test (affected) sites are classified and test site assessments are made (Resh *et al.* 2000).

## Metric Development

Multimetric indices are commonly used in the United States, to determine the extent of an impact on an aquatic system. Indices are comprised of several measures (metrics) that individually provide information on diverse biological attributes, and when integrated, provide an overall indication of biological condition (Barbour *et al.* 1995). These attributes are often based on best professional judgement, historical data, and geographical or physical attributes of the region (Barbour *et al.* 1995; Reynoldson *et al.* 1995).

Metrics were first developed for measuring the biotic integrity of fish communities in streams and rivers (Karr *et al.* 1986; Barbour *et al.* 1995). Development of benthic invertebrate metrics has followed. Examples now used include the invertebrate community index (ICI), rapid bioassessment protocols, and the benthic index of biotic integrity (IBI) (Barbour *et al.* 1995). Metrics were largely developed for lotic systems but some have recently being adapted to lentic systems such as wetlands (See Galatowitsch *et al.* 1999).

## Multivariate Techniques

Multivariate approaches, used predominantly in Canada, the United Kingdom, and Australia, also use several measures of the ecosystem to assess the similarity or difference between reference sites and affected (test) sites. However, instead of using historical information and best professional judgement to group reference sites *a priori*, various multivariate statistical analyses are used, e.g., principal components analysis, canonical correspondence analysis, and factor analysis, to determine the degree to which a test site is similar to a suite of reference sites.

Several methods of assessing anthropogenic impacts on river systems have been developed in the UK (RIVPACS), Australia (AUSRIVAS) and for the Laurentian Great Lakes of Canada (BEAST). The BEAST has also been adapted for use on the Fraser River in B.C. (Resh *et al.* 2000). Multivariate approaches have also been used for wetlands (Olsgard *et al.* 1998; Zimmer *et al.* 2000; King and Richardson 2002).

Resh *et al.* (2000) recommend that multimetric approaches should consider incorporating multivariate analyses for defining reference conditions and assessing impairment of test sites. For this research I will use multivariate techniques to construct a multimetric index based on data collected (both use similar data). The goal of this research is to produce an index of recovery that is straightforward enough to be used by oil sands environmental employees possessing a basic knowledge of aquatic ecology and invertebrate taxonomy.

## Chapter 2

## Physical and Biological Attributes of Constructed Wetlands in the Oil Sands Mining Region of Northeastern Alberta, Canada

### Introduction

The purpose of this chapter is to describe the physical and biological attributes of constructed wetlands in the Athabasca oil sands region. Constructed wetlands are defined as wetlands that result from anthropogenic modification of the substrates/groundwater such that wetland vegetation and soils characterize a site.

The circumpolar boreal forest stretches across North America and Eurasia as a continuous band of green. The boreal forest occupies more than 60% of the total area of forests in Canada and Alaska (Johnson *et al.* 1995). Much of this land surface is covered by wetlands. Approximately 75% of the prairie boreal region is covered in wetlands, with another 25% or more in the Northwest Territories (Johnson *et al.* 1995). Wetlands are classified many ways. In this thesis, the classification will follow that outlined by Vitt *et al.* (1996). The wetlands sampled for this study can be classified as swamp, freshwater marsh and saline wetlands.

### Physical and Biological Attributes Affecting Wetland Biota

Minshall (1984) surveyed the literature on insect responses to differences in the composition and size of substrates and produced several generalisations on substrate composition and size. First, aquatic plant substrates support higher densities of animals than mineral substrates. Secondly, large organic substrate particles are more productive than smaller ones. Thirdly, preferences for a substratum type differ among species.

**Wetland Soils**

Factors that influence benthic communities are characteristics of the wetland soil itself, such as grain particle size and organic content (see Minshall 1984). The substratum determines to a large extent the micro-environmental conditions under which the insects live, and thus profoundly affects their growth and survival (Minshall 1984).

Particle size has been reported to influence to habitat selection and distribution of several species of chironomid larvae. Sediment particle size affected the ease of substrate penetration for young larvae of *Heterotrissocladius marcidus* Walker *and Stictochironomus virgatus* Townes (Wiley, 1981 as cited in Francis and Kane 1995). Francis and Kane (1995) also reported that particle size might affect respiration because small particles have less room for oxygenated water in the interstitial space (Francis and Kane 1995).

The characteristics of the wetland soil and water not only affect the benthic community directly by influencing which taxa will inhabit a wetland, but also indirectly by influencing which vegetation can establish in the wetland (Barko and Smart 1983).

**Vegetation**

The vegetation present in a wetland can have a great influence on the macroinvertebrate community present. Angerilli and Beirne (1980) reported that ponds with vegetation (*Elodea canadensis* and *Utricularia minor*) had smaller larval mosquito populations. They also reported that vegetation might influence the presence/absence of predators such as notonectids and dytiscids.

Olson *et al.* (1995) compared the macroinvertebrate communities among vegetation types in a Minnesota prairie marsh. Several orders of invertebrates showed significant

differences in distribution between vegetation types and sites. *Typha*-dominated sites had the most diverse populations compared to open water sites dominated by filamentous algae.

deSzalay and Resh (2000) examined the factors that influence macroinvertebrate colonisation in seasonally flooded marshes. Through manipulation of the emergent plant cover, they concluded that the amount of emergent plant cover is important for invertebrate colonisation. In areas with dense emergent plants there was a more diverse macroinvertebrate community compared to open water areas. However, open water areas had higher total macroinvertebrate abundance with a less diverse macroinvertebrate community, dominated by midges (de Szalay and Resh 2000).

Vegetation provides habitat structure, and greater habitat diversity generally results in greater diversity of animals (Cyr and Downing 1988; Alcocer *et al.* 1998; Euliss *et al.* 1999; de Szalay and Resh 2000). Monocultures of cattail (*Typha latifolia*), for instance, provide less habitat heterogeneity than a wetland with a mixed vegetation structure including submergent and emergent vegetation (Heino 2000).

A combination of submergent and emergent plant species divides the habitat horizontally and vertically, thus providing microhabitats for chironomids (Driver 1977). Wrubleski (1987) reported that sago pondweed habitats, in Delta Marsh, MB, had the highest numbers of chironomids, due to the greater colonisable area of the plants' finely dissected leaves, compared to bulrush and cattail habitats.

Adult odonates have specific habitat requirements that are important in determining the presence/absence and abundance of species (Osborn 1996). Adult Libellulidae select suitable oviposition sites by the presence of certain types of vegetation and are attracted to smooth surfaces (Wildermuth 1992 in Osborn 1996). On the other hand, Aeshnidae (dragonfly) and Zygoptera (damselflies) select specific plants for endophytic oviposition

(Corbet, 1962 in Osborn 1996), and for perches (Meskin 1989, Buchwald 1992 in Osborn 1996). Large areas of submerged vegetation provide microhabitats for many weed-dwelling odonate larvae and their potential prey, as well as protection from predators, such as fish (Osborn 1996).

In southern Spain, Casa and Vilchez-Quero (1996) reported that the chironomid assemblage of a lagoon was determined most by the density of aquatic macrophytes (*Myriophyllum spicatum*) and secondly by the salinity of the water (Casas and Vilchez-Quero 1996).

The presence and composition of emergent and submergent macrophytes are expected to vary with wetland age. Newly created wetlands are expected to contain little, if any, vegetation. These new wetlands will be dominated by sediment dwelling animals such as sediment dwelling chironomids (See Barnes 1983). Older wetlands will contain a greater abundance of sediment dwelling species as well as predacious genera. Species diversity and richness are expected to rise through time due to the increase of habitat heterogeneity provided by macrophytes.

**Water Chemistry**

Various physico-chemical attributes can affect the benthic community. Properties of the water with greatest reported impact include salinity (Bayly and Williams 1966; Cannings and Scudder 1978; Bervoets 1996; Lovvorn *et al.* 1999) and dissolved oxygen (D.O.)(Saether 1979; Polonsky and Clements 1999).

In southeastern Wyoming wetlands, Lovvorn *et al.* (1999) reported a shift in the benthic community as a result of salinity. Snails and amphipods dominate low salinity (80-

800 μS/cm) wetlands but are absent (snails) or greatly reduced (amphipods) in more saline wetlands. In highly saline wetlands (800-3000 μS/cm), the community is dominated by chironomid larvae, insect predators (odonates and Hemiptera) and has higher densities of crustacean zooplankton (cladocerans and calanoid copepods) (Lovvorn *et al.* 1999). In two saline (7.4 g $L^{-1}$) crater lakes, in the state of Oriental, Mexico, chironomids were the most diverse group of macroinvertebrates, followed by amphipods, oligochaetes and leeches (Alcocer *et al.* 1998).

In Canada, Rawson and Moore (1944) sampled 60 saline lakes in central and southern Saskatchewan. Chironomid larvae, amphipods, sphaeriids, oligochaetes and molluscs dominated the benthic fauna. Most taxa exhibited a wide range of salinity tolerances. For example, leech abundance decreased with increasing salinity (up to 2250 mg $L^{-1}$), and gastropods were present in lakes with salinity up to 14,200 mg $L^{-1}$. However at around 3000 mg $L^{-1}$ gastropod abundance declined.

Cannings and Scudder (1978) studied the ecology of Chironomidae in saline lakes of the Chilcotin-Cariboo region of British Columbia. Low conductivity lakes (40-80 μS/cm) had a chironomid fauna that was distinct from meso-saline (400-2800 μS/cm) and more highly saline (4100 - 12,000 μS/cm) lakes. Interestingly, lakes whose conductivity was intermediate (400 - 2800 μS/cm) had higher phytoplankton productivity than less saline (40-80 μS/cm) lakes.

**Constituents in the Oil Sands**

Of particular importance in the wetlands of the Athabasca oil sands area is the presence of hydrocarbons (Mozley 1978) in the form of "lean oil sand" or tailings

containing un-recovered bitumen in the substrates, and the presence and elevated concentrations of naphthenic acids in the pore and recharge waters. In general, oil sands process material (OSPM) contains relatively high levels of salts (conductivity > 2000 μS/cm), naphthenic acids (20 - 100 mg $L^{-1}$), PAHs (associated with the bitumen fraction) and other potentially toxic or detrimental chemicals (Fine Tailings Fundamentals Consortium 1995a). Constituents within fresh OSPM are acutely toxic to a wide range of aquatic organisms in standard laboratory-based bioassays, with most of the acute response dissipated in as little as one year under natural ageing in an aerobic environment (Fine Tailings Fundamentals Consortium 1995a; Holowenko *et al.* 2002).

A variety of organisms have been studied to determine the effect of OSPM and tailings materials on aquatic biota. Wetlands containing fresh OSPM are unable to support amphibian populations due to low food sources and D.O. levels at the sediment interfaces, as well as relatively high and toxic concentrations of naphthenic acids and ammonia (Pollet and Bendell-Young 2000). Fresh OSPM also influences community composition of phytoplankton (Leung 2001) and lowers the diversity of benthic macroinvertebrates (Whelly 1999; Bendell-Young *et al.* 2000; Gould 2000).

**Artificial Substrate Use in Benthic Sampling**

Artificial substrates have been used to determine the rate of colonisation of periphyton (Cattaneo 1992), algae (Macan 1976), marine (Jacobi and Langevin 1996) and freshwater benthic invertebrates in both lotic systems (Meier *et al.* 1979; Ciborowski and Clifford 1984) and lentic systems (Benoit *et al.* 1998) . Samplers can be used to measure the abundance of animals living as sediment dwelling benthos (quantitative), epiphytic

habits (quantitative) and within the structure of emergent macrophytes (qualitative). This method can be easily used in a variety of areas. The length of time needed to reach colonisation saturation varies with the system observed and the sampler used.

The use of artificial substrates provides several advantages over other methods such as core samples and sweep samples. Artificial substrates allow data collection from locations that cannot be sampled effectively by other means, permit standardised sampling, reduce variability (compared to other sampling types), require less operator skill than other methods, and permit non-destructive sampling of an environment (Rosenberg and Resh 1982 in DeShon 1995).

The objective of this chapter is to describe the physical and biological attributes of "natural" and constructed wetlands and to determine how they compare to each other. Essentially, all wetlands in this study are constructed. Some wetlands are simply formed from borrow-pits (BM, H63I, and H63W) at the side of a roadway, while others are beaver ponds (TR0.8R, SBBP). For this thesis, "natural" wetlands are wetlands that have not been deliberately constructed for the purposes of studying detoxification of tailings (Test ponds, etc., see Table 2.2), for reclamation or habitat use (BL, PP), or for water storage and usage (LLW). It is presumed that natural reference wetlands are similar to one another in their physical and biological attributes, and could be distinct from constructed reference wetlands.

I compared the benthic community richness and abundance in three classes of wetlands of varying ages to determine which physico-chemical attributes most influence these benthic community attributes. Since the suite of wetlands selected represents a gradient across age ("new" to "mature"), salinity (low to high) and disturbance (OSPM-affected and non-affected), I predict that young OSPM-affected wetlands will have benthic

assemblages initially unique to this impact and their community attributes will converge toward high conductivity (salinity) reference wetlands.

## Methods

### Description of Study Sites

All study sites were located in the Fort McMurray region of northern Alberta. Wetlands were selected on the basis of accessibility and to provide a range of ages that was balanced with respect to wetland class. Location of wetlands ranged from 55° 44.377' N, 109° 28.576'W (Millennium Interceptor Ditch) in the south to 57° 11.349'N, 111° 35.945'W (Barge Marsh) in the north. Study site locations, in relation to lease boundaries, are shown in Figure 2.1 (1 cm = 2.5 km).

The dominant upland vegetation in this area is black spruce (*Picea mariana*) and trembling aspen (*Populus tremuloides*) trees, alder (*Alnus* sp.) and willow (*Salix* spp.) shrubs. Sedges (*Carex* spp.) often surrounded wetland edges, with cattails (*Typha latifolia*) often being the dominant emergent vegetation, followed by bulrush (*Scirpus* spp.). Submergent vegetation was composed of pondweed *(Potamogeton* spp.), hornwort or coontail (*Ceratophyllum demersum*), water smartweed (*Polygonum amphibium*) and stonewort or muskgrass (*Chara* spp., a colonial alga). Some sites also had dense mats of duckweed (*Lemna minor* and *L. triscula*) floating on the water surface.

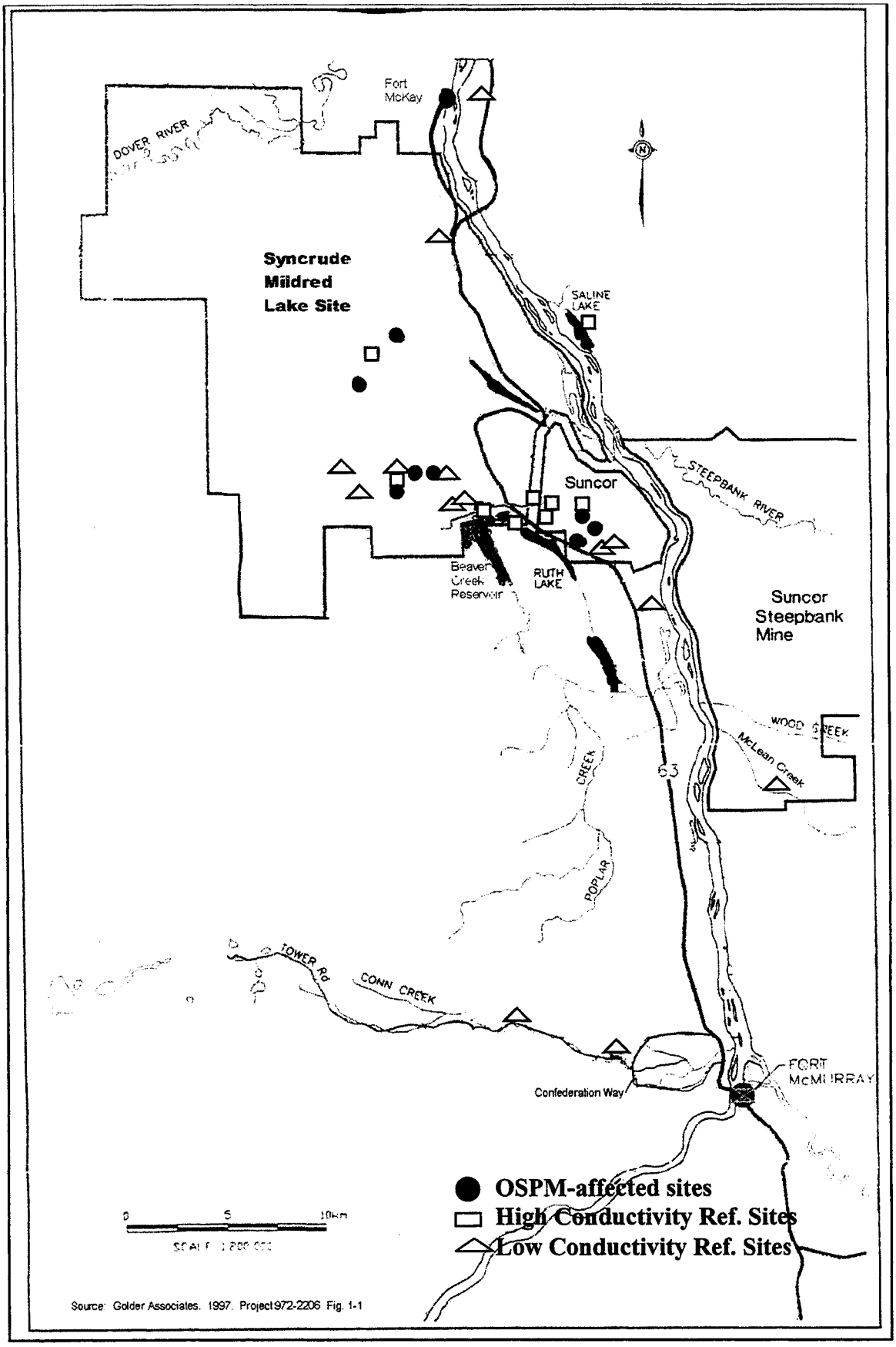



**Figure 2.1.** Map of lease sites and wetlands sampled in 2000 & 2001, showing relative position to lease site and other wetlands.

**Selection of Sampling Sites**

A total of 31 wetlands (sites) were sampled in 2000-2001 field seasons (Table 2.2). Wetland sites were selected from a set of 40+ wetlands previously sampled in the area by researchers from the University of Windsor (Ciborowski 1997; Whelly *et al.* 1998; Whelly 1999; J. Ciborowski, University of Windsor, pers. comm.). *A priori* classification of sites was based on physico-chemical data collected in 1997-1999. Eight sites were classified as being oil sands process material (OSPM) affected, 11 sites were classified as being higher conductivity (saline) and are constructed on waste materials (mining sands and/or consolidated tailings). Thirteen sites were classified as being lower conductivity (freshwater), containing natural surface waters and are not affected by OSPM or saline waters (see Table 2.1 and Table 2.2 for details). At each site the following suite of characteristics was evaluated (detailed in Table 2.3):

- water chemistry
- sediment characteristics
- benthic community (quantitative and qualitative sampling)
- aquatic vegetation (qualitative estimates)

**Experimental Design**

There are many ways to examine wetland succession. One way is to conduct a longitudinal study of a wetland from new to mature (Craft 1999). This approach is time-consuming, requiring monitoring over a period of several years. Alternatively, the effect of wetland age can be evaluated by simultaneously sampling wetlands that differ in age. Simultaneous sampling of wetlands of differing age can be used to examine the colonisation process and invertebrate succession in constructed wetlands (Ciborowski and

**Table 2.1.** Wetlands sampled in 2000 and 2001. Synoptic sampling refers to a single-visit measurement of environmental and biological variables. Intensive sampling refers to multiple collections of invertebrates (cores, sweep samples and artificial substrates) and measurements of environmental parameters.

| Name | Code | Lease Site | Class | Age in 2001 | Synoptic Sampling | Sediment | Intensive Sampling |
|---|---|---|---|---|---|---|---|
| CT Wetland | CTW | Suncor | OSPM | 3 | | x | x |
| CT Pond | CTP | Syncrude | OSPM | 4 | | x | x |
| Demo Pond | DP | Syncrude | OSPM | 8 | | x | x |
| Enviro - Test Pond 4 | ETP4 | Syncrude | OSPM | 5 | x | x | |
| Mature Fine Tailings-North | MFT-N | Suncor | OSPM | 10 | | x | x |
| Natural Wetland | NW | Suncor | OSPM | 15 | | x | x |
| Seepage Control Pond | SCP | Syncrude | OSPM | 26 | | x | x |
| Test Pond 2 | TP2 | Syncrude | OSPM | 12 | | x | x |
| Test Pond 5 | TP5 | Syncrude | OSPM | 12 | | x | x |
| Test Pond 9 | TP9 | Syncrude | OSPM | 8 | | x | x |
| Bill's Lake | BP | Syncrude | High Cond. | 5 | x | x | x |
| Crane Lake | CL | Suncor | High Cond. | 29 | | x | x |
| Crane Road Marsh | CRM | Suncor | High Cond. | 5 | | x | x |
| High Sulphate Pond | HS | Suncor | High Cond. | 16 | | x | x |
| Hydroline Wetland | HyW | Syncrude | High Cond. | | x | x | |
| Peat Pond | PP | Syncrude | High Cond. | 0 | | x | x |
| Saline Lake | SL | | High Cond. | | x | x | |
| Saline Marsh | SM | Suncor | High Cond. | 10 | | x | x |
| Sand Pit | S-PIT | Syncrude | High Cond. | 26 | | x | x |
| South Bison Pond | SB | Syncrude | High Cond. | 16 | x | x | x |
| South Hydroline East | So.HLE | Syncrude | High Cond. | | x | x | |
| South Hydroline West | So.HLW | Syncrude | High Cond. | | x | x | |
| Syncrude South Boundary | SBD | Syncrude | High Cond. | 26 | | x | x |
| Barge Marsh | BMR | | Low Cond. | 23 | | x | x |
| Hwy. 63 Intersection | H63I | | Low Cond. | 30 | | x | x |
| Hwy. 63 Wetland | H63W | | Low Cond. | 23 | | x | x |
| Leo's Pond | LP | Suncor | Low Cond. | 2 | | x | x |
| Loon Lake Wetland | LLW | Suncor | Low Cond. | 28 | | x | x |
| Millenuim Interceptor Ditch | MID | Suncor | Low Cond. | 1 | | x | x |
| N.W. Interceptor Ditch | NWID | Syncrude | Low Cond. | 23 | | x | x |
| Shallow Wetland | SW | Syncrude | Low Cond. | 8 | | x | x |
| South Boundary Beaver Pd. | SBBP | Syncrude | Low Cond. | 1 | | x | x |
| Test Pond 1 | TP1 | Syncrude | Low Cond. | 12 | | x | x |
| Tower Rd. 6.8R | TR 6.8R | | Low Cond. | 30 | | x | x |
| Tower Rd. 0.8R | TR 0.8R | | Low Cond. | 30 | | x | x |
| Tower Road 9.0L | TR 9.0L | | Low Cond. | 30 | x | x | |
| Tower Road 7.6L | TR 7.6L | | Low Cond. | 30 | x | x | |
| Tower Road 11.7L | TR11.7L7 | | Low Cond. | 30 | x | x | |
| West Interceptor Ditch | WID | Syncrude | Low Cond. | 26 | | x | x |

Clifford 1984; Jefferies 1989). The benefits of simultaneous sampling are that all wetlands are subject to the same annual conditions and all measurements are statistically independent of one another. If it is a low water year, then all wetlands will experience this, and changes in fauna will be reflected in all wetlands.

For this thesis, I selected, *a priori*, approximately equal numbers of wetlands in each of three classes (OSPM-affected, high conductivity reference, low conductivity reference) (Table 2.1 and 2.2). Several high conductivity wetlands exist on overburden material that contains lean oil sands and may have some properties of OSPM-affected wetlands, such as elevated naphthenic acid concentrations. However, the purpose of this research is to determine the length of time OSPM (tailings and tailings water) affects the zoobenthic community. Wetlands ranged in age from 0 to 30 years, the maximum age available in the lease area (oil sands mining began in the 1970's). Wetlands of varying ages were simultaneously sampled to allow for an inference into the change in the zoobenthic community with time.

Wetlands were sampled from June to July in 2000 and from June to August in 2001. Sampling of wetlands was based on accessibility of the area (within 1 km of a roadway) and availability of transportation during the season. I attempted to select a large number of young wetlands so that the early successional processes could be inferred, with as many older (> 10 years old) "one-time-addition" OSPM-affected wetlands as possible.

Intensive sampling of wetlands involved on-site physico-chemical data collection, sediment and water sample collection, chironomid collection for chromosome analysis (not used in this thesis), chlorophyll *a* analysis, and zoobenthic sample collection using cores, artificial substrates, and sweep samples. Synoptic sampling involved the same physico-

**Table 2.2** Summary of wetland site descriptions.

| Site | Code | Status | Age in 2001 | Wetland Type | Water Received |
|---|---|---|---|---|---|
| CT Wetland | CTW | OSPM | 3 | Constructed | OSPM |
| CT Pond | CTP | OSPM | 4 | Constructed | OSPM |
| Test Pond 9 | TP9 | OSPM | 8 | Constructed | OSPM |
| Demo Pond | DP | OSPM | 8 | Constructed | OSPM |
| Mature Fine Tailings-North | MFT-N | OSPM | 10 | Constructed | OSPM |
| Test Pond 5 | TP5 | OSPM | 12 | Constructed | OSPM |
| Test Pond 2 | TP2 | OSPM | 12 | Constructed | Surf. $H_2O$ / MFT release $H_2O$ |
| Natural Wetland | NW | OSPM | 15 | Natural | OSPM / Dyke seepage |
| Seepage Control Pond | SCP | OSPM | 26 | Constructed | OSPM / Dyke seepage |
| Peat Pond | PP | High Cond. Ref. | 0 | Constructed (reclamation) | Surface water |
| Bill's Lake | BL | High Cond. Ref. | 5 | Constructed (reclamation) | Surface water |
| Crane Road Marsh | CRM | High Cond. Ref. | 6 | Borrow Pit | Surface / Ground water |
| Saline Marsh | SM | High Cond. Ref. | 10 | Natural | Surface / Groundwater |
| South Bison Pond | SB | High Cond. Ref. | 16 | Constructed (reclamation) | Surface water |
| High Sulphate Pond | HS | High Cond. Ref. | 17 | Natural | Surface water |
| Sand Pit | S-PIT | High Cond. Ref. | 26 | Natural | Surface / Ground water |
| Syncrude South Boundary Ditch | SBD | High Cond. Ref. | 26 | Constructed (Diversion) | Surface water |
| Crane Lake | CL | High Cond. Ref. | 29 | Natural | Surface water |
| Saline Lake | SL | High Cond. Ref. | 45+ | Natural | Surface water |
| Millennium Interceptor Ditch | MID | Low Cond. Ref. | 1 | Creek diversion / Natural | Surface/Groundwater |
| South Boundary Beaver Pond | SBBP | Low Cond. Ref. | 1 | Constructed (beaver) | Surface water |
| Leo's Pond | LP | Low Cond. Ref. | 2 | Natural | Surface water |
| Shallow Wetland | SW | Low Cond. Ref. | 8 | Natural | Surface water |
| Barge Marsh | BM | Low Cond. Ref. | 10 | Borrow Pit | Surface water |
| Test Pond 1 | TP1 | Low Cond. Ref. | 12 | Constructed (Ref. to TP's) | Surface water |
| Hwy. 63 Wetland | H63W | Low Cond. Ref. | 23 | Borrow Pit | Surface water |
| NW Interceptor Ditch Wetland | NWID | Low Cond. Ref. | 23 | Borrow Pit | Surface water |
| West Interceptor Ditch | WID | Low Cond. Ref. | 26 | Creek diversion / Natural | Surface water |
| Loon Lake Wetland | LLW | Low Cond. Ref. | 28 | Constructed (Water use) | Surface water |
| Hwy. 63 Intersection | H63I | Low Cond. Ref. | 30 | Natural | Surface water |
| Tower Rd. 6.8R | TR 6.8R | Low Cond. Ref. | 30 | Natural (Beaver pond) | Surface water |
| Tower Rd. 0.8R | TR 0.8R | Low Cond. Ref. | 30 | Natural (Beaver pond) | Surface water |

Wetlands listed as "Natural" have formed in depressions in the ground and are not borrow pits or constructed pits.

Wetlands listed as "Constructed" had basins anthropogenically created specifically for the purpose of creating a wetland to study effects of OSPM (note exceptions in table).

Wetlands listed as "Natural (Beaver pond)" are beaver ponds.

Wetlands listed as "Constructed (Reclamation)" are wetlands constructed solely for reclamation purposes following the Alberta Environment Constructed Wetland Manual.

chemical collection as for intensive sampling. However, only sweep samples of the benthos were collected.

## Field Methods

### Physico-Chemical Data

### General Environmental Features

Qualitative field notes were taken at each site. Wetlands were sampled at varying times of the day (e.g., between 9 a.m. and 6 p.m.). General characteristics of each wetland were noted. Subjective estimates of terrestrial vegetation included percent composition and relative abundance of terrestrial plants surrounding the site, riparian cover, and dominant aquatic macrophytes. A qualitative measure (score from 0 - 5) of macrophyte development was recorded for each wetland. Wetlands with abundant macrophyte cover (> 75%) were scored as 5, wetlands with no or very little (<10 %) macrophyte cover were scored as 0. Table 2.3 provides a summary of environmental and biological attributes measured during synoptic surveys.

### Water Chemistry

At each site, measurements of pH, conductivity, salinity, temperature and dissolved oxygen (D.O.) concentration were measured. Salinity is defined as the concentrations of $Ca^{+2}$, $Mg^{+2}$, $Na^{+}$, $K^{+}$, $HCO_3^{-}$, $CO_3^{-2}$, $SO_4^{-2}$, and $Cl^{-}$ (Wetzel 1983). Water was obtained from the wetland by submersing an inverted 4-L bailer 10-cm below the surface of the water and filling it. Measurements were then taken from this sample (these readings pertained to surface of the wetland). pH was measured with an Orion QuiKchecK™ model 106 • ATC pocket meter. Oxidative-reduction potential (ORP) was measured using an Orion

QuiKchecK™ model 108 ORP pocket meter. Salinity and conductivity were measured using a YSI Model 33 - Salinity-Conductivity-Temperature (S-C-T) Meter. Temperature was measured using a pocket thermometer. Dissolved oxygen was measured using a YSI Model 51B oxygen meter. Where wetland depth was sufficient, D.O. concentration and temperature were also measured at 5 cm above the sediment surface.

On one day each field season, a 500-mL sample of water, from each wetland, was collected in a pre-cleaned glass bottle using a pole-mounted sampler. The bottle was secured in the sampler, the cap removed and the rubber stopper of the sampler placed to cover the opening of the bottle. The bottle was then lowered into the water column approximately 2 m from shore, to a depth of 50 cm (if wetland depth allowed; otherwise depth was one-half of water column) and filled. These samples were kept cool and shipped to the Syncrude Canada Ltd. Research Laboratories in Edmonton for analysis of naphthenic acids (quantification using methylene chloride extraction and FTIR method, (Jivraj *et al.* 1996)), ions (major cations by multi-element ICP-OES, anions and ammonia by Ion Chromatography, and alkalinity by automated titration), and trace metals (multi-element ICP-OES). Results of these analyse are summarised in Appendix A.2.

**Chlorophyll *a* Analysis**

Analysis of Chlorophyll *a* concentration in water samples measures estimates of algal biomass. A 500-mL Nalgene bottle was held 20-30 cm below the water surface and the cap was then removed, allowing the water to fill the container. Several drops of saturated magnesium chloride ($MgCl_2$) solution were added to prevent degradation of the

**Table 2.3.** Summary of environmental and biological attributes measured during synoptic surveys.

| Phase | Attribute | Method Used |
|---|---|---|
| General | Size | Qualitative |
| | Canopy Cover | Qualitative |
| | Macrophytes | Qualitative |
| | Chlorophyll *a* (mg $L^{-1}$) | Filtration/acetone extraction |
| Water | Dissolved Oxygen (mg $L^{-1}$) | YSI meter |
| | Conductivity (µS/cm) | YSI meter |
| | Salinity (‰) | YSI meter |
| | pH | Orion Quickcheck® |
| | Oxidative-reduction potential (mV) | Orion Quickcheck® |
| Sediment | Texture/Odour | Qualitative |
| | Loss on Ignition (%) | 440°C 3 h. |
| | Median Particle Size (phi) | Seiving |
| Benthos | Community | Cores n=5 |
| | | Artificial substrates n=5 |
| | | Composite sweep sample |

chlorophyll. The water was then filtered in the shade, under vacuum (Nalgene hand pump at 15 PSI) through a 45-mm diameter Whatman GF/C glass fibre filter mounted on a Millipore™ vacuum filtration apparatus. The filter was wrapped in aluminum foil, labelled and stored on ice until return to the laboratory. The volume of water filtered was recorded to the nearest mL.

**Sediment Characteristics**

Sediment was collected from each site using a small trowel. Sediment was scooped from the upper layers (5-10 cm depth) of the wetland substrate and placed in a plastic pan. The sediment was mixed and passed through a 1-mm plastic mesh sieve to exclude large particles and fibrous masses of organic material. Approximately 500 mL was placed in a labelled soil bag. Samples were kept cool in the field (using a cooler and ice pack) and then frozen in a -10°C chest freezer to later be analysed for sediment organic content (loss on ignition) and particle size distribution using standard ASTM methods D 2974-87, D 421-85, C136-96a, and D1140-97.

**Benthic Invertebrate Community Analysis**

**Quantitative Benthic Samples**

Benthic samples were collected using three different methods. Core samples provide an estimate of the infaunal (sediment-dwelling) community, which can reach very high densities. Cores provide a quantitative measure of natural densities of infauna. Cores are preferable to samples that collect larger volumes of sediment (e.g., Ekman and Ponar grabs) because they require less time to process and sort. However, the large zoobenthos that occur at low densities tend not to be sampled.

Artificial substrates collect large, mobile animals, and can be sorted relatively quickly compared to core or grab samples, because relatively little detritus accumulates on the substrates. However, the fauna collected may not be truly representative of the entire zoobenthic community. Sweep net (dip net) samples generate composite collections acquired over a broad area and across diverse microhabitats. However, sweep samples are only semi-quantitative and take a long time to sort because they collect large numbers of animals and lots of organic material.

**Artificial Substrate Samples**

Artificial substrates were used to provide an estimate of the epibenthic (at and above the sediment surface) and epiphytic (living on plants) animals in each wetland. The design of the artificial substrates is modified from Benoit *et al.* (1998).

Artificial substrates were constructed using 17.7 x 17.7 cm (8 x 8 inch) ceramic tiles, silicon caulking and plastic aquarium plants (artificial macrophytes) mimicking the genus *Elodea* (=*Anacharis*). Ceramic tiles were purchased at a local home improvement store, and were glazed white on one side, off-white on the unglazed side. Nine artificial macrophyte sections (each approx. 10 cm long) were affixed, equidistant from each other, to the dry, unglazed side of the tile using silicon caulking designed for use underwater.

A retrieval box was constructed from pieces of wood (2 cm x 2 cm) cut so that the box sat just inside the edge of the tile when placed atop the tile. The box was 18 cm on all sides. Mesh netting, purchased from a local fabric store (approx. 0.5 cm mesh diameter), was stretched tautly over 5 sides of the box, with one face left open for the tile to fit into. All seams and junctions (corners, etc.) were then sealed with caulking to prevent loss of animals when used for artificial substrate collection.

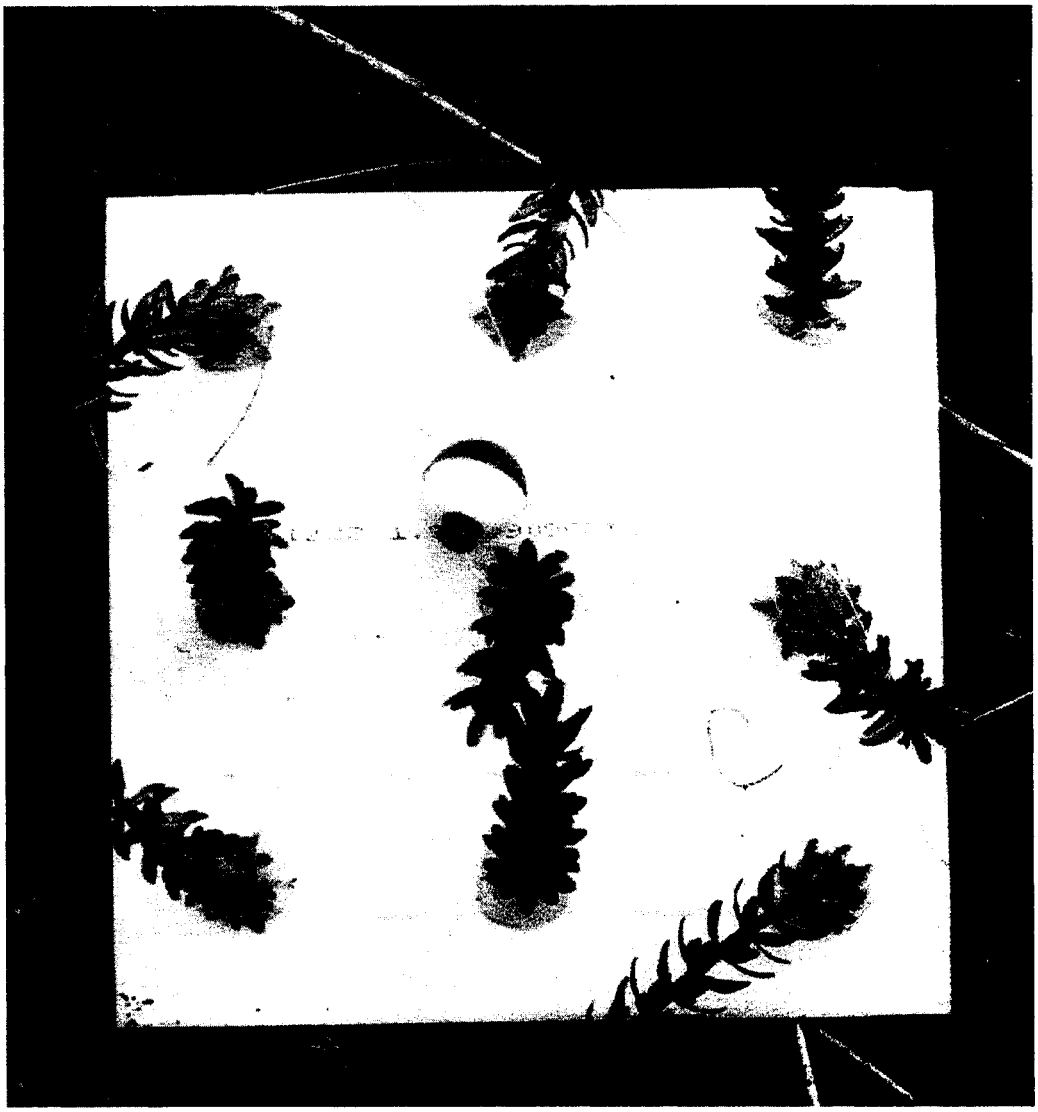


**Figure 2.2.** Photograph of the artificial substrates used in this study. Design modified from Benoit *et al.* (1998). The artificial substrate is composed of 17.7 x 17.7 cm ceramic tiles with nine - 10 cm sections of plastic macrophyte (genus *Elodea* = *Anacharis*) affixed using aquarium caulking. A red and white fishing float was affixed to the tile using a harness of monofilament fishing line to facilitate location and retrieval eight days later.

Five tiles were placed approximately equidistant around the perimeter of a wetland to best estimate each type of habitat (emergent vegetation, submergent vegetation and open water to 1-m depth). The slope and stability of the substrate dictated the location of the tile and its distance from the shore. Tile location within the wetland was marked using a red and white fishing float attached to an anchoring harness, made of monofilament fishing line, around the tile. Water depth above the tile, distance from shore, and compass triangulation for each tile was taken. Tiles were left in the wetland for 8 days.

After 8 days each tile was removed using a retrieval box and a 12–L rinse bucket. Prior to removal of each tile, approximately 8-L of pond water was poured through a 250-μm sieve into a 12-L plastic pail. A 1-L Nalgene squeeze bottle was then filled with the sieved water for rinsing the tile and retrieval box.

A tile was retrieved from the wetland by carefully placing the box over top of the tile in a manner that created as tight a seal as possible and prevented the macrophytes from protruding. Once the box was lowered in place, the collector's hands were carefully slid under the tile and the tile and box apparatus was lifted from the substrate. As the box was raised, excess water drained through the sides of the box. The tile and box apparatus was then placed above a 12-L pail and the tile was allowed to fall into the pail. The box was rinsed of any debris left on the mesh. The tile was agitated, in the bucket, in an up-and-down motion to remove any large debris and sediment.

Once the tile was sufficiently cleared of large debris, the squeeze bottle was used to remove smaller animals adhering to the tile and the artificial macrophytes. The debris and water in the bucket were then poured through the 250-μm sieve bucket to remove excess water and retain any organism larger than 250 μm. The sieve bucket was agitated, in the

wetland, in an up-and-down and twisting motion, until the sample was sufficiently rinsed of fine sediments (the water draining from the bucket was relatively clear).

**Core Samples**

To provide a measure of the infauna (animals living in the sediment) of the wetland, benthic cores were taken near each tile at the times of placement and removal 8 days later (5 replicate samples taken twice at each wetland for a total of 10 core samples per wetland per season).

The core apparatus consisted of a 25.3-cm length of clear polyvinyl carbonate (PVC) tube (inside diameter 6.5 cm). The tube was fitted with a plunger of equal length, whose external diameter measured slightly less than 6.5 cm, providing a snug fit between the core and plunger. A core was taken by inserting the PVC tube into the substrate as deeply as possible. The top of the tube was then sealed by firmly inserting a plastic yogurt cup. The collector's hand was slid under the substrate and capped the base of the tube. The tube was then removed from the water. The top of the tube was then held over a 250-μm sieve bucket as the plunger was carefully slid into the bottom of the tube.

Once the plunger was secured, the plastic cup was removed and the sediment and water were forced upward by applying pressure from the bottom with the plunger. The water and top 5 cm of sediment extruded from the top of the tube (166 $cm^3$ = 166 mL) were deposited in the sieve bucket. The sediment was then rinsed several times to remove particles and animals smaller than 250 μm.

**Qualitative Benthic Samples:**

**Sweep Net Samples**

A single composite, qualitative sweep sample was collected at each wetland visited. Invertebrates were collected by moving a D-frame net (30-cm opening, 500-μm Nytex mesh; BioQuip Inc., Sacramento, CA) through emergent and submergent vegetation and across the surface of the wetland substrate. The sweep was conducted along a transect length of a few metres up to 1 metre from the shoreline. Material collected in the net was gently rinsed in an up-down motion to remove fine sediment and with care taken to not lose any animals. The rinsed material was then placed in a plastic tray for qualitative observation and recording of the fauna present. The sample was then decanted back into the net, allowed to drain and placed in a labelled polyethylene soil bag.

**Sample Field Processing and Preservation**

Each sample was field-rinsed in a 250-μm mesh sieve bucket to remove fine particles and retain organisms larger than 250 μm. The material retained by the sieve and a location label were placed in a 4-L polyethylene soil bag and preserved with formalin-ethanol solution (30 parts water (added in field): 15 parts 95% ethanol: 6 parts 100% formalin) and sealed with a twist tie. Samples were stored upright, in a plastic tote until processed in the laboratory.

## Laboratory Methods

### Physico-chemical Data

### General Environmental Features

Data were transcribed from field notes and entered into Excel® and STATISTICA® database tables.

### Water Chemistry

Field measurements of water chemistry were compiled and entered into Excel® and STATISTICA® databases. Water samples (500 mL) were analysed by the Syncrude Canada Ltd. Research laboratory for major ions (Na, Ca, K, Mg, F, Cl, $SO_4$, $CO_3$ and $HCO_3$), trace metals (Al, B, Ba, Cd, Co, Cu, Fe, Li, Mn, Ni, Pb, Sb, Se, Sr, Si, Ti, V, and Zn), naphthenic acid concentration, total dissolved solids, and laboratory measures of temperature, pH, and conductivity using SCL standard methods.

### Sediment Analysis

Sediment samples were processed in the laboratory to determine organic content, through loss on ignition, and particle size distribution. Methods used are based on ASTM designations D-2974-87, D-2977-71, and C-136-96a, with modifications for hand sieving.

Frozen sediments were thawed for 24 hours at room temperature. Excess water was poured off and wet mass of the sample (approximately 100 g) measured. The sediment was then spread on a sheet of aluminum foil, air-dried for 48 hours, oven dried at 105°C for 24 hours, cooled in a desiccator, and weighed (dry mass). Water content was calculated from oven dried sample using the equation:

$$\text{Water content (\%)} = \frac{[(A - B) * 100]}{A}$$

Where A is sample wet mass (g) and B is sample dry mass (g). The oven-dried sample was transferred to a ceramic crucible and incinerated in a muffle furnace, at 440°C for at least 2 hours, until a uniform colour was reached. The incinerated sample was cooled to room temperature in a desiccator and weighed to the nearest milligram. Loss on ignition was determined by first calculating % ash content using the equation:

$$\text{Ash Content \% (D)} = \frac{C \bullet 100}{B}$$

Where C is sediment ashed mass (g) and B is oven dried weight (g). To determine loss on ignition (LOI) ash content (%) is subtracted from 100.

**Particle Size Distribution**

Ashed sediment samples were used to determine the particle size distribution (particles greater than 90 μm). Samples were ground, with a ceramic mortar and pestle, and hand sieved through a standard brass sieve series. Mesh sizes were 8.0, 4.0, 2.0, 1.0, 0.5, 0.25, 0.125, and 0.090 mm. Material was passed through the sieves using a side-to-side and up-down motion for 2 minutes. Material retained in each sieve was re-ground and re-sieved until all possible material had been retained.

Median particle size distribution was determined by interpolation after plotting cumulative sample mass against Wentworth Scale value ($\log_2$ (particle diameter)). In the summer of 2002, storage freezer failure resulted in the loss of sediment samples collected in 2001. Sediment values for wetland sites that had been sampled in previous years (1998-99) were substituted for the analysis.

**Chlorophyll *a* Analysis from Water Samples**

Chlorophyll *a* samples collected in 2001 were lost due to freezer failure in the summer of 2002 and will not presented.

**Benthic Invertebrate Community Sample Processing**

Samples from the 33 sites studied in 2000-01 (qualitative and quantitative samples) were processed following the methodology of Ciborowski (1991). A nested sieve series (4.00 mm, 1.00 mm, 0.500 mm, 0.250 mm) was placed in a large container (we used a 25 L plastic bucket). The preservative and sample were gently decanted into the sieves. The sample was repeatedly rinsed with water and decanted until the odour of formalin was negligible. Formalex® (S&S Company of GA, Inc.) was then added to the decanted preservative to break down the formalin. This liquid was then discarded.

Samples were gently rinsed with tap water until a sufficient amount of material was rinsed through the sieves. Samples were also "gold-panned" to separate organic material from inorganic material. Material retained in each sieve was placed in a petri dish or, if the sample was particularly large, separated using Ludox ™ (DuPont). Ludox is a colloidal silica polymer with a specific gravity of 1.15 g $cm^{-3}$. de Jonge and Bouwman (1977) reported that this product reduced sorting time of estuarine benthos by one-third by floating the benthos and allowing the sediment to settle to the base of a separatory receptacle .

Samples were sorted using a grid-etched petri dish and a dissecting microscope. Four-mm and 1-mm fractions were scanned twice under 6X power. The 500-μm and 250-μm fractions were scanned once under 12X and once at 25X. All dishes were scanned twice and all animals were removed from each portion of the sample. If samples contained

large quantities of organic material or animals (such as micro-crustacea) only a one-quarter subsample was sorted such that each sample took up to 2 hours to process. However, some samples contained substantial organic matter in the larger fractions (4-mm and 1-mm) which increased processing time up to 8 hours. The coarse organic material (detritus >1mm) that remained after the sample was sorted, was dried in a drying oven at 60°C for 48 hours, and dry mass recorded to the nearest milligram.

Animals were enumerated and identified to genus (excluding chironomids and oligochaetes) using the keys summarised in Table 2.4. Identified animals were placed in individually labelled vials. Chironomids and oligochaetes were mounted on microscope slides for identification to genus and species, respectively.

Chironomids collected in 2000 were mounted using CMC -9AF aqueous mounting medium (Masters Company, Inc., Wood Dale, IL). Groups of up to 5 individuals were mounted beneath a single coverslip, with a maximum of 2 cover slips used per microscope slide. Head capsules were removed and placed ventral side up to facilitate viewing the mouthparts. Chironomids were identified using the keys of Wiederholm (1983) and Oliver and Roussel (1983). Chironomids from samples collected in 2000 were identified to genus, specimens from 2001 were identified to only sub-family or tribe.

Oligochaetes were slide mounted using CMC-10 aqueous mounting medium (Masters Company, Inc., Wood Dale, IL). A proportion of the oligochaetes collected were identified to genus, where possible, using the keys of Kathman and Brinkhurst (1999) and Klemm (1985). However, identification to genus was difficult because of immature specimens, and thus oligochaete data presented will be for the class Oligochaeta only.

**Table 2.4.** List of taxonomic keys used for identification of wetland invertebrates

| Taxon | | Taxonomic Reference |
|---|---|---|
| **Aquatic Stages** | | |
| Annelida | Oligochaeta | Kathman & Brinkhurst (1999)<br>Klemm (1985) |
| Crustacea | Amphipoda<br>Other taxa | Clifford (1991), Peckarsky et al. (1990), Pennak (1978) |
| Hexapoda | Ephemeroptera | Clifford, (1991), Edmunds et al. (1976) |
| | Trichoptera | Wiggins (1998) |
| | Odonata | Westfall & May (1996), Needham et al. (2000) |
| | Diptera - Chironomidae | Wiederholm (1983), Oliver & Roussel (1983), Coffman & Ferrington (1996) |
| All Other Orders | | Clifford (1991), Peckarsky et al. (1990), Pennak (1978), Merritt & Cummins (1996) |

## Statistical Analyses

### Physico-Chemical Data

The physical, chemical and biological features measured in environmental studies are typically intercorrelated. Principle components analysis (PCA) was used to reduce the physico-chemical data set to a smaller number of statistically independent variables against which to ordinate environmental characteristics of the wetlands sampled. Cluster analysis was used on the PCA scores to provide information on the similarity of sites within the generalised PCA groupings. Cluster groups were generated using Ward's method, based on Euclidean distances among site attributes. The results allowed us to compare OSPM-affected sites to environmentally similar reference sites, in terms of responses measured at the community level.

Chemical properties (total salinity, conductivity, naphthenic acid concentration (all $\log_e$ +1 transformed) and pH) and sediment (LOI, detritus, median particle size (all $\log_e$ +1 transformed), pH and ORP) characteristics were used in the PCA. Where meter measures where suspect (e.g., pH had jumped from 8 to 11 in 24 h), values were replaced with values previously obtained at each site in 1998-99.

Data were analysed using the STATISTICA® software package. Principal components analysis was performed on the correlation matrix generated from the data set using varimax rotation of the derived factors.

### Benthic Invertebrate Community Data

Benthos for each sampling type (core, artificial substrate, and sweep) were analysed independently. For core samples, two cores from the time of substrate placement and two

cores from the time of substrate removal were processed, for a total of four cores per wetland. The mean number of animals found in each core was calculated to give a single value for each wetland.

The mean number of animals from two artificial substrate samples was calculated to provide a single value for each wetland. Sweep samples were only collected once per wetland.

Data for each of the core samples, artificial substrate samples, and sweep samples were tabulated in raw form (numbers recovered per sieve size fraction in each sample, summed), and in estimated total numbers per sample (numbers recovered/proportion of sample sorted, summed over size fractions) for each taxon identified (see Appendix A.1 for details).

For analysis of benthic community data, abundances of taxa were summarised in three ways to determine which method best measured the community composition. Abundance, $\log_e + 1$ transformed, is a measure of the total number of individuals present at a wetland site. Relative abundance, determined by dividing the number of representatives of a taxon by the total number of organisms in each sample, was used to provide a measure of the contribution of that taxon to the overall community. $\text{Log}_2$ relative abundance was used as well. This transformation converts the relative abundance values to octaves and gives more weight to rarer taxa (Gauch 1984).

Richness (a measure of diversity) and abundance were determined by calculating the mean number of taxa per sample. Microcrustacea (Cladocera, Copepoda, and Ostracoda) and planktonic and non-benthic organisms (gerrids, terrestrial invertebrates) were excluded from tallies as the objective was to observe the patterns in benthic macroinvertebrate fauna.

Multivariate analyses are unduly influenced by rare taxa, thus only 'common' groups of organisms were used. To be included in the multivariate analyses, a taxon had to meet the following two criteria:

1) present at 18 percent or more of the wetlands (6 or more sites)

2) have a total mean abundance ($\log_{10} + 1$ transformed) value of greater than 0.1 per sample unit

A total of 20 taxa met these criteria for core samples, 20 taxa for artificial substrate samples and 23 taxa for sweep samples. Seventeen of these taxa were common to all three sampling techniques (Table 2.8 A, B, C).

**Comparing "Natural" and Constructed Reference Wetlands**

Cluster analysis was used to determine how similar reference wetlands on lease sites (some constructed (TP1, PP, SB etc.), and some more natural (SM, CRM, HS, etc.)) were to reference wetlands off lease sites (such as borrow pits that fill with water, e.g., BM, H63W, or beaver ponds, TR0.8R) both physico-chemically and biologically. For biological data, relative abundance was used for each sampling method. A cluster analysis using Ward's method and Euclidean distances was used to generate a dendrogram of wetland sites. An *a posteriori* ANOVA technique (Green and Vascotto, 1979) was used to determine to which variables contributed to the differences among the groups observed in the cluster analysis. The null hypothesis ($H_0$) for this section is that reference wetlands were equivalent, biologically and physico-chemically, regardless of their proximity to the lease sites.

### Comparing Benthic Invertebrate Richness and Abundance Changes with Wetland Age

To determine how taxonomic richness and abundance changed with wetland age, I plotted richness vs. age and ($\log_{10}$+1) abundance vs. age. Each group of points for each wetland class was fitted with a line using the "$\log_{10}$ fit option" of Statistica®. A one-way analysis of covariance (ANCOVA) and F- ratios were then used to determine if the adjusted differences in richness or abundance among wetland classes were statistically significant.

### Physico-chemical Factors Affecting Benthic Invertebrate Richness and Abundance

Multiple regression was used to determine which physico-chemical factors (pH and ORP of the water and sediment, conductivity and naphthenic acid concentration of the water, detritus, sediment organic content and median particle size of the sediment, wetland area, and macrophyte development) most influenced benthic invertebrate richness and abundance for each sampling method. Abundance values were $\log_e$+1 transformed for this analysis. Salinity, ORP-water, conductivity (water), naphthenic acid concentration, LOI, detritus and wetland area were $\log_e$+1 transformed.

## Results

### Physico-chemical Data

### Summary of General Environmental Features

In Table 2.5, the range of environmental variables at reference and OSPM-affected sites are summarised. Minimum, median and maximum values were determined for qualitative parameters (See Appendix A.2 for raw data).

**Table 2.5.** Range of values at reference and OSPM-affected sites in 2000 & 2001

| From 2000 data | Low Conductivity Reference | | | | High Conductivity Reference | | | | OSPM-affected | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Min. | Median | Max. | n | Min. | Median | Max. | n | Min. | Median | Max. | n |
| Cond. ($\mu$S/cm) | 440 | 600 | 1050 | 5 | 662 | 1480 | 2000 | 5 | 1050 | 2550 | 4850 | 7 |
| Salinity (‰) | 0 | 0.02 | 0.03 | 5 | 0.3 | 1.3 | 1.7 | 5 | 0.8 | 1.8 | 3.5 | 7 |
| pH (water) | 8.18 | 8.9 | 10.4 | 5 | 8.1 | 8.84 | 10.05 | 5 | 8.3 | 8.9 | 9.2 | 7 |
| Naphthenic Acid (mg/L) | 1.0 | 1.7 | 2.5 | 5 | 3.1 | 12.18 | 15.3 | 5 | 7.2 | 45.5 | 65.2 | 7 |
| LOI (%) | 1.81 | 5.6 | 19.9 | 5 | 3.1 | 8.99 | 18.5 | 5 | 3.8 | 5.8 | 9.6 | 7 |
| Median Particle (phi) | 2.5 | 3.0 | 4.0 | 5 | 2.0 | 3.0 | 4.0 | 5 | 3 | 3.7 | 4.1 | 7 |

| From 2001 data | Low Conductivity Reference | | | | High Conductivity Reference | | | | OSPM-affected | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Min. | Median | Max. | n | Min. | Median | Max. | n | Min. | Median | Max. | n |
| Cond. ($\mu$S/cm) | 176 | 331.5 | 933 | 8 | 768 | 1903 | 5030 | 6 | 2020 | 2240 | 5050 | 3 |
| Salinity (‰) | 0 | 0 | 0.4 | 8 | 0.3 | 1.3 | 3.0 | 6 | 1.1 | 1.5 | 3.8 | 3 |
| pH (water) | 6.8 | 7.7 | 9.05 | 8 | 7.01 | 7.8 | 8.9 | 6 | 8.5 | 8.5 | 8.9 | 3 |
| Naphthenic Acid (mg/L) | 1.0 | 1.25 | 1.9 | 8 | 0.5 | 5.25 | 8.7 | 6 | 52.3 | 58.6 | 61.1 | 3 |
| LOI (%) | 2.7 | 5.3 | 10.4 | 8 | 4.5 | 14.4 | 28.4 | 6 | 4.6 | 5.8 | 6.2 | 3 |
| Median Particle (phi) | 2 | 2.9 | 4 | 8 | 3.4 | 4 | 4.5 | 6 | 3 | 4 | 4.1 | 3 |

In 2000, OSPM-affected wetlands had the highest measured values for salinity, conductivity, and naphthenic acid concentration, while also having lower sediment organic content (LOI) than high conductivity reference wetlands (Table 2.5). Water pH was similar across all wetland classes. Low conductivity reference wetlands had conductivity values that over-lapped with high conductivity values (Table 2.5). However, low conductivity reference wetlands had the lowest salinity values, and surprisingly the lowest sediment organic content (Table 2.5).

In 2001, the OSPM-affected wetlands again had the highest measured values for conductivity and naphthenic acid concentration (Table 2.5). However, this year the salinity values were more comparable to high conductivity reference wetlands (Table 2.5). The sediment organic content was lowest of all wetland classes (Table 2.5). High conductivity reference wetlands had higher conductivity values compared to 2000. Naphthenic acid concentrations were lower in 2001 compared to 2000, due to the lower naphthenic acid concentrations in the wetlands sampled in 2001 (Table 2.5, Appendix A.2). High conductivity reference wetlands had the highest sediment organic content of all wetland classes (Table 2.5). Low conductivity reference wetlands had conductivity values that overlapped with high conductivity reference wetlands. However, low conductivity reference wetlands also had the lowest salinity and naphthenic acid concentrations (Table 2.5).

## Multivariate Analysis of Environmental Features

### Principal Components Analysis

Principal components analysis accounted for 71.1 % of the variation seen in the environmental data set (Table 2.6). Four principle components removed significant amounts of variation.

Values of salinity, conductivity and naphthenic acid concentration (all $\log_e$+1transformed) were strongly positively associated with the first principal component (PC-I). Macrophyte development was weakly negatively correlated with PC I. Median particle size was also weakly associated with PC-I (Table 2.6). Thus, wetlands having high scores for PC-I were saline, with high conductivity and high concentration of naphthenic acids, and had finer grained sediment. This component accounted for 29% of environmental variation among wetland variables measured.

Values of sediment organic content (LOI), median particle size, and detritus (coarse organic material) were strongly positively associated with the second principal component (PC-II). Water pH was strongly negatively associated with PC-II (Table 2.6). Thus, wetlands having high scores for PC-II had higher amounts of coarse organic material (detritus), finer particles, and higher sediment organic content, while having more alkaline water (Table 2.6, Figure 2.2). This component accounted for 15% of environmental variation among wetland variables measured.

Macrophyte development and sediment pH were positively associated with the third principal component (PC-III) (Table 2.6). Sediment ORP had a strong negative association with PC-III. Wetlands having high scores in PC-III were characterised by having > 50% macrophyte coverage, and displaying slightly alkaline sediments, that were strongly

**Table 2.6.** Principal component scores of environmental variables on four principal components derived for 34 wetlands. Loadings represent the correlation between the value of the variable and the score of each principal component. Bold faced values are significant at $p<0.01$. "Prop. Explained" is the proportion of the original variance among the 10 environmental variables accounted for by each principal component. Note that values for salinity, conductivity [naphthenic acid], LOI, and wetland area were $\log_e +1$ transformed prior to use in the PCA to meet the multivariate assumptions of normality and linearity.

| Variable | PC-I | PC-II | PC-III | PC-IV |
|---|---|---|---|---|
| Conductivity | **0.958** | 0.087 | 0.051 | 0.097 |
| Salinity | **0.955** | 0.096 | 0.036 | -0.097 |
| [Naphthenic Acid] | **0.798** | -0.302 | -0.178 | 0.237 |
| Median Particle Size | **0.452** | **0.450** | 0.328 | 0.325 |
| Sediment Organic Content (LOI) | 0.211 | **0.789** | 0.106 | 0.148 |
| Detritus | -0.293 | **0.524** | -0.344 | -0.198 |
| pH (Water) | 0.174 | **-0.610** | 0.150 | 0.352 |
| Macrophyte Dev. | **-0.410** | 0.227 | **0.688** | 0.019 |
| pH (Sediment) | -0.214 | 0.132 | **0.533** | -0.245 |
| ORP (Sediment) | -0.328 | 0.213 | **-0.734** | 0.067 |
| Wetland Area | -0.033 | -0.015 | 0.064 | **-0.905** |
| Prop. Explained | 0.287 | 0.154 | 0.145 | 0.125 |
| Cumulative Prop. | 28.7% | 44.1% | 58.6% | 71.1% |
| Eigenvalue | 3.317 | 1.755 | 1.528 | 1.093 |

reduced (has a very negative OPR value = anoxic). PC-III accounted for 15% of variation among measurements.

Wetland size (area) was the only environmental variable associated with PC-IV (Table 2.6). Wetlands with negative values for PC-IV were larger in size.

The pattern of environmental similarity among 33 wetland sites included in the multivariate analysis is summarised in a bivariate scatterplot of the principal component scores for PC-I and PC-II (Figure 2.3). Wetlands with high values in PC-I were saline, with high conductivity and salinity, and few macrophytes. Most young OSPM-affected wetlands, along with a few young (PP) saline reference wetlands (SB, SM, and S-Pit) are located here. Wetlands low in PC-I are fresh water with many macrophytes. Low conductivity reference wetlands (e.g. TR0.8R, TR6.8R, MID) have these characteristics.

Wetlands with large values of PC-II have alkaline water, organic sediment and more detritus. Most reference wetlands with organic sediments (e.g. SBD, SM, SB, and PP) characterise this group. Wetlands with smaller values of PC-II have circumneutral water, less detritus and mineral sediment (e.g. LLW, S-Pit, and H63W).

**Cluster Analysis**

Cluster analysis was performed using the four PCA-derived variables to determine the Euclidean distance relationship among 33 wetland sites (See Table 2.5 for the range of values; see Table 2.6 for PC scores; See Appendix A.2 for detailed water chemistry values). Ward's method was used to cluster sites that were most similar to one another. An analysis of variance (ANOVA) approach (Green and Vascotto 1978) was used to determine which PC's had the most influence on each group. F-ratios were calculated using the ANOVA module of Statistica V6.0™ using four groups.

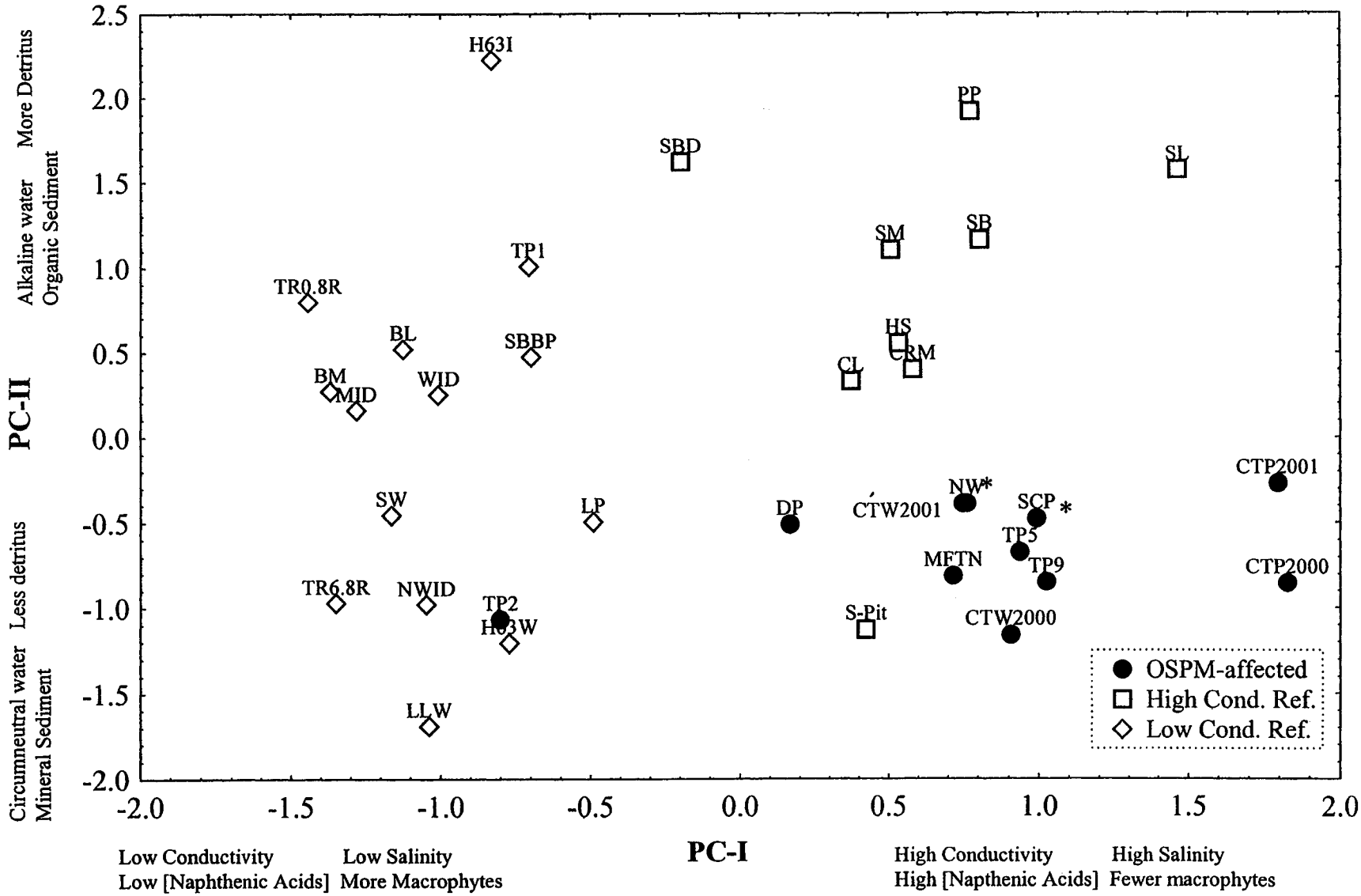



**Figure 2.3**. Bivariate scatterplot of the principal component scores for PC-I and PC-II showing the pattern of environmental similarity among 34 wetland sites. Asterisks represent wetlands that receive periodic OSPM inputs.

Group A was separated from Group B by water chemistry (PC I (F=27.41) and PC II (F=7.06)). Wetlands in Group A had higher conductivity, salinity, and concentrations of naphthenic acid ($F_{(4,12)}$=27.23; $p< 0.001$). Wetlands in Group B had lower conductivity, salinity and concentrations of naphthenic acid (Figure 2.4).

Wetlands in Subgroup A1 were separated from Subgroup A2 by PC-II (F=-17.39, $p<0.001$) and PC-III (F= 4.71, $p<0.05$) (F=8.67). Subgroup A1 consisted of eight OSPM-affected wetlands. Of these, five were from the Syncrude lease (CTP2000, CTP2001, TP9, SCP, DP) and one was from the Suncor lease (CTW2000, CTW2001). All of the A1 sites were relatively small in size and had high conductivity (2240-5050 µS/cm). Naphthenic acid concentrations (42.1 - 65.2 mg/L) and salinity (1.2 - 4.2 ‰) were high. These wetlands also had more mineral sediment (4.6-9.61%), less detritus (8.26-39.6 g), and more alkaline water pH (8.4-9) compared to sub-cluster A2 (Figure 2.4).

Subgroup A2 consisted of two OPSM-affected wetland (TP5, NW), one off-lease low conductivity reference wetland (H63I), and eight high conductivity reference wetlands (CRM, HS, CL, SL, PP, SM, SB, SBD) (Figure 2.4). These wetlands had a lower range of conductivity (565-5530 µS/cm), salinity (0-4.0 ‰) and naphthenic acid concentration (0.5-57.8 mg/L) than wetlands in subgroup A1. These wetlands also had more organic sediment (5.3-28.4%), more detritus (16.3-93.21 g) and circumneutral water pH (7.01-8.97) (Figure 2.4 and Appendix A.2).

Subgroup B1 contained one OSPM-affected wetland (TP2), one high conductivity reference wetland (BL) and six low conductivity reference wetlands (LP, SW, NWID, H63W, TP1, and WID). Wetlands in this subgroup were separated from subgroup B2 by PC-III (F = 6.76, $p<0.05$) and PC-IV (F= 12.25, $p<0.01$) (F=5.73). Wetlands in B1 had

moderate levels of conductivity (378-1050 μS/cm), a range of salinity (0-0.4 ‰) and naphthenic acid concentrations between 1.0-3.1 mg/L. Wetlands in subgroup B1 had more alkaline sediment pH (7-9.6), in a more reduced state (-84 to-309 mV), and were wetlands of moderate size (Figure 2.4).

Subgroup B2 contained one high conductivity reference wetland (S-Pit) and six low conductivity reference wetlands (LLW, TR6.8R, SBBP, and BM, MID, TR0.8R). Wetlands in subgroup B2 had higher levels of conductivity (176-1480 μS/cm), a higher salinity (0- 1.3 ‰) and higher naphthenic acid concentration (1.0-12.2 mg/L). Wetlands in subgroup B2 had more circumneutral sediment pH (7.1 - 8.5), in a more oxidised state (+3 to-129 mV), and were wetlands of larger size (Figure 2.4).

## Comparing "Natural" and Constructed Reference Wetlands

### Physico-chemical

A great physical distance (approx. 100 km from the furthest south to the furthest north wetland - See Figure 2.1) separates reference wetlands from each other. To determine how similar (or different) reference wetlands are to each other cluster analysis was performed using Ward's method and Euclidean distance to graphically represent the groups of wetlands. Subsequently, an ANOVA was calculated, *a posteriori*, using four groups, following the approach of Vascotto and Green (1978). F-ratios were calculated using the ANOVA module of Statistica V6.0™ using four groups, to identify which variables were most different among the groups identified by the cluster analysis.

The null hypothesis ($H_o$) for this analysis was that reference wetlands were equivalent physico-chemically, regardless of their proximity to the lease sites. That is,

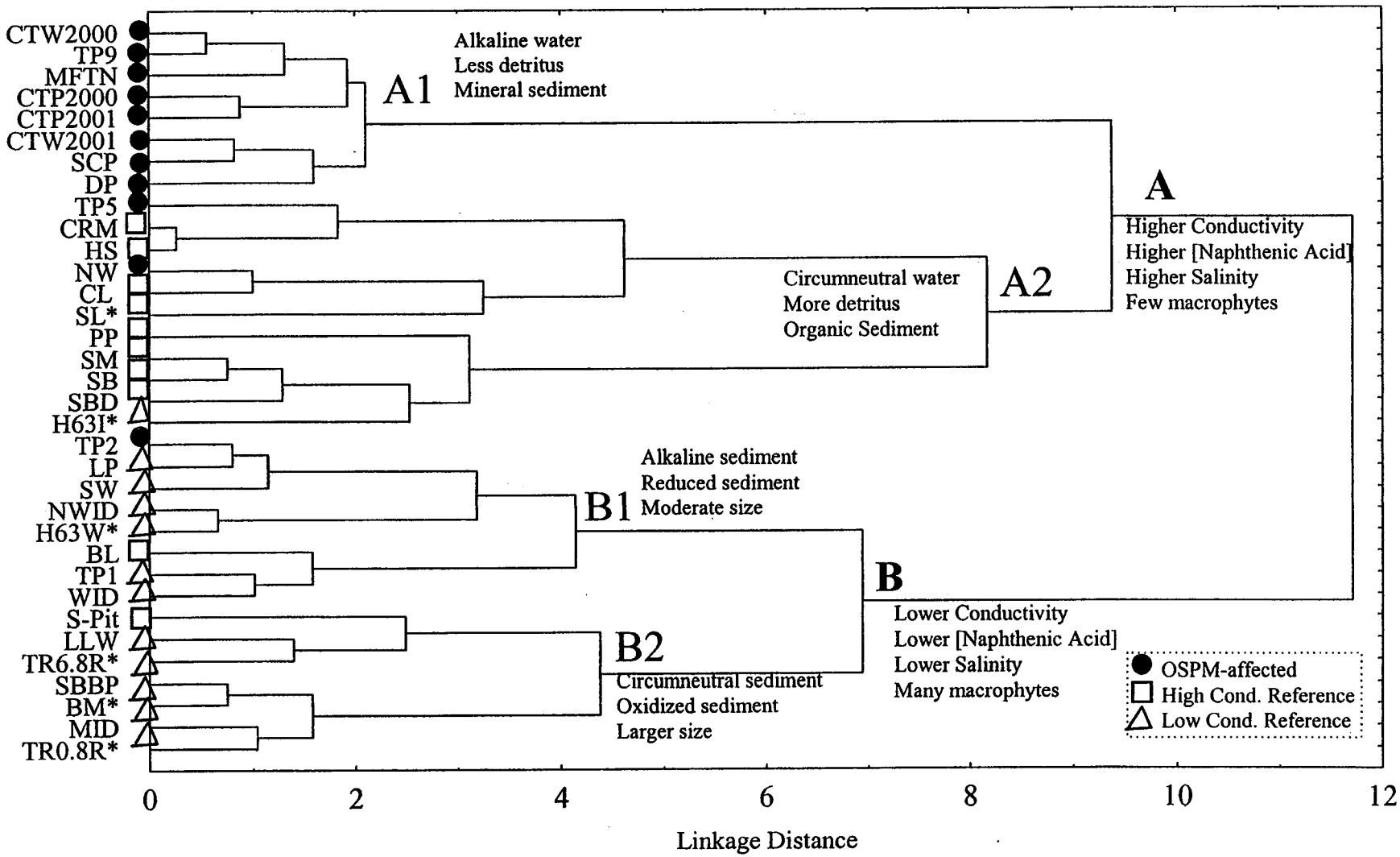



**Figure 2.4**. Dendrogram of wetland sites using three PCA-derived variables. Ward's method and Euclidean distances used. Off-lease sites indicated with an asterisk. Groups A & B are separated from each other by characteristics of water chemistry with PC I ($F_{3,33}$=27.41; $p < 0.001$) and PC II ($F_{3,33}$=7.06; $p < 0.001$) contributing to the differences.

location of a wetland relative to oil sands lease areas does not influence whether a wetland will be classified into one group or another. Cluster analysis of reference wetlands using physico-chemical data is shown in Figure 2.5. Sites marked with an asterisk are located off lease of Syncrude and Suncor.

Wetlands in Groups A and B were separated based on sediment ORP values ($F=144.24$, $p<0.001$) and conductivity ($F= 4.67$, $p<0.05$) ($F=12.93$). Wetlands in Group B have higher conductivity (675-5030 μS/cm) than wetlands in Group A (176-3750 μS/cm) (Figure 2.5, Appendix A.2). Wetlands in Group B also have more reduced sediment (-179 to -309 mV) than wetlands in Group A (+71 to -122 mV) (Figure 2.5).

Group A contains two sub-groups. Subgroup A1 differs from Subgroup A2 because of differences in water pH ($F = 6.05$, $p<0.05$) and sediment ORP ($F = 24.50$, $p<0.001$) ($F=3.58$). Wetlands in Subgroup A1 have more oxidised sediment (+71 to -39 mV), as well as more alkaline water pH (6.8-8.9) compared to Subgroup A2 (-73 to -122mV, 7.4-10 pH).

While it might be expected that wetland sites located off-lease would cluster as a single group with similar physico-chemical attributes, this was not the case (Figure 2.5). Three off-lease wetlands (TR0.8R. TR6.8R, H63I) clustered together with other wetlands having similar attributes. Off-lease wetlands in subgroup A1 & A2 have similar salinity, and naphthenic acid concentrations to each other and to on-lease reference wetlands (Figure 2.5). Interestingly, the four off-lease sites in Group A are made of the two southern most (TR0.8R, TR6.8R) and northern most (H63I, BM) wetlands (Figure 2.1 and Figure 2.5). One off-lease wetland (H63W) clustered most similarly to on-site reference wetlands in close proximity to it (~ 5 km). Another off-lease wetland (SL) also clustered closely with

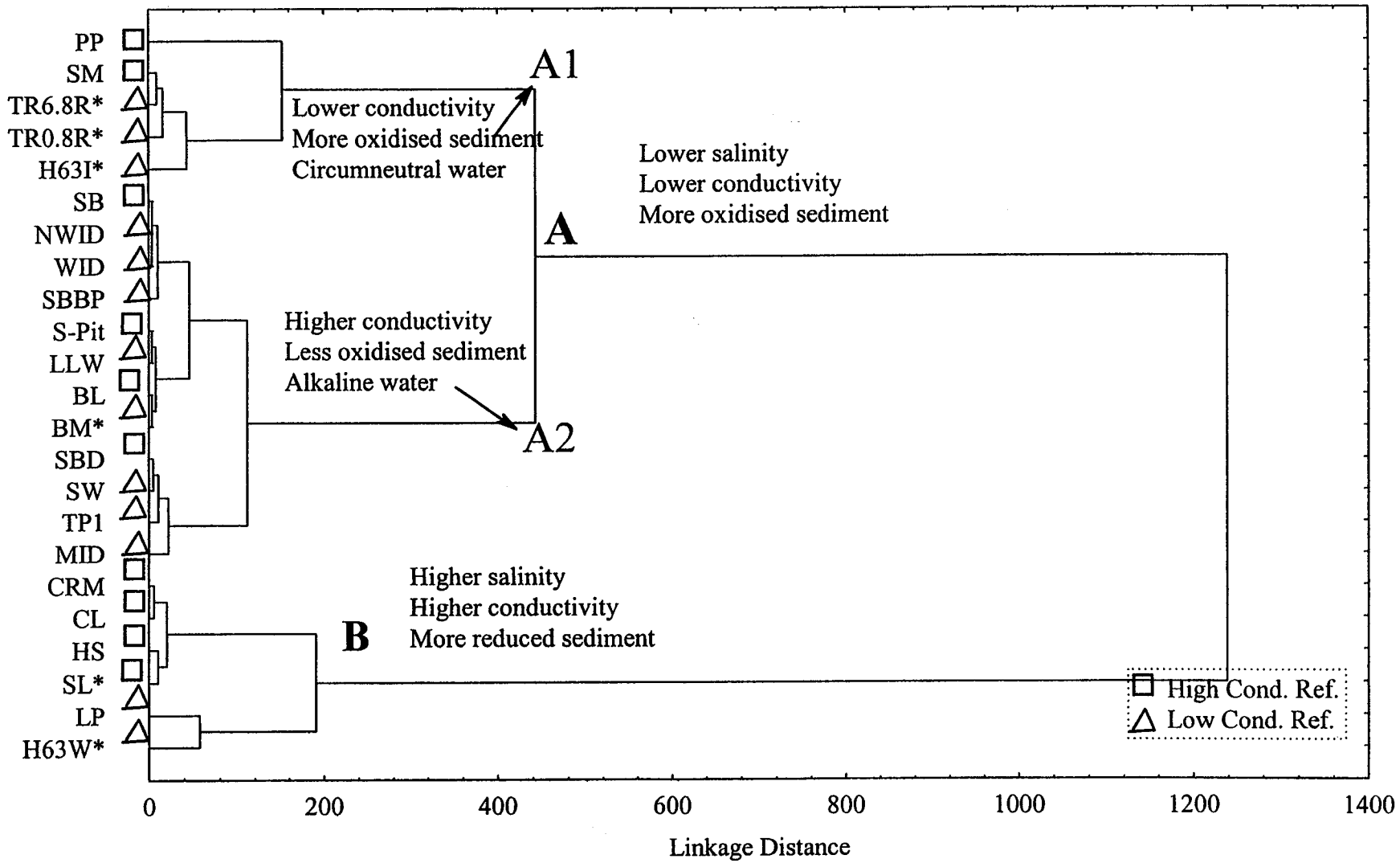



**Figure 2.5**. Cluster analysis of reference sites using physico-chemical data. Wetlands located off lease areas are indicated by an asterisk. Ward's method and Euclidean distance used. Groups A & B are significantly separated by conductivity ($F = 4.67$, $p<0.05$) and sediment ORP ($F = 144.24$, $p<0.001$). Subgroups A1 & A2 are separated by water pH ($F = 6.05$, $p<0.05$) and sediment ORP ($F = 24.50$, $p<0.001$).

on-site reference wetlands. This analysis indicates that off-lease wetlands are physico-chemically similar to on-lease reference wetlands.

**Biological**

To determine how similar (or different) reference wetlands are to each other a cluster analysis was performed using Ward's method and Euclidean distance to graphically represent the biological groups of wetlands. An ANOVA was then calculated, *a posteriori*, using three groups, following the approach of Vascotto and Green (1978). F-ratios were calculated using the ANOVA module of Statistica V6.0™ using four groups, to identify which variables were most different among the groups identified by the cluster analysis.

The null hypothesis ($H_o$) for this analysis is the same as for the previous analysis. Cluster analysis of reference wetlands using biological data is shown in Figure 2.6. Sites marked with an asterisk are located off lease of Syncrude and Suncor.

Relative abundances of selected taxa were used in this analysis (19 taxa for core samples, 21 taxa for artificial substrate samples, and 23 taxa for sweep samples). Cluster analysis of reference wetlands using relative abundance of benthic invertebrate data is shown in Figure 2.6-2.8. Sites marked with an asterisk are located off lease of Syncrude and Suncor.

For core samples, Group A was separated from Group B by relative abundances of Tanytarsini ($F = 20.96$), Oligochaetes ($F = 68.8$) and leptocerid caddisflies ($F = 7.59$). Wetlands in Group A (PP, SBBP, H63I, SL, CRM, SPit, HS, TR0.8R, WID, SBD, CL, NWID, H63W) had lower relative abundance of Tanytarsini and higher relative abundances of oligochaete and leptocerid caddisflies compared to Group B (SM, TR6.8R, LLW, MID, SW, TP1, LP, BM, SB, BL) (Figure 2.6).

The relative abundance of oligochaetes ($F = 71.28$, $p < 0.001$) and leptocerid caddisflies ($F = 8.77$, $p < 0.01$) separated Subgroup B1 from Subgroup B2 (Figure 2.6). Wetlands in Subgroup B1 had lower relative abundances of oligochaetes and leptocerid caddisflies compared to wetlands in Subgroup B2 (Figure 2.6).

The six off-lease reference wetlands used in this analysis clustered amongst the on-lease reference wetlands indicating that off-lease wetlands were not biologically dissimilar to each other. For artificial substrate samples, Group A was separated from Group B by relative abundances of orthoclad midges ($F = 54.34$), Haliplid beetles ($F = 7.6$), Chironomini midges ($F = 5.83$), and Tanytarsini midges ($F = 3.91$) (Figure 2.7). Wetlands in Group A (PP, HS, H63W, TP1, BL, SW, CRM, SM, TR0.8R, SBD, WID, SB) had lower relative abundance of Orthocladiinae, Chironomini, and Tanytarsini midges, and haliplid beetles compared to Group B (SPit, CL, LP, LLW, SBBP, TR6.8R, NWID, BM, MID, H63I) ($F = 32.64$).

The relative abundance of Tanytarsini ($F = 7.56$, $p < 0.001$) and haliplid beetles ($F = 4.71$, $p < 0.05$) separated Subgroup A1 from Subgroup A2 ($F = 3.04$)(Figure 2.6). The relative abundance of Orthocladiinae ($F = 79.74$, $p < 0.01$) and haliplid beetles ($F = 15.84$, $p < 0.001$) separated Subgroup B1 from Subgroup B2 ($F = 60.76$) (Figure 2.7).

Off-lease reference wetlands co-occur in both Group A and Group B, but are not clustered separately from on-lease reference wetlands (Figure2.7). This indicates that artificial substrate samples off-lease wetlands are not biologically dissimilar to each other.

For sweep samples, Group A was separated from Group B by relative abundances of Tanytarsini midges ($F = 47.5$), caenid mayflies ($F = 14.3$) and Tanypodinae midges ($F = 7.2$) (Figure 2.8). Wetlands in Group A (PP, TR6.8R) had higher relative abundance of Tanytarsini and Tanypodinae midges, as well as caenid mayflies compared to Group B

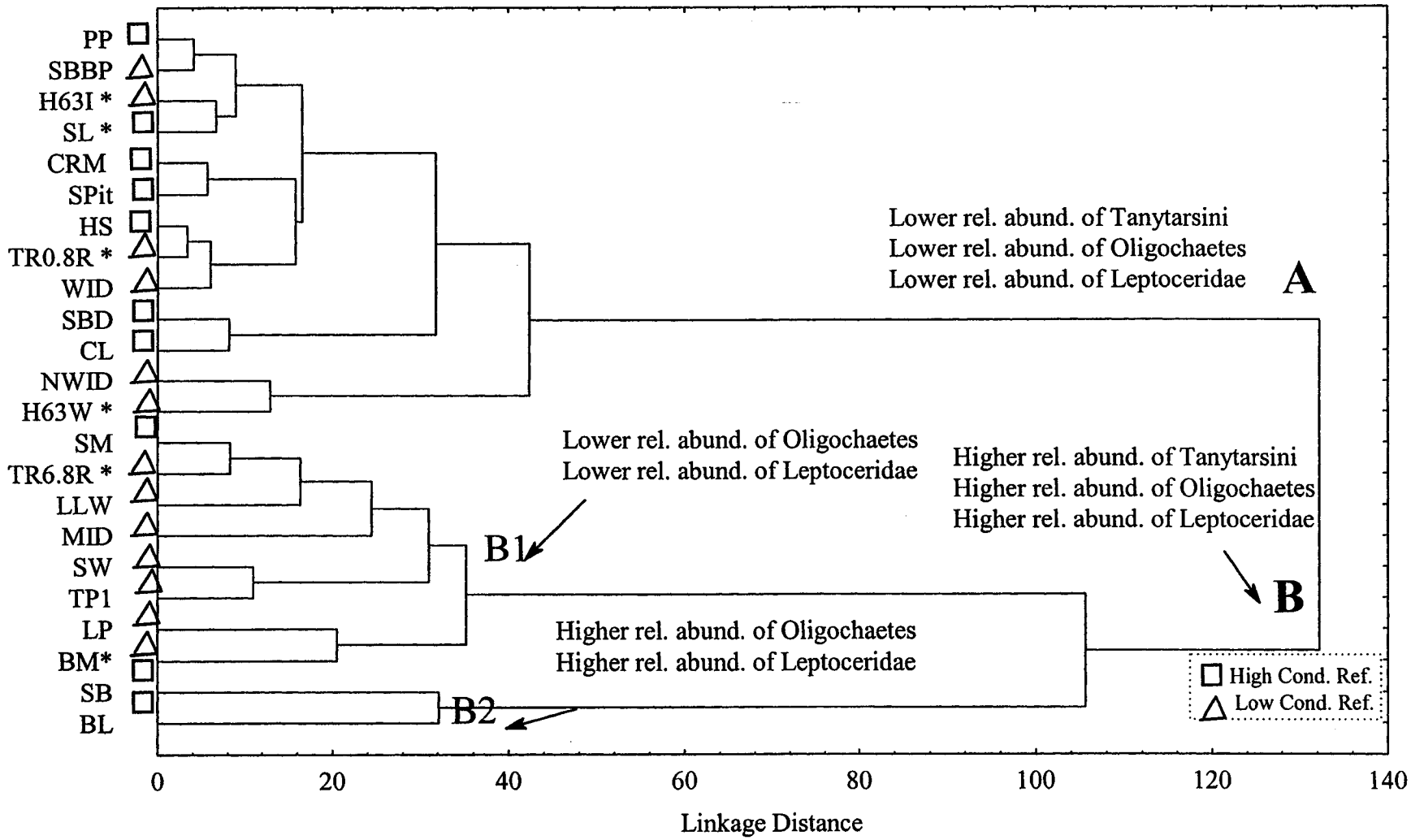



**Figure 2.6.** Cluster analysis for core samples from 22 reference wetlands using relative abundance for 19 selected benthic taxa. Wetlands located off-lease are indicated with an asterisk. Groups A & B are significantly ($p<0.05$) separated by relative abundance of Tanytarsini ($F = 20.96$), Oligochaeta ($F = 68.80$) and Leptoceridae ($F = 7.59$). Subgroups A1 & A2 are significanlty separated ($p< 0.01$) by relative abundance of Oligochaeta ($F = 71.28$) and Leptocerdiae ($F = 8.77$).

(CRM, H63W, CL, SBBP, MID, HS, BL, WID, TR0.8R, SM, SW, S-Pit, BM, LLW, LP, TP1, SL, SB, NWID, H63I).

Off-lease reference wetlands co-occur in the different groups and are not clustered separately from on-lease reference wetlands. This indicates that sweep samples from off-lease wetlands are not biologically dissimilar from one another.

**Benthic Invertebrate Community Analysis**
**Core Samples**

Richness (the number of families present at a wetland) at low conductivity reference wetlands was initially fairly high (6 - 10 families in young wetlands) and was only slightly greater (6-12 families in mature wetlands) in older wetlands (Fig. 2.9). Young high conductivity reference wetlands had high richness (7-10 families). A reduced number of families (6-8 families) occurred in saline wetlands of increasing age (Figure 2.9). Initially, young OSPM-affected wetlands had few families (3-5 families) and only had 6-7 taxa in the two oldest wetlands (Figure 2.9).

F-ratios calculated between OSPM-affected wetlands and high and low conductivity reference wetlands revealed that there was not a significant difference in richness ($F=1.65$, $p>0.05$) between OSPM-affected wetlands and reference wetlands. It is noteworthy, that two wetlands (indicated by asterisks in Figure 2.9) receive periodic inputs of OSPM, and this may set the benthic community back to levels that are only slightly above those of young OSPM-affected wetlands, as the toxic constituents in fresh OSPM have not had sufficient time to degrade.

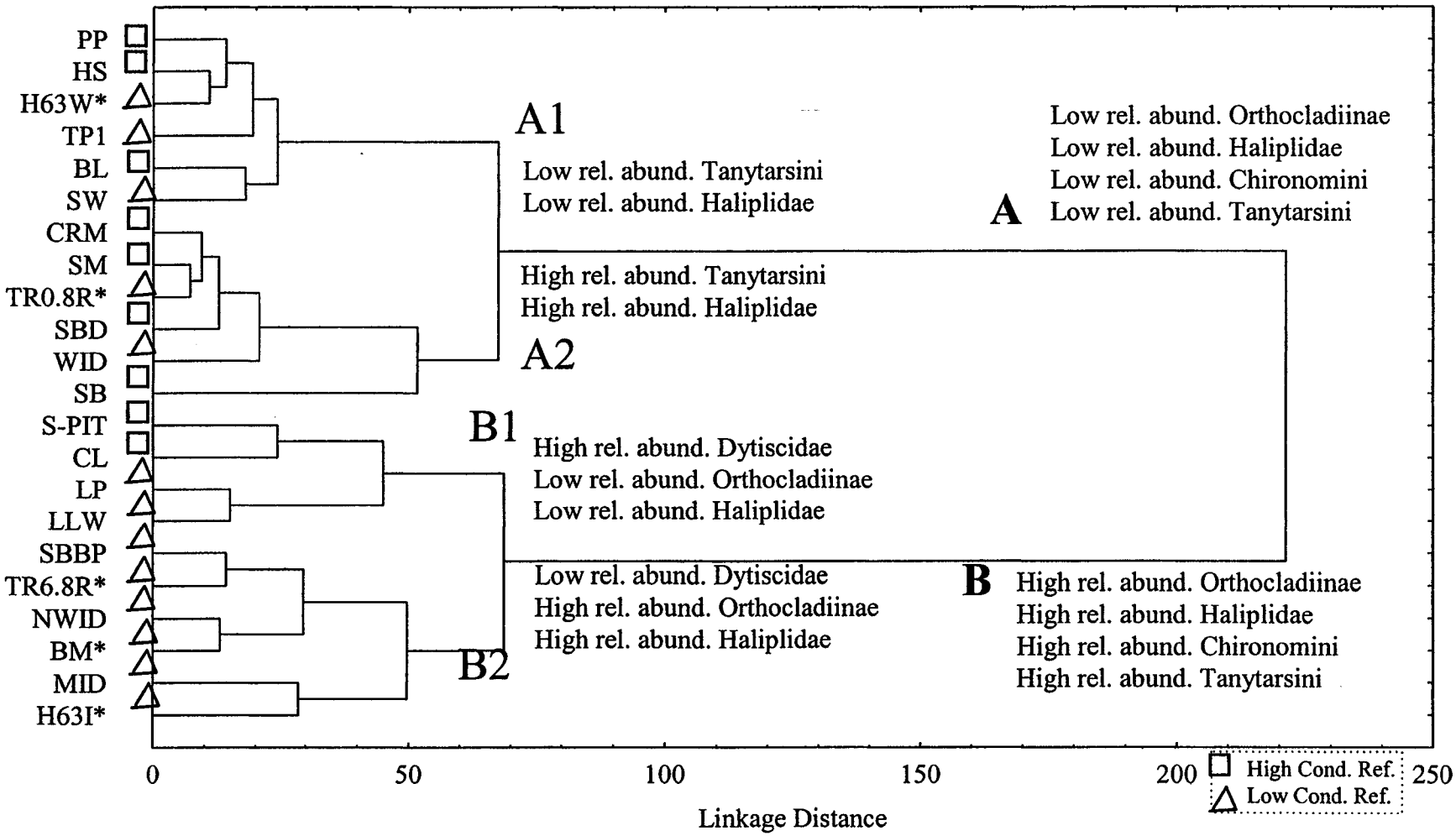



**Figure 2.7.** Cluster analysis for artificial substrates, from 22 reference sites, using relative abundances for 20 selected taxa. Sites located off lease are indicated with an asterisk. Ward's method and Euclidean distances used. Groups A & B are significantly ($p < 0.05$) separated by relative abundance of Orthocladiinae midges ($F=54.30$), Haliplidae ($F=7.6$), Chironomini midges ($F=5.83$) and Tanytarsini midges ($F=3.91$). Subgroups A1 &A2 are significanlty ($p<0.05$) separated by relative abundance of Tanytarsini midges ($F=7.56$) and haliplid beetles (4.71). Subgroups B1 & B2 are significantly ($p<0.01$)separated by relative abundances of Orthocladiinae midges ($F=79.74$) and haliplid beetles ($F=15.84$).

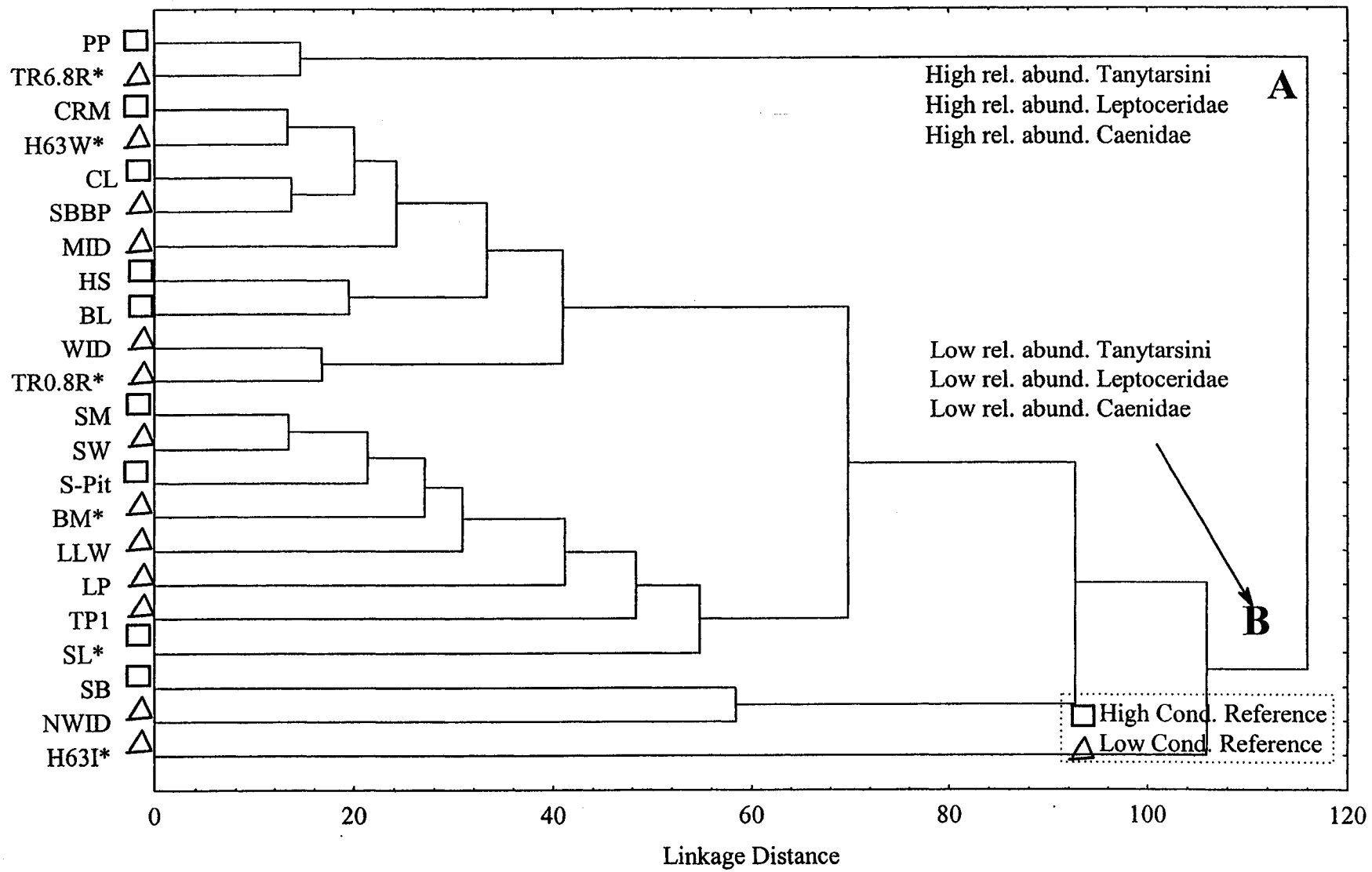



**Figure 2.8**. Cluster analysis for sweep samples, from 22 reference sites only, using relative abundance for 23 selected taxa. Wetlands indicated with * are located off-site. Groups A & B are significantly separated by relative abundance of Tanytarsini midges (F = 47.5), Caenid mayflies (F = 14.3) and Leptocerid caddisflies (F = 7.2).

Abundance at low conductivity reference wetlands was high (181 -330 individuals per core) in young wetlands and showed a slight decreasing trend in older wetlands (Fig.2.10). Abundance at high conductivity reference wetlands was high (403 - 1100 individuals per core) in young wetlands but showed a trend toward decreasing abundance in older wetlands (Figure 2.10). Young OPSM-affected wetlands had benthic invertebrate abundances similar to those of young reference wetlands and showed a trend toward increasing abundance in older wetlands (Figure 2.10). F-ratios calculated between OSPM-affected wetlands and low and high conductivity reference wetlands showed that there was no significant difference in abundance between OSPM-affected wetlands and reference wetlands ($F=1.59$, $p>0.05$).

**Artificial Substrate Samples**

Richness at young low conductivity reference wetlands was high (8-13 families) and only slightly greater (10-18 families) in mature wetlands (Fig. 2.11). Young high conductivity reference wetlands had moderately high richness (6-20 families). There were slightly fewer families (12-18 families) in mature wetlands (Figure 2.11). Young OSPM-affected wetlands had few families (2-10 families). Fifteen taxa were found in the two oldest wetlands (Figure 2.11).

F-ratios calculated between OSPM-affected wetlands and high and low conductivity reference wetlands revealed that there was not a significant difference in richness ($F=1.58$, $p>0.05$) between OSPM-affected wetlands and reference wetlands. As previously noted two OSPM-affected wetlands (indicated by asterisks in Figure 2.11) receive periodic inputs of OSPM.

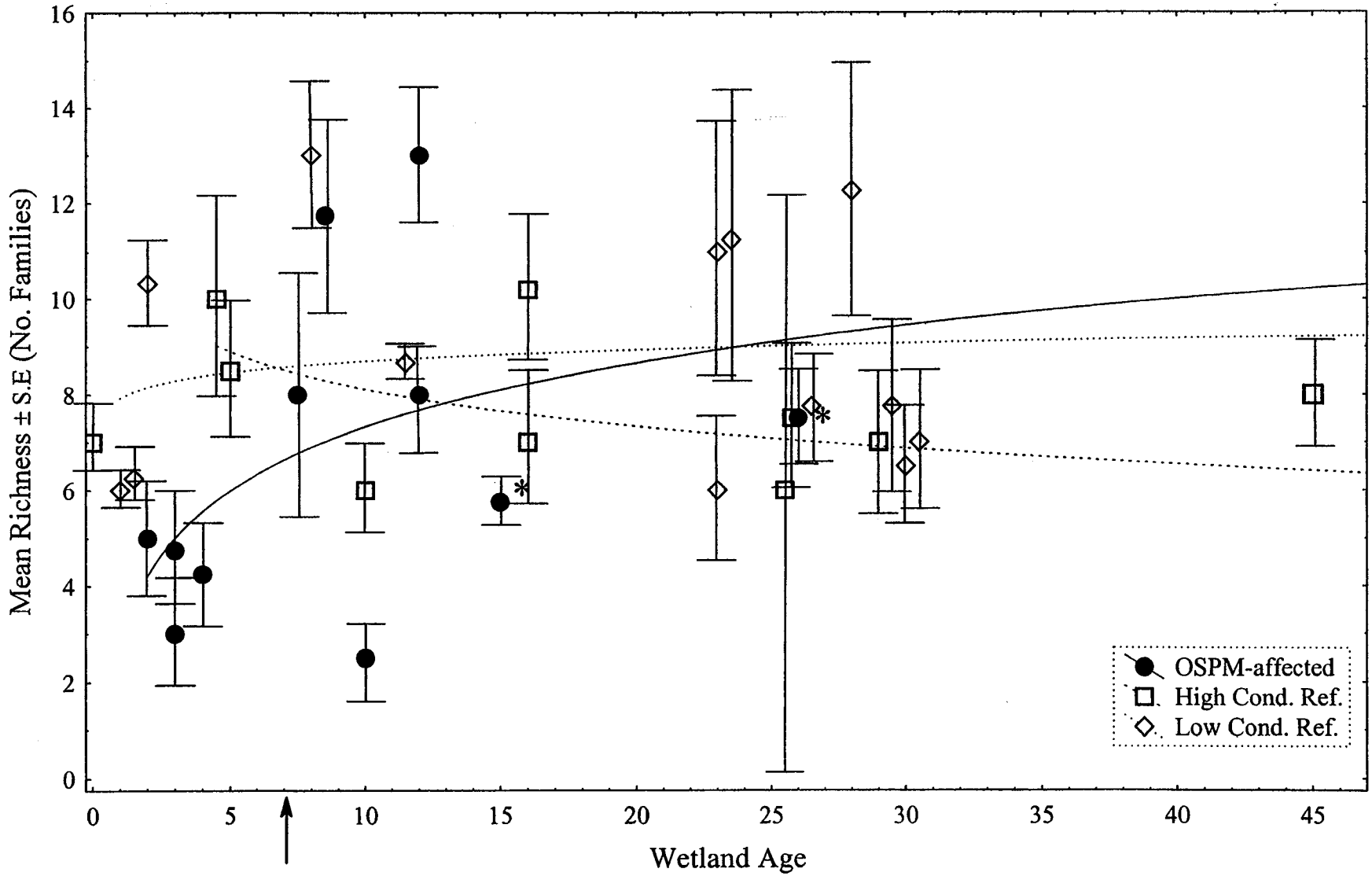



**Figure 2.9.** Mean richness (± S.E.) of benthic invertebrates compared to wetland age for core samples. OSPM-affected wetlands did not initially have significantly fewer taxa than reference wetlands ($p > 0.05$). Wetlands marked with an asterisk receive periodic OSPM inputs. The arrow represents the approximate age where richness of OSPM-affected wetlands reaches an asymptote.

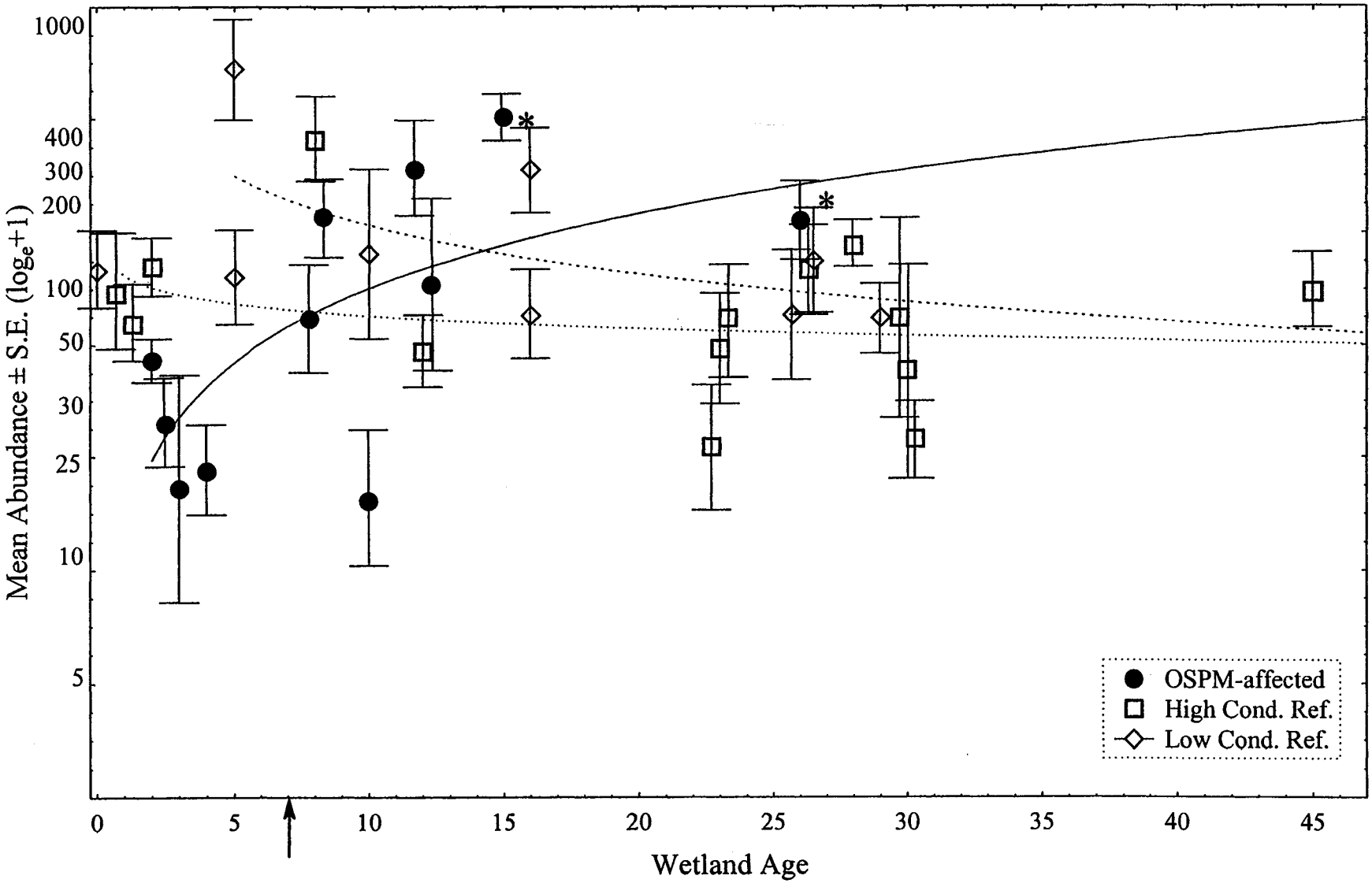



**Figure 2.10.** Mean abundance ± S.E. ($log_e$+1 transformed) versus wetland age for core samples. Abundances of benthic invertebrates were not significanlty lower at OSPM-affected wetlands ($p > 0.05$) compared to reference wetlands. The arrow represents approximate age where abundnace of OSPM-affected wetlands reaches an asymptote.

Abundance at young low conductivity reference wetlands was high (123 -735 individuals per artificial substrate) and only slightly great in mature wetlands (Fig.2.12). Abundance at young high conductivity reference wetlands was high (735 -individuals per artificial substrate). There was slightly lower abundance in mature wetlands (Figure 2.12). Young OPSM-affected wetlands had benthic invertebrate abundances comparable that of young reference wetlands. Mature OSPM-affected wetlands had slightly greater abundance (Figure 2.12).

F-ratios calculated between OSPM-affected wetlands and low and high conductivity reference wetlands showed that there was a significant difference in abundance between OSPM-affected wetlands and reference wetlands ($F=4.13$, $p<0.05$). The apparent similarity of benthic invertebrate abundances of two groups of OSPM-affected wetlands may have been due the high numbers of midge larvae capable of inhabiting OPSM-affected wetlands.

**Sweep Samples**

Richness at young low conductivity reference wetlands was fairly high (14-30 families), and only slightly less (3-22 families) in mature wetlands (Figure 2.13). Young high conductivity reference wetlands had moderately high richness (10-17). There were slightly more families (14-20 families) in mature wetlands (Figure 2.13). Young OSPM-affected wetlands had few families (2-10 families). Only 13-19 taxa were found in the two oldest wetlands (Figure 2.13).

F-ratios calculated between OSPM-affected wetlands and high and low conductivity reference wetlands revealed that there was a significant difference in richness ($F = 3.41$, $p<0.05$) between OSPM-affected wetlands and reference wetlands. As previously noted

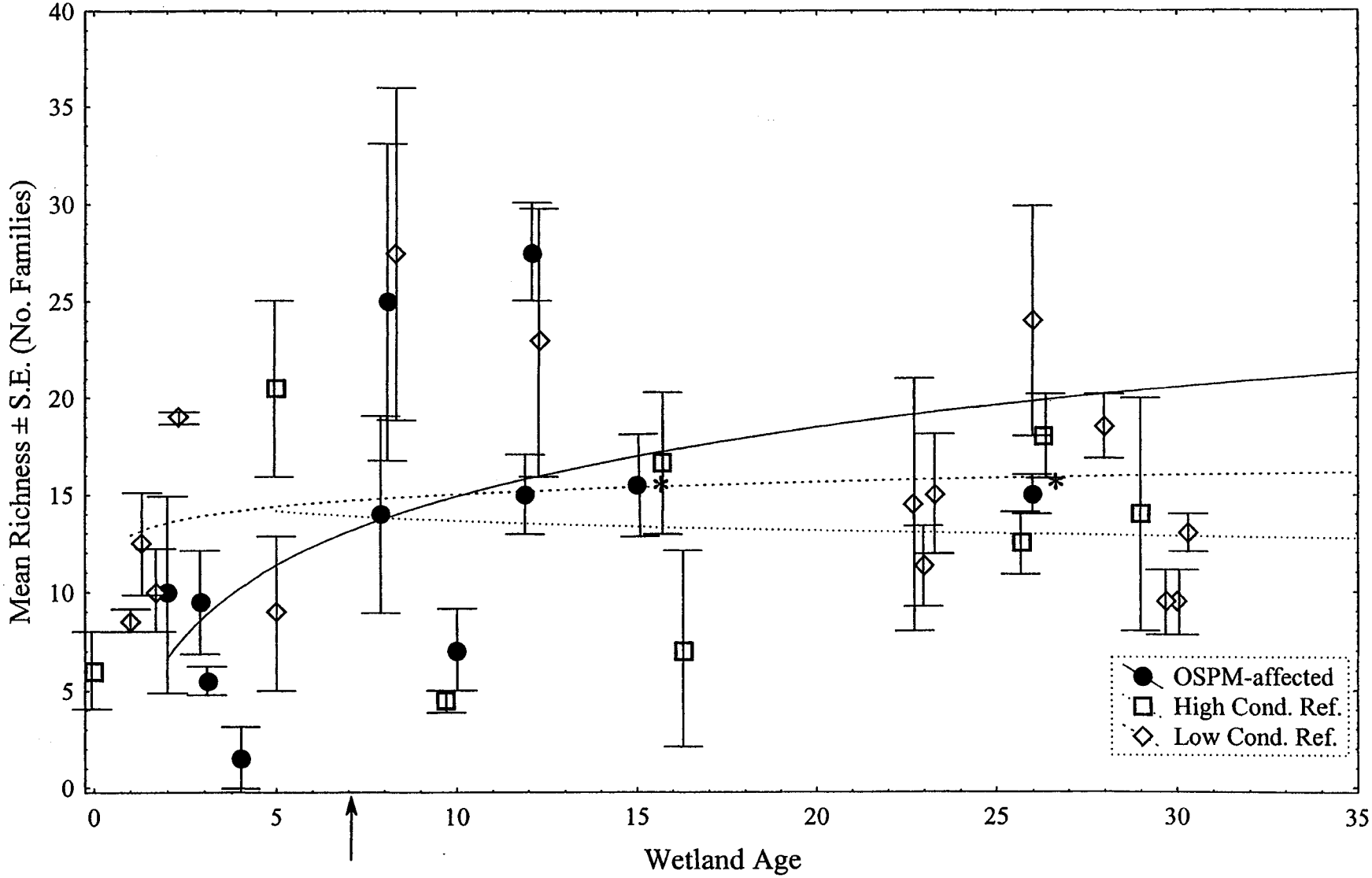



**Figure 2.11.** Mean richness ± S.E. versus wetland age for artificial substrate samples. Richness was not significanlty lower at OSPM-affected wetlands ($p > 0.05$). Wetlands marked with an asterisk receive periodic OSPM inputs. The arrow represents approximate age where richness in OSPM-affected wetlandsreaches an asymptote.

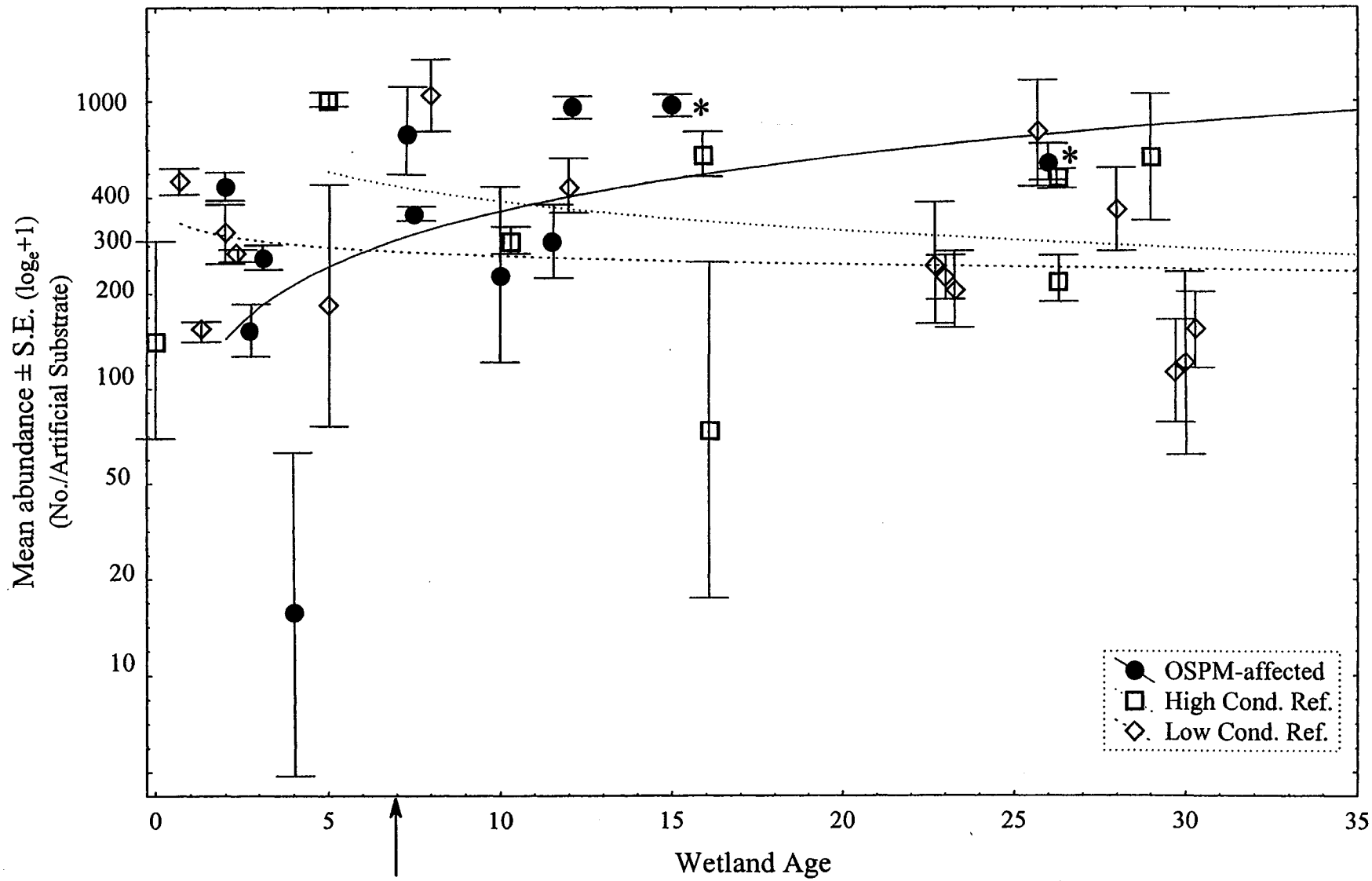



**Figure 2.12.** Mean abundance ± S.E. ($log_e$+1) versus wetland age for pooled artificial substrate samples. Abundance was significanlty lower at OSPM-affected wetlands ($p < 0.05$). Wetlands marked with an asterisk receive periodic OSPM inputs. The arrow represents approximate age where abundance in OSPM-affected wetlands reaches an asymptote.

two wetlands (indicated by asterisks in Figure 2.12) receive periodic inputs of OSPM.

Abundance at young, low conductivity reference wetlands was moderate (330 - 602 individuals per sweep) and slightly decreased in mature wetlands (Figure 2.14). Abundance at young high conductivity reference wetlands was also moderate (1100 individuals per sweep). Mature high conductivity reference wetlands had slightly greater abundance (Figure 2.14). Young OPSM-affected wetlands had benthic invertebrate abundances comparable that of young reference wetlands (403 individuals per sweep). Mature OSPM-affected wetlands had slightly greater abundance (Figure 2.14).

F-ratios calculated between OSPM-affected wetlands and low and high conductivity reference wetlands showed that there was no significant difference in abundance between OSPM-affected wetlands and reference wetlands ($F=2.37$, $p>0.05$). I believe that OSPM-affected wetlands have similar abundance of benthic invertebrates due the high numbers of midge larvae capable of inhabiting OPSM-affected wetlands.

For all of the analyses above, there appeared to be an overall convergence trend in richness and abundance of taxa in OSPM-affected wetlands starting around 8 years of age and reaching a maximum around 15 years of age. This general trend led to designating wetlands 7 years or younger as "young" wetlands, and wetlands older than 8 years as being "mature". The arrow in Figures 2.9 to 2.14 represents the age at which there should be a transition of the young benthic community to a mature benthic community.

**Physico-chemical Factors Affecting Benthic Invertebrate Richness and Abundance**

To determine which physico-chemical factors most strongly affected the richness and abundance of benthic invertebrates, forward step-wise multiple regression was used. Physicochemical factors (independent variables) were regressed against abundance ($\log_e+1$

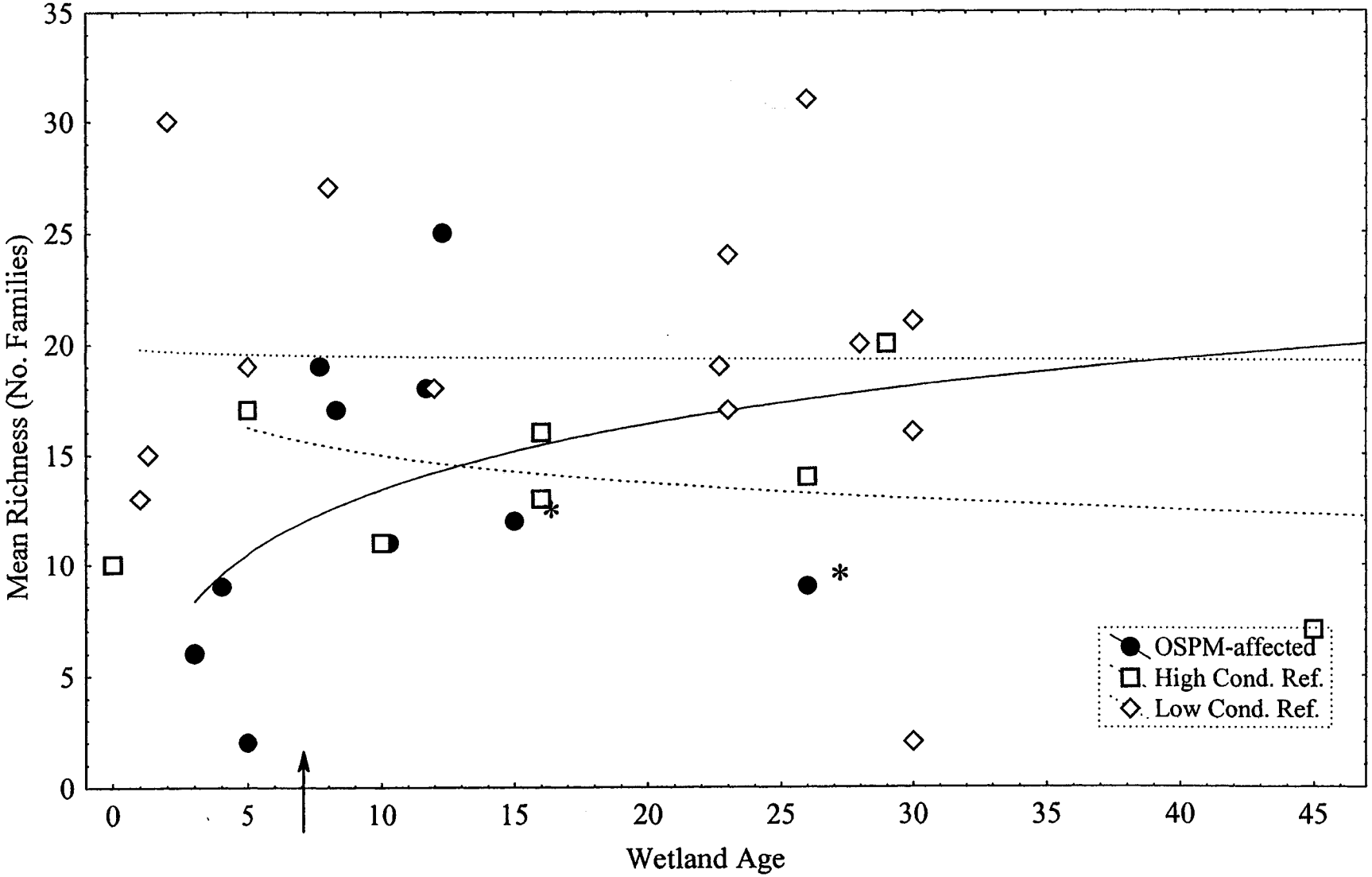



**Figure 2.13.** Family richness of benthic invertebrates compared to wetland age for pooled sweep samples. OSPM-affected wetlands initially had significantly fewer taxa than reference wetlands ($p < 0.05$). Asterisks represent wetlands that receive period OSPM input. The arrow represents the approximate age where richness of OSPM-affected wetlands eraches an asymptote.

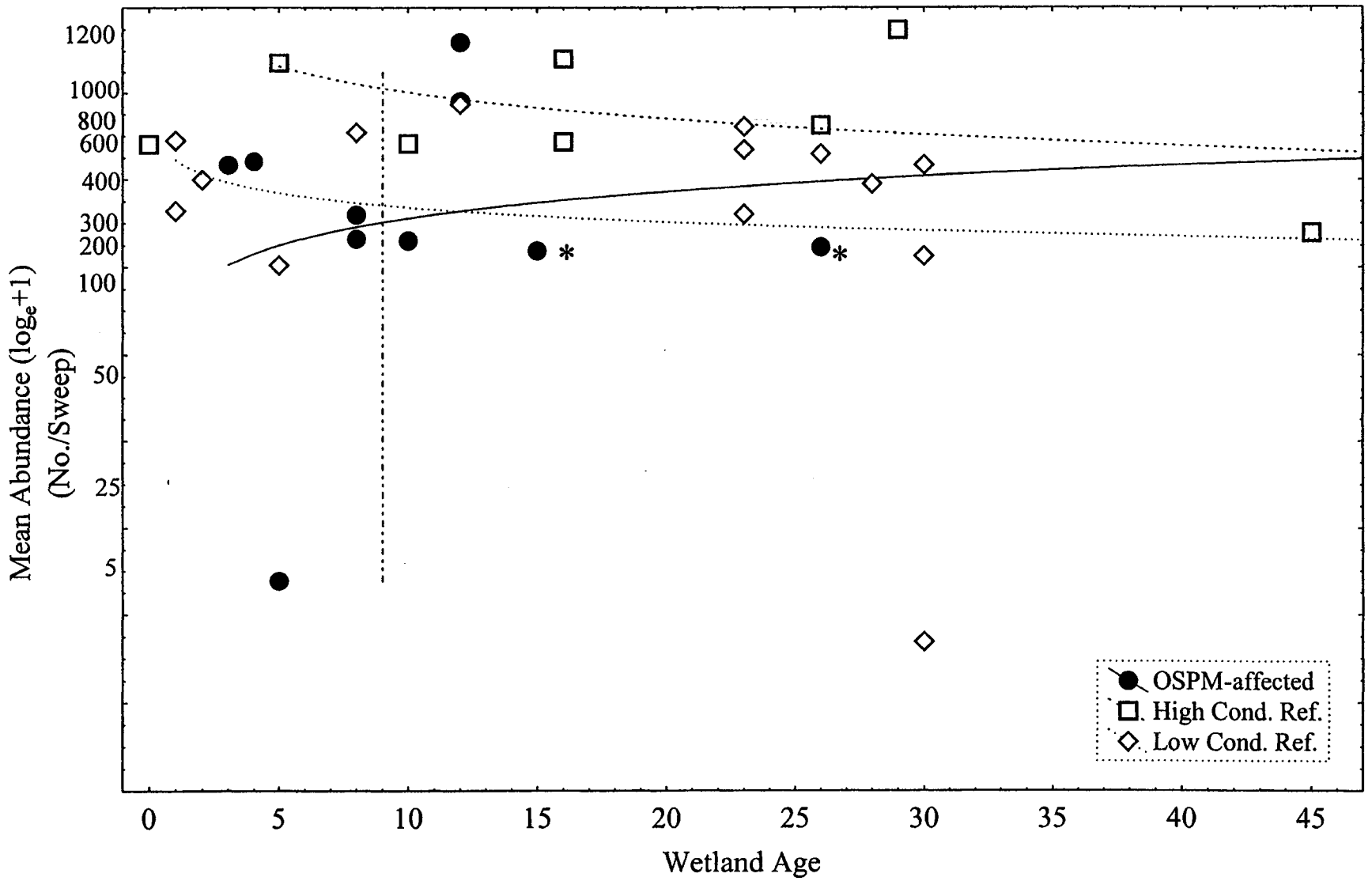



**Figure 2.14.** Abundance ($log_e$+1) versus wetland age for sweep samples. OSPM-affected wetlands did not have significantly lower abundance than reference wetlands ( $p > 0.05$). The arrow represetns the approximate age where abundance reaches an asymptote in OSPM-affected wetlands.

transformed) and richness (dependent variables) for each sampling method. Wetland age was used as a covariate in these analyses. Eleven physical variables (pH - water and sediment, conductivity, salinity, naphthenic acid concentration, sediment ORP, sediment organic content (LOI), median particle size, detritus, macrophyte development and wetland area) were used to start the analysis. Forward stepwise regression admitted fewer than 11 variables. The significant variables admitted for each regression are summarised in Table 2.7, 2.8, 2.9.

**Core Samples**

Macrophyte development was the only physicochemical factor that significantly influenced benthic invertebrate abundance ($R^2$=0.15, $p < 0.05$; Table 2.7). Wetlands, of any age, with few macrophytes had fewer benthic invertebrates than wetlands with more macrophytes (Figure 2.15 A). Young OSPM-affected wetlands had the lowest abundance of invertebrates as well as having the least amount of macrophytes (Figure 2.15 A).

Water pH ($p < 0.05$; $R^2$= 0.16) and naphthenic acid concentration ($p < 0.01$; $R^2$ =0.29) had significant influences on the richness of the benthic invertebrates found in core samples (cumulative $R^2$=0.574). Wetlands with the highest concentrations of naphthenic acid (generally young OSPM-affected) also had the fewest numbers of families compared to wetlands with lower concentrations of naphthenic acid (Figure 2.15 B). Water pH only seemed to decrease richness in young OSPM-affected wetlands (Figure 2.15 C). This could be the result of the acid fraction of the OSPM (naphthenic acids) causing some sort of toxicity to wetland biota.

**Table 2.7.** Summary of results for multiple regression of physico-chemical factors against abundance (log+1) and richness for core samples.

| Variable | Step-wise Regression (Abundance) | | | |
|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | p |
| Intercept | 4.00 | 0.313 | | ** |
| Macrophyte Dev. | 0.252 | 0.108 | 0.144 | * |
| Total | | | 0.144 | |

* p<0.05 **p< 0.01 ns = not significant

| Variable | Step-wise Regression (Richness) | | | |
|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | p |
| Intercept | -1.069 | 4.614 | | ns |
| [Naphthenic Acid] | -1.592 | 0.303 | 0.280 | ** |
| pH (Water) | 1.499 | 0.560 | 0.165 | ** |
| Sediment ORP | -0.007 | 0.004 | 0.037 | ns |
| Wetland Area | -0.429 | 0.291 | 0.036 | ns |
| Salinity | 2.299 | 1.444 | 0.021 | ns |
| Sed. org. cont. (LOI) | -1.001 | 0.698 | 0.034 | ns |
| Total | | | 0.574 | |

* p<0.05 **p< 0.01 ns = not significant

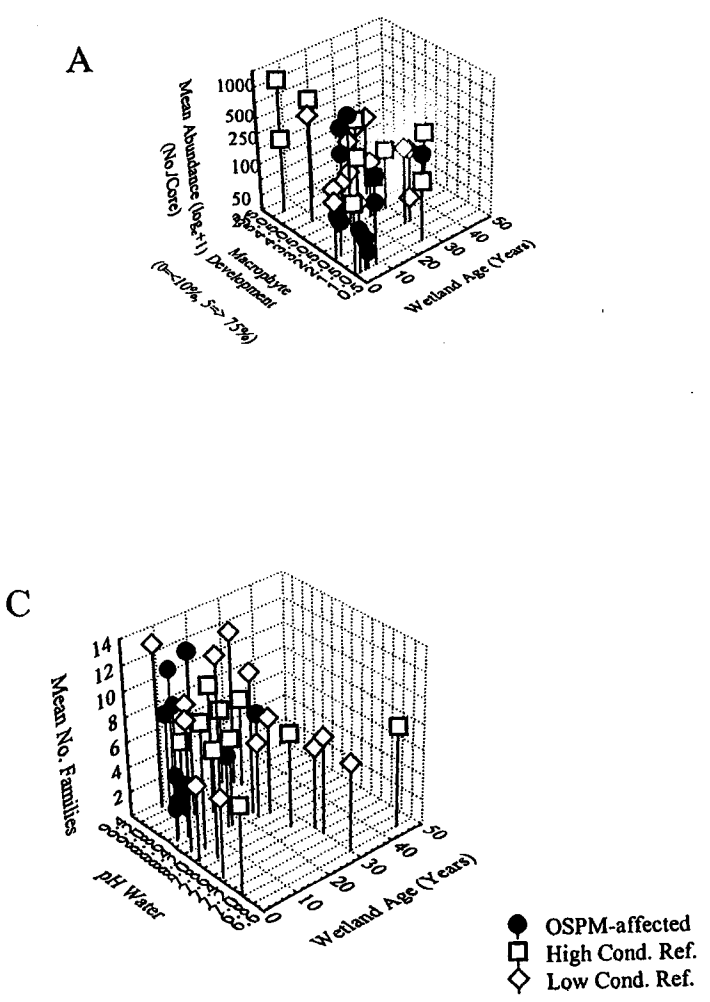



**Figure 2.15.** Graphical representation of significant multiple regression results for core samples. Macrophyte development explained 15% of the total variation of invertebrate abundance ($p<0.05$) (A). Naphthenic acid concentration explained 30% of the total variation of invertebrate abundance ($p<0.01$) (B), while water pH explained 17% of the total variation of invertebrate richness ($p<0.01$) (C).

**Artificial Substrate Samples**

Conductivity had a significant positive effect ($R^2$=0.22, $p<0.01$) on the abundance of benthic invertebrates found on artificial substrates (Table 2.8). Generally, wetlands with lower conductivity had fewest benthic invertebrates regardless of wetland age (Figure 2.16 A).

Conductivity ($R^2$ = 0.16, $p<0.01$), pH water ($R^2$ = 0.15, $p<0.01$), wetland area ($R^2$ = 0.08, $p<0.001$) and detritus ($R^2$= 0.23, $p<0.01$) significantly influenced benthic invertebrate richness for artificial substrate samples (cumulative $R^2$=0.65; Table 2.8). Young wetlands, Figure 2.16 (A,B,C) of all three classes, had lower richness, and less detritus than older wetlands (Figure 2.16 B).

Wetlands with lower conductivity, and young OPSM-affected wetlands (highest conductivity), had the lowest richness (Figure 2.16 C). Wetlands with the lowest pH values (circumneutral pH) had fewer families than wetlands with more alkaline water (Figure 2.16 D). The pH and conductivity of the water, affecting the richness of the benthos, suggests that these attributes might be correlated with naphthenic acid concentration, even though naphthenic acid concentration was not a significant factor in this regression (wetlands with high naphthenic acid concentration also have high conductivity).

Wetlands with smaller surface area also had lower richness than larger wetlands (Figure 2.16 E). Young, small wetlands had fewer families than large, or older wetlands, suggesting that wetland size may not be the only factor in determining richness. Wetlands with larger surface area also generally had larger drainage basins. This could result in greater allochthonus nutrient input, which could contribute to greater detritus inputs.

**Table 2.8.** Summary of results for multiple regression of physico-chemical factors against abundance (log+1) and richness for artificial substrate samples.

| Variable | Step-wise Regression (Abundance) | | | |
|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | p |
| Intercept | -3.581 | 3.208 | | ns |
| Conductivity | 0.314 | 0.314 | 0.216 | ** |
| pH (Sediment) | 0.354 | 0.211 | 0.048 | ns |
| [Naphthenic Acid] | 0.304 | 0.224 | 0.033 | ns |
| Sediment ORP | 0.005 | 0.002 | 0.030 | ns |
| pH (Water) | 0.454 | 0.259 | 0.052 | ns |
| Macrophyte Dev. | 0.181 | 0.140 | 0.038 | ns |
| Total | | | 0.417 | |

* $p<0.05$ **$p< 0.01$ ns = not significant

| Variable | Step-wise Regression (Richness) | | | |
|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | p |
| Intercept | 31.629 | 19.524 | | ns |
| Detritus | 4.180 | 0.946 | 0.229 | ** |
| Conductivity | -9.080 | 3.076 | 0.157 | ** |
| pH (Water) | 4.878 | 1.258 | 0.153 | ** |
| Wetland Area | -2.302 | 0.802 | 0.076 | ** |
| Salinity | 7.989 | 5.096 | 0.032 | ns |
| Total | | | 0.647 | |

* $p<0.05$ **$p< 0.01$ ns = not significant

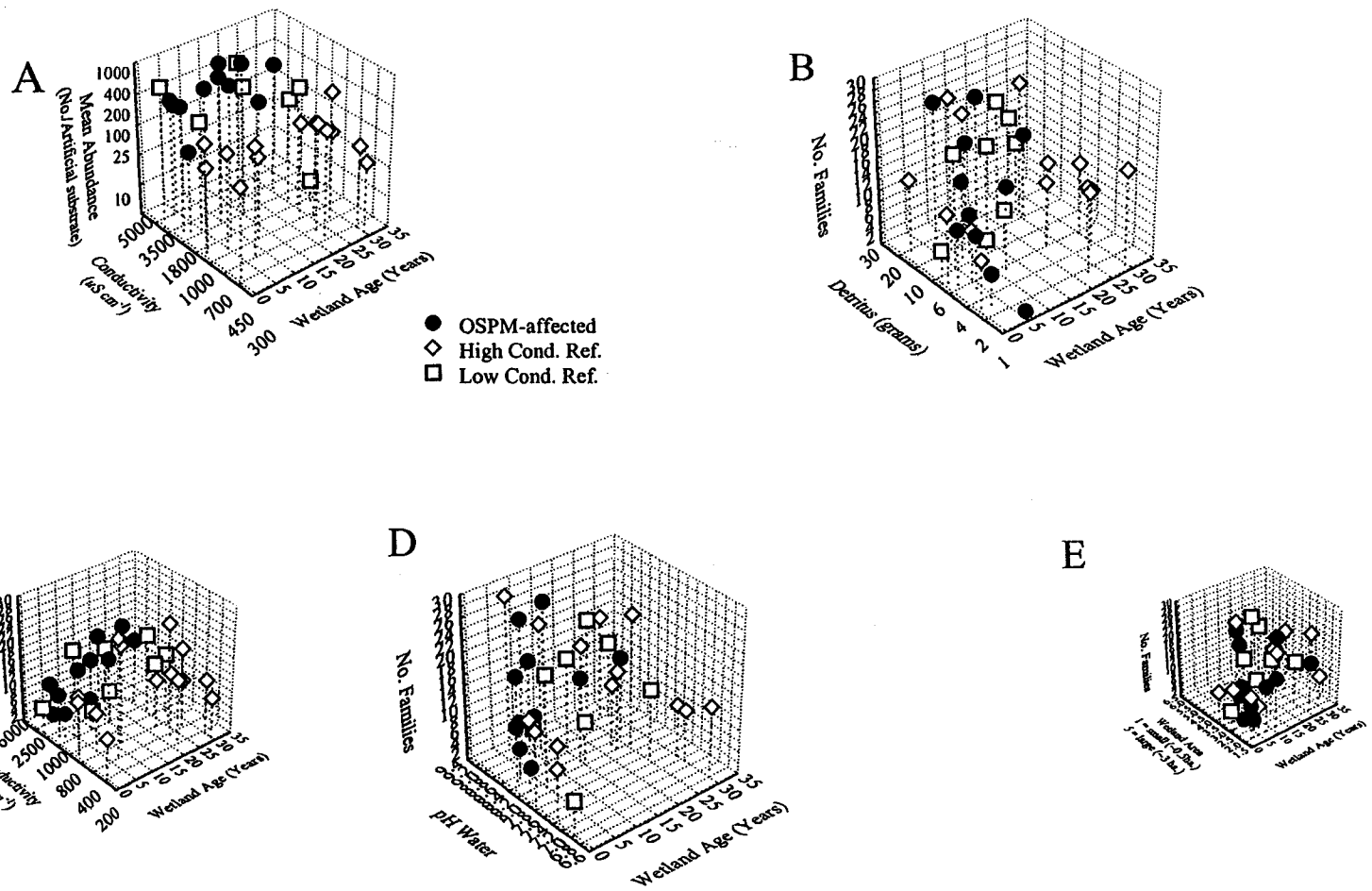



**Figure 2.16.** Graphical representation of significant multiple regression results for artificial substrate samples. Conductivity explained 22% of the total variation of invertebrate abundance ($p<0.01$) (A). Detritus explained 23% ($p<0.01$) (B), conductivity 16% ($p<0.01$) (C), water pH 15% ($p<0.01$) (D), and wetland size (surface area) 8% ($p<0.01$) (E) of the total variation of invertebrate richness.

**Sweep Samples**

Detritus was related to abundance of benthic invertebrates in sweep samples ($R^2$=0.20, $p<0.01$; Table 2.9). Wetlands with less detritus had fewer invertebrates than wetlands with more detritus (Figure 2.17 A). This suggests that inputs of organic material may ultimately be important in determining the abundance of invertebrate taxa.

Salinity ($R^2$=0.32, $p<0.01$), sediment ORP ($R^2$=0.16, $p<0.05$) and, detritus ($R^2$=0.09) were significantly related to benthic invertebrate richness for sweep samples (cumulative $R^2$=0.62). More saline wetlands had fewer families than less saline wetlands of the same age (Figure 2.17 B). Wetlands with more reduced sediments (anoxic = more negative) had more families than wetlands with less reduced sediments (Figure 2.17 C). Younger OPSM-affected wetlands (<10 years) also had the lowest richness compared to similar aged wetlands (Figure 2.17 D). This suggests that characteristics of OSPM-affected sediment influence richness.

**Discussion**

**Physico-chemical Attributes of Wetland Sites**

Salinity of wetlands in the oil sands region ranged from 0 to 3.8 ‰, conductivity ranged from 176 to 5050 µS/cm, and pH ranged from 6.8 to 10.4. These values are within the range reported in saline lakes of other North American regions.

Topping and Scudder (1977) characterised the physico-chemical composition of 33 lakes in the interior of British Columbia. They reported water conductivity ranging from 41 to 55,932 µS/cm, and pH ranging from 8.1 to 10.2 (Topping and Scudder 1977). Saline crater lakes in salt pans of Oriental, Mexico have salinity ranging from 6.0 to 7.4 ‰, pH ranging from 8.4 to 9.0, and sediment organic matter ranging from 2.8 to 8.4%

**Table 2.9.** Summary of results for multiple regression of physico-chemical factors against abundance (log+1) and richness for sweep samples.

| Variable | Step-wise Regression (Abundance) | | | |
|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | p |
| Intercept | 8.651 | 4.494 | | ns |
| Detritus | 0.789 | 0.276 | 0.182 | ** |
| Sediment ORP | -0.005 | 0.003 | 0.082 | ns |
| Condcutivity | -1.280 | 0.719 | 0.067 | ns |
| pH (Water) | 0.519 | 0.331 | 0.039 | ns |
| Salinity | 1.527 | 1.232 | 0.035 | ns |
| Total | | | 0.405 | |

* p<0.05 **p< 0.01 ns = not significant

| Variable | Step-wise Regression (Richness) | | | |
|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | p |
| Intercept | 5.490 | 9.711 | | ns |
| Salinity | -8.616 | 1.711 | 0.322 | ** |
| Sediment ORP | -0.032 | 0.012 | 0.168 | ** |
| Detritus | 2.828 | 1.056 | 0.094 | * |
| Sed. org. con. (LOI) | -1.715 | 1.533 | 0.020 | ns |
| Sediment pH | 1.231 | 1.127 | 0.018 | ns |
| Total | | | 0.622 | |

* p<0.05 **p< 0.01 ns = not significant

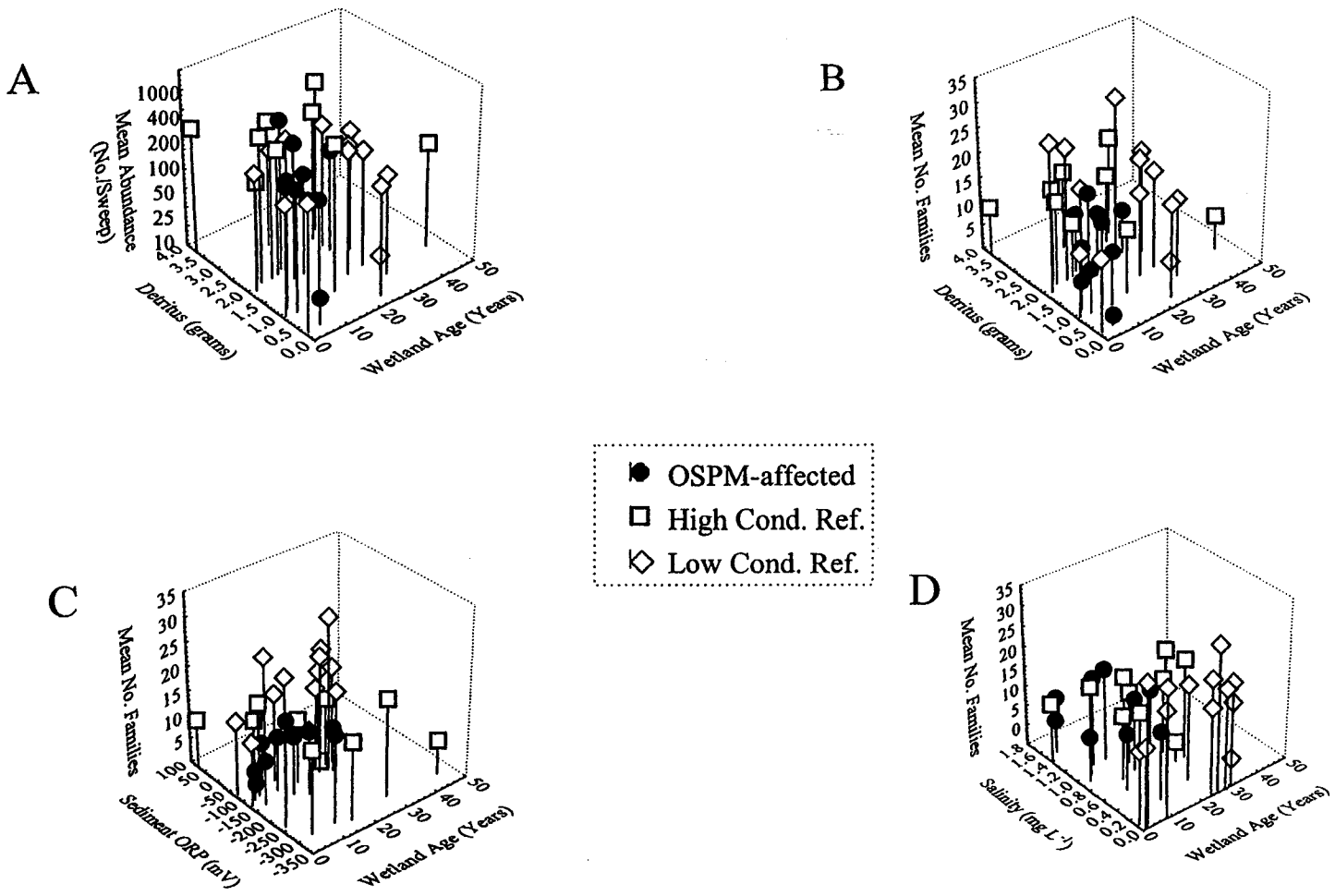



**Figure 2.17**. Graphical representation of significant multiple regression results for sweep samples. Detritus explained 20% of the variation of invertebrate abundance ($p<0.05$) (A). Salinity explained 33% ($p<0.01$) (B), sediment ORP explained 16% ($p<0.01$) (C), and detritus explained 9% ($p<0.05$) (D) of the total variation of invertebrate richness.

(Alcocer *et al.* 1998). High plains wetlands of southeast Wyoming had salinity values from 80 to 3000 μS/cm (Lovvorn *et al.* 1999).

**Comparing On-lease and Off-lease Reference Sites Physico-chemically**

Most of the wetlands included in the present study were close to each other (most within 5 km). However, several wetlands were located at distances as much as 100 km apart. The oil deposits, and presumably the saline-sodic overburden, of this area are unevenly distributed in space and depth (Strobl *et al.* 1997; Wightman *et al.* 1997). The McMurray/Wabiska stratigraphic interval contains the surface mineable oil sands, and has deposits under varying depths overburden (soil and clay horizons) (Strobl *et al.* 1997; Wightman *et al.* 1997). With wetlands located as much as 50 km from the centre of the test area (centred at current active oil sand lease developments) a range of physico-chemical features could be expected and these could distinguish them from sites located on leases. If the physico-chemical factors were different enough, the flora and fauna of these off-lease wetlands would likely be distinct. Since some off-lease wetlands were included as reference sites for this study, it was important to demonstrate the unique or common features of these wetlands.

The null hypothesis of the present study was that a wetland's location, on or off the oil sands leases, would have no effect on group membership when performing cluster analyses. If one were to reject this hypothesis then one would expect that location would be a factor for group membership, and that all off-site wetlands would cluster in a single group. The results showed that off-lease reference sites did not cluster together in a single physico-chemical group and that on-lease reference sites were members of each group (See

Figure 2.3). The patchy distribution of the bitumen deposits and the chemistry of the underlying soils would prevent the off-lease reference wetlands from clustering alone. The water and soil chemistry that results from wetlands forming over saline reclamation soils would be similar to a wetland that formed over saline soils at an off-lease area, except for areas where lean oil sands occurs in overburden deposits.

**Taxonomic Resolution**

There is an ongoing debate as to the level of taxonomic identification necessary to detect differences between reference and test (OSPM-affected) areas. The level of taxonomy used should reflect the nature of the question being asked of the biological data (Bowman 1997; Bailey *et al.* 2001). Several studies have indicated that family level identification was sufficient to detect changes in the benthic community in response to a stressor (Ferraro and Cole 1990; James *et al.* 1995; Olsgard *et al.* 1998). King and Richardson (2002) suggested using a two tiered-taxonomic approach, with non-Chironomidae identified to family and Chironomidae identified to species.

For the purposes of this study, and the usefulness of the results of this research to oil sands environmental planning needs, family level identification of taxa was considered adequate. This level of identification is appropriate when limited taxonomic expertise is available, the goal is to determine relatively large between-site differences, and when sampling in areas that have low taxa richness (Lenat and Resh 2001). Lower diversity of invertebrates may occur in the oil sands region, than elsewhere, due to harsh winters, fluctuating water levels, and changing chemical conditions of the water, especially in small wetlands that have small drainage basins.

In 2000, most taxa were initially identified to genus (including the Chironomidae and Oligochaeta). However, time constraints in 2001 only permitted identification of the insect taxa to genus, chironomid taxa to sub-family/tribe and oligochaetes to class. Consequently, only family level of identification was used for data analysis (see Appendix A1 for complete classification tables).

The only exception to the family rule was the Chironomidae. The Chironomidae are considered by some to be a difficult group to identify to genus. They also require additional time to properly prepare specimens for identification. Rabeni and Wang (2001) suggested that monitoring programs could eliminate Chironomidae and use resources to analyse more sites. However, Chironomidae make up a significant proportion of benthos in boreal wetlands and can provide valuable information on water quality and chemistry (Cannings and Scudder 1978; Bervoets 1996; Ciborowski 1997). Alberta Environment (Anderson 1990) lists chironomid identification to subfamily as the highest level of identification recommended for routine monitoring studies. As will be presented in the following chapter, chironomids identified to subfamily or tribe level yielded more useful information than at the family level of identification. Chironomid identification to this level can be easily performed on animals retained on 0.500 mm sieves and larger using a stereo-dissecting microscope, a good taxonomic key, and some practice.

**Measures of the Benthic Community and the Effect of OSPM**

Most of the taxa identified from Fort McMurray area wetlands are ubiquitous in their distribution, capable of aerial dispersal, and are good colonisers (Clifford 1991; Merritt and Cummins 1996). Taxa commonly found in these wetlands include the Chironomidae (Tribes Tanytarsini & Chironomini, subfamilies Orthocladiinae &

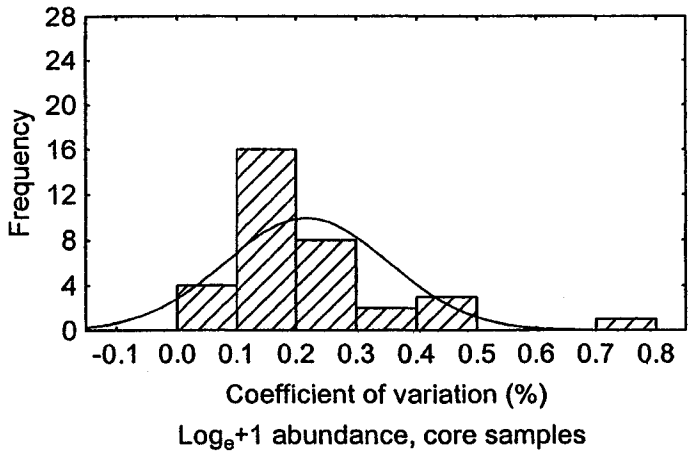
Tanypodinae), amphipods (*Hyallela* sp), corixids, dytiscid and haliplid beetles. Lymnaeid and planorbid snails, and oligochaetes are ubiquitous in their distribution. However, they possess limited dispersal abilities.

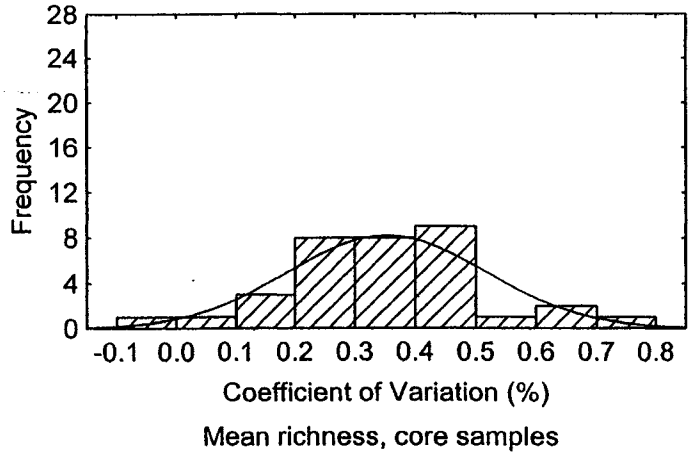
New wetlands in the oil sands region were colonised within a year of creation (Figures 2.9 - 2.14). The number of families in new reference wetlands ranged from 6-20 for wetlands < 5 years old (Figures 2.9, 2.11, 2.13). These young wetlands were dominated by taxa capable of aerial dispersal (midges, beetles). There was a wide range of variability in the richness and abundance of invertebrates collected in the study wetlands. However, the variability of the sample relative to the mean of the sample (coefficient of variation) indicated that most wetlands had a coefficient of variation less than 50% (Figure 2.18).

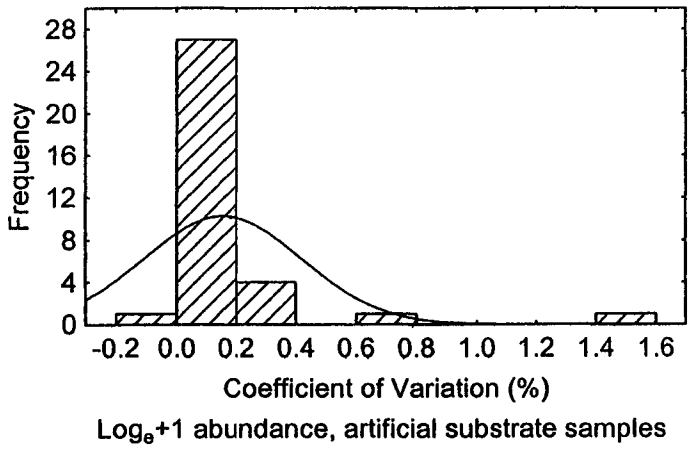
Aquatic and semi-aquatic insects mate near shorelines, and disperse only short distances (up to several kilometres; Kovats *et al.* (1996)). Since wetlands occur throughout the region, distance from source wetlands will unlikely be a factor limiting the colonisation of newly created wetlands. Wetlands located on the lease sites are not considered to be the only source of insects from which other wetlands are colonised. Insects can disperse via flight and further with wind assistance. Kovats *et al.* (1996) found the mean inland dispersal distance from shore for small riverine caddisflies ranged from 650-1845 m. Also, they found that some mated females could travel inland as far as 5 km. If the taxa found in the wetlands sampled are capable dispersing in the range indicated by Kovats *et al.* (1996), then colonisation of adjacent wetlands is not expected to be of concern.

**Factors Limiting Benthic Community Development**

Water pH, naphthenic acid concentration, detritus, and sediment ORP all significantly influenced the richness (number of families) of benthic invertebrates in the



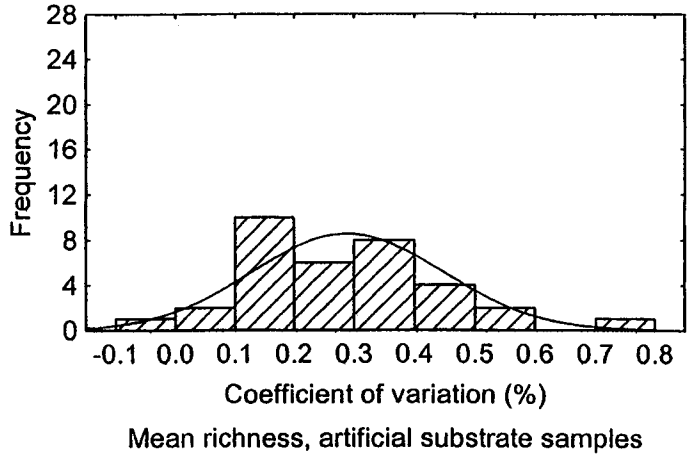






**Figure 2.18.** Coefficient of variation calculated for artificial substrate and cores samples. Calculated using v = s/ X where "v" is the coefficient of variation, "s" is the standard deviation, and "X" is the sample mean.

studied wetlands. Detritus, water conductivity, and macrophyte development were significantly related to the abundance (number of individuals) of benthic invertebrates in a wetland. Young wetlands, regardless of class, had less negative ORP values (the sediment is less reduced) than older wetlands. Reducing conditions prevail in sediments, and lower redox potentials (more anoxic sediment) are observed in lakes systems with high concentrations of humic compounds (Wetzel 1983), suggesting that lack of detritus, via macrophyte senescence, may influence the richness of invertebrates in these wetlands.

Higher naphthenic acid concentration appeared to limit the richness of benthic invertebrates in wetlands. Young OSPM-affected wetlands had fewer invertebrate families than young reference wetlands (See Appendix A.1). These differences are more likely due to habitat suitability rather than to colonisation potential. Whelly (1999) used water-filled trays to estimate the colonisation potential of OSPM-affected wetlands. He compared three OSPM-reference pairs of wetlands and reported no consistent evidence that chironomid adults avoided trays filled with OSPM water. Also, he reported that chironomid oviposition activity did not appear to be inhibited by OSPM, per se. Rather, patterns were determined by the different peaks of insect emergence at different wetlands.

The OSPM-affected wetlands used in his study were all relatively mature (> 7 years old), and either had well-developed macrophyte presence or had relatively organic sediments. Naphthenic acids are degraded through microbial activity, thus reducing their toxicity in wetlands with lots of macrophytes, detritus or organic sediment, as organic material provides a substrate for the formation of biofilms (Nix and Martin 1992; Herman *et al.* 1994; Fine Tailings Fundamentals Consortium 1995b; Gould 2000, Holowenko *et al.*, 2002). Naphthenic acid toxicity decreases rapidly in OSPM-affected wetlands (Fine Tailings Fundamentals Consortium 1995b). However, it takes longer to form a well-

developed macrophyte community (up to 5 years; personal observation). Thus, the development of the macrophyte community is more important for invertebrates than is toxicity.

Sediment ORP, detritus and water conductivity are interrelated. The decomposition of organic material releases $CO_2$, lowering pH in natural systems with well-developed organic soils (Wetzel 1983). A thick layer of organic substrate may isolate the underlying material, such as OSPM, from surface water, leading to a decrease in conductivity and naphthenic acid concentration. Wetlands with fewer macrophytes have less detritus, resulting in less habitat for invertebrates (Driver 1977; Barnes 1983; Friday 1987; Cyr and Downing 1988; Olson *et al.* 1995; Lovvorn *et al.* 1999; Zimmer *et al.* 2000). Streever *et al.* (1996) compared the dipteran community from 10 natural wetlands and 10 created in Florida's phosphate mining area. They reported that pH, conductivity and sediment quality were weakly related to dipteran community structure and concluded that there is no difference in natural and created wetland dipteran communities (Streever *et al.* 1996). However, they stressed that a lack of consistent differences between created and natural wetlands does not indicate complete functional similarity (Streever *et al.* 1996).

Sweep samples showed that in reference wetlands of equivalent age there were significantly fewer taxa (families) present in the young OSPM-affected wetlands. This trend did not seem to decrease with wetland age, i.e., older OSPM-affected wetlands did not have more families. This is could be due to the nature of the sampling technique. Sweep samples collect taxa from many habitats as well as collecting large, mobile taxa such as odonate larvae. Also, reduced richness could be a result of OSPM toxicity. The two oldest OSPM-affected wetlands, Natural Wetland (15 y) and Seepage Control Pond (25 y), both receive periodic or continuous seepage of OSPM through ongoing seepage of waters

contained within the retaining sand dykes (dyke seepage waters) of the settling basin (MLSB). If OSPM or other tailings material are added as a one time addition to the wetland, the toxic constituents in these materials (such as ammonia, but mainly naphthenic acids) break down with time, such that the only residual toxicity is likely due to salinity (Herman *et al.*. 1994; Fine Tailings Fundamentals Consortium 1995b; Lai *et al.* 1996; Whelly 1999; Bendell-Young *et al.* 2000; Gould 2000; Oil Sands Wetlands Working Group 2000; Leung 2001; Leung *et al.* 2003, Holowekno *et al.* 2002). The benthic community of these wetlands would then be expected to converge with those found in high conductivity (salinity) wetlands.

Examination of the relative abundance of selected taxa from core and sweep samples showed that OSPM-affected wetlands did develop similar numbers of families as reference wetlands, even with initially fewer families than similar aged reference wetlands (See Figures 2.8, 2.10, 2.12). OSPM-affected wetlands also had similar abundance of benthic invertebrates as reference wetlands regardless of wetland age (See Figure 2.9, 2.13). Abundance of invertebrates was significantly lower for artificial substrate samples only. This could be because artificial substrates collect taxa that are epiphytic, and generally OSPM-affected wetlands have less epiphytic taxa.

It is not surprising to find that OSPM-affected wetlands had similar benthic invertebrate abundances (number of individuals) as reference wetlands. OSPM-affected wetlands, especially younger ones, have a benthic community dominated by chironomid larvae. Fugitive species are quick colonisers that build up large population densities (Kajak 1964 cited in Cantrell and Lachlan 1977). Chironomids are often the first invertebrates to colonise mud habitats (Cantrell and Lachlan 1977), and tolerate elevated salinity and conductivity (Cannings and Scudder 1978; Bervoets 1996; Colbo 1996; Lovvorn *et al.*

1999). While older OSPM-affected wetlands take longer to develop a diverse benthic community, chironomid larvae provide the bulk of the benthic community in young OSPM-affected wetlands.

Naphthenic acids occur naturally within crude oil and are a diverse group that can include many carboxylated cyclic and non-cyclic alkanes, with similar surfactant properties. They represent a group of organic acid components of bitumen that are released from the bitumen under alkaline conditions ((Lai *et al.* 1996; Holowenko *et al.* 2002)). Naphthenic acids act as surfactants and when exposed to biological membranes, can inhibit the exchange of gases across respiratory surfaces of aquatic organisms. The disruption of membrane exchange efficiency by naphthenic acids is believed to be the prime reason that fresh OSPM is toxic to all aquatic organisms.

Herman *et al.* (1994) determined that there are indigenous communities of microbes that break down bitumen and its constituents. This has been shown in OSPM waters (Holowenko *et al.* 2002) and in OSPM-affected wetlands (Moore *et al.* 2002). These naphthenate-degrading bacteria are located in the sediment, at interfaces and in biofilms attached to surfaces such as plant stalks and detritus (Nix *et al.*. 1994). Lai *et al.* (1996) examined the factors that affect the degradation of naphthenic acids and found that temperature, dissolved oxygen and phosphate increase the rate of breakdown of naphthenic acids. Other studies on the toxicity of contaminants in wetlands found that DOC and suspended solids decrease bioavailability of metals through sorption and changes in water chemistry (Polonsky and Clements 1999), and that nutrient addition enhances the activity of indigenous microbiota facilitating breakdown of oil in sediments (Lin *et al.* 1999).

Improving the development of the benthic community in OSPM-affected wetlands may be facilitated through the addition of organic material amendments to these wetlands.

Depositing a layer of organic material, as an amendment in OSPM-affected wetlands, would probably be effective at mitigating negative impacts and aid in facilitating the development of the microbiological and macrophyte communities. In addition, constructing larger, shallow (depth < 3m) wetlands may also speed the breakdown of toxic constituents in the water or sediment. Continued monitoring of the benthic invertebrate communities in such aquatic environments would be advised, as it will be important to determine how the water chemistry and zoobenthic communities of "one-time-addition" OSPM-affected wetlands change over time.

**Recommended Sampling Protocol**

Artificial substrates have been used when sampling streams and rivers (DeShon 1995), and for lakes and wetlands (Rosenberg *et al.* 1977; Benoit *et al.* 1998). This technique provides a semi-quantitative method of assessing the benthic fauna. However, artificial substrate samples require more time to collect as two trips are needed - one to set them in place and one to collect them. The length of time that they are left in the waterbody may also influence the taxa found on them (see DeShon 1995, Barbour *et al.* 1999b, and Benoit *et al.* 1998) for a summary of advantages and disadvantages of artificial substrates).

Artificial substrate samplers are not routinely used in Rapid Biological Protocols (RPBs) in streams because it is thought that up to eight weeks is required for colonisation (Barbour *et al.* 1999b). However, Benoit *et al.* (1998), reported that substrates, in lakes, had reached saturation density and abundance by 8 days after placement.

This study has shown that 5 samplers, left for 8 days, were adequate for an estimate of the zoobenthic community in these wetlands. (Benthic invertebrates were collected from 5 artificial substrates, with 2 artificial substrate samples processed (40% of total samples

collected) for each wetland, and a significant difference was found between young and mature reference wetlands).

Sweep samples require the least time to collect of all the sampling methods used. Sweep samples are also the recommended method of sampling for RBPs due to the ease of collection. However, maintaining a consistent technique among personnel collecting these samples is difficult (personal observation) and may result in collector bias. Therefore, if a quantitative assessment of the benthic fauna is desired, the use of artificial substrates is recommended over sweep samples.

**Summary**

In general, the findings of this study indicate that older, one-time-addition OSPM-affected wetlands were physico-chemically similar to reference wetlands. Off-lease site reference wetlands were similar to on-site reference wetlands both physicochemically and biologically. Within five years of inception the zoobenthic abundance in OSPM-affected wetlands was similar to that of reference wetlands. Within 5-7 years of creation the richness in the zoobenthic community of OSPM-affected wetlands was similar to reference wetlands. However, if a wetland received periodic OSPM inputs, richness was lower than for similarly aged reference wetlands. Initially, toxicity accounts for the biological differences between OSPM-affected and reference wetlands. Later, the development of the macrophyte community, the quantity of detritus (coarse organic material), and sediment organic content appear to influence the biological differences between OSPM-affected and reference wetlands.

# Chapter 3

## Assessing Wetland Zoobenthic Community Development in the Oil Sands Mining Region of Northern Alberta

### Introduction

One purpose of environmental assessment and management is the maintenance of biological integrity (Reynoldson *et al.* 1995). Historically, chemical monitoring has been used to assess the efficacy of pollution abatement programs on the biota of water bodies. Increasingly, benthic macroinvertebrates and fish, are being used in routine monitoring and assessment programs (see Karr *et al.* 1986). Benthic macroinvertebrates are often used because of their close association with sediments, and potential contaminants, through their feeding and behavioural activities (Reynoldson *et al.* 1995). Benthic invertebrates form an integral link between the terrestrial and aquatic food ecosystems and provide an indication of the ecological status of aquatic systems (Ciborowski *et al.* 1995).

Biota respond to environmental stressors in three common ways: reduction in species richness, changes in species composition to a community dominated by opportunistic species, and by a reduction in the mean size of organisms (Gray 1989 cited in Barbour *et al.* 1995). Each feature of the community responds differently to stressors, and an approach that incorporates many attributes into assessment of the biota is preferred (Barbour *et al.* 1995).

### Biomonitoring

Biological monitoring, or biomonitoring, is the 'systematic use of biological responses to evaluate changes in the environment' (Karr 1991; Norris 1995; Gerritsen *et al.*

1998). In this broad sense, evaluating the establishment of the benthic invertebrate community of constructed wetlands and the community-level changes that occur is biomonitoring. Biomonitoring is integral to the measurement of the total ecological health of a water body, and is becoming increasingly important in water quality monitoring and assessment (Gerritsen *et al.* 1998).

Biomonitoring incorporates several elements: biosurveys, bioassays, and chemical monitoring (Barbour *et al.* 1999a). Biosurveys, such as rapid bioassessment protocols (RBPs), are used to detect aquatic life impairments and assess the relative severity of these impairments (Barbour *et al.* 1999a). The biological community reflects overall ecological integrity of a water body. Historical stresses are integrated over time and provide a measure of fluctuating environmental conditions (Barbour *et al.* 1999a).

Bioassays and chemical monitoring are integrated with biosurvey data to help determine the specific stress agents causing impact, limit the specific source of these agents and to design appropriate treatment (Barbour *et al.* 1999a).

Often, benthic macroinvertebrates are used for biomonitoring and biosurveys because of their limited dispersal habits, and are presumed to reflect site specific impacts (Barbour *et al.* 1999a; Rabeni and Wang 2001). Macroinvertebrates integrate effects of short-term variations, and sensitive stages will respond quickly to stress, whereas the overall community will respond more slowly (Barbour *et al.* 1999a). Invertebrates have also been used because they are relatively easy to identify to family, assemblages constitute a range of trophic levels and/or pollution tolerances, and sampling is relatively easy (Barbour *et al.* 1999a).

**Biocriteria**

Biocriteria are numerical values or narrative expressions that describe the reference biological condition of aquatic communities (Gerritsen *et al.* 1998). Biocriteria provide the only direct assessment of water body condition and are sensitive to a broader range of human influences than are chemical criteria alone (Karr1991, USEPA 1991b, in Gerritsen *et al.* 1998). Biocriteria describe the best possible community condition expected in a natural aquatic community relatively free from anthropogenic influence (Gerritsen *et al.* 1998).

The USEPA outlines the selection and implementation of biocriteria for lakes and reservoirs (Gerritsen *et al.* 1998). Effective biocriteria provide for scientifically sound evaluations, protect the most sensitive biological value, and support and strive for protection of biotic integrity (Gerritsen *et al.* 1998). Biocriteria share several characteristics: they include specific assemblage characteristics, they are clearly written and easily understood, they are defensible in a court of law, and they maintain the philosophy and policy of anti-degradation of water resource quality (Gerritsen *et al.* 1998).

Some of the steps outlined for the biocriteria process by Gerritsen *et al.* (1998, Table 10-3) include:

- preliminary classification of sites to determine reference condition.
- characterisation of the reference condition through historical data, survey of reference sites and expert consensus.
- testing the preliminary classification of sites and any necessary revisions.
- development of a metric evaluation and index through data analysis and interpretation.

- development of biocriteria through adjustment of physical and chemical covariates.

**Importance of Reference Sites**

Biotic integrity is the ability of a system to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organisation comparable to that of natural habitat of the region (Karr and Dudley 1981 in Karr *et al.* 1986). A major concern for oil sands companies is the remediation or reclamation of areas that have been adversely affected by mining activity. Remediation targets require precise definition to establish when sufficient action has been taken (Reynoldson and Metcalfe-Smith 1992).

The best method to measure whether an anthropogenically disturbed area has returned, or is returning, to pre-disturbance conditions is to compare it to other local areas that are relatively undisturbed (reference sites). The reference site, or best available condition, is representative of a minimally disturbed site (or sites) organised by selected physical, chemical and biological characteristics (Reynoldson *et al.* 1997). The reference condition refers to a suite of environmental characteristics only. The biota found in these wetlands are the type of community that reflects the reference condition.

Comparing degraded or disturbed sites to reference sites is common in field biological studies (see Somers 1998; Galatowitsch *et al.* 1999; Barbour and Yoder 2000; Resh *et al.* 2000; Ferraro and Cole 1990; James *et al.* 1995; Bowman 1997; Reynoldson and Wright 2000; Bailey *et al.* 2001; King and Richardson 2002). Various attributes of the biological community can be used to determine the extent to which a site has been affected by human activity.

To establish when the benthic community of OSPM-affected wetlands is comparable to reference wetlands, it is necessary to develop an index or measure. Multimetric and multivariate approaches that use benthic invertebrates employ similar data collection methods, but differ in the way reference sites are selected, test (impaired) sites are classified, and test site assessments are made (Resh *et al.* 2000).

**Metric Development**

Multimetric indices for bioassessment are commonly used, in the United States, to determine the extent of an impact on an aquatic system. "Metrics" refers to attributes of the biological assemblage, such as taxa richness, that are combined to produce a multimetric index (Fore *et al.* 1996). For this chapter the terms metric and multimetric are used in the context of biological assessment. Indices are comprised of several measures (metrics) that individually provide information on diverse biological attributes, and when integrated, provide an overall indication of biological condition (Barbour *et al.* 1995). Often, these attributes are based on best professional judgement, historical data, and geographical or physical attributes of the region (Barbour 1995; Reynoldson et al. 1995).

Biological integrity metrics were first developed for measuring the biotic condition of fish communities in streams and rivers (Karr *et al.* 1986; Barbour *et al.* 1995). Development of benthic invertebrate metrics has followed, and currently, they are used in the invertebrate community index (ICI), rapid bioassessment protocols, and the benthic index of biotic integrity (IBI) (Barbour *et al.* 1995). Metrics have been largely developed for lotic systems and have recently been adapted to lentic systems such as wetlands (Galatowitsch *et al.* 1999).

An ideal metric is relatively insensitive to natural variation, responds to stressors of concern, and is stable over time (Barbour *et al.* 1995). Candidate metrics are selected based on knowledge of aquatic systems, flora, and fauna, literature reviews, and historical data (Barbour *et al.* 1995). Candidate metrics can include relative abundance, number of species (richness), number of intolerant species, and percent abundance of different functional feeding groups.

In a multimetric approach, candidate metrics are evaluated by comparing the expected value of each metric at a reference site to the value at a degraded site and assigning a numeric score corresponding to 'good', 'fair', or 'poor' environmental condition (Gerritsen *et al.* 1998). Metrics that are redundant, that don't vary in concert with the stressor of interest, or that are generally less robust are rejected, leaving the "core metrics". Core metrics are those that best discriminate among sites of good or poor environmental condition (Barbour *et al.* 1995). Core metrics are selected to represent diverse aspects of structure, composition, individual health, or processes of the aquatic biota, forming the foundation for a sound integrated analysis of the biotic condition (Barbour *et al.* 1995). The scores of all metrics are summed to produce a total score for the assemblage studied (Gerritsen *et al.* 1998).

Development of an index of biotic integrity follows from the results of multimetric development, often for each assemblage sampled, e.g., macrophytes, benthic invertebrates, fish (Gerritsen *et al.* 1998). Three steps for the development of an index of biotic integrity include characterisation of reference condition, evaluation and selection of metrics, and multimetric index building (Gerritsen *et al.* 1998).

Characterisation of reference condition is accomplished by graphical analysis using box and whisker plots. Inter-quartile range is used to evaluate whether a real difference

exists between two areas, and if a metric is a good candidate for use in assessment (Gerritsen *et al.* 1998).

Metrics are typically evaluated using box and whisker plots and examining the interquartile coefficient - the ratio of the interquartile range to the "scope for detection" (Gerritsen *et al.* 1998). The inter-quartile coefficient is a non-parametric analogue to the coefficient of variation and is used similarly. However, it is bi-directional (Gerritsen *et al.* 1998). Comparing reference to test (impaired) sites is used to evaluate the response of metrics to stressors (Gerritsen *et al.* 1998). Box and whisker plots are used here.

A responsive metric will have a statistically significant difference in central tendency or variance between reference and test (degraded) sites (Gerritsen *et al.* 1998). Responsive metrics are evaluated for redundancy by examining correlations between metrics. If a pair of metrics has a correlation coefficient greater than 0.9, examination of the need for both metrics is warranted (Gerritsen *et al.* 1998).

**Multimetric Development in Wetlands**

Over the past several years several multimetric indices have been proposed for use in wetlands. The Minnesota Pollution Control Agency (2002) has developed the Invertebrate Index of Biological Integrity (IBI) for Wetlands. Burton *et al.* (1999) developed a preliminary Invertebrate IBI for Lake Huron Coastal wetlands. Galatowitsch *et al.* (1999) developed community metrics to measure recovery of Minnesota wetlands.

Galatowitsch *et al.* (1999) evaluated the use of metrics based on species assemblages related to land use patterns in three ecoregions. In examining the plant, fish, bird, invertebrate and amphibian community at 116 wetlands, they found 5 metrics useful for many wetlands: proportion of wetland birds, wetland bird richness, proportion of

insectivorous birds, importance of *Carex*, and importance of invasive perennials (Galatowitsch *et al.* 1999). The invertebrate metrics, that were selected, included abundance of Corixidae, number of crustacean taxa, abundance of Dytiscidae, number of gastropod taxa, total invertebrate abundance, number of insect taxa, invertebrate taxa richness, proportion of gastropods, proportion of dipterans, and proportion of Ephemeroptera. They suggested that monitoring recovery in wetlands with community indicators would require different metrics depending on the wetland type and ecoregion.

Burton *et al.* (1999) examined six coastal wetlands on Lake Huron, three of which were relatively pristine, and three that were relatively degraded. Twenty-four potential metrics were identified, in an attempt to find community components that could ordinate wetlands according to the degree of anthropogenic disturbance. Fourteen metrics separated the wetland sites, including odonate richness and relative abundance, Crustacea plus Mollusca taxa richness, total richness, relative abundance of gastropods, relative abundance of Sphaeriidae, and relative abundance of Amphipoda. They separated the metrics according to the vegetation zone in each wetland. Four measures of the overall vegetation zones included total taxa richness, evenness, Shannon diversity index and Simpson index.

The Minnesota Pollution Control Agency (Helgen, 2001) has developed the Invertebrate Index of Biological Integrity for Depressional Wetlands. Ten metrics have been developed for these wetlands using data from two dip net samples and 10 activity trap samples. Metrics were tested against water and sediment chemistry and human disturbance. Metrics include chironomid genera (number of genera), proportion of Corixidae, number of ETSD (number of genera of Ephemeroptera, Trichoptera, and presence of Sphaeriidae, and dragonflies), and gastropod taxa (number of genera and species). In addition, they identified tolerant and intolerant taxa in these wetlands and have

included them as a metric - number of intolerant taxa, and tolerant taxa proportion of sample count. Tolerant taxa included amphipods, Corixidae, *Enallagma* (a coenagrionid damselfly), *Erpobdella* (a leech), and *Physa* (a snail). Intolerant taxa were *Leucorrhinia* and *Libellula* (libellulid dragonflies), *Tanytarsus* and *Procladius* (Tanytarsini and Tanypodinae chironomid larvae, respectively), *Triaenodes* and *Oecetis* (leptocerid caddisflies), and Sphaeriidae (fingernail clams).

**Multivariate Techniques**

Multivariate approaches, used predominantly in Canada, Great Britain and Australia, also use several measures of the ecosystem to assess the similarity or difference between a reference site and a potentially degraded (test) site. However, instead of using historical information and best professional judgement to group reference sites *a priori*, multivariate approaches identify associations between physical attributes and the biota. Test (degraded) sites are evaluated comparing these sites to reference sites selected *a priori*. Various multivariate statistical analyses are used in multivariate analysis, e.g., principal components analysis, canonical correspondence analysis, and factor analysis, to determine the degree to which the biota actually at a test site are similar to the biota expected to occur there based on the environmental characteristics of the test site.

Several methods of assessing anthropogenic impacts on river systems have been developed in the UK (RIVPACS; Wright 1995), Australia (AUSRIVAS; Parsons and Norris 1996) and for the Laurentian Great Lakes of Canada (BEAST; Reynoldson *et al.* 1995). The BEAST has also been adapted for use on the Fraser River in B.C. (Resh *et al.* 2000). Multivariate approaches have been used for benthic invertebrates in the North Sea

(Olsgard *et al.* 1998), Florida phosphate mines (King and Richardson 2002), and for prairie wetlands in Minnesota (Zimmer *et al.* 2000).

**Developing Predictive Models**

The basis for multivariate analysis lies in the British approach proposed by Wright *et al.* (1984), and Norris (1995). A predictive model is developed in several steps. The first step involves the *a priori* selection of a large number of sites (assumed to be unimpaired). The biota are sampled at these sites, along with a wide range of environmental and physico-chemical variables. The sites are then grouped, using the biota, into similar classes by multivariate classification techniques, such as cluster analysis. Sites are not necessarily in adjacent areas or in the same region (Reynoldson *et al.* 1995; Bailey 1998; Resh *et al.* 2000; Reynoldson *et al.* 2000b). Based on similarities of the biota among sites, site groups are defined. The environmental characteristics that can best separate these groups are then quantified using discriminant function analysis (DFA) (Norris 1995). A subset of environmental variables, little affected by human activities, which best discriminate among the groups of sites, is chosen (Norris 1995). These environmental variables make the predictive classification model.

The predictive model is used to classify 'test' sites. The environmental variables identified above are measured and biota sampled. The environmental variables are used to match test sites with the most environmentally similar group of reference sites based on probabilities of group membership (Norris 1995; Reynoldson *et al.* 1997; Bailey 1998; Resh *et al.* 2000; Reynoldson and Wright 2000; Reynoldson *et al.* 2000b). If a test site has been classified with a reference group, the taxa that should occur at this type of site are predicted. The taxa collected at the test site are compared with the predicted taxa. The

degree of environmental impact is determined based on the deviation of predicted from observed (Norris 1995; Reynoldson *et al.* 1997; Bailey 1998; Resh *et al.* 2000; Reynoldson and Wright 2000; Reynoldson *et al.* 2000b). Depending on the model, these comparisons may be based only on the presence and absence of taxa (Norris 1995), or on relative abundances (Reynoldson *et al.* 1997; Resh *et al.* 2000).

**Other Multivariate Techniques and Uses**

Multivariate techniques can also be used to analyse data independently of a predictive model. Since environmental problems generally involve many variables, they should be analysed using multivariate statistics (Green 1979 cited in (Norris 1995). Commonly used ordination techniques include Detrended Correspondence Analysis (DCA), Principal Components Analysis (PCA), and Principal Co-ordinates Analysis (PCoA). These techniques all are effective for showing relationships among species, by grouping similar species near each other (Gauch 1984).

Zimmer *et al.* (2000) used cluster analysis, canonical correspondence analysis and principal components analysis to determine if the presence of fathead minnows and drainage history contributed to differences in benthic communities of 19 semi-permanent prairie wetlands (Zimmer *et al.* 2000). Green and Vascotto (1978) recommend classification analysis of biological data followed by multiple discriminant analysis of species- assemblage groups on environmental variables for pollution studies.

**Combining Multimetric Approaches and Multivariate Techniques**

Some researchers in the United States view multivariate techniques as too difficult to develop, calculate, and explain to environmental managers and the general public (see

(Gerritsen 1995; Fore *et al.* 1996). Such researchers suggest that multivariate analyses are best used for exploratory purposes when the researcher has limited knowledge of the system and wants to generate testable hypotheses (Gerritsen 1995; Fore *et al.* 1996). However, multivariate methods can facilitate the objective selection of indicators to be used in multimetric applications (Resh *et al.* 2000). Resh *et al.* (2000) recommend that multimetric approaches should consider incorporating multivariate analyses for defining reference conditions and assessing impairment of test sites.

Several studies have incorporated the multimetric indices and multivariate techniques. Spieles and Mitsch (2000) used two biotic indices (Chandler Biotic Score and the Hilsenhoff Biotic Index) and the Invertebrate Community Index along with principal components analysis, to extract trends in variability for both environmental parameters and invertebrate indices and metrics (Spieles and Mitsch 2000). Resh *et al.* (2000) evaluated 44 metrics from 17 sites in the Fraser River, including measures of richness, numbers of individuals, functional feeding groups, and a biotic index. Richness metrics consistently had the lowest error rates (misclassification) of all metrics examined. They recommended that multimetric biological collections be supplemented with recording of similar environmental measurements required for multivariate techniques, and that multimetric and multivariate analyses be used together so that one could base the decision of site impairment on both approaches (Reynoldson *et al.* 1997; Resh *et al.* 2000)

For the current research I have used multivariate techniques (Principal Components Analysis and Discriminant Function Analysis) to construct a multimetric index based on biological and physico-chemical data collected from three classes of wetlands in the oil sands region of northeastern Alberta. The goal of this approach was to produce an invertebrate-based index of community composition that is straightforward enough to be

used by oil sands environmental employees possessing a basic knowledge of aquatic ecology and invertebrate taxonomy.

**Methods**

A detailed outline of data collection and sample processing methods is provided in Chapter 2. A total of 31 wetlands was sampled in 2000 and 2001. In 2001, two wetlands were re-sampled and were treated as independent sites for data analysis, giving a total of 33 wetlands used in the data analysis. For each wetland, benthic invertebrates were enumerated from four core samples, two artificial substrate samples and one sweep sample. Data for each sampling method was analysed separately, because each sampling method assesses different aspects of the wetland benthic macroinvertebrate community. Core samples provide an estimate of the infaunal (sediment dwelling) community, which can reach very high densities. Cores provide a quantitative measure of natural densities of infauna. Artificial substrates collect large, mobile animals. Sweep net (dip net) samples generate composite collections acquired over a broad area and across diverse microhabitats.

**Taxonomic Resolution**

For this analysis, all taxa were initially identified to genus, where possible, and grouped back to family level of classification. Chironomids, collected in 2000, were initially identified to genus. Chironomids, in samples from 2001, were identified to subfamily or tribe. Chironomid taxa used for this analysis were identified at the tribe or subfamily level.

**Statistical Methods**

For all analyses in this chapter, the Statistica version 6.0® software package (Statsoft Inc., 2001) was used, including factor analysis by principal components, and discriminant and canonical analysis.

For each sampling method (i.e., core, artificial substrate, or sweep) benthos were tabulated and analysed separately. Data for each core sample, artificial substrate sample and sweep sample was summarised in raw form (numbers recovered per sieve size fraction in each sample), and expressed as estimated total numbers per sample (numbers recovered/proportion of sample sorted, summed over size fractions) for each taxon identified (see Appendix A1 for details).

The biological processes responsible for abundance of species are exponential (Gauch 1984). Thus, only a few dominant species control the results of multivariate analyses (Gauch 1984). Logarithmic transformation of these data puts species abundance on a more equal footing (Gauch 1984). Gauch (1984) recommends that abundance values fall within an intermediate range of 0 to 10. This allows qualitative and quantitative information to be expressed without either dominating the other. Compression from a greater range can be accomplished through logarithmic transformations, such as the octave scale or $\log_2$ transformation. $Log_2$ relative abundance was calculated for each taxon and used in the analyses for this chapter. This method converts the relative abundance values to octaves and gives more weight to rare taxa and counteracts the effects of outliers (Gauch 1984). Data were also trimmed to further reduce the undue effects of statistically significant outliers in the PCA. Outlier relative abundances were replaced by the next highest value observed in the data set.

**Determining Which Taxa Best Define Wetland Class**

Ordination and classification techniques, such as principal components analysis, organise community data on species abundances, exclusive of environmental data (Gauch 1984). Results of taxa ordination place distributionally similar taxa close together in dimensional space, and dissimilar taxa far apart (Gauch 1984).

Principal components analysis reduces the number of variables and detects structure in the relationships between variables. Since a large number of taxa were identified from the relatively small number of wetlands sampled, it was necessary to reduce the number of taxa to a manageable group. Principal Components Analysis (PCA) was used to reduce the number of independently distributed families and to determine which families of taxa were most likely to occur together.

Principal components analysis calculates the line (component) that extracts the maximum amount of statistical variance from a cloud of points (Fore *et al.* 1996). The number of dimensions through which the line passes is equal to the number of taxa collected (Fore *et al.* 1996). PCA, like regression, is unduly influenced by departures from multivariate normality (normality assumes that data is a random sample of the underlying population and the scores for one taxon are independent of those for all other taxa) (Tabachnick and Fidell 1989). Outliers, such as sites with high abundances or many zeroes can also cause problems. To correct for departure from normality and to control the effect of outliers, data was transformed using $\log_2$(relative abundance) and trimmed where necessary.

Discriminant function analysis (DFA) is used to determine which variables best discriminate between two or more groups identified by the investigator. A DFA will determine whether groups differ with regard to the mean of a variable and one can use that variable to predict group membership. If the group means for a variable are significantly different, then we can say that this variable discriminates among the groups.

In Chapter 2, it was shown that "young" wetlands had fewer families than wetlands 7 or more years old. Consequently, these latter wetlands were operationally defined as "mature". DFA was used to determine which PC scores (based on families of zoobenthos) best discriminate between "young" and "mature" reference wetlands. The goal was to determine, which taxa differ most in relative abundance between relative ages of wetlands, i.e., young reference wetlands and mature reference wetlands? The taxa that compose each PC factor are likely to occur together and best characterise wetlands of a specific age class, e.g., "mature" reference wetlands.

**Ordination of Biological Data**

Principal components analysis (PCA) was first used to determine which groups of taxa collected by each sampling method co-occurred in the 33 wetlands. Only relatively common groups of taxa were included in a PCA (those that were collected in 6 or more wetlands and that had a minimum relative abundance of at least 0.1). The number of principal components (PC's) used in subsequent analyses were those that had eigenvectors greater than 1.0. A scree plot of each analysis was examined to confirm that the PC's selected explained disproportionately high amounts of variation in the original data (StatSoft 2001). Principal component loadings were ascertained after varimax rotation (this

maximises the variances of the squared raw PC loading across variables for each component).

Each PC represents a group of taxa whose relative abundances are correlated with one another. The first PC accounts for the greatest amount of variation in relative abundances of taxa among the wetlands in the study. Each subsequent PC explains additional variation, but its contribution is proportionately less than the previous PC.

The numerical values (eigenvalues) for each taxon associated with each PC indicate how strongly the relative abundance of that taxon contributes to the overall score of that PC. The sign (positive or negative) of a taxon's eigenvalue indicates if its relative abundance is positively or negatively associated with the score of that PC. For example, taxa that have large positive eigenvalues associated with a PC strongly co-occur and are most abundant at wetlands that have the highest scores for that PC. Taxa that have large negative eigenvalues for that same PC also co-occur, but the relative abundances of these taxa are negatively associated with the scores of that PC.

**Core Samples**

For core samples, principal components analysis (PCA) was used to determine which groups of 20 taxa co-occurred in 33 wetlands (Table 3.2). A list of the $\log_2$ (relative abundance) for taxa selected can be found in Table 3.1A.

**Artificial Substrate Samples**

For artificial substrate samples, PCA was used to determine which groups of 20 taxa co-occurred in 33 wetlands (Table 3.3). A list of the $\log_2$ (relative abundance) for taxa selected can be found in Table 3.1B.

**Sweep Samples**

For sweep samples, principal components analysis (PCA) was first used to determine which groups of 22 taxa co-occurred in 31 wetlands (Table 3.4). A list of the $\log_2$ (relative abundance) for taxa selected can be found in Table 3.1C.

**Multiple Regression Analyses**

The relationship between relative abundance of the zoobenthic taxa (PC scores) and 11 physical variables was examined using (forward step-wise) multiple regression. This technique was used by Corkum and Ciborowski (1988) to examine the relationship between the relative abundance of biological groups and environmental variables (independent PCs) and detects non-linear trends by including quadratic terms. This approach was used to determine which independent PCs (groups of taxa) were influenced by physico-chemical factors and whether the relationship was positive or negative (as determined by the sign of the t-value).

**Classification of Biological Data**

Discriminant function analysis is used to determine which variables, or PCs (groups of taxa), discriminate between two or more (*a priori* defined) groups.

For the initial classification only reference wetlands were used. Mature reference wetlands represent the benchmark against which all other wetlands are measured. Wetlands

**Table 3.1 A.** $Log_2$ (mean relative abundance) of taxa selected for use from core samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest.

| | OSPM-affected | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | CTW2000 | CTW2001 | CTP2000 | CTP2001 | DP | TP9 | MFTN | TP5 | TP2 | NW | SCP |
| Baetidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.36 | 0.00 | 0.31 | 0.47 | 0.16 | 0.00 |
| Caenidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.32 | 0.36 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ceratopogonidae | 0.00 | 0.74 | 1.72 | 3.29 | 0.17 | 2.45 | 0.00 | 0.00 | 1.34 | 0.00 | 0.62 |
| Chironomini | 0.00 | 0.00 | 0.82 | 1.17 | 0.90 | 0.00 | 1.17 | 0.00 | 2.03 | 0.66 | 0.18 |
| Coenagrionidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.36 | 0.00 | 0.00 | 0.11 | 0.00 | 0.00 |
| Corixidae | 0.00 | 0.00 | 0.00 | 2.58 | 0.17 | 0.64 | 0.00 | 0.00 | 0.21 | 0.00 | 0.00 |
| Dytiscidae | 0.00 | 0.74 | 0.82 | 1.81 | 0.32 | 0.88 | 0.00 | 0.30 | 0.94 | 0.16 | 0.00 |
| Empididae | 1.62 | 0.00 | 0.82 | 0.00 | 1.52 | 0.36 | 0.00 | 0.00 | 0.94 | 0.00 | 2.69 |
| Haliplidae | 0.00 | 0.00 | 0.00 | 0.00 | 1.17 | 0.88 | 0.00 | 0.00 | 1.43 | 0.00 | 0.74 |
| Hirudinea | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Hydrophilidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 0.47 | 0.00 | 0.00 |
| Leptoceridae | 0.00 | 0.00 | 0.00 | 2.25 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Libellulidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.39 | 0.00 | 0.00 |
| Lymnaeidae | 0.00 | 0.00 | 0.00 | 0.00 | 1.32 | 0.00 | 0.00 | 0.00 | 0.94 | 0.00 | 0.00 |
| Orthocladiinae | 5.07 | 5.12 | 2.02 | 2.58 | 0.58 | 3.82 | 2.58 | 0.77 | 1.85 | 3.66 | 1.30 |
| Planorbidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.58 | 1.08 | 0.00 | 2.18 | 0.00 | 1.93 | 1.05 |
| Talitridae | 0.00 | 0.00 | 0.00 | 1.17 | 0.90 | 0.00 | 0.00 | 2.02 | 1.00 | 0.00 | 0.00 |
| Tanypodinae | 4.14 | 2.82 | 0.82 | 2.25 | 0.32 | 2.03 | 5.77 | 0.96 | 1.85 | 2.84 | 4.11 |
| Tanytarsini | 1.62 | 5.03 | 3.11 | 4.00 | 4.36 | 5.30 | 4.63 | 4.61 | 4.26 | 4.06 | 1.76 |
| Abund. ($log_e$+1) | 3.89 | 3.64 | 3.52 | 3.04 | 5.31 | 4.50 | 3.04 | 4.96 | 5.79 | 6.06 | 5.24 |
| Est. Abund. | 25.00 | 0.00 | 23.25 | 0.00 | 545.17 | 0.00 | 0.00 | 138.50 | 647.25 | 554.75 | 130.75 |
| No. Families | 2.67 | 3.00 | 4.75 | 4.25 | 11.75 | 8.00 | 2.25 | 8.00 | 13.00 | 5.75 | 7.50 |
| % Chironomini | 0.00 | 0.00 | 13.33 | 5.00 | 9.50 | 0.00 | 2.94 | 0.00 | 19.40 | 4.48 | 0.00 |
| % Orthocladiinae | 58.90 | 47.17 | 24.44 | 20.00 | 9.50 | 73.04 | 5.88 | 1.71 | 2.99 | 5.68 | 7.84 |
| % Tanypodinae | 36.99 | 8.49 | 0.00 | 15.00 | 5.03 | 0.00 | 63.24 | 0.00 | 5.47 | 19.28 | 79.41 |
| % Tanytarsini | 4.11 | 44.34 | 62.22 | 60.00 | 75.98 | 26.96 | 27.94 | 98.29 | 72.14 | 70.57 | 12.75 |

**Table 3.1 A**.cont. $Log_2$ (mean relative abundance) of taxa selected for use from core samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest.

| | High Conductivity Reference | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Taxon | PP | CRM | BL | SM | SB | HS | SBD | SPit | CL |
| Baetidae | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Caenidae | 0.27 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Ceratopogonidae | 1.62 | 0.00 | 0.00 | 0.00 | 1.27 | 1.19 | 0.00 | 0.00 | 0.00 |
| Chironomini | 0.70 | 2.06 | 3.60 | 0.16 | 0.36 | 0.60 | 4.17 | 1.79 | 3.48 |
| Coenagrionidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.36 | 0.00 | 0.00 | 0.00 | 0.00 |
| Corixidae | 0.00 | 0.00 | 1.13 | 0.79 | 0.00 | 0.09 | 0.69 | 0.00 | 0.00 |
| Dytiscidae | 1.17 | 1.32 | 1.00 | 0.97 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 |
| Empididae | 0.00 | 0.23 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 |
| Haliplidae | 0.00 | 0.00 | 0.48 | 0.00 | 0.64 | 0.00 | 0.55 | 0.00 | 0.00 |
| Hirudinea | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.21 | 0.00 | 0.00 |
| Hydrophilidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.69 | 0.40 |
| Leptoceridae | 0.00 | 0.04 | 0.00 | 0.00 | 1.82 | 0.09 | 0.00 | 0.69 | 0.00 |
| Libellulidae | 0.87 | 0.16 | 0.26 | 0.57 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Lymnaeidae | 0.00 | 0.76 | 0.00 | 0.00 | 0.64 | 0.00 | 0.00 | 0.00 | 0.00 |
| Oligochaeta | 0.27 | 1.25 | 5.26 | 0.00 | 5.99 | 2.10 | 3.72 | 0.00 | 3.39 |
| Orthocladiinae | 1.80 | 0.68 | 3.32 | 2.63 | 0.36 | 0.66 | 1.43 | 2.02 | 1.18 |
| Planorbidae | 0.00 | 3.04 | 0.85 | 0.00 | 0.00 | 0.97 | 1.92 | 3.26 | 2.26 |
| Talitridae | 0.00 | 0.00 | 0.00 | 0.45 | 3.91 | 0.00 | 0.39 | 0.00 | 0.00 |
| Tanypodinae | 0.70 | 1.23 | 1.00 | 1.14 | 1.09 | 0.47 | 1.73 | 1.16 | 0.71 |
| Tanytarsini | 3.08 | 1.54 | 2.26 | 5.11 | 2.95 | 0.25 | 2.03 | 2.83 | 0.71 |
| Abund. (loge+1) | 4.80 | 6.77 | 4.84 | 5.34 | 4.50 | 5.74 | 5.09 | 4.41 | 4.38 |
| Est. Abund. | 0.00 | 689.50 | 0.00 | 0.00 | 0.00 | 1372.63 | 0.00 | 199.25 | 107.25 |
| No. Families | 7.00 | 10.00 | 8.50 | 6.00 | 7.00 | 10.20 | 7.50 | 6.00 | 7.00 |
| % Chironomini | 5.56 | 73.51 | 44.80 | 0.30 | 3.33 | 21.89 | 71.26 | 34.16 | 56.52 |
| % Orthocladiinae | 22.22 | 20.52 | 36.00 | 12.95 | 3.33 | 44.21 | 5.75 | 66.46 | 6.52 |
| % Tanypodinae | 5.56 | 5.97 | 4.00 | 3.01 | 13.33 | 21.46 | 12.07 | 0.00 | 4.35 |
| % Tanytarsini | 66.67 | 0.00 | 15.20 | 83.73 | 80.00 | 12.45 | 10.92 | 0.00 | 4.35 |

**Table 3.1 A**.cont. $Log_2$ (mean relative abundance) of taxa selected for use from core samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest.

| | Low Conductivity Reference Wetlands | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | SBBP | MID | LP | SW | NWID | TP1 | BM | H63W | WID | LLW | H63I | TR6.8R | TR0.8R | Freq. |
| Baetidae | 0.00 | 0.00 | 0.37 | 0.00 | 1.30 | 0.00 | 0.00 | 0.36 | 0.00 | 0.00 | 0.27 | 0.99 | 0.00 | 10 |
| Caenidae | 0.00 | 0.00 | 0.00 | 0.00 | 2.43 | 0.00 | 1.76 | 1.26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.73 | 7 |
| Ceratopogonidae | 0.00 | 0.00 | 0.90 | 0.34 | 1.30 | 0.68 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 14 |
| Chironomini | 1.21 | 4.37 | 1.96 | 2.69 | 2.52 | 2.93 | 2.31 | 1.26 | 1.67 | 1.67 | 1.62 | 1.57 | 1.65 | 29 |
| Coenagrionidae | 0.00 | 0.00 | 0.00 | 0.12 | 0.45 | 0.00 | 0.00 | 0.88 | 0.00 | 0.24 | 0.50 | 0.00 | 0.00 | 8 |
| Corixidae | 0.29 | 1.40 | 0.37 | 0.00 | 0.00 | 0.00 | 1.37 | 0.36 | 0.00 | 0.24 | 0.00 | 0.00 | 0.00 | 14 |
| Dytiscidae | 0.00 | 0.00 | 0.90 | 0.24 | 1.30 | 0.67 | 0.84 | 0.00 | 1.00 | 0.45 | 0.00 | 1.57 | 0.00 | 21 |
| Empididae | 0.00 | 0.00 | 3.98 | 0.62 | 0.00 | 1.14 | 0.00 | 0.00 | 1.26 | 2.73 | 0.00 | 0.00 | 0.00 | 13 |
| Haliplidae | 0.00 | 0.00 | 0.37 | 0.12 | 0.45 | 0.00 | 1.37 | 0.36 | 0.00 | 0.24 | 0.50 | 0.00 | 0.00 | 14 |
| Hirudinea | 0.00 | 0.41 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.64 | 0.00 | 0.45 | 0.27 | 0.00 | 0.00 | 6 |
| Hydrophilidae | 0.00 | 0.00 | 0.36 | 0.12 | 0.45 | 0.67 | 0.00 | 0.00 | 0.26 | 0.00 | 0.00 | 0.00 | 0.00 | 9 |
| Leptoceridae | 0.00 | 0.00 | 0.36 | 0.24 | 0.00 | 0.00 | 0.00 | 0.36 | 0.00 | 0.45 | 0.00 | 0.00 | 0.41 | 11 |
| Libellulidae | 0.00 | 0.00 | 0.00 | 0.00 | 1.97 | 0.00 | 0.00 | 1.08 | 0.00 | 0.79 | 0.00 | 0.99 | 0.00 | 9 |
| Lymnaeidae | 0.00 | 1.72 | 0.00 | 0.00 | 0.45 | 0.00 | 0.00 | 0.00 | 2.07 | 0.79 | 0.00 | 0.00 | 0.00 | 8 |
| Oligochaeta | 0.73 | 2.79 | 4.39 | 2.02 | 2.99 | 3.41 | 3.86 | 2.38 | 2.84 | 3.70 | 2.89 | 2.56 | 2.31 | 27 |
| Orthocladiinae | 0.00 | 0.99 | 2.83 | 1.73 | 3.49 | 1.14 | 4.08 | 2.12 | 0.85 | 0.00 | 1.52 | 2.30 | 1.80 | 31 |
| Planorbidae | 0.91 | 1.99 | 0.00 | 3.52 | 0.45 | 3.95 | 0.00 | 2.21 | 1.76 | 2.60 | 0.50 | 0.00 | 0.00 | 20 |
| Talitridae | 0.29 | 0.00 | 0.00 | 0.44 | 4.06 | 0.00 | 1.76 | 4.24 | 0.00 | 0.45 | 1.03 | 0.00 | 0.87 | 15 |
| Tanypodinae | 1.68 | 1.57 | 2.34 | 2.28 | 2.43 | 1.77 | 3.50 | 1.93 | 0.48 | 1.96 | 2.04 | 0.99 | 1.21 | 33 |
| Tanytarsini | 3.12 | 5.16 | 4.51 | 4.56 | 3.49 | 4.01 | 4.33 | 1.70 | 0.00 | 5.14 | 2.93 | 4.83 | 0.41 | 32 |
| Abund. (loge+1) | 4.74 | 4.34 | 4.75 | 5.92 | 4.24 | 4.03 | 3.48 | 4.50 | 4.84 | 4.93 | 4.80 | 3.28 | 5.03 | 33 |
| Est. Abund. | 0.00 | 0.00 | 189.00 | 948.13 | 0.00 | 96.50 | 0.00 | 0.00 | 175.38 | 214.25 | 0.00 | 0.00 | 0.00 | 16 |
| No. Familes | 6.00 | 6.25 | 10.33 | 13.00 | 11.00 | 8.67 | 6.00 | 11.25 | 7.75 | 12.25 | 7.75 | 6.50 | 7.00 | 33 |
| % Chironomini | 11.76 | 34.29 | 12.50 | 27.89 | 16.05 | 18.68 | 8.06 | 14.29 | 0.00 | 27.04 | 15.15 | 5.71 | 34.21 | 27 |
| % Orthocladiinae | 0.00 | 1.71 | 29.69 | 15.87 | 34.57 | 0.00 | 32.26 | 34.29 | 100.00 | 0.00 | 13.64 | 11.43 | 39.47 | 30 |
| % Tanypodinae | 19.61 | 3.43 | 18.75 | 19.50 | 14.81 | 0.00 | 20.97 | 28.57 | 0.00 | 3.70 | 22.73 | 2.86 | 21.05 | 27 |
| % Tanytarsini | 68.63 | 60.57 | 39.06 | 36.73 | 34.57 | 81.32 | 38.71 | 22.86 | 0.00 | 69.26 | 48.48 | 80.00 | 5.26 | 30 |

**Table 3.1 B.** $Log_2$(mean relative abundance) of selected taxa from artificial substrate samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest. Bracketed values beside taxon name indicates trimmed maximum or minimum values used in the analysis, i.e., an extreme value was replaced by the next most extreme value in the analysis (Shown in brackets).

| | OSPM-affected | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | CTW2000 | CTW2001 | CTP2000 | CTP2001 | DP | TP9 | MFTN | TP5 | TP2 | NW | SCP |
| Baetidae (0.41) | 0.00 | 0.00 | 0.00 | 0.00 | 0.50 | 0.00 | 0.00 | 1.07 | 0.47 | 0.00 | 0.00 |
| Caenidae (0.45) | 0.00 | 0.30 | 0.00 | 0.00 | 1.23 | 0.20 | 0.25 | 0.45 | 0.07 | 0.00 | 0.12 |
| Ceratopogonidae (1.76) | 2.75 | 0.00 | 3.21 | 0.00 | 0.96 | 0.80 | 0.00 | 1.68 | 0.86 | 0.00 | 0.43 |
| Chironomini | 0.63 | 2.61 | 2.14 | 0.00 | 2.20 | 0.00 | 0.00 | 3.48 | 0.77 | 0.00 | 0.00 |
| Coenagrionidae (1.53) | 0.41 | 0.00 | 0.00 | 0.00 | 0.91 | 0.00 | 1.96 | 0.45 | 0.61 | 0.41 | 0.12 |
| Corduliidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 |
| Corixidae (1.11) | 0.00 | 0.55 | 0.98 | 0.00 | 0.08 | 0.00 | 0.25 | 0.00 | 0.13 | 0.07 | 0.89 |
| Dytiscidae | 0.15 | 1.11 | 1.97 | 0.00 | 0.82 | 0.53 | 0.97 | 0.94 | 0.90 | 0.19 | 0.23 |
| Haliplidae | 0.15 | 0.00 | 0.00 | 0.00 | 0.30 | 0.67 | 0.25 | 0.24 | 0.83 | 0.00 | 0.12 |
| Hirudinea (0.93) | 4.27 | 0.00 | 0.00 | 0.00 | 2.06 | 0.00 | 0.00 | 0.00 | 0.13 | 0.00 | 0.00 |
| Lestidae (0.32) | 0.00 | 1.51 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.19 | 0.12 |
| Libellulidae (0.54) | 0.00 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.00 |
| Lymnaeidae (2.61) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.59 | 1.11 | 0.00 | 0.00 | 0.00 | 0.00 |
| Oligochaeta | 0.83 | 0.00 | 0.00 | 0.00 | 4.17 | 2.32 | 0.00 | 0.94 | 2.24 | 0.00 | 4.67 |
| Orthocladiinae | 3.41 | 5.07 | 3.21 | 4.94 | 1.16 | 2.59 | 5.85 | 2.44 | 2.94 | 3.68 | 1.35 |
| Physidae (0.66) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.82 | 0.00 | 0.07 | 0.00 | 0.00 |
| Planorbidae (1.23) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 |
| Talitridae (2.68) | 0.00 | 0.00 | 0.00 | 0.00 | 2.40 | 0.53 | 0.00 | 0.63 | 1.08 | 0.00 | 0.00 |
| Tanypodinae (3.94) | 1.95 | 3.94 | 2.42 | 2.23 | 2.14 | 4.96 | 3.35 | 1.19 | 1.43 | 3.86 | 4.91 |
| Tanytarsini | 4.57 | 5.80 | 5.02 | 3.07 | 4.82 | 5.07 | 3.65 | 4.80 | 3.50 | 4.75 | 1.94 |
| Abun. ($log_{10}$+1) | 6.12 | 5.38 | 4.64 | 2.67 | 6.75 | 5.83 | 5.57 | 5.61 | 6.94 | 6.96 | 6.37 |
| Est. Abund. | 727.50 | 0.00 | 0.00 | 0.00 | 1233.13 | 307.00 | 0.00 | 777.00 | 1152.50 | 1507.50 | 901.00 |
| No. Families | 10.00 | 5.50 | 9.50 | 1.50 | 25.00 | 14.00 | 7.00 | 15.00 | 27.50 | 15.50 | 15.00 |
| % Chironomini (70.68) | 1.54 | 4.78 | 7.14 | 0.00 | 10.12 | 0.00 | 0.00 | 23.71 | 3.61 | 0.00 | 0.00 |
| % Orthocladiinae (75.00) | 26.85 | 30.43 | 17.35 | 72.73 | 3.48 | 7.33 | 73.20 | 10.34 | 34.37 | 23.11 | 4.62 |
| % Tanypodinae (79.59) | 8.02 | 13.48 | 9.18 | 9.09 | 9.62 | 43.97 | 3.02 | 26.43 | 26.26 | 30.43 | 8.72 |
| % Tanytarsini (30.44) | 63.58 | 51.30 | 66.33 | 18.18 | 76.78 | 47.41 | 14.89 | 62.93 | 53.30 | 50.46 | 8.46 |

**Table 3.1 B cont.**. $Log_2$(mean relative abundance) of taxa selected for use from artificial substrate samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest. Bracketed values beside taxon name indicates trimmed maximum or minimum values used in the analysis, i.e., an extreme value was replaced by the next most extreme value in the analysis (Shown in brackets)..

| | High Conductivity Reference | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Taxon | PP | CRM | BL | SM | SB | HS | SBD | S-PIT | CL |
| Baetidae (0.41) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 |
| Caenidae (0.45) | 0.00 | 0.00 | 0.00 | 0.00 | 1.54 | 0.00 | 0.00 | 0.38 | 0.00 |
| Ceratopogonidae (1.76) | 0.00 | 1.27 | 0.00 | 0.00 | 1.76 | 1.24 | 0.00 | 0.00 | 0.09 |
| Chironomini | 0.00 | 1.75 | 2.25 | 1.82 | 2.27 | 1.83 | 2.26 | 4.06 | 4.99 |
| Coenagrionidae (1.53) | 0.00 | 0.06 | 0.67 | 0.00 | 3.26 | 0.00 | 0.00 | 0.14 | 0.00 |
| Corduliidae | 0.00 | 0.19 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 |
| Corixidae (1.11) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 1.11 | 0.49 | 0.00 |
| Dytiscidae | 1.05 | 0.97 | 0.67 | 0.26 | 0.00 | 0.38 | 0.90 | 0.68 | 0.18 |
| Haliplidae | 0.00 | 0.50 | 0.67 | 0.00 | 0.00 | 0.68 | 0.00 | 0.00 | 0.00 |
| Hirudinea (0.93) | 0.00 | 0.35 | 0.00 | 0.00 | 0.56 | 0.38 | 0.37 | 0.00 | 0.18 |
| Lestidae (0.32) | 0.00 | 0.30 | 0.26 | 0.00 | 0.00 | 0.32 | 0.00 | 0.00 | 0.00 |
| Libellulidae (0.54) | 0.44 | 0.00 | 0.67 | 0.26 | 0.00 | 0.00 | 0.37 | 0.14 | 0.00 |
| Lymnaeidae (2.61) | 0.00 | 1.50 | 3.42 | 0.00 | 1.54 | 0.85 | 0.00 | 0.85 | 0.00 |
| Oligochaeta | 0.78 | 0.40 | 0.00 | 0.00 | 3.26 | 3.03 | 3.10 | 0.00 | 3.03 |
| Orthocladiinae | 3.79 | 1.71 | 2.31 | 2.04 | 1.28 | 3.20 | 2.50 | 3.08 | 2.73 |
| Physidae (0.66) | 0.00 | 0.00 | 1.92 | 0.00 | 0.97 | 0.00 | 0.66 | 0.00 | 0.00 |
| Planorbidae (1.23) | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.77 | 2.94 | 0.14 | 0.09 |
| Talitridae (2.68) | 0.00 | 0.00 | 0.00 | 0.00 | 5.18 | 0.00 | 1.97 | 2.52 | 0.18 |
| Tanypodinae (3.94) | 1.80 | 2.72 | 0.67 | 1.12 | 1.95 | 2.76 | 1.46 | 3.69 | 2.31 |
| Tanytarsini | 3.59 | 1.95 | 4.28 | 1.24 | 1.54 | 2.69 | 1.60 | 4.11 | 2.06 |
| Abun. ($log_{10}$+1) | 4.95 | 7.00 | 5.54 | 5.55 | 4.66 | 6.50 | 5.15 | 6.21 | 6.61 |
| Est. Abund. | 0.00 | 1954.00 | 0.00 | 0.00 | 0.00 | 1892.33 | 0.00 | 642.50 | 1011.50 |
| No. Families | 6.00 | 20.50 | 9.00 | 4.50 | 7.00 | 16.67 | 12.50 | 18.00 | 14.00 |
| % Chironomini (70.68) | 0.00 | 18.12 | 14.07 | 1.40 | 38.10 | 11.64 | 30.95 | 30.59 | 70.68 |
| % Orthocladiinae (75.00) | 48.65 | 17.42 | 14.81 | 71.93 | 14.29 | 37.21 | 38.10 | 14.51 | 12.96 |
| % Tanypodinae (79.59) | 30.43 | 23.14 | 68.89 | 1.75 | 19.05 | 9.10 | 16.67 | 20.95 | 13.96 |
| % Tanytarsini (30.44) | 9.46 | 42.51 | 2.22 | 24.91 | 28.57 | 26.26 | 14.29 | 23.14 | 9.10 |

**Table 3.1 B cont.**. $Log_2$(mean relative abundance) of taxa selected for use from artificial substrate samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest. Bracketed values beside taxon name indicates trimmed maximum or minimum values used in the analysis, i.e., an extreme value was replaced by the next most extreme value in the analysis (Shown in brackets).

| | Low Conductivity Reference | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | SBBP | MID | LP | SW | NWID | TP1 | BM | H63W | WID | LLW | H63I | TR6.8R | TR0.8R | Freq. |
| Baetidae (0.41) | 0.00 | 0.00 | 0.00 | 0.16 | 0.00 | 0.40 | 0.27 | 0.00 | 0.00 | 0.33 | 0.00 | 1.23 | 1.23 | 10 |
| Caenidae (0.45) | 0.27 | 0.00 | 0.17 | 0.36 | 2.46 | 0.00 | 0.27 | 0.93 | 0.00 | 0.18 | 0.00 | 1.23 | 1.70 | 18 |
| Ceratopogonidae (1.76) | 0.00 | 0.00 | 0.70 | 1.03 | 0.00 | 3.18 | 0.00 | 0.24 | 1.11 | 0.48 | 0.00 | 0.00 | 0.54 | 18 |
| Chironomini | 3.87 | 4.56 | 3.21 | 3.77 | 3.09 | 0.40 | 2.82 | 3.17 | 0.41 | 4.09 | 2.36 | 2.34 | 1.89 | 27 |
| Coenagrionidae (1.53) | 0.00 | 2.15 | 0.00 | 0.16 | 2.00 | 0.15 | 1.53 | 0.24 | 0.00 | 0.18 | 0.94 | 0.00 | 0.00 | 19 |
| Corduliidae | 0.00 | 0.00 | 0.00 | 0.11 | 0.00 | 0.28 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 6 |
| Corixidae (1.11) | 0.50 | 0.00 | 0.70 | 0.31 | 0.00 | 0.00 | 0.27 | 0.24 | 0.00 | 0.00 | 0.00 | 2.52 | 1.49 | 17 |
| Dytiscidae | 0.39 | 0.00 | 1.25 | 0.44 | 0.00 | 0.62 | 0.27 | 0.00 | 0.41 | 0.61 | 0.00 | 0.00 | 0.00 | 25 |
| Haliplidae | 0.79 | 0.58 | 0.00 | 0.40 | 1.14 | 0.52 | 0.27 | 0.45 | 0.22 | 0.18 | 0.55 | 0.74 | 0.00 | 21 |
| Hirudinea (0.93) | 0.00 | 0.58 | 0.00 | 0.21 | 0.93 | 0.62 | 0.00 | 0.45 | 0.08 | 0.18 | 1.72 | 0.00 | 0.00 | 16 |
| Lestidae (0.32) | 0.00 | 0.00 | 0.00 | 0.06 | 0.00 | 0.15 | 0.27 | 1.40 | 0.00 | 0.18 | 0.00 | 0.00 | 0.54 | 12 |
| Libellulidae (0.54) | 0.00 | 0.00 | 0.00 | 0.26 | 0.68 | 0.15 | 1.42 | 1.67 | 0.15 | 0.00 | 0.00 | 0.00 | 0.54 | 14 |
| Lymnaeidae (2.61) | 0.00 | 1.98 | 1.39 | 0.21 | 2.61 | 0.15 | 0.00 | 0.00 | 0.08 | 1.28 | 0.00 | 0.00 | 0.00 | 14 |
| Oligochaeta | 3.31 | 2.78 | 4.89 | 2.84 | 3.60 | 3.57 | 4.27 | 2.65 | 4.01 | 4.75 | 0.00 | 3.64 | 1.49 | 24 |
| Orthocladiinae | 4.64 | 5.12 | 3.09 | 1.45 | 4.60 | 1.84 | 4.54 | 2.61 | 0.47 | 1.28 | 5.11 | 5.06 | 1.23 | 33 |
| Physidae (0.66) | 1.52 | 0.99 | 0.00 | 0.06 | 0.38 | 0.00 | 0.27 | 0.24 | 0.08 | 0.00 | 0.55 | 0.00 | 0.00 | 13 |
| Planorbidae (1.23) | 0.14 | 3.88 | 0.00 | 0.11 | 0.00 | 1.45 | 0.51 | 0.00 | 1.52 | 0.00 | 3.93 | 1.23 | 1.49 | 15 |
| Talitridae (2.68) | 0.39 | 0.00 | 0.00 | 0.82 | 2.00 | 0.28 | 3.32 | 3.02 | 0.00 | 0.94 | 0.94 | 0.74 | 2.68 | 18 |
| Tanypodinae (3.94) | 1.67 | 2.68 | 3.21 | 2.57 | 2.21 | 1.13 | 2.59 | 1.90 | 0.82 | 1.28 | 2.08 | 2.13 | 0.93 | 33 |
| Tanytarsini | 1.57 | 2.15 | 4.03 | 4.09 | 4.28 | 4.08 | 4.01 | 3.08 | 1.03 | 4.61 | 4.64 | 2.13 | 1.70 | 33 |
| Abun. ($log_{10}$+1) | 6.18 | 4.64 | 5.59 | 7.13 | 5.12 | 6.14 | 5.48 | 5.22 | 6.81 | 5.95 | 4.70 | 4.32 | 4.72 | 33 |
| Est. Abund. | 751.20 | 0.00 | 377.00 | 1879.50 | 0.00 | 995.00 | 0.00 | 0.00 | 1431.50 | 820.00 | 0.00 | 0.00 | 0.00 | 17 |
| No. Families | 12.50 | 8.50 | 13.00 | 27.50 | 15.00 | 23.00 | 14.50 | 11.33 | 24.00 | 18.50 | 9.50 | 9.50 | 13.00 | 33 |
| % Chironomini (70.68) | 32.59 | 34.59 | 20.95 | 35.75 | 14.29 | 1.61 | 12.55 | 34.38 | 13.04 | 37.96 | 6.38 | 9.38 | 37.50 | 27 |
| % Orthocladiinae (75.00) | 57.46 | 51.88 | 19.05 | 4.88 | 44.00 | 12.90 | 45.89 | 21.88 | 15.22 | 3.40 | 51.77 | 75.00 | 18.75 | 33 |
| % Tanypodinae (79.59) | 4.73 | 5.26 | 5.91 | 30.43 | 6.86 | 3.40 | 31.17 | 32.03 | 11.91 | 9.46 | 36.88 | 7.81 | 31.25 | 33 |
| % Tanytarsini (30.44) | 5.22 | 8.27 | 20.95 | 13.96 | 6.86 | 5.91 | 10.39 | 11.72 | 30.43 | 3.40 | 4.96 | 7.81 | 12.50 | 33 |

**Table 3.1 C.** $Log_2$ (mean relative abundance) of taxa selected for use from sweep samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest. Bracketed values beside taxon name indicates trimmed maximum or minimum values used in the analysis, i.e., an extreme value was replaced by the next most extreme value in the analysis (shown in brackets).

| | OSPM-affected | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | CTW 2001 | CTP2000 | CTP2001 | DP | TP9 | MFTN | TP5 | TP2 | NW | SCP |
| Baetidae (3.29) | 0.00 | 0.00 | 0.00 | 0.62 | 0.57 | 0.00 | 0.00 | 3.25 | 0.07 | 0.00 |
| Caenidae (0.96) | 0.00 | 0.00 | 0.00 | 3.90 | 1.78 | 0.00 | 0.38 | 0.51 | 0.00 | 0.00 |
| Ceratopogonidae (1.47) | 0.00 | 2.01 | 0.00 | 0.00 | 0.57 | 0.00 | 0.00 | 0.63 | 0.00 | 3.72 |
| Chironomini | 1.81 | 1.01 | 0.00 | 1.05 | 0.00 | 0.00 | 0.77 | 2.32 | 0.00 | 0.00 |
| Coenagrionidae (1.37) | 0.00 | 0.00 | 0.00 | 2.40 | 0.00 | 1.99 | 1.07 | 1.36 | 0.49 | 0.00 |
| Corixidae (5.67) | 0.00 | 4.78 | 6.25 | 1.65 | 4.21 | 2.58 | 1.49 | 2.32 | 3.07 | 1.08 |
| Dytiscidae (3.42) | 1.42 | 1.93 | 0.00 | 2.54 | 3.42 | 1.58 | 0.14 | 1.46 | 0.00 | 1.31 |
| Haliplidae (1.56) | 0.00 | 0.00 | 0.00 | 0.62 | 1.56 | 0.58 | 0.26 | 0.74 | 0.00 | 0.00 |
| Hydrophilidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Leptoceridae (0.88) | 0.00 | 0.00 | 0.00 | 3.33 | 2.42 | 0.00 | 0.59 | 0.51 | 0.00 | 0.00 |
| Lestidae | 2.26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.58 | 0.59 | 0.00 | 1.95 | 0.00 |
| Libellulidae | 0.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.93 | 1.67 | 1.26 | 0.00 |
| Lymnaeidae (3.42) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Notonectidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.58 | 1.00 | 1.18 | 1.79 | 0.00 |
| Oligochaeta | 0.00 | 0.00 | 0.00 | 3.79 | 2.14 | 0.58 | 0.26 | 3.15 | 0.00 | 5.40 |
| Orthocladiinae | 5.92 | 3.44 | 0.00 | 1.65 | 1.30 | 2.90 | 2.14 | 2.32 | 0.93 | 2.71 |
| Physidae (2.29) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.38 | 0.00 | 0.00 |
| Planorbidae | 0.27 | 0.00 | 0.00 | 2.25 | 0.00 | 0.00 | 0.00 | 0.89 | 0.00 | 0.00 |
| Sphaeriidae (0.19) | 0.00 | 0.00 | 0.00 | 1.05 | 0.00 | 0.00 | 0.00 | 1.53 | 0.00 | 0.00 |
| Talitridae (4.41) | 0.00 | 0.00 | 0.00 | 3.73 | 0.00 | 0.00 | 0.59 | 4.41 | 0.00 | 0.00 |
| Tanypodinae | 4.22 | 2.01 | 0.00 | 1.05 | 2.55 | 1.58 | 2.85 | 1.22 | 0.00 | 3.91 |
| Tanytarsini | 2.87 | 5.69 | 0.00 | 2.99 | 3.77 | 3.24 | 5.06 | 4.09 | 3.27 | 2.12 |
| Abund. ($Log_e$+1) | 7.17 | 7.21 | 2.39 | 6.23 | 6.33 | 6.30 | 7.90 | 8.41 | 8.60 | 6.60 |
| Est. Abund. | 0.00 | 560.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5494.00 | 0.00 |
| No. Families | 6.00 | 9.00 | 2.00 | 19.00 | 17.00 | 11.00 | 18.00 | 25.00 | 12.00 | 9.00 |
| % Chironomini | 2.93 | 1.56 | 0.00 | 9.52 | 0.00 | 0.00 | 1.64 | 15.75 | 0.00 | 0.00 |
| % Orthocladiinae | 69.27 | 15.31 | 0.00 | 19.05 | 7.69 | 38.24 | 7.98 | 15.75 | 9.42 | 24.19 |
| % Tanypodinae | 20.49 | 4.69 | 0.00 | 9.52 | 25.64 | 11.76 | 14.55 | 5.25 | 0.00 | 61.29 |
| % Tanytarsini | 7.32 | 78.44 | 0.00 | 61.90 | 66.67 | 50.00 | 75.82 | 63.25 | 90.58 | 14.52 |

**Table 3.1 C.** cont. $Log_2$ (mean relative abundance) of taxa selected for use from sweep samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest. Bracketed values beside taxon name indicates trimmed maximum or minimum values used in the analysis, i.e., an extreme value was replaced by the next most extreme value in the analysis (shown in brackets).

| | High Conductivity Reference | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Taxon | PP | BL | CRM | SM | SB | HS | S-Pit | CL |
| Baetidae (3.29) | 0.00 | 1.21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Caenidae (0.96) | 0.00 | 0.00 | 0.00 | 0.00 | 0.64 | 0.00 | 0.48 | 0.06 |
| Ceratopogonidae (1.47) | 0.00 | 0.73 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Chironomini | 1.99 | 1.21 | 1.66 | 0.99 | 0.00 | 3.07 | 2.07 | 3.92 |
| Coenagrionidae (1.37) | 0.00 | 0.73 | 0.00 | 0.00 | 0.00 | 0.00 | 0.62 | 0.12 |
| Corixidae (5.67) | 2.36 | 2.49 | 0.87 | 3.63 | 1.42 | 0.00 | 2.72 | 0.80 |
| Dytiscidae (3.42) | 3.31 | 2.49 | 0.92 | 2.26 | 0.64 | 0.71 | 1.22 | 0.52 |
| Haliplidae (1.56) | 0.00 | 0.00 | 0.76 | 0.22 | 0.00 | 0.00 | 0.18 | 0.06 |
| Hydrophilidae | 0.22 | 1.86 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Leptoceridae (0.88) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Lestidae | 0.00 | 1.57 | 0.65 | 1.86 | 0.00 | 1.19 | 0.00 | 0.00 |
| Libellulidae | 0.41 | 1.21 | 0.00 | 2.04 | 0.00 | 0.00 | 0.00 | 0.06 |
| Lymnaeidae (3.42) | 0.00 | 0.00 | 0.00 | 0.00 | 3.05 | 3.12 | 0.00 | 0.00 |
| Notonectidae | 0.00 | 0.00 | 0.09 | 2.26 | 0.00 | 0.00 | 0.85 | 0.00 |
| Oligochaeta | 0.73 | 0.00 | 3.38 | 0.00 | 2.84 | 0.21 | 0.18 | 1.41 |
| Orthocladiinae | 3.40 | 4.07 | 3.05 | 2.95 | 1.08 | 1.90 | 4.08 | 1.54 |
| Physidae (2.29) | 0.00 | 3.26 | 0.00 | 0.00 | 0.00 | 1.96 | 0.00 | 0.00 |
| Planorbidae | 0.00 | 3.89 | 1.22 | 0.00 | 0.64 | 4.32 | 0.00 | 0.00 |
| Sphaeriidae (0.19) | 0.00 | 0.00 | 0.00 | 0.00 | 1.69 | 0.00 | 0.00 | 0.00 |
| Talitridae (4.41) | 0.00 | 1.86 | 0.00 | 0.00 | 5.74 | 0.00 | 0.00 | 1.52 |
| Tanypodinae | 2.16 | 1.57 | 0.76 | 0.99 | 1.69 | 1.62 | 4.03 | 0.83 |
| Tanytarsini | 5.86 | 1.86 | 0.97 | 4.30 | 2.59 | 1.77 | 3.77 | 0.83 |
| Abund. ($Log_e$+1) | 7.41 | 6.02 | 8.36 | 7.41 | 6.19 | 7.44 | 7.62 | 8.74 |
| Est. Abund. | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 886.00 | 0.00 | 0.00 |
| No. Families | 10.00 | 19.00 | 17.00 | 11.00 | 13.00 | 16.00 | 14.00 | 20.00 |
| % Chironomini | 4.07 | 6.06 | 19.43 | 3.59 | 0.00 | 50.00 | 6.78 | 80.25 |
| % Orthocladiinae | 78.05 | 12.12 | 8.57 | 68.26 | 60.00 | 16.30 | 26.84 | 4.44 |
| % Tanypodinae | 13.12 | 72.73 | 65.71 | 24.55 | 13.33 | 18.48 | 33.90 | 10.86 |
| % Tanytarsini | 4.75 | 9.09 | 6.29 | 3.59 | 26.67 | 14.13 | 32.49 | 4.44 |

**Table 3.1 C.** cont. $Log_2$ (mean relative abundance) of taxa selected for use from sweep samples. Wetlands are ordered, from left to right, as OSPM-affected, high conductivity reference and low conductivity reference. Within in each group, wetlands are ordered (left to right) from youngest to oldest. Bracketed values beside taxon name indicates trimmed maximum or minimum values used in the analysis, i.e., an extreme value was replaced by the next most extreme value in the analysis (shown in brackets).

| | Low Conductivity Reference | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | SBBP | MID | LP | SW | NWID | TP1 | BM | H63W | WID | LLW | H63I | TR6.8R | TR0.8R | Freq. |
| Baetidae (3.29) | 0.44 | 0.00 | 3.78 | 0.19 | 0.96 | 3.42 | 1.64 | 0.00 | 2.05 | 1.36 | 0.00 | 1.98 | 3.29 | 15.00 |
| Caenidae (0.96) | 0.00 | 0.00 | 0.00 | 0.19 | 0.96 | 0.00 | 1.37 | 0.80 | 0.00 | 0.00 | 0.00 | 3.12 | 0.00 | 12.00 |
| Ceratopogonidae (1.47) | 0.00 | 0.00 | 1.46 | 2.21 | 1.31 | 0.00 | 0.00 | 0.00 | 0.45 | 0.00 | 0.00 | 1.47 | 0.70 | 11.00 |
| Chironomini | 3.59 | 0.97 | 2.39 | 2.70 | 1.23 | 0.00 | 0.43 | 1.68 | 0.45 | 1.62 | 0.00 | 1.98 | 0.27 | 23.00 |
| Coenagrionidae (1.37) | 0.00 | 0.21 | 0.00 | 0.37 | 0.00 | 0.00 | 1.37 | 1.98 | 0.00 | 0.00 | 0.00 | 0.67 | 2.31 | 14.00 |
| Corixidae (5.67) | 2.82 | 4.34 | 2.25 | 3.21 | 0.86 | 5.29 | 2.57 | 1.31 | 2.11 | 2.81 | 5.67 | 2.19 | 1.17 | 29.00 |
| Dytiscidae (3.42) | 0.78 | 0.97 | 4.76 | 2.67 | 0.74 | 0.27 | 0.00 | 1.51 | 1.31 | 0.60 | 0.00 | 0.67 | 1.03 | 27.00 |
| Haliplidae (1.56) | 0.00 | 0.00 | 0.00 | 1.01 | 0.74 | 0.00 | 0.00 | 0.80 | 1.51 | 0.33 | 5.67 | 0.00 | 0.00 | 15.00 |
| Hydrophilidae | 0.00 | 0.00 | 0.81 | 0.00 | 0.00 | 0.00 | 0.00 | 0.45 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.00 |
| Leptoceridae (0.88) | 0.00 | 0.00 | 0.00 | 0.37 | 0.00 | 0.39 | 0.23 | 0.00 | 0.45 | 0.00 | 0.00 | 1.47 | 0.88 | 10.00 |
| Lestidae | 0.44 | 0.40 | 0.00 | 2.06 | 0.18 | 0.00 | 1.46 | 3.13 | 3.03 | 0.00 | 0.00 | 0.00 | 1.30 | 16.00 |
| Libellulidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.86 | 0.00 | 1.72 | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 | 1.30 | 12.00 |
| Lymnaeidae (3.42) | 0.00 | 2.01 | 3.42 | 1.85 | 5.61 | 0.00 | 0.00 | 1.08 | 3.03 | 2.22 | 0.00 | 0.00 | 0.00 | 9.00 |
| Notonectidae | 0.00 | 0.00 | 1.58 | 2.16 | 0.86 | 0.00 | 1.37 | 1.68 | 0.24 | 0.00 | 0.00 | 0.00 | 0.00 | 13.00 |
| Oligochaeta | 1.05 | 1.62 | 3.24 | 2.88 | 1.60 | 3.32 | 0.00 | 0.00 | 3.10 | 4.41 | 0.00 | 1.13 | 0.70 | 22.00 |
| Orthocladiinae | 0.78 | 2.17 | 3.00 | 3.36 | 3.66 | 2.86 | 4.06 | 2.11 | 0.00 | 0.00 | 0.00 | 2.52 | 0.00 | 26.00 |
| Physidae (2.29) | 2.82 | 0.00 | 0.00 | 1.20 | 0.00 | 0.00 | 0.23 | 1.31 | 1.60 | 2.29 | 0.00 | 0.00 | 0.00 | 10.00 |
| Planorbidae | 1.28 | 0.71 | 0.00 | 2.25 | 0.34 | 3.71 | 0.00 | 0.00 | 3.48 | 2.75 | 0.00 | 1.13 | 1.90 | 16.00 |
| Sphaeriidae (0.19) | 0.00 | 0.00 | 0.00 | 0.19 | 0.49 | 0.00 | 0.00 | 0.00 | 4.39 | 0.00 | 0.00 | 1.13 | 4.04 | 8.00 |
| Talitridae (4.41) | 0.00 | 0.00 | 0.00 | 2.67 | 3.72 | 0.27 | 3.37 | 0.00 | 0.00 | 1.36 | 0.00 | 0.00 | 2.19 | 12.00 |
| Tanypodinae | 0.78 | 0.21 | 2.58 | 1.91 | 1.78 | 0.70 | 1.64 | 0.45 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 24.00 |
| Tanytarsini | 3.59 | 0.97 | 3.35 | 3.92 | 3.03 | 3.94 | 5.09 | 0.80 | 0.80 | 4.32 | 0.00 | 5.71 | 0.27 | 29.00 |
| Abund. ($Log_e$+1) | 6.64 | 7.45 | 6.99 | 7.54 | 7.61 | 7.87 | 7.34 | 6.60 | 7.30 | 6.95 | 1.69 | 6.13 | 7.17 | 31.00 |
| Est. Abund. | 0.00 | 0.00 | 412.00 | 913.00 | 0.00 | 774.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.00 |
| No. Families | 13.00 | 15.00 | 30.00 | 27.00 | 24.00 | 18.00 | 17.00 | 19.00 | 31.00 | 20.00 | 2.00 | 16.00 | 21.00 | 31.00 |
| % Chironomini | 46.97 | 17.14 | 16.83 | 17.35 | 5.99 | 0.00 | 0.69 | 33.33 | 33.33 | 9.88 | 0.00 | 5.00 | 50.00 | 23.00 |
| % Orthocladiinae | 3.03 | 62.86 | 27.72 | 29.22 | 51.50 | 29.41 | 30.58 | 50.00 | 0.00 | 0.00 | 0.00 | 8.00 | 0.00 | 26.00 |
| % Tanypodinae | 3.03 | 2.86 | 19.80 | 8.68 | 10.78 | 2.94 | 4.12 | 5.56 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 24.00 |
| % Tanytarsini | 46.97 | 17.14 | 36.63 | 44.75 | 31.74 | 67.65 | 64.60 | 11.11 | 66.67 | 90.12 | 0.00 | 87.00 | 50.00 | 29.00 |

were classified as "mature" if they were 7 years or older and "young" if they were younger than 7 years. Seven years was picked as the cut-off as zoobenthic abundance and richness appears to be at an asymptote among wetlands aged 7 to 15 years (See Chapter 2).

DFA was performed on core samples using 3 variables (PCs) in 22 reference wetlands. DFA was performed on artificial substrate and sweep samples using 5 variables in 22 reference wetlands (artificial substrate samples) and 21 wetlands (one sweep sample was lost prior to processing).

Forward step-wise DFA was used. This method builds a model to best predict to which group a case (wetland) belongs, by evaluating all variables and progressively including ones that contribute most to the discrimination between groups (StatSoft 2001). The most important factors were evaluated by looking at the standardised canonical coefficients for each variable. Variables that had the largest coefficients were judged to be most important.

A discriminant function 'score' for each wetland was generated by summing the value of each of the products of raw canonical coefficient and the corresponding mean relative abundance (PC score) for that variable. The DFA-equation for reference wetlands was composed of the DFA 'score' (e.g., $Root_i$ x $Var_i$) for each significant principal component in the model and a constant, e.g., constant + ($Root1_1$xVar1)-($Root1_2$xVar2). This equation (for reference wetlands) was used to predict the class to which OSPM-affected wetlands should belong based upon their taxonomic composition.

**Summary of Data Analysis**

PCA was used to organise the data into manageable groups of co-occurring taxa. Each principal component (PC) for the biological data was used in a multiple regression

with physico-chemical variables to determine the nature of the relationship between each independent group of taxa (PC) and various physico-chemical parameters. The resulting PC scores were used to produce a discriminant function model to determine which PC factors best discriminate between two groups ("young" or "mature" reference) of wetlands. The DFA-equation for reference wetlands was developed. From this, an equation was developed to classify OSPM-affected wetlands. By plotting the values from this equation against wetland maturity class ("young" or "mature"), the taxa were determined which characterize "young" or "mature" reference and OSPM-affected wetlands. By plotting the values from this equation against actual wetland age, it is possible to visually infer when the trajectories of the wetland classes converge.

**Developing a Multimetric Indicator of Concordance with Mature Reference Wetlands**

To determine which taxa would be best candidates to include in a "metric" for reference wetlands, DFA was used on the taxa that had been identified as important for distinguishing between young and mature reference wetlands (the taxa that are contained in the important PC factors determined in the previous section). Also included in the analysis were relative abundances (percent) of chironomid tribes and subfamilies within the family Chironomidae, mean richness and $\log_e + 1$ mean abundance of benthic taxa (per sample unit). Relative abundances of chironomid tribes and subfamilies were chosen because of the pattern that emerged when chironomid data were examined (Appendix A.4).

A forward stepwise DFA was performed using reference wetlands only. Variables that had the highest coefficients were judged to be most important. A discriminant function 'score' ("metric score") was generated from the value of the raw canonical coefficients multiplied by the value for that variable (PC). The DFA-equation for reference wetlands is

composed of multiple DFA 'scores' and a constant, e.g., constant + $Root1_1$xVar1 - $Root1_2$xVar2. This equation is used to predict whether OSPM-affected wetlands are equivalent to similar aged reference wetlands based upon their taxonomic composition.

**Testing the Metric**

Often, proposed metrics or indices are tested on data that are collected from the same site in previous years or in years following (e.g., Burton *et al.* (1999)). Correct classification of the same sites in different years is seen as confirmation that the metric works. Researchers suggest that this proposed metric be tested on other areas to determine its range of applicability. The proposed metric was tested by applying it to data from the same region, collected in 1997 (Whelly 1999; Table 3.4). The purpose of this was to determine how well wetlands that were not included in the 2000-2001 study were classified with this metric. Some wetlands had been sampled at both time points (1997 and 2000-01) and were expected to classify similarly.

**Results**

For this section the $\log_2$ (relative abundance) values, or octaves, are used for the invertebrate taxa unless otherwise indicated. Richness is the mean number of families present in a wetland, and mean abundance is the "total number of individuals" per sample unit, $\log_e$+1 transformed. The percent composition of the chironomid tribes or subfamilies represents the percent of that subfamily/tribe within the family Chironomidae. For example, a wetland with a value of 66 for %Tanytarsini variable would have two thirds of the total chironomid taxa in the tribe Tanytarsini (see Appendix A.1 for raw data). Tables 3.1 A, 3.1 B, and 3.1C shows the $Log_2$ (relative abundance) values for the taxa selected for

each of core, artificial substrate and sweep sample analyses. The results for the three sampling methods are presented in the following section. Overall, young reference wetlands were generally characterised by similar taxa. Generally, mature reference wetlands were characterised by similar taxa for the different sampling methods. Artificial substrate and sweep samples had a more similar zoobenthic community in young wetlands than core samples.

**Core Samples**

Principal components analysis of octaves of the core samples explained 41.7% of the variation in relative abundance of 20 families with 3 components (Table 3.2). PC1 accounted for 16.3% of variation in relative abundance among wetlands. Wetlands exhibiting high scores for PC1 had high relative abundances of damselflies (Coenagrionidae), amphipods (Talitridae), mayflies (Caenidae and Baetidae), and dragonflies (Libellulidae).

PC2 accounted for 13.8% of the variation in relative abundance among wetlands. High scores for this component indicated wetland with high relative abundance orthoclad midges, no-see-ums (Ceratopogonidae), and dytiscid beetles, and low relative abundance of Chironomini midges and planorbid snails (Table3.2).

PC3 accounted for 11.6% of the variation in relative abundance among wetlands. High scores for this component indicated wetland dominance by hydrophilid and haliplid beetles, worms, lymnaeid snails, and dance fly larvae (Empididae) (Table 3.2).

The results of the multiple regression between the PCs for the biological data and physico-chemical parameters are shown in Table 3.3. The taxa associated with PC1 had low relative abundances when naphthenic acid concentration was high. The taxa in PC2,

**Table 3.2.** Principal component (PC) scores for core samples collected from 33 wetlands. Groups of taxa associated with each PC co-occur if their values are the same sign. Opposite signs indicate taxa that exhibit negative associations. Taxa are $\log_2$(mean relative abundance) transformed. Bold loadings are significant at $p < 0.01$. "Prp. Expl." is the proportion of the original variance (among 20 variables) accounted for by each principal component. Cumulative proportion is also calculated.

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| Coenagrionidae | **0.790** | -0.039 | 0.053 |
| Talitridae | **0.774** | 0.004 | -0.003 |
| Caenidae | **0.724** | 0.101 | -0.044 |
| Hirudinea | **0.584** | -0.561 | -0.101 |
| Baetidae | **0.564** | 0.319 | 0.086 |
| Libellulidae | **0.461** | 0.149 | 0.045 |
| Corixidae | **0.296** | 0.109 | -0.073 |
| Leptoceridae | **0.280** | -0.279 | 0.050 |
| | | | |
| Orthocladiinae | 0.086 | **0.647** | -0.338 |
| Ceratopogonidae | 0.049 | **0.612** | 0.170 |
| Dytiscidae | 0.017 | **0.602** | 0.156 |
| Tanytarsini | 0.191 | **0.386** | -0.082 |
| Tanypodinae | 0.080 | **0.314** | -0.250 |
| Chironomini | 0.081 | **-0.415** | 0.366 |
| Planorbidae | -0.120 | **-0.687** | 0.126 |
| | | | |
| Hydrophilidae | -0.102 | -0.017 | **0.702** |
| Oligochaeta | 0.272 | -0.050 | **0.665** |
| Lymnaeidae | 0.011 | -0.261 | **0.608** |
| Empididae | -0.260 | 0.077 | **0.581** |
| Haliplidae | 0.499 | 0.302 | **0.516** |
| | | | |
| % Explained | 0.163 | 0.138 | 0.116 |
| Cumul. % Expl. | 16.3% | 30.1% | 41.7% |
| Eigenvalue | 3.387 | 2.870 | 2.084 |

**Table 3.3**. Summary of results for multiple regression of three PC (independent groups of taxa) against physico-chemical factors for core samples. Taxa included in each PC are listed in Table 3.2. Forward step-wise regression admitted less than 11 physico-chemical variables. Sign of t-value indicates whether the relationship is positive or negative.

| Variable | PC1 | | | | |
|---|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | t | p |
| Intercept | 0.655 | 0.310 | | | * |
| [Naphthenic Acid] | -0.315 | 0.127 | 0.166 | -2.48 | ** |
| Total | | | 0.166 | | |

| Variable | PC2 | | | | |
|---|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | t | p |
| Intercept | 0.876 | 1.130 | | | ns |
| [Naphthenic Acid] | 0.260 | 0.141 | 0.169 | 1.84 | * |
| Wetland Area | -0.278 | 0.129 | 0.083 | -2.16 | * |
| Macrophyte Dev. | -0.146 | 0.129 | 0.060 | | ns |
| Sediment Organic Content (LOI) | -0.412 | 0.279 | 0.036 | | ns |
| Detritus | 0.269 | 0.249 | 0.027 | | ns |
| Total | | | 0.375 | | |

| Variable | PC3 | | | | |
|---|---|---|---|---|---|
| | Reg. Coeff. | S.E. | $R^2$ | t | p |
| Intercept | 3.733 | 3.336 | | | ns |
| [Naphthenic Acid] | -0.621 | 0.139 | 0.154 | -4.48 | * |
| Detritus | -0.384 | 0.255 | 0.111 | -1.50 | * |
| Wetland Area | -0.173 | 0.124 | 0.054 | | ns |
| Macrophyte Dev. | -0.138 | 0.120 | 0.051 | | ns |
| pH Water | 0.194 | 0.261 | 0.042 | | ns |
| Particle Size | 0.470 | 0.234 | 0.029 | | ns |
| Sediment Organic Content (LOI) | -0.509 | 0.290 | 0.040 | | ns |
| pH Sediment | -0.330 | 0.215 | 0.046 | | ns |
| Total | | | 0.527 | | |

* $p < 0.05$ ** $p < 0.01$ ns = not significant

with positive PC values, had a slight increase in relative abundance when naphthenic acid concentrations were higher, suggesting that these taxa are less sensitive to the effects of naphthenic acids. However, the taxa in PC2 with negative PC values (Chironomini and Planorbidae) were negatively associated with increasing naphthenic acid concentration, that is their relative abundances decreased. PC3 had a very strong negative relationship with naphthenic acid concentration and a less negative association with the amount of detritus. The taxa in PC3 showed a decreased relative abundance when naphthenic acid concentration was high and detrital abundance was low.

The results of the DFA separating young and mature reference wetlands are shown in Table 3.4. Two factors (PC1 and PC2) did not distinguish "young" from "mature" reference wetlands ($p > 0.05$). All 22 wetlands were classified as "mature", with no "young" wetlands correctly classified and all "mature" wetlands correctly classified.

Figure 3.1 summarises the grouping of wetlands according to PCA factor scores for relative abundances of families within the 22 reference wetlands. Correctly classified young reference wetlands would be located above the horizontal line, if present. However all of the wetlands classified as "young", *a priori*, were classified as mature in the DFA analysis. Generally, libellulid dragonflies, amphipods, coenagrionid dragonflies, and mayflies dominated mature reference wetlands (Figure 3.1).

From Table 3.4, PC 1 and PC2 were used to develop a 'DFA score' equation for reference wetlands only. The equation for the analysis of core samples is

$$(PC1 * 0.820) - (PC2 * 0.543) - 0.307 \qquad (1)$$

Figure 3.2 summarises the results of applying equation (1) to all 3 wetland classes, (including OSPM-affected wetlands). This allowed the determination of where the zoobenthic composition in OSPM-affected sites falls, with respect to reference sites.

**Table 3.4.** Discriminant function analysis summary for core samples using 22 reference wetlands. Wetlands were grouped by age class (young (≤ 7 yrs.) or mature (> 7 yrs.)). The principal components listed are taken from Table 3.2, and represent the $\log_2$(mean relative abundance) values for 20 selected taxa. The overall p-value for the model is shown in bold, as well as significant p-values for each variable contributing to the model. Wilks' lambda represents the discriminatory power of the current model, and partial Wilks' lambda is the unique discriminatory contribution of each variable (3.3A). The variables not in the model are also shown (3.3B). Included is the classification matrix (3.3C). The DFA-score equation is developed from the variables in the model and is calculated by multiplying each variable in the model by its raw canonical coefficient and adding a constant.

A) Variables in the model

| | Wilks' Lambda | Partial Lambda | p | Toler. | 1-Toler. | Raw Canon. Coeff. |
|---|---|---|---|---|---|---|
| PC1 | 0.984 | 0.931 | 0.251 | 0.987 | 0.013 | 0.820 |
| PC2 | 0.938 | 0.977 | 0.511 | 0.987 | 0.013 | -0.543 |
| Constant | | | | | | -0.307 |

Wilks' Lambda: 0.916 approx. $F_{(2,19)}$= 0.869 **p < 0.436**

B) Variables not in the model

| | Wilks' Lambda | Partial Lambda | p-level | Toler. | 1-Toler. |
|---|---|---|---|---|---|
| PC3 | 0.910 | 0.993 | 0.725 | 0.931 | 0.069 |

C) Classification Matrix

| Actual Group | Young | Mature | Total | Percent Correct |
|---|---|---|---|---|
| Young | 0 | 6 | 6 | 0 |
| Mature | 0 | 16 | 16 | 100 |
| Total | 0 | 22 | 22 | 73 |

DFA-score equation: (PC1 * 0.820) - (PC2 * 0.543) - 0.307

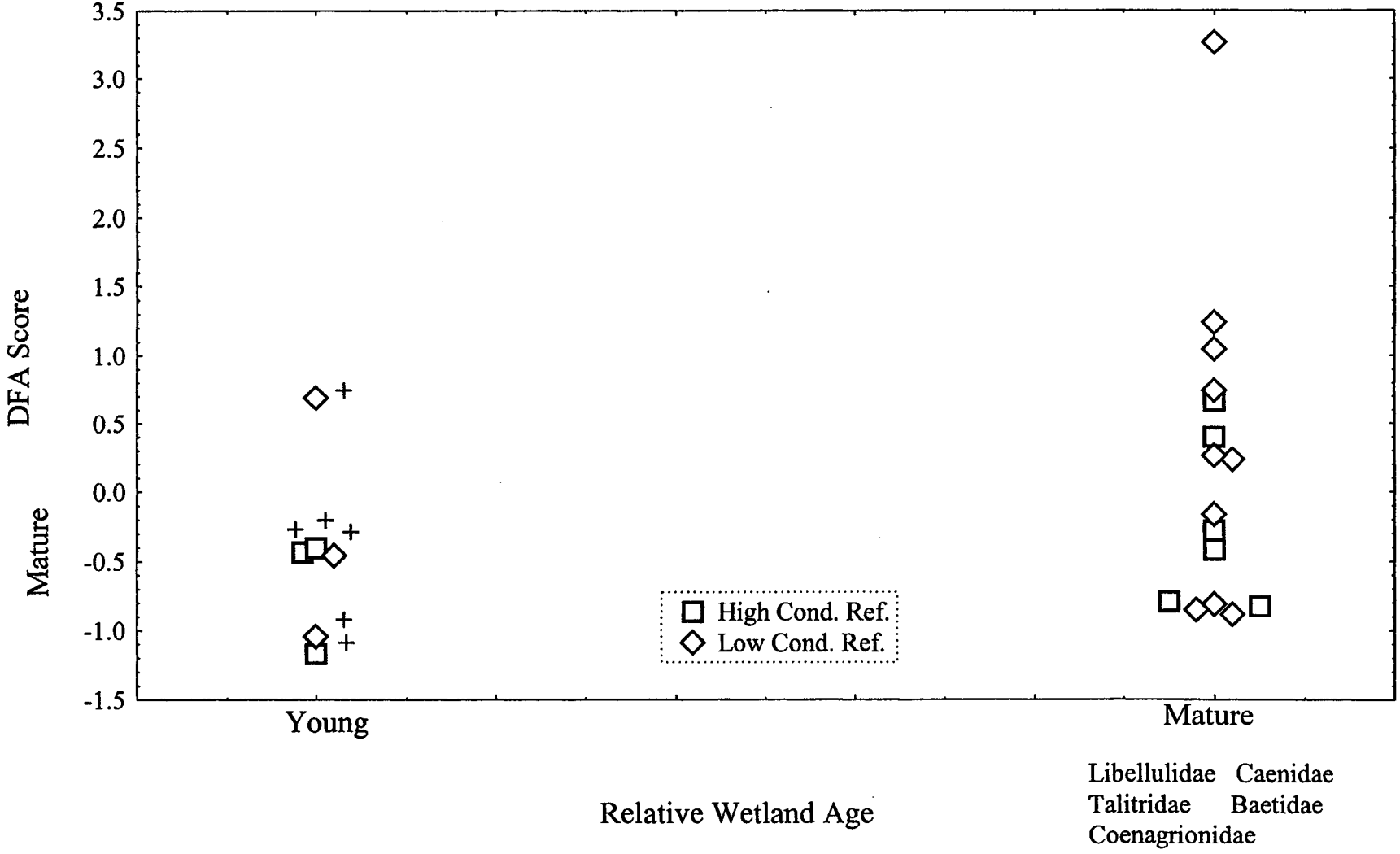



**Figure 3.1.** Taxa best distinguishing young from mature reference wetlands using 20 taxa from 22 reference wetlands, for core samples. Wetlands classified as young by the DFA are listed above the horizontal line. Wetlands classified as mature are below. Taxa listed above the line are generally characteristic of young reference wetlands. Taxa listed below the line are generally charcteristic of mature reference wetlands. Wetlands indicated with a cross were misclassified in the DFA analysis.

Dytiscid beetles, orthoclad midges, and biting midges dominate core samples at all young OSPM-affected sites (Figure 3.2). Six "young" reference wetlands were classified as "mature" by the DFA analysis. Examination of Table 3.1A shows that these wetlands had high relative abundance odonate and mayfly larvae that characterise "mature" reference wetlands, as well as high relative abundance of Dytiscidae that characterise "young" OSPM-affected wetlands. Several OSPM-affected wetlands classified, *a priori*, as "mature" (NW, MFTN, SCP, TP9) had taxa in relative abundances that were intermediate between young OSPM-affected wetlands and mature reference wetlands (Figure 3.2).

Plotting the results of the DFA equation against wetland age (in 2001) provides a visual tool to determine when the successional trajectories of the different wetland classes converge. Several OSPM-affected wetland communities appear to reach an asymptote similar to that of mature reference wetlands (Figure 3.3). These wetlands have high relative abundance of dytiscids beetles (characteristic of "young" wetlands) but also have moderate relative abundances of caenid mayflies and amphipods (See Table 3.1A)

There are several OSPM-affected wetlands classified as "mature" that have a zoobenthic community intermediate to "young" and "mature" wetlands (TP5, NW) (Figure 3.3). Two "mature" OSPM-affected wetlands (MFTN and SCP) lack the taxa identified in the DFA as being important to separating "young" from "mature". Examining Table 3.1A reveals that non-Chironomini midges dominate the benthic community of these "mature" wetlands (10 yr. and 25 yr. old respectively). However, these taxa were not identified as being important in distinguishing "young" from "mature" reference wetlands.

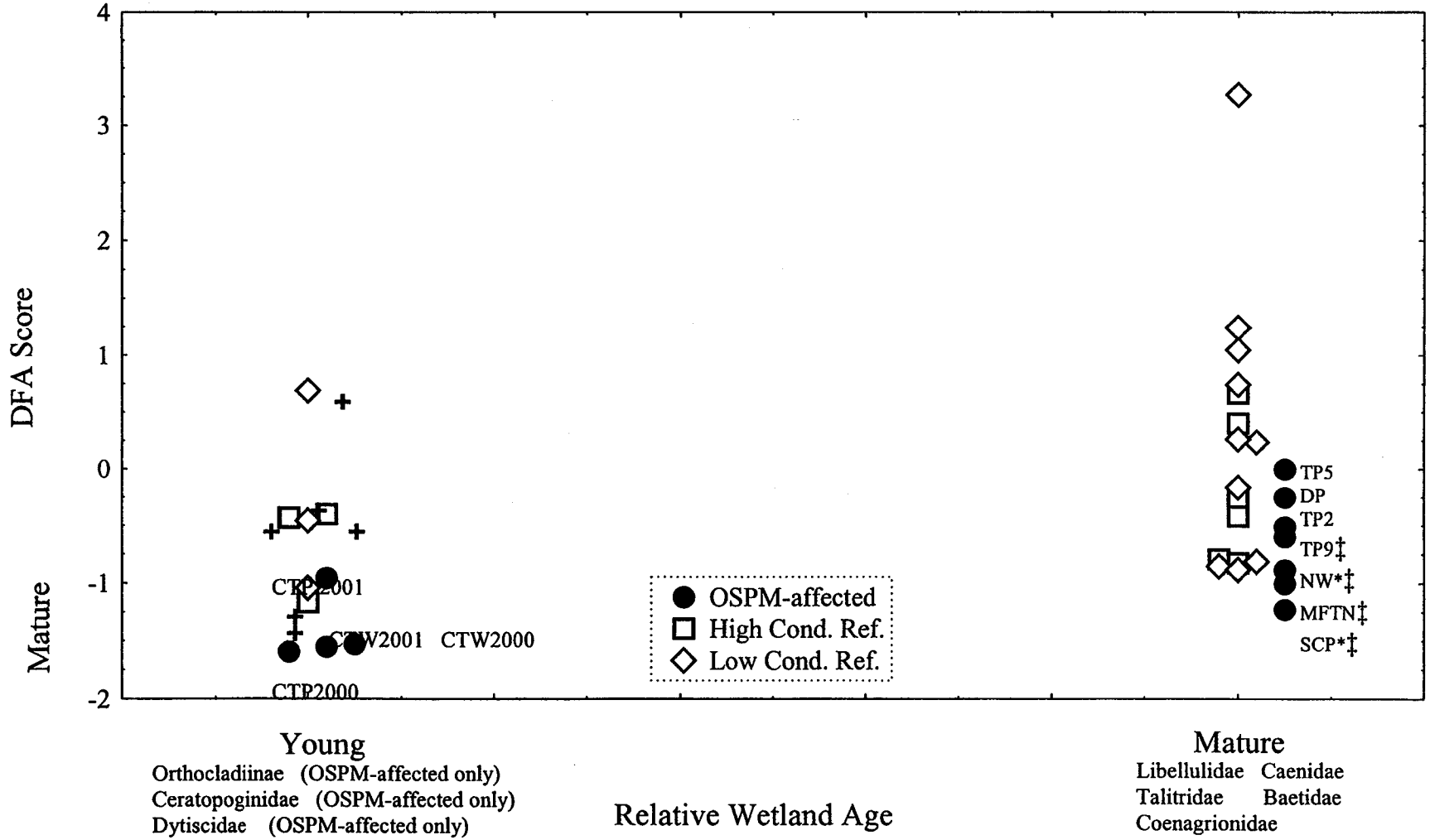



**Figure 3.2.** Taxa best distinguishing young and mature wetlands using core samples from 33 wetlands. Only OSPM affected wetlands are labelled to best illustrate their location relative to reference wetlands. Wetlands classified as young by the DFA are located above the line, mature wetlands are located below. Taxa listed above the line are characteristic of young reference wetlands, taxa listed below the line are characteristic of mature reference wetlands. Wetlands marked with + are misclassified in the DFA analysis. Wetlands marked with * receive ongoing OSPM inputs. Wetlands marked with ‡ have benthic taxa intermediate to young and mature wetlands.

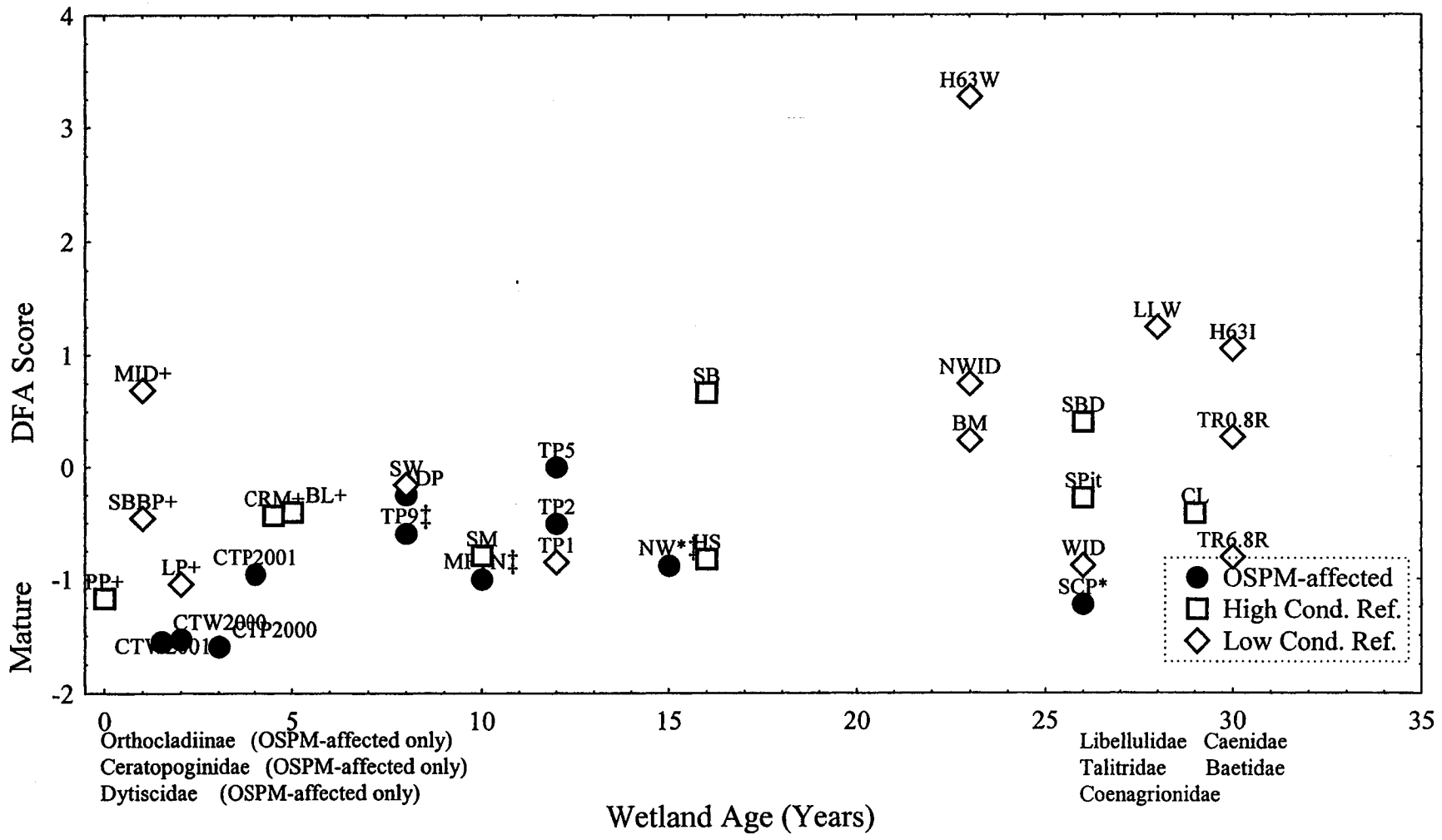



**Figure 3.3.** Taxa best distinguishing wetlands at any age using core samples. Wetlands correctly classified by the DFA as young should be located above the horizontal line. Wetlands correctly classified as mature by the DFA should be located below the line. Taxa listed above the line are generally characteristic of young reference wetlands. Taxa listed below the line are generally characterisitic of mature reference wetlands. Wetlands marked with + are misclassified in the DFA analysis. Wetlands marked with * receive ongoing OSPM inputs. Wetlands marked with ‡ have benthic taxa intermediate ot young and mature wetlands.

**Artificial Substrate Samples**

Principal components analysis of octaves of the artificial substrate samples explained 60.4% of the variation in relative abundance of 20 families with 5 components (Table 3.5). PC1 accounted for 14% of variation in relative abundance among wetlands. Wetlands exhibiting high scores for PC-I had very high relative abundances of mayflies (Caenidae and Baetidae), amphipods (Talitridae), Chironomini midges and worms.

For samples collected by the artificial substrate method, PC2 accounted for 13.8% of the variation in relative abundance among wetlands. High scores for PC2 indicated wetlands with high relative abundance of coenagrionid damselflies, leeches, haliplid beetles and snails (Lymnaeidae). Water boatmen (Corixidae) were negatively associated with PC-II (Table 3.5).

PC3 accounted for 12.7% of the variation in relative abundance among wetlands. High scores for this component indicated wetlands dominated by orthoclad midge larvae and physid snails (Table 3.5). Wetlands with high scores for this component had low abundances of corduliid dragonfly and no-see-um midge (Ceratopogonidae) larvae.

PC4 accounted for 11.5% of the variation in relative abundance among wetlands. High scores for this component indicated wetland dominance by planorbid snails. Tanypod and Tanytarsini midge larvae and predacious diving beetles (Dytiscidae) were negatively associated with PC-4 (Table 3.5).

The fifth PC accounted for 8.5% of the variation in relative abundance among wetlands. High scores for this component indicated wetland dominance by libellulid dragonflies and lestid damselflies (Table 3.5).

**Table 3.5.** Principal component (PC) scores for artificial substrate samples collected from 33 wetlands. Groups of taxa associated with each PC co-occur if their values are the same sign. Opposite signs indicate taxa that exhibit negative associations. Taxa are $\log_2$(mean relative abundance) transformed. Bold loadings are significant at $p < 0.01$. "Prp. Expl." is the proportion of the original variance (among 20 variables) accounted for by each principal component. Cumulative proportion is also calculated.

| | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| Caenidae | **0.864** | 0.039 | 0.001 | -0.165 | 0.053 |
| Talitridae | **0.832** | 0.135 | -0.001 | 0.140 | 0.201 |
| Baetidae | **0.544** | -0.095 | -0.526 | 0.098 | 0.073 |
| Chironomini | **0.529** | 0.096 | 0.159 | 0.056 | -0.120 |
| Oligochaeta | **0.407** | 0.125 | -0.293 | 0.374 | -0.160 |
| | | | | | |
| Coenagrionidae | 0.285 | **0.731** | 0.273 | -0.074 | -0.088 |
| Hirudinea | 0.096 | **0.705** | -0.223 | 0.156 | -0.097 |
| Haliplidae | 0.068 | **0.643** | 0.004 | 0.125 | 0.093 |
| Lymnaeidae | -0.012 | **0.601** | 0.168 | -0.240 | 0.024 |
| Corixidae | 0.327 | **-0.650** | 0.182 | 0.186 | -0.014 |
| | | | | | |
| Orthocladiinae | -0.126 | 0.154 | **0.701** | -0.192 | -0.096 |
| Physidae | 0.099 | 0.502 | **0.575** | 0.291 | -0.121 |
| Corduliidae | -0.491 | 0.129 | **-0.496** | 0.061 | 0.436 |
| Ceratopogonidae | -0.065 | 0.098 | **-0.827** | -0.100 | -0.198 |
| | | | | | |
| Planorbidae | -0.013 | 0.035 | -0.038 | **0.759** | 0.103 |
| Dytiscidae | -0.103 | -0.363 | -0.182 | **-0.508** | -0.186 |
| Tanypodinae | -0.135 | -0.090 | 0.275 | **-0.570** | -0.042 |
| Tanytarsini | 0.082 | 0.151 | -0.113 | **-0.802** | 0.097 |
| | | | | | |
| Lestidae | 0.017 | -0.065 | -0.088 | -0.054 | **0.869** |
| Libelullidae | 0.316 | -0.013 | 0.287 | 0.109 | **0.719** |
| | | | | | |
| % Total | 0.139 | 0.138 | 0.127 | 0.115 | 0.085 |
| Cumul. % Expl. | 13.9 % | 27.7 % | 40.4 % | 51.9 % | 60.4 % |
| Eigenvalue | 2.755 | 3.346 | 2.302 | 2.019 | 1.644 |

**Table 3.6.** Summary of results for multiple regression of five PCs (independent groups of taxa) against physico-chemical variables for artificial substrate samples. Taxa included in each PC are listed in Table 3.5. Forward step-wise regression admitted less than 11 physico-chemical variables. Sign of t-value indicates whether the relationship is positive or negative.

| | PC1 | | | | |
|---|---|---|---|---|---|
| Variable | Reg. Coeff. | S.E. | $R^2$ | t | p |
| Intercept | -12.569 | 2.405 | | | ** |
| pH Sediment | 0.954 | 0.175 | 0.404 | 5.448 | ** |
| pH Water | 0.529 | 0.205 | 0.036 | | ns |
| Salinity | -1.104 | 0.479 | 0.041 | | ns |
| Sediment Org. Cont. (LOI) | 0.639 | 0.240 | 0.049 | | ns |
| Detritus | -0.323 | 0.146 | 0.068 | -2.221 | * |
| [Naphthenic Acid] | 0.263 | 0.189 | 0.028 | | ns |
| Total | | | 0.626 | | |
| | PC2 | | | | |
| Variable | Reg. Coeff. | S.E. | $R^2$ | | p |
| Intercept | 4.737 | 3.137 | | | ns |
| Conductivity | -1.068 | 0.500 | 0.255 | -2.135 | * |
| Particle Size | -0.356 | 0.224 | 0.061 | | ns |
| pH Water | 0.493 | 0.231 | 0.058 | 2.133 | * |
| Salinity | 1.487 | 0.814 | 0.044 | | ns |
| Macrophyte Dev. | -0.179 | 0.117 | 0.038 | | ns |
| [Naphthenic Acid] | -0.275 | 0.224 | 0.030 | | ns |
| Total | | | 0.486 | | |
| | PC3 | | | | |
| Variable | Reg. Coeff. | S.E. | $R^2$ | | p |
| Intercept | 1.295 | 2.827 | | | ns |
| pH Water | -0.570 | 0.261 | 0.135 | -2.182 | * |
| Wetland size (Area) | 0.254 | 0.134 | 0.055 | | ns |
| Detritus | -0.267 | 0.187 | 0.043 | | ns |
| pH Sediment | 0.347 | 0.227 | 0.032 | | ns |
| [Naphthenic Acid] | 0.207 | 0.137 | 0.057 | | ns |
| Total | | | 0.323 | | |
| | PC4 | | | | |
| Variable | Reg. Coeff. | S.E. | $R^2$ | | p |
| Intercept | 3.547 | 1.806 | | | ns |
| [Naphthenic Acid] | -0.393 | 0.101 | 0.360 | -3.902 | ** |
| Sediment Org. Cont. (LOI) | 0.495 | 0.221 | 0.116 | 2.242 | * |
| pH Water | -0.449 | 0.202 | 0.076 | -2.220 | * |
| Total | | | 0.552 | | |
| | PC5 | | | | |
| Variable | Reg. Coeff. | S.E. | $R^2$ | | p-level |
| Intercept | -0.217 | 0.638 | | | ns |
| Macrophyte Dev. | 0.671 | 0.114 | 0.385 | 5.906 | ** |
| Particle Size | -0.446 | 0.172 | 0.064 | | ns |
| ORP Sediment | 0.005 | 0.002 | 0.050 | | ns |
| [Naphthenic Acid] | 0.285 | 0.118 | 0.087 | | ns |
| Total | | | 0.586 | | |

* $p < 0.05$ ** $p < 0.01$ ns = not significant

The results of the multiple regression between the PCs for the biological data and physico-chemical parameters are shown in Table 3.6. The taxa associated with PC1 increased in relative abundance with increasing sediment pH (alkaline sediment). These same taxa also decreased in relative abundance with a decrease in the amount of detritus in a wetland (Table 3.6). Taxa in PC2, with positive scores, decreased in relative abundance with increasing conductivity, and increased with more alkaline water. Corixidae had a negative PC value and were insensitive to the effects of increased conductivity and water pH. Orthoclad midges and planorbid snails (PC3) had a decrease in relative abundance with increasing water pH (Table 3.6). Planorbid snails (PC4) decreased in relative abundance when naphthenic acid concentration was high, water pH was alkaline, and sediment organic content was low (Table 3.6). Dytiscid beetles, and tanypod and Tanytarsini midges had high relative abundances when naphthenic acid concentration was high, sediment organic content was low, and water pH was alkaline (Table 3.6). Odonate taxa (Lestidae and Libellulidae) had a strong positive relationship with macrophyte development (Table 3.6).

The results from the DFA separating "young" and "mature" reference wetlands are shown in Table 3.7. Three factors (PC1, PC3, and PC4) contributed to distinguishing "young" from "mature" reference wetlands ($p < 0.05$), while classifying (82%) wetlands to the correct group (Table 3.7 C).

Figure 3.4 summarises the grouping of wetlands according to PCA scores for families within the 22 reference wetlands. Correctly classified "young" reference wetlands should be located above the horizontal line. "Young" reference wetlands have a benthic community characterised by high relative abundance of Tanytarsini and tanypod midges, corixids, biting midges, and dytiscid beetles (Figure 3.4).

**Table 3.7**. Discriminant function analysis summary for artificial substrate samples using 22 reference wetlands. Wetlands were grouped by age class (young (≤ 7 yrs.) or mature (> 7 yrs.)). The principal components listed are taken from Table 3.5, and represent the $\log_2$(mean relative abundance) values for 20 selected taxa. The overall p-value for the model is shown in bold, as well as significant p-values for each variable contributing to the model. Wilks' lambda represents the discriminatory power of the current model, and partial Wilks' lambda is the unique discriminatory contribution of each variable (3.7A). The variables not in the model are also shown (3.7B). Included is the classification matrix (3.7C). The DFA-score equation is developed from the variables in the model and is calculated by multiplying each variable in the model by its raw canonical coefficient and adding a constant.

A) Variables in the model

| | Wilks' Lambda | Partial Lambda | p | Toler. | 1-Toler. | Raw Canon. Coeff. |
|---|---|---|---|---|---|---|
| PC1 | 0.858 | 0.694 | **0.011** | 0.750 | 0.250 | -1.049 |
| PC3 | 0.752 | 0.792 | **0.043** | 0.803 | 0.197 | 0.817 |
| PC4 | 0.698 | 0.853 | 0.095 | 0.897 | 0.103 | -0.721 |
| Constant | | | | | | 0.350 |
| Wilks' Lambda: 0.595 | | approx. $F_{(3,18)} = 4.081$ | | **p < 0.023** | | |

B) Variables not in the model

| | Wilks' | Partial | p-level | Toler. | 1-Toler. |
|---|---|---|---|---|---|
| PC2 | 0.590 | 0.992 | 0.716 | 0.941 | 0.059 |
| PC5 | 0.594 | 0.998 | 0.840 | 0.930 | 0.070 |

C) Classification Matrix

| Actual Group | Young | Mature | Total | Percent Correct |
|---|---|---|---|---|
| Young | 4 | 2 | 6 | 66.7 |
| Mature | 2 | 14 | 16 | 87.5 |
| Total | 6 | 16 | 22 | 81.8 |

DFA-score Equation: - (PC1*1.049) + (PC3*0.817) - (PC4*0.721) + 0.350

Correctly classified "mature" reference wetlands should be located below the horizontal line. These wetlands were generally dominated by high relative abundance of caenid and baetid mayflies, amphipods, Chironomini and orthoclad midges, physid and planorbid snails and worms (Figure 3.4). Two "young" reference wetlands were classified as having "mature" benthic communities. Two "mature" reference wetlands were classified as having a benthic community characteristic of a "young" wetland.

From Table 3.7, PC1, PC3 and PC4 were used to develop an equation for reference wetlands only. The equation for the analysis of artificial substrate samples is

$$-(PC1*1.049) + (PC3*0.817) - (PC4*0.721) + 0.350 \qquad (2)$$

Figure 3.5 summarises the results of applying equation (2) to all 3 wetland classes, (including OSPM-affected wetlands) allowing determination of where the zoobenthic composition of OSPM-affected sites falls, with respect to reference sites. Generally, "young" OSPM-affected sites were characterised by high relative abundance of Tanytarsini and tanypod midges, dytiscid beetles, biting midges, and corixids (Figure 3.5). Correctly classified "young" reference wetlands had moderately high relative abundances of Tanytarsini and tanypod midges, dytiscid beetles, biting midges, and corixids (Figure 3.5).

"Mature" OPSM-affected wetlands had lower relative abundance of mayflies, amphipods, Chironomini midges, and snails compared to "mature" reference wetlands (Table3.1B). "Mature" OSPM-affected wetlands had higher relative abundance of dytisicds and corixids compared to "mature" reference wetlands (Table 3.1B). Three OSPM-affected wetlands classified, *a priori*, as "mature" (NW, MFTN, SCP) have a benthic community more characteristic of "young" wetlands (Figure 3.5).

Plotting the results of the DFA equation against wetland age in 2001 provides a visual tool to determine when the successional trajectories of the different wetland classes

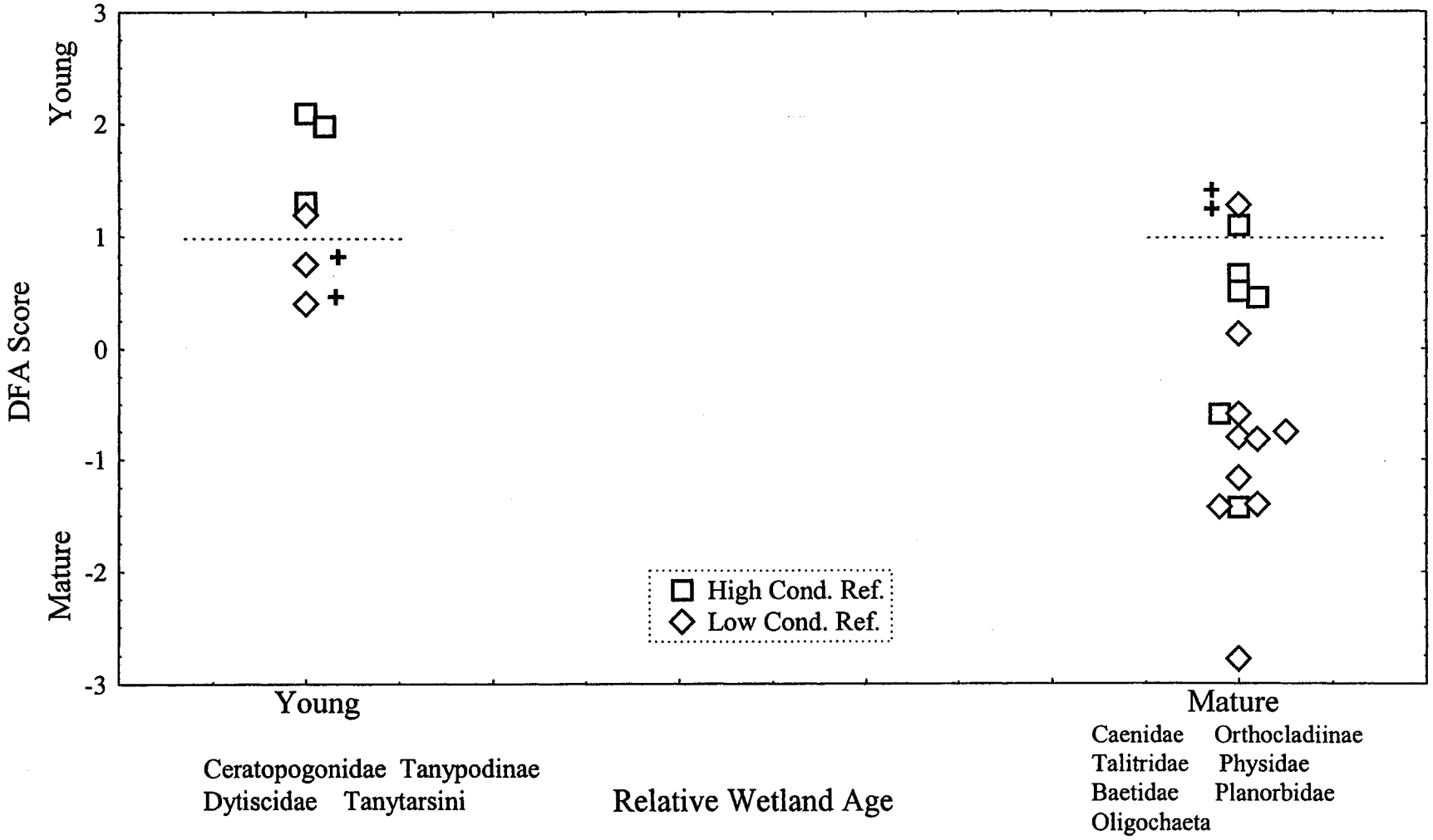



**Figure 3.4.** Taxa best discriminating young from mature reference wetlands for 20 taxa collected from 22 reference wetlands for artificial substrate samples. Wetlands classified as young wetlands should be located above the horizontal line. Wetlands classified as mature should be located below the line. Taxa listed on the left of the x-axis are generally characteristic of young reference wetlands. Taxa listed on the right of the x-axis are generally characteristic of mature reference wetlands. Wetlands marked with + are misclassified in the DFA analysis.

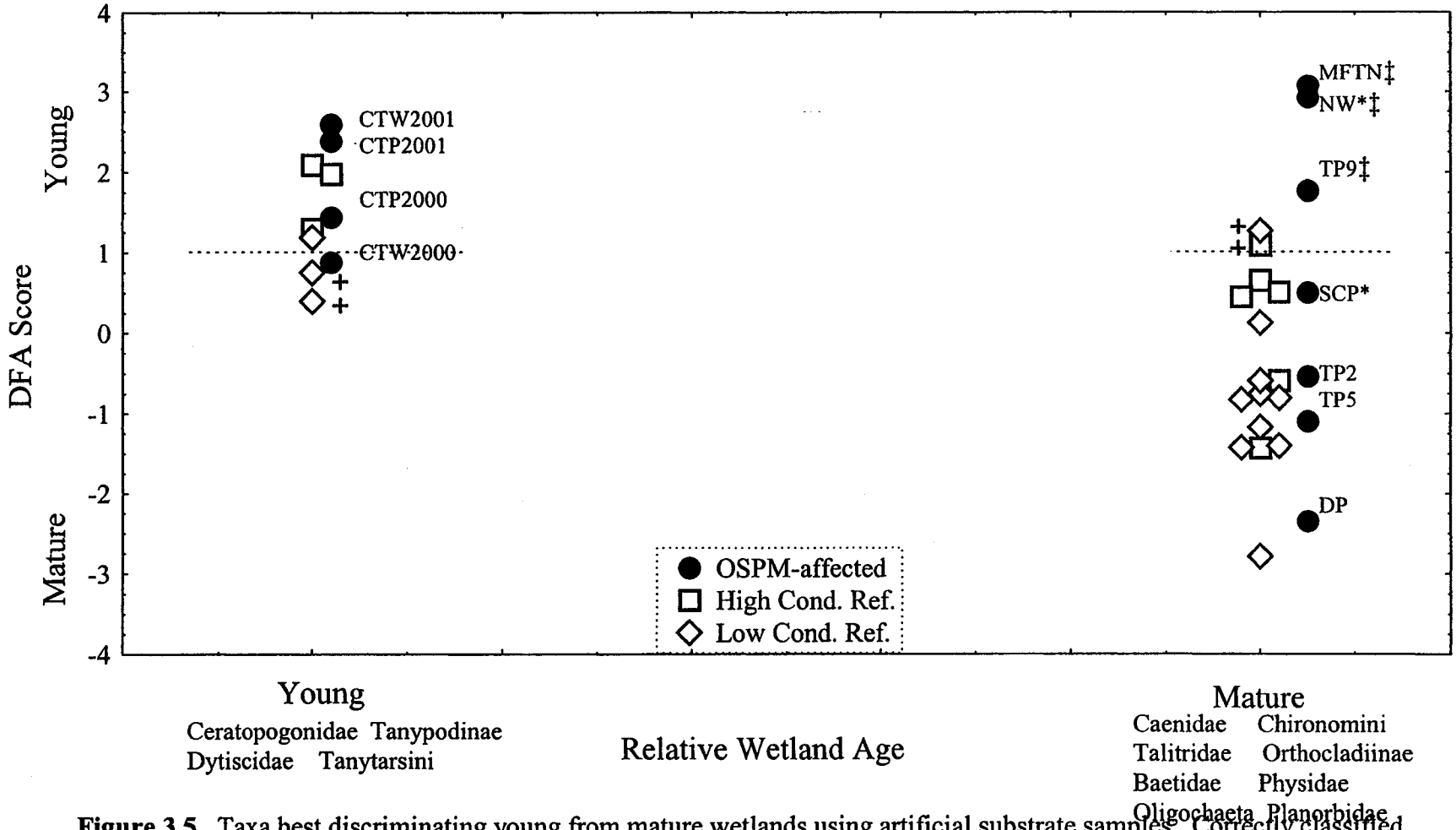



**Figure 3.5.** Taxa best discriminating young from mature wetlands using artificial substrate samples. Correctly classified young wetlands should be located above the horizontal line. Correctly classified mature wetlands should be located below the line. Taxa listed on the left of the x-axis are generally characteristic of young reference wetlands. Taxa listed on the right of the x-axis are generally characteristic of mature reference wetlands. OSPM-affected wetlands are labelled to better distinguish their position relative to reference wetlands. Wetlands marked with + are misclassified in the DFA analysis. Wetlands marked with * receive periodic OSPM unputs. Wetlands marked with ‡ have benthic taxa common to young and mature wetlands.

converge. Several OSPM-affected wetland communities are beginning to reach an asymptote similar to that of mature reference wetlands (Figure 3.6). One "mature" OSPM-affected (SCP) wetland is classified as having taxa similar to "mature" reference, however examination of the $\log_2$ (mean relative abundance) values for this wetland (Table 3.1B), shows that it possesses a great number of worms, which classifies as "mature" reference (Figure 3.6). From Figure 3.6, it is indicated that there are a few mature reference wetlands whose communities are more similar to "young" reference wetlands than to "mature" reference wetlands, or may be at an intermediate benthic community between "young" and "mature" reference wetlands.

The arrow on the x-axis is the approximate (inferred) transition point for a "young" wetland to be classified as a "mature" wetland based on the zoobenthic community. Two "young" reference wetlands (LP, SBBP) are misclassified because of the large number of semi-aquatic oligochaetes (Enchytraeidae - LP; Chironomini midges - SBBP) in these wetlands and should still be considered to be "young". H63I is misclassified because of high relative abundance of Orthocladiinae, but this wetland also has high relative abundance of Corixidae, so it may have a benthic community intermediate between young and mature reference. Saline Marsh (SM) is misclassified because of moderate relative abundances of Tanytarsini and tanypod midges (Table3.1B).

**Sweep Samples**

Principal components analysis of octaves of sweep samples explained 60.1% of the variation in relative abundance of 22 families with 5 components (Table 3.8). PC1 accounted for 16% of variation in relative abundance among wetlands. Wetlands

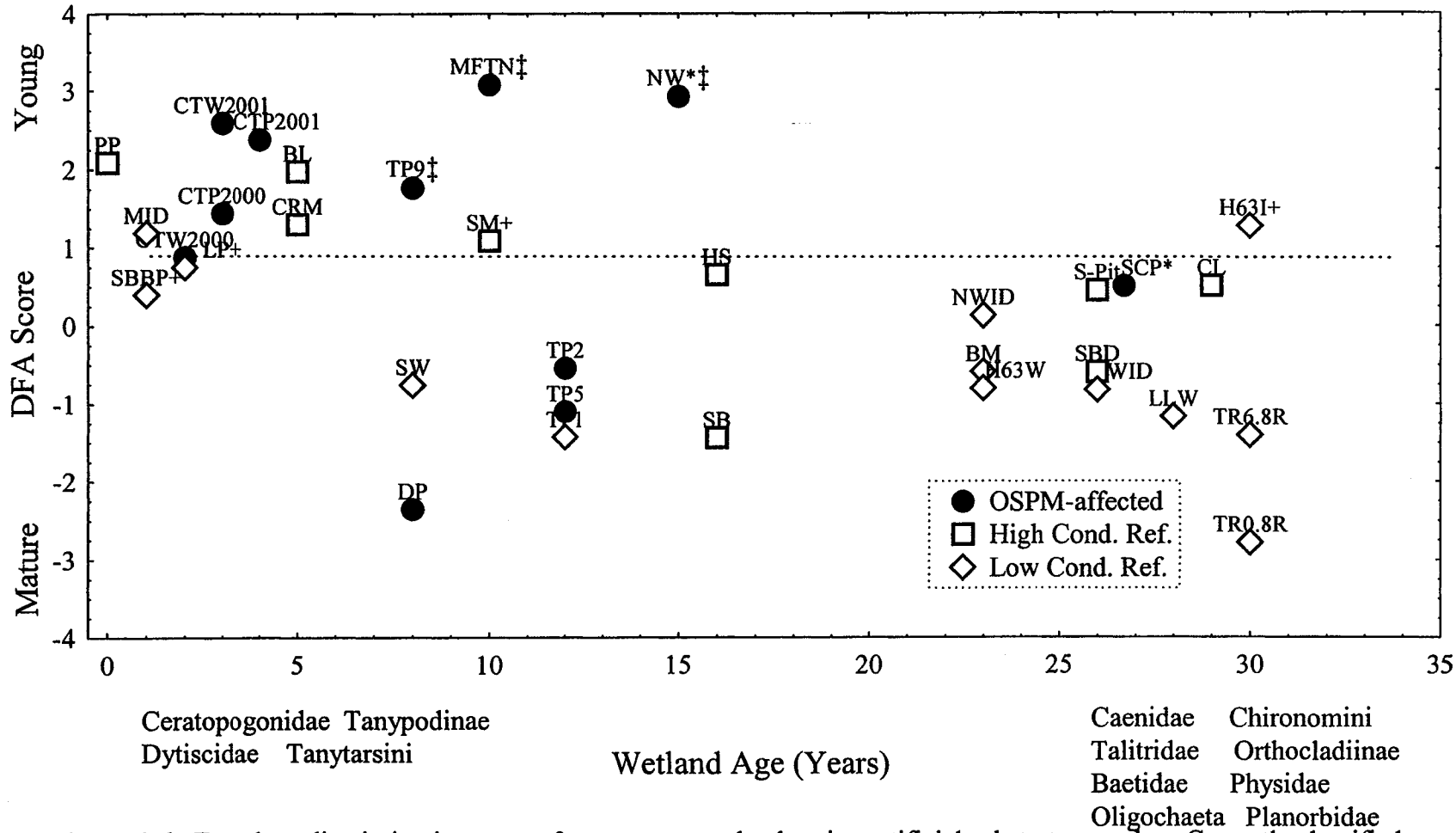



**Figure 3.6.** Taxa best discriminating young from mature wetlands using artificial substrate samples. Correctly classified young wetlands should be located above the horizontal line Correctly classified mature wetlands should be located below the line. Taxa listed on the left of the x-axis are generally charactersitic of young reference wetlands. Taxa listed on the right of the x-axis are generally characteristic of mature reference wetlands. Wetlands marked with + are misclassified in hte DFA analysis. Wetlands marked with * receive periodic OSPM inputs. The dashed line represents the extrapolation of the line for wetlands with benthos more characteristic of young wetlands. Wetlands marked with ‡ have taxa common to young and mature wetlands.

exhibiting high scores for PC-I had high relative abundances of fingernail clams (Sphaeriidae), leptocerid caddisflies, amphipods and caenid and baetid mayflies (Table 3.8).

In the samples collected by the sweep method, PC2 accounted for 13% of the variation in relative abundance among wetlands. High scores for this component indicated wetland with very high relative abundance of midges (Orthocladiinae, Tanypodinae, Tanytarsini), dytiscid beetles, and biting midges (Table 3.8).

PC3 accounted for 11.5% of the variation in relative abundance among wetlands. High negative scores for this component indicated wetlands dominated by damselflies (Lestidae, Coenagrionidae), backswimmers (Notonectidae) and dragonflies (Libellulidae) (Table 3.7). Oligochaetes are negatively associated with PC3 (Table3.8).

PC4 accounted for 10.8% of the variation in relative abundance among wetlands. High scores for this component indicated wetland dominance by physid and planorbid snails, and hydrophilid beetles (Table 3.8).

PC5 accounted for 8.8% of the variation in relative abundance among wetlands. High scores for this component indicated wetland dominance by lymnaeid snails and Chironomini midges. Water boatmen (Corixidae) were negatively associated with PC5 and generally did not occur with the other taxa (Table 3.8).

The results of the multiple regression between the PCs for the biological data and physico-chemical parameters are shown in Table 3.9. The taxa in PC1 have lowered relative abundance when conductivity increases (Table 3.9). Taxa in PC2 have slightly lowered relative abundance when sediment organic content is low. Taxa in PC3 have lowered relative abundance when naphthenic acid concentration is high or wetlands size is small. Worms (PC3) are not affected by naphthenic acid concentration (Table 3.9). Taxa

**Table 3.8.** Principal component (PC) scores for sweep samples collected from 31 wetlands. Groups of taxa associated with each PC co-occur if their values are the same sign. Opposite signs indicate taxa that exhibit negative associations. Taxa are $\log_2$ (mean relative abundance) transformed. Bold loadings are significant at $p < 0.01$. "Prp. Expl." is the proportion of the original variance (among 22 variables) accounted for by each principal component. Cumulative proportion is also calculated.

| | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| Sphaeriidae | **0.861** | -0.110 | -0.051 | 0.056 | 0.211 |
| Leptoceridae | **0.761** | -0.095 | 0.075 | 0.009 | -0.330 |
| Talitridae | **0.708** | 0.042 | 0.188 | -0.017 | 0.207 |
| Caenidae | **0.650** | 0.144 | 0.166 | **-0.391** | 0.110 |
| Baetidae | **0.568** | 0.014 | 0.058 | **0.500** | -0.329 |
| Haliplidae | **0.322** | **-0.309** | -0.156 | -0.102 | 0.193 |
| | | | | | |
| Orthocladiinae | -0.139 | **0.817** | 0.224 | -0.064 | 0.102 |
| Tanypodinae | -0.120 | **0.782** | -0.087 | -0.251 | 0.131 |
| Tanytarsini | 0.173 | **0.613** | 0.102 | -0.119 | -0.266 |
| Dytiscidae | 0.126 | **0.601** | -0.151 | 0.255 | -0.006 |
| Ceratopogonidae | **0.401** | **0.491** | -0.270 | 0.211 | -0.073 |
| | | | | | |
| Libellulidae | 0.159 | 0.115 | **0.782** | 0.092 | -0.100 |
| Coenagrionidae | 0.432 | -0.026 | **0.686** | -0.033 | -0.060 |
| Lestidae | -0.098 | -0.146 | **0.545** | 0.268 | 0.490 |
| Notonectidae | 0.095 | 0.243 | **0.523** | -0.099 | 0.273 |
| Oligochaeta | **0.456** | 0.036 | **-0.618** | 0.158 | 0.050 |
| | | | | | |
| Planorbidae | 0.191 | -0.245 | -0.132 | **0.775** | 0.117 |
| Physidae | -0.146 | -0.177 | 0.034 | **0.698** | 0.377 |
| Hydrophilidae | -0.157 | 0.319 | 0.225 | **0.668** | -0.035 |
| | | | | | |
| Lymnaeidae | 0.191 | -0.045 | -0.376 | 0.257 | **0.606** |
| Chironomini | -0.105 | **0.317** | -0.105 | 0.233 | **0.444** |
| Corixidae | -0.189 | -0.252 | -0.116 | -0.034 | **-0.660** |
| | | | | | |
| % Total | 0.160 | 0.130 | 0.115 | 0.108 | 0.088 |
| Cumul. % Expl. | 16.0 % | 29.0 % | 40.5 % | 51.3 % | 60.1 % |
| Eigenvalue | 3.569 | 3.044 | 2.379 | 2.622 | 1.610 |

**Table 3.9.** Summary of results for multiple regression of five PCs (Independent groups of taxa) against physico-chemical factors for sweep samples. Taxa included in each PC are listed in Table 3.8. Forward step-wise regression admitted less than 11 physico-chemical variables. Sign of t-value indicates whether the relationship is positive or negative.

| | PC1 | | | | |
|---|---|---|---|---|---|
| Variable | Reg. Coeff. | S.E. | $R^2$ | t | p-level |
| Intercept | 7.507 | 3.584 | | | * |
| Conductivity | -1.767 | 0.562 | 0.257 | -3.142 | * |
| pH Water | 0.543 | 0.242 | 0.064 | | ns |
| Salinity | 1.912 | 0.937 | 0.044 | | ns |
| Wetland Size (Area) | -0.241 | 0.146 | 0.060 | | ns |
| Total | | | 0.425 | | |

| | PC2 | | | | |
|---|---|---|---|---|---|
| Variable | Reg. Coeff. | S.E. | $R^2$ | t | p-level |
| Intercept | 0.957 | 0.679 | | | ns |
| Detritus | 0.497 | 0.204 | 0.085 | | ns |
| Sediment Organic Content (LOI) | -0.549 | 0.286 | 0.116 | -1.920 | * |
| Macrophyte Dev. | -0.236 | 0.127 | 0.091 | | ns |
| Total | | | 0.292 | | |

| | PC3 | | | | |
|---|---|---|---|---|---|
| Variable | Reg. Coeff. | S.E. | $R^2$ | t | p-level |
| Intercept | 1.510 | 0.825 | | | ns |
| [Naphthenic Acid] | -0.306 | 0.119 | 0.194 | -2.566 | * |
| Wetland Size (Area) | -0.305 | 0.117 | 0.124 | -2.606 | * |
| Macrophyte Dev. | 0.223 | 0.118 | 0.086 | | ns |
| Sediment Organic Content (LOI) | -0.429 | 0.251 | 0.045 | | ns |
| Detritus | 0.289 | 0.178 | 0.052 | | ns |
| Total | | | 0.501 | | |

| | PC4 | | | | |
|---|---|---|---|---|---|
| Variable | Reg. Coeff. | S.E. | $R^2$ | t | p-level |
| Intercept | -1.753 | 0.726 | | | * |
| Macrophyte Dev. | 0.484 | 0.150 | 0.181 | 3.228 | * |
| ORP Sediment | 0.004 | 0.002 | 0.054 | | ns |
| [Naphthenic Acid] | 0.209 | 0.150 | 0.040 | | ns |
| Wetland Size (Area) | 0.177 | 0.133 | 0.046 | | ns |
| Total | | | 0.321 | | |

| | PC5 | | | | |
|---|---|---|---|---|---|
| Variable | Reg. Coeff. | S.E. | $R^2$ | t | p-level |
| Intercept | -4.586 | 2.168 | | | * |
| Macrophyte Dev. | 0.324 | 0.126 | 0.170 | 2.573 | * |
| pH Water | 0.391 | 0.248 | 0.070 | | ns |
| Wetland Size (Area) | 0.135 | 0.134 | 0.028 | | ns |
| Total | | | 0.268 | | |

* $p < 0.05$ ** $p < 0.01$ ns = not significant

in PC4 and PC5 have increased relative abundance with increased macrophyte development (Table 3.9).

The results from the DFA separating young and mature reference wetlands are shown in Table 3.10. Three principal components (PC1, PC2, PC4 and PC3) contributed to distinguishing "young" from "mature" reference wetlands ($p > 0.05$), while correctly classifying (86%) wetlands to the correct group (Table 3.10).

Figure 3.7 summarises the grouping of wetlands according to PCA factor scores for relative abundances of families within the 21 reference wetlands. Correctly classified "young" reference wetlands should be located below the horizontal line. "Young" reference wetlands were dominated by midges (Tanytarsini, Tanypodinae and Orthocladiinae), dytiscid beetles and a lower relative abundance of Chironomini compared to "mature" reference wetlands (Table 3.1C, Figure 3.7).

Correctly classified "mature" reference wetlands should be located above the line. These wetlands were dominated by high relative abundances of physid and planorbid snails, libellulid dragonflies, lestid and coenagrionid damselflies, and backswimmers (Notonectidae) (Figure 3.7). Two "young" reference wetlands were classified as having "mature" benthic communities. One "mature" reference wetland was classified as having a benthic community characteristic of a "young" wetland.

From Table 3.10, PC1, PC2, PC4 and PC3 were used to develop an equation for reference wetlands only. The equation for the analysis of artificial substrate samples is

$$-(PC1*0.945) + (PC2*0.652) + (PC4*0.493) - (PC3*0.388) - 0.109 \quad (3)$$

Figure 3.8 summarises the results of applying equation (3) to all 3 wetland class, (including OSPM-affected wetlands) allowing the determination of where the composition of OSPM-affected sites falls, with respect to reference sites.

**Table 3.10.** Discriminant function analysis summary for sweep samples using 21 reference wetlands. Wetlands were grouped by age class (young (≤ 7 yrs.) or mature (> 7 yrs). The principal components listed are taken from Table 3.8, and represent the $log_2$(mean relative abundance) values for 22 selected taxa. The overall p-value for the model is shown in bold, as well as significant p-values for each variable contributing to the model (3.10A). The variables not in the model are also shown (3.10B). Included is the classification matrix (3.10C). The DFA-score equation is developed from the variables in the model and is calculated by multiplying each variable in the model by its raw canonical coefficient and adding a constant.

A) Variables in the model

| | Wilks' Lambda | Partial Lambda | p | Toler. | 1-Toler. | Raw Canon. Coeff. |
|---|---|---|---|---|---|---|
| PC1 | 0.750 | 0.749 | **0.034** | 0.949 | 0.051 | -0.945 |
| PC2 | 0.678 | 0.829 | 0.088 | 0.923 | 0.077 | 0.652 |
| PC4 | 0.640 | 0.878 | 0.155 | 0.969 | 0.031 | 0.493 |
| PC3 | 0.600 | 0.936 | 0.312 | 0.946 | 0.054 | -0.388 |
| Constant | | | | | | -0.109 |

Wilks' Lambda: 0.562 approx. $F_{(4,16)} = 3.123$ **$p < 0.045$**

B) Variables not in the model

| | Wilks' | Partial | p-level | Toler. | 1-Toler. |
|---|---|---|---|---|---|
| PC5 | 0.542 | 0.966 | 0.478 | 0.971 | 0.029 |

C) Classification Matrix

| Actual Group | Young | Mature | Total | Percent Correct |
|---|---|---|---|---|
| Young | 4 | 2 | 6 | 67 |
| Mature | 1 | 14 | 15 | 93 |
| Total | 5 | 16 | 21 | 86 |

DFA-score Equation: - (PC1*0.945) + (PC2*0.652) + (PC4*0.493) - (PC3*0.388) - 0.109

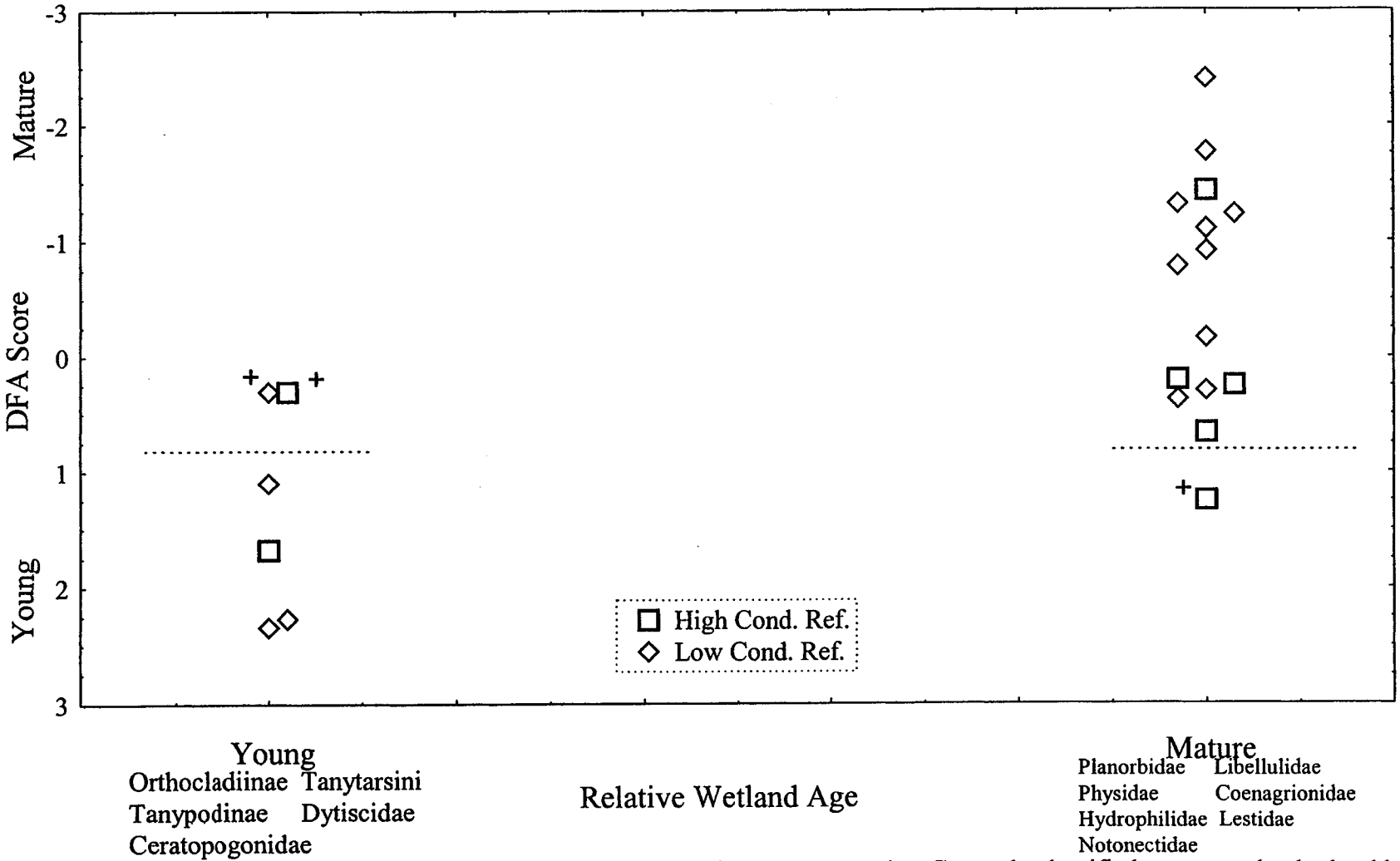



**Figure 3.7.** Taxa best distinguishing 21 reference wetlands using sweep samples. Correctly classified young wetlands should be located below the line Correctly classified mature wetlands should be located above. Taxa listed on the left of the x-axis are generally characteristic of young reference wetlands. Taxa listed on the right of the x-axis are generally characterstic of mature reference wetlands. Wetlands marked with + are misclassified in the DFA analysis. Note that y-axis scaling is reversed.

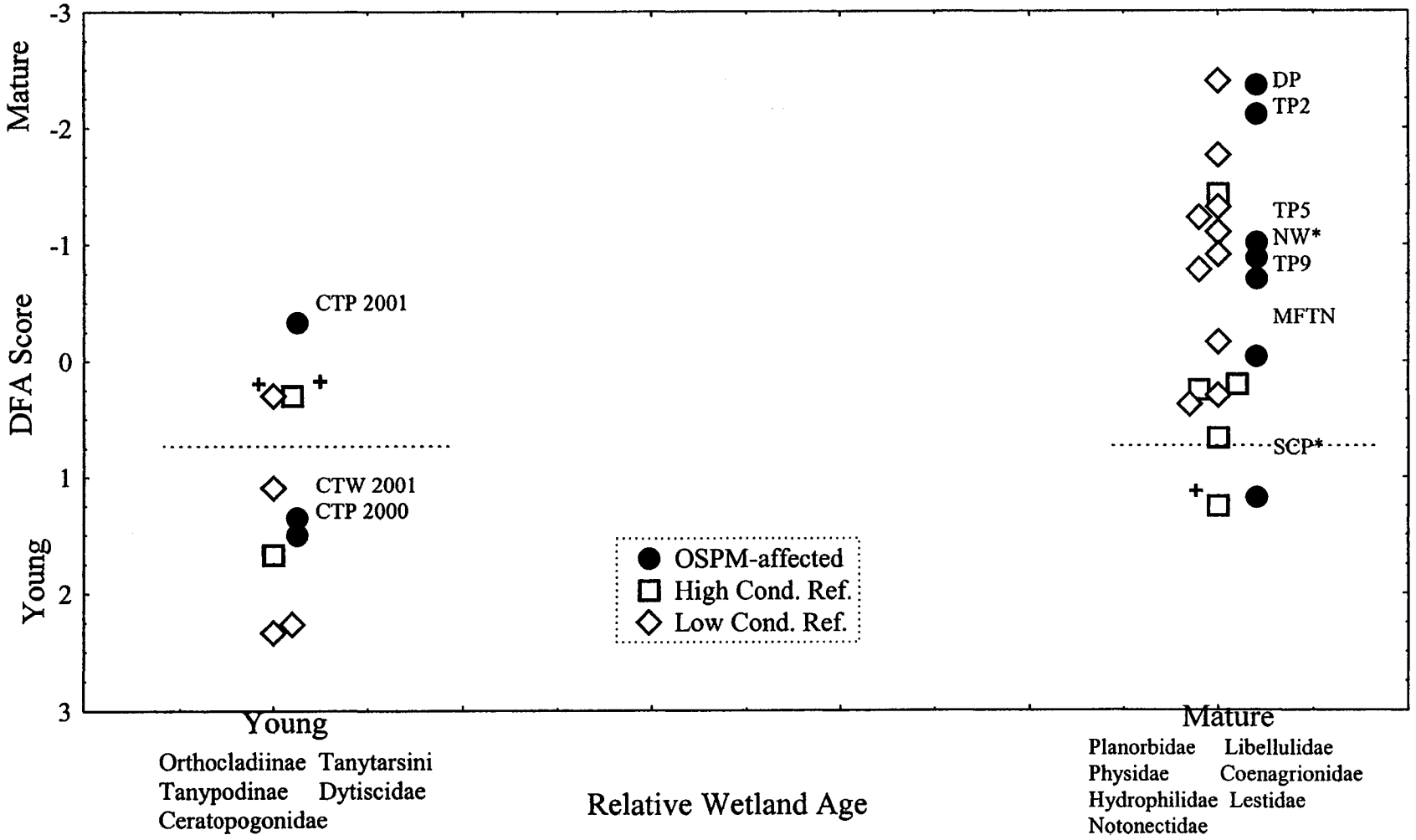



**Figure 3.8.** Taxa best distinguishing young from mature wetlands using sweep samples. Correctly classified young wetlands should be located below the line. Correctly classified mature wetlands should be located above. Taxa listed on the left of the x -axis are generally characteristic of young reference wetlands. Taxa listed on the right of the x-axis are generally characterstic of mature reference wetlands. Wetlands marked with + are misclassified in the DFA analysis. Wetlands marked with * receive periodic OSPM inputs. Note that y-axis scaling is reversed.

"Young" OSPM-affected sites, are characterised high relative abundance of non-Chironomini midges, dytiscid beetles and biting midges (Figure 3.8). "Mature" OSPM-affected wetlands have low relative abundance of physid and planorbid snails, libellulid, coenagrionid and lestid odonates and notonectids compared to "mature" reference wetlands (Table3.1C). "Mature" OSPM-affected wetlands also have higher relative abundance of non-Chironomini midges and dytiscid beetles than "mature" reference wetlands (Table 3.1C).

Plotting the results of the DFA equation against wetland age in 2001 provides a visual tool to infer when the successional trajectories of three wetland classes converge. One OSPM-affected wetlands classified, *a priori*, as "mature" have taxa that are more characteristic of "young" wetlands (SCP) (Figure 3.9). The remaining six mature OSPM-affected wetlands have a benthic community that is intermediate to "young" and "mature" reference (Figure 3.9).

One OSPM-affected wetland (SCP) had a very positive DFA score. This wetland contained no taxa in the DFA equation (it only had large number of corixids) and should have classified lower on the axis.

**Developing a Metric**

**Core Samples**

No taxa successfully distinguished "young" from "mature" reference wetlands using PCA and DFA for zoobenthic data collected, using core samples. Thus, zoobenthic taxa from core samples will not be considered for developing a metric.

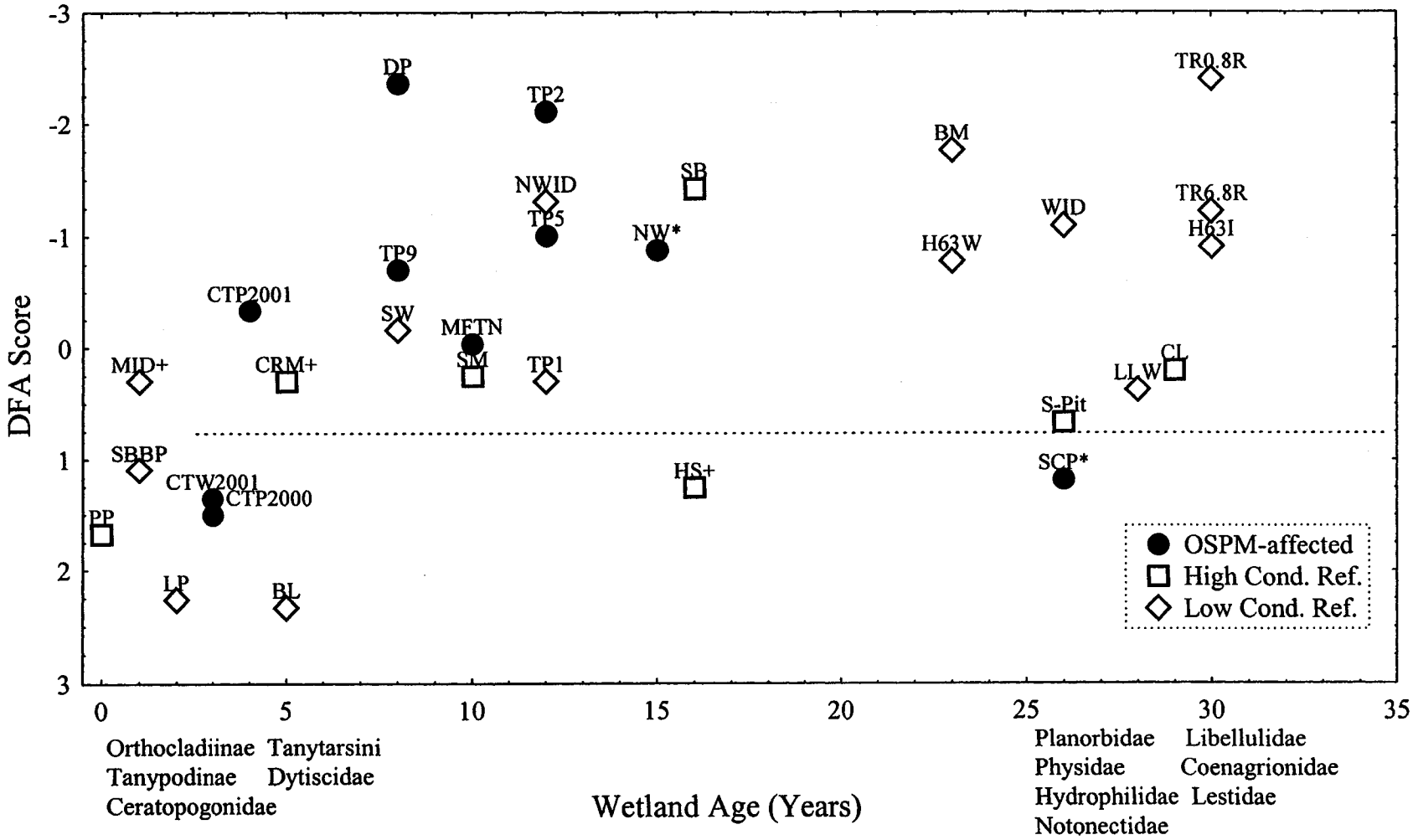



**Figure 3.9.** Taxa best distinguishing 33 wetlands using sweep samples. Correctly classified young wetlands should be located below the line. Correctly classified mature wetlands should be located above. Taxa listed on the left of the x-axis are generally characteristic of young reference wetlands. Taxa listed on the right of the x-axis are generally characterstic of mature reference wetlands. Wetlands marked with + are misclassified in the DFA analysis. Wetlands marked with * receive periodic OSPM inputs. Wetlands marked with & have taxa that are more characteristic of young wetlands.

**Artificial Substrate Samples**

The taxa comprising PC1, PC3 and PC2 for artificial substrate samples (Table 3.5) were used in a DFA to determine which taxa would be best suited for developing a metric to distinguish relative wetland age for reference wetlands. Richness (mean number of families per sample unit), $\log_e + 1$ mean abundance (mean number of individuals per sample unit) and percent composition of each chironomid tribe / subfamilies within the chironomidae were also used.

Five variables were identified as significantly discriminating ($p < 0.05$) between "young" and "mature" reference wetlands (Table 3.11 A). These five variables also correctly classified 95% of reference wetlands (Table 3.11 C). One wetland classified, *a priori*, as "young" were classified as "mature" by the DFA (Table 3.11 C).

The five significant variables in the model for reference wetlands ($\log_2$ (relative abundance)Talitridae, Physidae, Dytiscidae, and Caenidae, as well as, Richness) were used to classify OSPM-affected wetlands. A "metric equation" was developed the same way as for the previous DFA. The equation consists of the raw coefficients of canonical variables multiplied by each variable and a constant. The metric equation for artificial substrate samples is

$$-(\text{Talitridae} * 1.478) + (\text{Physidae}*3.255) + (\text{Dytiscidae}*3.242) + (\text{Caenidae}*5.298) - (\text{Richness}*0.093) - 0.256 \quad (5) \quad \text{(Table 3.11).}$$

Figure 3.10 illustrates the separation of "young" and "mature" wetlands for all classes. A regression between 'metric score' (dependent variable) and wetland age (independent variable) was performed because the change in zoobenthic community represents a continuum from young to mature. However, the 95% confidence intervals are

**Table 3.11**. Discriminant Function Analysis Summary for development of proposed metric for artificial substrate samples using $\log_2$(mean relative abundance) for selected taxa. The overall p-value for the model is in bold, as are the significant variables in the model (3.11A). Variables not in the model are shown for comparison (3.11B). Classification matrix shows the percent correct classification for each group, as well as the number of misclassified wetlands (3.11C). Standardized coefficients for canonical variables show the contribution of each variable in the model to each root. (3.11D) Eigenvalues for each root explain the magnitude and direction of the unique contributions of each root to the canonical function. The metric equation is developed by multiplying each variable in the model by its corresponding raw canonical coefficient and adding a constant.

A) Variables in the Model

| | Wilks' Lambda | Partial Lambda | p | Toler. | 1-Toler. (R-sqr.) | Raw Canon. Coeff. |
|---|---|---|---|---|---|---|
| Taltiridae | 0.542 | 0.553 | **0.002** | 0.319 | 0.681 | -1.478 |
| Physidae | 0.440 | 0.683 | **0.015** | 0.538 | 0.462 | 3.255 |
| Dytiscidae | 0.555 | 0.541 | **0.002** | 0.524 | 0.476 | 3.242 |
| Caenidae | 0.395 | 0.759 | **0.039** | 0.337 | 0.663 | 5.298 |
| Richness | 0.356 | 0.843 | 0.103 | 0.730 | 0.270 | -0.093 |
| Constant | | | | | | -0.256 |
| Wilks' Lambda: 0.300 | | approx. $F_{(5,16)} = 7.466$ | | | **p< 0.0009** | |

B) Variables not in the model

| | Wilks' Lambda | Partial Lambda | p | Toler. | 1-Toler. (R-sqr.) |
|---|---|---|---|---|---|
| Chironomini | 0.296 | 0.988 | 0.676 | 0.827 | 0.173 |
| Orthocladiinae | 0.292 | 0.974 | 0.536 | 0.746 | 0.254 |
| Tanytarsini | 0.300 | 1.000 | 0.942 | 0.893 | 0.107 |
| Tanypodinae | 0.285 | 0.950 | 0.390 | 0.861 | 0.139 |
| Ceratopogonidae | 0.299 | 0.995 | 0.797 | 0.737 | 0.263 |
| Oligochaeta | 0.282 | 0.941 | 0.348 | 0.799 | 0.201 |
| Planorbidae | 0.298 | 0.995 | 0.782 | 0.782 | 0.218 |
| Baetidae | 0.299 | 0.997 | 0.846 | 0.764 | 0.236 |
| Corduliidae | 0.300 | 0.998 | 0.883 | 0.455 | 0.545 |
| Abundance ($\log_e$+1) | 0.299 | 0.998 | 0.859 | 0.279 | 0.721 |
| % Chironomini | 0.294 | 0.981 | 0.595 | 0.872 | 0.128 |
| % Orthocladiinae | 0.294 | 0.980 | 0.587 | 0.532 | 0.468 |
| %Tanytarsini | 0.295 | 0.983 | 0.621 | 0.766 | 0.234 |
| %Tanypodinae | 0.292 | 0.972 | 0.522 | 0.808 | 0.192 |

**Table 3.11 cont.**. Discriminant Function Analysis Summary for development of proposed metric for artificial substrate samples using $\log_2$(mean relative abundance) for selected taxa. The overall p-value for the model is in bold, as are the significant variables in the model (3.11A). Variables not in the model are shown for comparison (3.11B). Classification matrix shows the percent correct classification for each group, as well as the number of misclassified wetlands (3.11C). Standardized coefficients for canonical variables show the contribution of each variable in the model to each root. (3.11D) Eigenvalues for each root explain the magnitude and direction of the unique contributions of each root to the canonical function. The metric equation is developed by multiplying each variable in the model by its corresponding raw canonical coefficient and adding a constant.

C) Classification Matrix

| Actual Group | Young | Mature | Total | Percent Correct |
|---|---|---|---|---|
| Young | 5 | 1 | 6 | 83 |
| Mature | 0 | 16 | 16 | 100 |
| Total | 5 | 17 | 22 | 95 |

D) Standardised Coefficients for Canonical Variables

| | Root 1 |
|---|---|
| Talitridae | -1.414 |
| Physidae | 0.918 |
| Dytiscidae | 1.119 |
| Caenidae | 1.010 |
| Richness | -0.555 |
| Eigenvalue | 2.333 |
| Cum.Prop | 1.000 |

Metric Equation:

- (Talitridae*1.478) + (Physidae*3.255) + (Dytiscidae*3.242) + (Caenidae*5.298) - (Richness*0.093) - 0.256

the prediction limits for a given point along the regression line. They represent the 95% confidence interval for any estimated value along the regression line (Sokal and Rohlf 1981). This method generates a broader confidence interval than for the observed sample mean (Sokal and Rohlf 1981). Wetlands that fall outside of the confidence limit had a benthic community that was significantly different ($p < 0.05$) from the community found within the confidence limit. The arrow on the x-axis represents the age at which richness and abundance reach asymptotic levels, has been shown to occur (See Ch. 2).

Young wetlands (< 7 yr.) within the confidence limit had higher relative abundance of Dytiscidae, and lower richness compared to older wetlands (Figure3.10, Appendix A.3). Mature wetlands (>7 yr.) within the confidence limit had higher relative abundance of Talitridae, physid snails, caenid mayflies and higher richness (Figure 3.10). Mature wetlands above the confidence limit higher abundance of Dytisicdae compared to wetlands within the confidence limit (Figure 3.10, Appendix A.3).

**Sweep Samples**

The taxa comprising PC1, PC2, PC4 and PC3 for sweep samples (Table 3.7) were used in a DFA to determine which taxa would be best suited for developing a metric to distinguish relative wetland age for reference wetlands. Richness (mean number of families per sample unit), $\log_e + 1$ mean abundance (mean number of individuals per sample unit) and percent composition of each chironomid tribe / subfamilies within the chironomidae were also used.

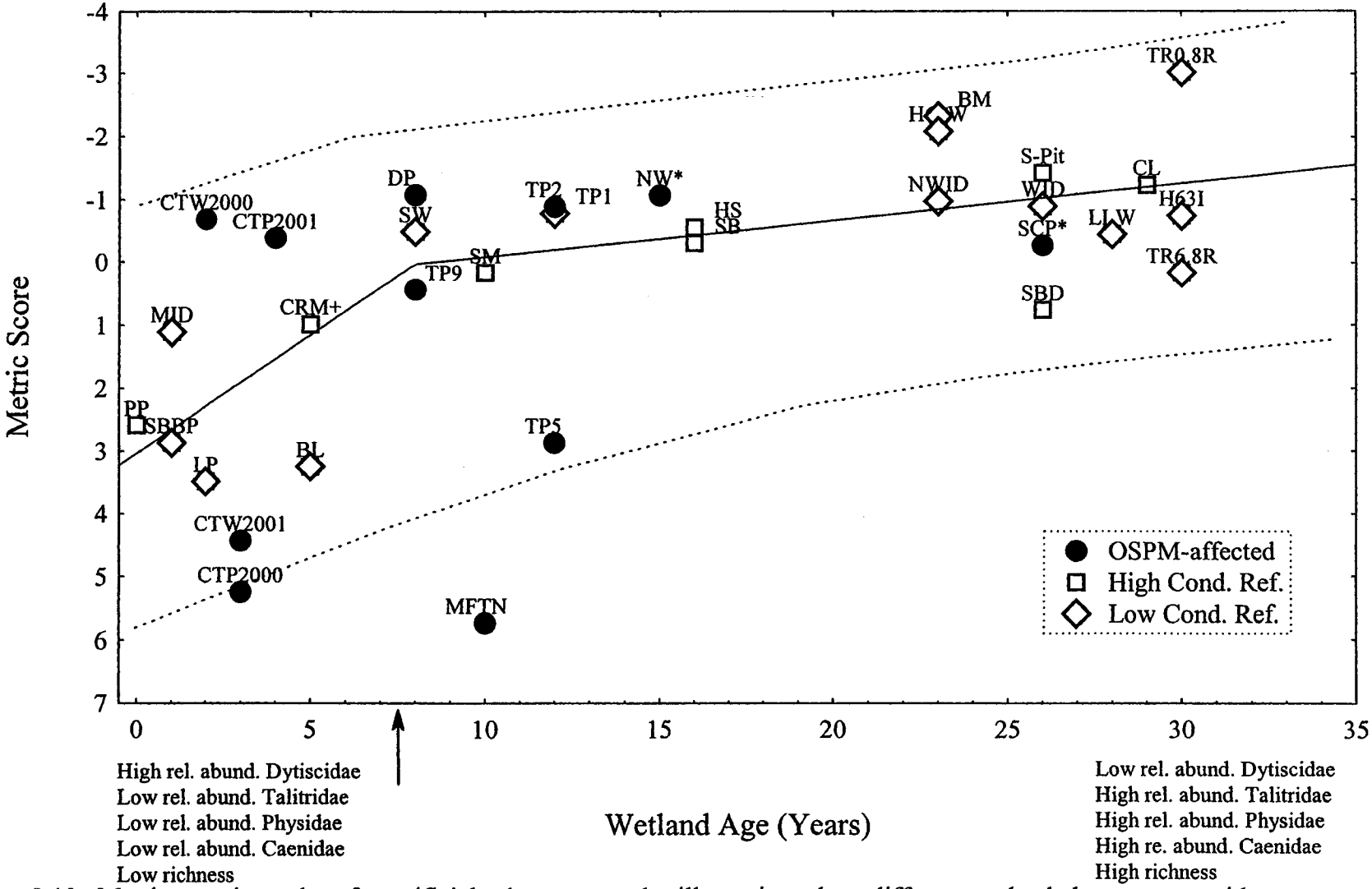



**Figure 3.10.** Metric equation values for artificial substrate samples illustrating where different wetland classes occur with respect to each other for any age. 95% predictive limits are shown (dashed lines). The arrow represents the transition from "young" to "mature" reference wetland. Wetlands marked with * receive periodic OSPM inputs. Wetlands marked with + are mislassified in the DFA analysis.

Nine variables were identified as discriminating ($p < 0.05$) between "young" and "mature" reference wetlands (Table 3.12 A). These nine variables also classified correctly all of the reference wetlands (Table 3.12 C).

The nine significant variables in the model for reference wetlands ($\log_2$ (relative abundance) Dytiscidae, Notonectidae, Planorbidae, Physidae, Caenidae, Baetidae, Tanytarsini, and %Orthocladiinae) were then used to classify OSPM-affected wetlands. A "metric equation" was developed the same way as for the previous DFA. The equation consists of the raw coefficients of canonical variables multiplied by each variable and a constant. The metric equation for sweep samples is

-(Dytiscidae*0.631) + (Notonectidae*2.220) - (%Orthocladiinae*0.094) -
(Sphaeriidae*5.757) + (Planorbidae*1.755) - (Physidae*1.682) + (Caenidae*5.045) -
(Baetidae*0.654) - (Tanytarsini*0.488) + 1.859 (6) (Table 3.12)

Figure 3.11 illustrates the separation of "young" and "mature" wetlands for all classes. Regression between 'metric score' (dependent variable) and wetland age (independent variable) was performed because the change in zoobenthic community represents a continuum from young to mature. The 95% confidence intervals are the prediction limits for a given point along the regression line. They represent the 95% confidence interval for any estimated value along the regression line (Sokal and Rohlf 1981). This method generates a broader confidence interval than for the observed sample mean (Sokal and Rohlf 1981). Wetlands that fall outside of the confidence limit had a benthic community that was significantly different ($p<0.05$) from the community found within the confidence limit. The arrow on the x-axis represents the age at which richness

**Table 3.12.** Discriminant Function Analysis Summary for development of proposed metric for sweep samples using $\log_2$(mean relative abundance) for selected taxa. The overall p-value for the model is in bold, as are the significant variables in the model. Wilks' lambda represents the discriminatory power of the current model, and partial Wilks' lambda is the unique discriminatory contribution of each variable (3.12A). Variables not in the model are shown for comparison (3.12B). Classification matrix shows the percent correct classification for each group, as well as the number of misclassified wetlands (3.12C). Standardized coefficients for canonical variables show the contribution of each variable in the model to each root (3.12D). Eigenvalues for each root explain the magnitude and direction of the unique contributions of each root to the canonical function. The metric equation is developed by multiplying each variable in the model by its corresponding raw canonical coefficient and adding a constant.

A) Variables in the model

| | Wilks' Lambda | Partial Lambda | p | Toler. | 1-Toler. (R-sqr.) | Raw. Canon. Coeff. |
|---|---|---|---|---|---|---|
| Dytiscidae | 0.159 | 0.889 | 0.266 | 0.392 | 0.608 | -0.631 |
| Notonectidae | 0.375 | 0.377 | **0.001** | 0.231 | 0.769 | 2.220 |
| %Orthocladiinae | 0.388 | 0.364 | **0.001** | 0.182 | 0.818 | -0.094 |
| Spaheriidae | 0.154 | 0.918 | 0.343 | 0.400 | 0.600 | -5.757 |
| Planorbidae | 0.320 | 0.441 | **0.003** | 0.090 | 0.910 | 1.755 |
| Physidae | 0.248 | 0.570 | **0.015** | 0.198 | 0.802 | -1.682 |
| Caenidae | 0.242 | 0.582 | **0.017** | 0.153 | 0.847 | 5.045 |
| Baetidae | 0.193 | 0.732 | 0.070 | 0.475 | 0.525 | -0.654 |
| Tanytarsini | 0.192 | 0.735 | 0.072 | 0.367 | 0.633 | -0.488 |
| Constant | | | | | | 1.859 |
| Wilks' Lambda: 0.141 | | approx. $F_{(9,11)} = 7.436$ | | | **p< 0.002** | |

B) Variables not in the model

| | Wilks' Lambda | Partial Lambda | p | Toler. | 1-Toler. (R-sqr.) |
|---|---|---|---|---|---|
| Orthocladiinae | 0.140 | 0.994 | 0.818 | 0.109 | 0.891 |
| Tanypodinae | 0.141 | 1.000 | 0.965 | 0.571 | 0.429 |
| Ceratopogonidae | 0.140 | 0.994 | 0.806 | 0.296 | 0.704 |
| Oligochaeta | 0.141 | 0.999 | 0.925 | 0.658 | 0.342 |
| Libellulidae | 0.129 | 0.914 | 0.355 | 0.775 | 0.225 |
| Lestidae | 0.137 | 0.970 | 0.592 | 0.307 | 0.693 |
| Coenagrionidae | 0.140 | 0.990 | 0.753 | 0.566 | 0.434 |
| Leptoceridae | 0.139 | 0.987 | 0.724 | 0.344 | 0.656 |
| Haliplidae | 0.141 | 0.999 | 0.930 | 0.639 | 0.361 |
| Talitridae | 0.133 | 0.944 | 0.459 | 0.629 | 0.371 |
| Abundance ($\log_e$+1) | 0.140 | 0.992 | 0.788 | 0.789 | 0.211 |
| Richness | 0.140 | 0.995 | 0.825 | 0.343 | 0.657 |
| %Chironomini | 0.130 | 0.919 | 0.369 | 0.443 | 0.557 |
| %Tanytarsini | 0.137 | 0.972 | 0.601 | 0.189 | 0.811 |
| %Tanypodinae | 0.141 | 0.997 | 0.868 | 0.758 | 0.242 |

**Table 3.12. cont.** Discriminant Function Analysis Summary for development of proposed metric for sweep samples using $\log_2$(mean relative abundance) for selected taxa. The overall p-value for the model is in bold, as are the significant variables in the model (3.12A). Variables not in the model are shown for comparison (3.12B). Classification matrix shows the percent correct classification for each group, as well as the number of misclassified wetlands (3.12C). Standardized coefficients for canonical variables show the contribution of each variable in the model to each root (3.12D). Eigenvalues for each root explain the magnitude and direction of the unique contributions of each root to the canonical function. The metric equation is developed by multiplying each variable in the model by its corresponding raw canonical coefficient and adding a constant.

C) Classification Matrix

| Actual Group | Young | Mature | Total | Percent Correct |
|---|---|---|---|---|
| Young | 6 | 0 | 6 | 100 |
| Mature | 0 | 15 | 15 | 100 |
| Total | 6 | 15 | 21 | 100 |

D) Standardised Coefficients for Canonical Variables

| | Root 1 |
|---|---|
| Dytiscidae | -0.574 |
| Notonectidae | 1.773 |
| %Orthocladiinae | -2.016 |
| Sphaeriidae | -0.488 |
| Planorbidae | 2.695 |
| Physidae | -1.590 |
| Caenidae | 1.786 |
| Baetidae | -0.810 |
| Tanytarsini | -0.917 |
| Eigenval | 6.084 |
| Cum.Prop | 1.000 |

Metric Equation:

- (Dytiscidae*0.631) + (Notonectidae*2.220) - (%Orthocladiinae*0.094) - (Sphaeriidae*5.757) + (Planorbidae*1.755)- (Physidae*1.682) + (Caenidae*5.045) - (Baetidae*0.654) - (Tanytarsini*0.488) + 1.859

and abundance reach asymptotic levels has been shown to occur (See Ch. 2).

Young wetlands (< 7 yr.), within the confidence limit, had high relative abundance of Dytiscidae, moderate % Orthocladiinae, and low relative abundance of Notonectidae, snails, mayflies and fingernail clams (Figure 3.11, Appendix A.3). Note that CTP2001 contains no taxa in the metric (it only has Corixidae), and is placed at its spot because of the constant (1.859) in the metric equation.

Mature wetlands (>7 yr.) within the confidence limit generally had low relative abundance of Dytiscidae, moderate to high relative abundance of Planorbidae, Physidae, Caenidae, Baetidae, Sphaeriidae and Notonectidae, as well as lower % Orthocladiinae compared to young wetlands within the confidence limit (Figure3.11, Appendix A.3). Three "mature" OSPM-affected wetlands fall on the confidence limits, indicating that these sites may not be equivalent to "mature" reference wetlands, based on their taxonomic composition.

**Testing the Metric**

Using data of Whelly (1999), the metric proposed for sweep samples (see above for details) was applied it to his data. The taxa in nine significant variables identified for sweep samples, and the corresponding raw canonical values, were used to calculate a "metric score" (Table 3.13 AppendixA.3). Eight wetlands in this data set were sampled both in 1997 and 2000-2001 (TR1 = TR0.8R, TR5 = TR6.8R, SYN = SCP, SW, CL, NW, SB, HS). The metric score calculated from sweep samples in 2000-01 correctly classified all of the 12 reference wetlands, and one would expect that wetlands sampled in 1997 would

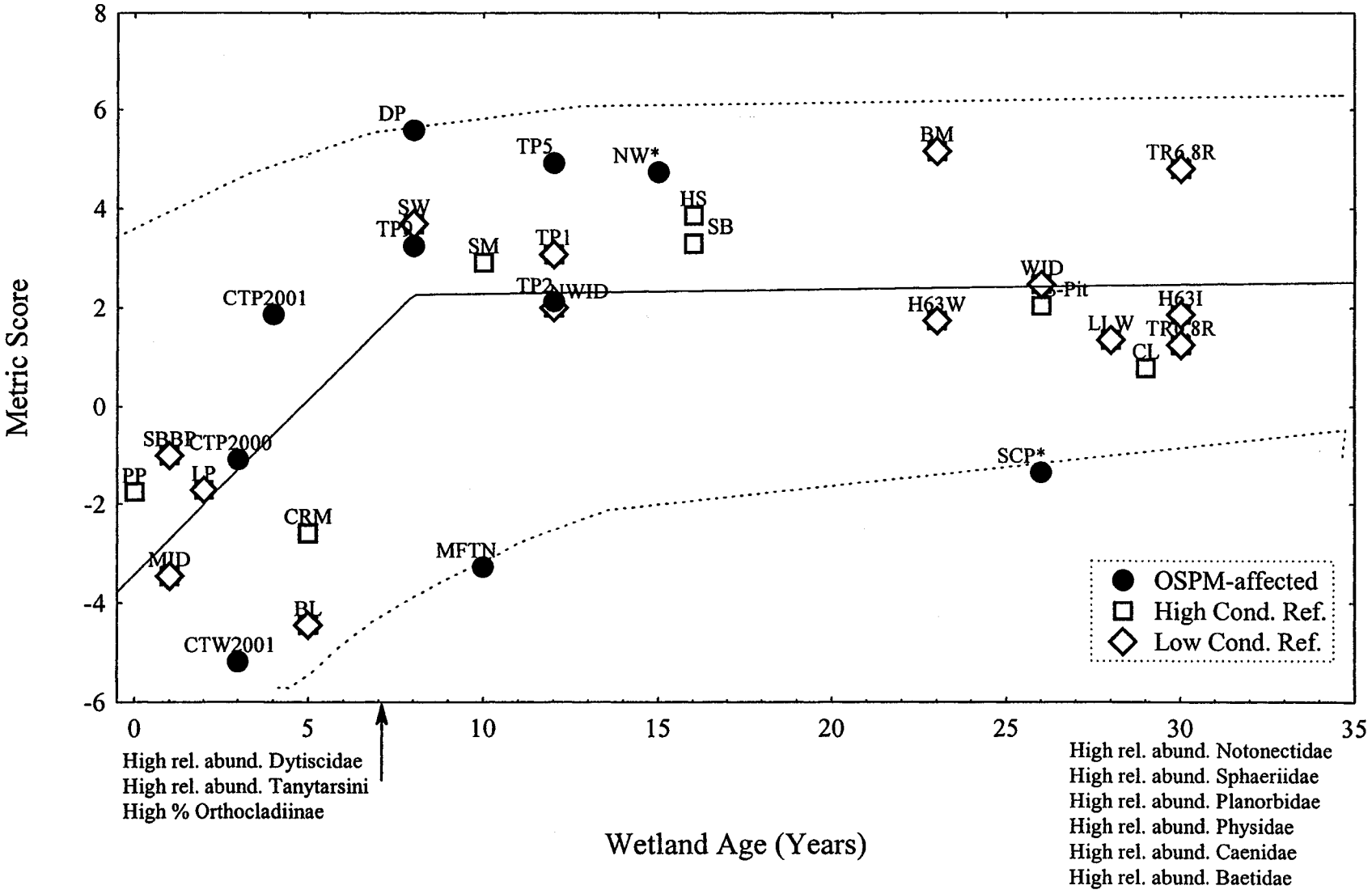



**Figure 3.11.** Metric equation values for sweep samples illustrating where different wetland classes occur with respect to each other for any age. 95% predicitive limits are shown (dashed lines). The arrow represents the inferred transition from "young" to "mature" reference wetland. Wetlands marked with * receive periodic OSPM inputs.

either have similar 'metric scores' or have similar community composition to the same wetlands sampled in 2000-01.

Caution must be used when comparing these two data sets as there were some differences between the data sets. Collector bias, using the same sampling technique, may also influence the conclusions drawn from the zoobenthic community data.

Figure 3.12 and Table 3.13, shows that the wetlands sampled in 1997 generally fell within similar ranges as for wetland sampled in 2000-01. Wetland samples within the confidence limits had low relative abundance of Dytiscidae, moderate to high relative abundance of Planorbidae, Physidae, Caenidae, Baetidae, Sphaeriidae and Notonectidae, as well as lower % Orthocladiinae compared to young wetlands within the confidence limit (Figure 3.12).

Wetlands located above the regression line had very high relative abundance of dytiscids, Tanytarsini and %Orthocladiinae, compared to similar aged "young" wetlands (Figure 3.12). Wetlands located below the regression line (and in the lowest part of the confidence interval) had very low relative abundance of notonectids, and snails, as well as having very high relative abundance of (%) Orthocladiinae (Figure 3.12).

SW was classified as 'young' in 1997, and was classified as 'mature' in 2001. This site had an increase in the relative abundance of notonectids and snails as well as an increase in mayfly taxa. This suggests that increasing relative abundances of these taxa is part of the successional processes in this wetland.

Part of assessing how well the calculated metric predicts the status of OSPM-affected wetlands involves the use of 'best professional judgement'. Best professional judgement, based on the authors' knowledge of the area and visual assessment of the

**Table 3.13.** Results of applying the metric equation developed for sweep samples (at bottom) to data from Whelly (1999).

| 1997 Site | 2001Site | Class | Metric Score (1997) | Metric Score (2001) | Age Class (0 = Young 1 = Mature) | Wetland Age (1997) |
|---|---|---|---|---|---|---|
| HW | | OSPM-affected | -5.653 | | 0 | 9 |
| NW | NW | OSPM-affected | -0.602 | 4.738 | 0 | 11 |
| SYN | SCP | OSPM-affected | 6.960 | -1.344 | 1 | 21 |
| CL | CL | High Cond. Ref. | 2.130 | 0.779 | 1 | 25 |
| HS | HS | High Cond. Ref. | 1.264 | 3.864 | 0 | 12 |
| SB | SB | High Cond. Ref. | 0.343 | 3.309 | 0 | 12 |
| MR | | Low Cond. Ref. | -1.169 | | 1 | 26 |
| PCR | | Low Cond. Ref. | 1.554 | | 1 | 26 |
| RL | | Low Cond. Ref. | -3.151 | | 1 | 22 |
| SP | | Low Cond. Ref. | -2.060 | | 1 | 26 |
| SW | SW | Low Cond. Ref. | 8.709 | 3.695 | 0 | 4 |
| TR1 | TR0.8R | Low Cond. Ref. | 6.599 | 1.245 | 1 | 26 |
| TR3 | | Low Cond. Ref. | -10.102 | | 1 | 26 |
| TR4 | | Low Cond. Ref. | 2.411 | | 1 | 26 |
| TR5 | TR6.8R | Low Cond. Ref. | -0.491 | 4.805 | 1 | 26 |

Metric Equation:

- (Dytiscidae*0.631) + (Notonectidae*2.220) - (%Orthocladiinae*0.094) - (Sphaeriidae*5.757) + (Planorbidae*1.755)- (Physidae*1.682) + (Caenidae*5.045) - (Baetidae*0.654) - (Tanytarsini*0.488) + 1.859

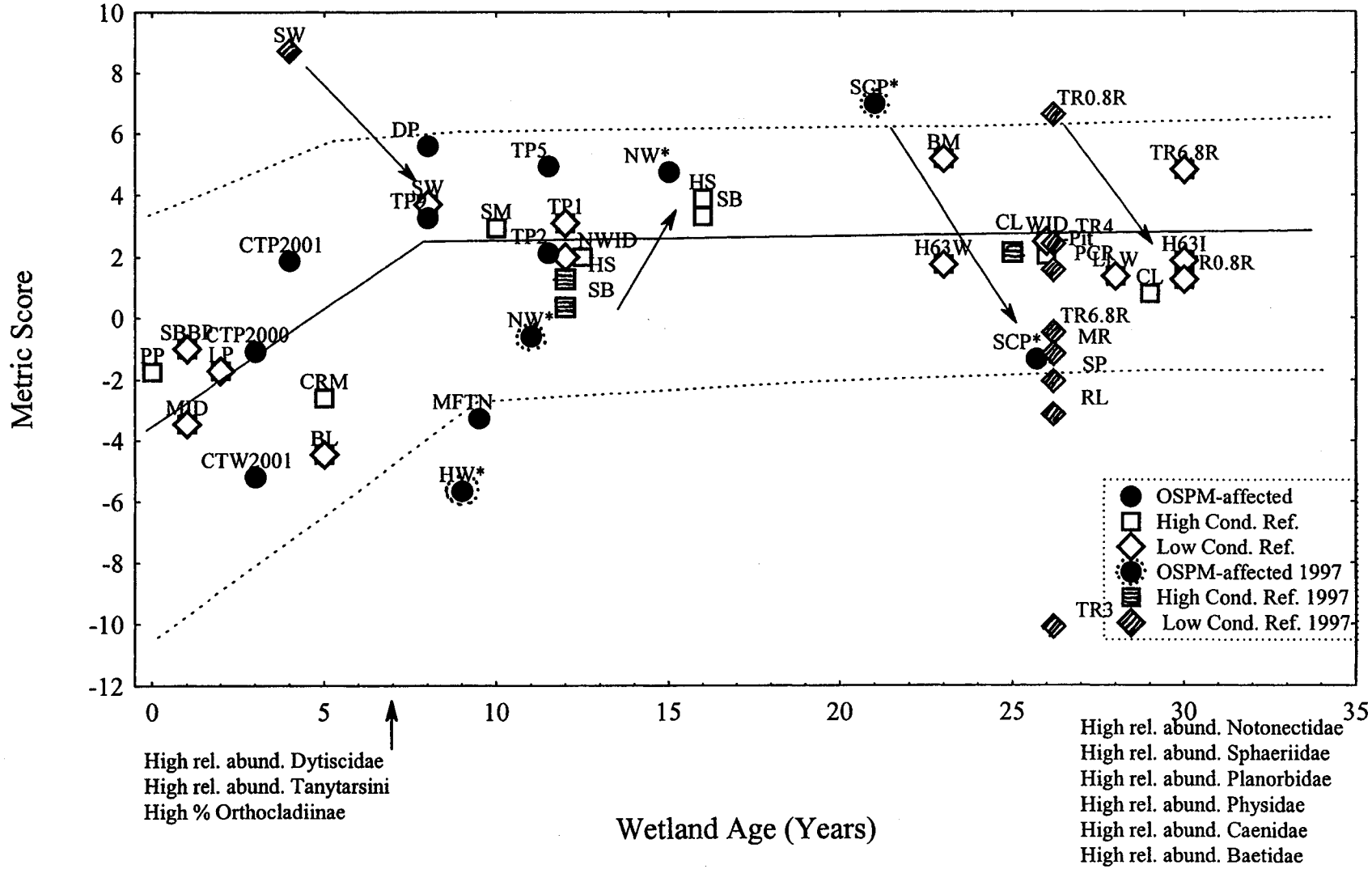



**Figure 3.12.** Metric score values for sweep samples illustrating where different wetland classes occur with respect to each other for any age. Data from Whelly (1999) (in grey) over-laid on data from 2000-2001. No confidence limits are shown as they fall outside of the graph area. The arrow on the x-axis represents the inferred transition from "young" to "mature" reference wetland. Wetlands marked with * receive periodic OSPM inputs. Arrows on the graph show the successional trajectories of wetlands sampled in 1997 and 2001.

wetlands, would predict that three OSPM-affected wetlands would not have a zoobenthic community that would be considered equivalent to reference (Table 3.14).

However, the metric calculated to these wetlands, based on PCA and DFA of the zoobenthic community of reference wetlands, would consider these three wetlands to have a zoobenthic community equivalent to reference (Table 3.14). This indicates that the metric is not predicting OSPM-affected wetland status very well. The predictive ability of the metric may be improved through further processing of samples within each wetland or through the assessment of a greater number of reference wetlands, both on and off the lease areas or by using a narrower predictive interval than 95%.

**Discussion**

From the analyses conducted for this chapter, the components of the benthic community have been identified that can best be used to assess the development of "young" wetland communities compared to "mature" reference wetland communities. The benthic communities of the reference condition were then used to predict the benthic community in OSPM-affected (test) wetlands. If best professional judgement and metric classification (calculated based on reference condition) were in concordance with each other, with respect to an OSPM-affected wetlands' status, then the metric is said to have worked.

Data from artificial substrate samples indicated that the $\log_2$ (relative abundance) of Talitridae, Physidae, Planorbidae, Dytiscidae, Caenidae, and richness could be used to determine whether a "young" wetland is becoming more similar to a "mature" reference wetland (Figure 3.10).

Young wetlands within the 95% confidence limits had lower relative abundance of amphipods, snails, mayflies, and richness than mature reference wetlands within the

**Table 3.14.** Assessment of how well metric assesses the status of OSPM-affected wetlands with respect to reference wetlands. 'Best professional judgement' represents the authors' estimation of wetlands' status based on knowledge of the area and visual surveys of the wetland habitat. 'Metric classification' represents the numerical assignment of wetland status based on PCA and DFA analysis of the zoobenthic community. Lack of concordance between the expected wetland status (best professional judgement) and calculated wetland status (metric classification) indicates that further refinement of the metric is necessary.

| | Equivalent to mature reference wetland? | | | | |
|---|---|---|---|---|---|
| OSPM-affected wetland | Best Professional Judgement | | | Metric classification | |
| | Yes | Maybe | No | Sweep | Artificial Substrate |
| CTW | | X | | Y | Y |
| CTP | | | X | Y | Y |
| DP | | X | | M | Y |
| TP9 | | | X | Y | Y |
| MFTN | | X | | M | N |
| TP5 | X | | | Y | M |
| TP2 | | X | | Y | Y |
| NW | X | | | Y | Y |
| SCP | | | X | M | Y |

Y = yes, M = maybe, N = no

confidence limit (Figure 3.10). Mature wetlands within the 95% confidence limit had higher richness and relative abundance of snails, amphipods, and mayflies than young wetlands within the 95% confidence limit. Thus, wetlands within the confidence limit could be considered to be the "ideal" mature wetlands. Wetlands above the 95% confidence limit had a community composition more characteristic of a "younger" community. This measure of the zoobenthic community is fairly robust, with the absence, or very low abundance, of snails being indicative of an OSPM-affected wetland, and the relative abundance of amphipods (Talitridae) being indicative of an older wetland.

Data from sweep samples indicated that the $\log_2$ (relative abundance) of Dytiscidae, Notonectidae, Planorbidae, Physidae, Caenidae, Baetidae, and Tanytarsini could be used to determine whether a "young" wetland (reference or OSPM-affected) is becoming more similar to that of a "mature" reference wetland (Figure 3.11).

"Young" wetlands within the 95% confidence limit had high relative abundance of dytiscids and Tanytarsini midges, low relative abundance of notonectids and snails than older wetlands, and moderate % Orthocladiinae (Figure 3.11). "Mature" wetlands within the 95% confidence limit had moderate to low relative abundance of dytiscids and moderate to high level of snails, mayflies and backswimmers compared to young wetlands within the 95% confidence limit. Thus, wetlands within the confidence limit could be considered to be the mid-range mature wetlands. Wetlands at or above the 95% confidence limit had a community composition more characteristic of a "younger" benthic community, while wetlands at or below the 95% confidence limit had a poor benthic community than wetlands within or above the 95% confidence limit. This measure of the benthic community was the most robust thus far and is recommended for use in further monitoring studies.

**Relative Merits of the Sampling Approaches**

When selecting a sampling method, it is important to have a clear definition of the objective of the sampling effort as well as an understanding of the shortcomings of the method chosen (DeShon 1995). In streams, Kerans and Karr (1992) point out that monitoring programs may reach biased conclusions if the biological condition is assessed by sampling a single habitat. The same can be presumed about wetlands. A variety of habitats exists within a wetland (vegetated or not, deep or shallow water), and within each habitat there are a variety of microhabitats (e.g., emergent vs. submergent vegetation). Replicated and quantitative sampling in multiple habitat types with a variety of biological attributes provides the strongest assessment of biological condition (Kerans *et al.* 1992). In the present study, three sampling methods were used to evaluate the zoobenthic community of these wetlands. Each method sampled a different part of the community and, as a result, included different taxa for each proposed metric.

Core samples collect the infauna of wetlands, which are dominated by chironomids, oligochaetes and ostracods (Whelly 1999). Examination of the factors significant in discriminating between wetland classes revealed that PC 1 and PC2 were the only factors whose individual contribution were useful (See Table 3.4). Taxa defining these components included Hirudinea (leeches), odonates, mayflies and dytiscid beetles. It may be that finer taxonomic resolution for leeches (identification to genus or species) would better identify the taxa characterising different wetland classes. It may also be that collecting epibenthic fauna is a matter of chance (they swim by or are there when you sample) with this technique. Using core samples to determine the state of the zoobenthic

community is not recommended, unless one wanted to focus on the changes in the Chironomidae and Oligochaeta.

Artificial substrates focus on the relatively mobile epibenthic and epiphytic taxa inhabiting the wetland. Young wetlands evaluated using this sampling were characterised by the presence of mobile taxa (dytiscid beetles and midges) (Figure 3.4-Figure 3.6). Mature wetlands were characterised by periphyton grazers (snails and Chironomini midges), and detritivores (amphipods, mayflies and worms). The taxa used in the DFA equation correctly classified 95% of young from mature reference wetlands (Table 3.7). This collection technique samples a wider range of taxa in the community than do cores, and is relatively easy to use. Artificial substrates are recommended for use, where a more quantitative estimate of the zoobenthic community is required, or the estimate the colonisation potential of a wetland.

Sweep samples collect large, rare and mobile taxa, as well as common organisms, while sampling a variety of habitats. Young wetlands were characterised by the presence of predators (dytiscid beetles), and tube building scraping or detritus/micro-organism feeder (Tanytarsini and Orthocladiinae chironomids). Mature wetlands had zoobenthic communities characterised by detritivorous or grazer taxa (snails - Planorbidae and Physidae, amphipods - Talitridae, chironomini midges) (Figure 3.7, Figure 3.12).

The taxa that were analysed for sweep samples (Table 3.12) and used to develop the metric equation were then tested on wetlands sampled by Whelly (1997). Ideally, the equation developed would classify wetlands sampled in 1997 similarly to wetlands sampled in 2000-01 (the metric score would be similar or the relative position of the wetlands within the 95% confidence limits would be similar).

Generally, the metric scores calculated for Whelly's data were similar to those calculated in 2000-01 (Table 3.13, Appendix A.3). The taxa sampled in six common reference wetlands indicate that most of these wetlands have a zoobenthic community that is undergoing successional changes, and is increasing the relative abundances of taxa characteristic of mature wetlands.

**Converting Metric Equations into Something Useful**

In developing an index of recovery for OSPM-affected wetlands multivariate techniques were applied to determine which taxa characterised young or mature wetlands. The objective was to create a simple index for the continued monitoring of OSPM-affected wetlands in the Fort McMurray oil sands region.

Diversity and absolute abundance indices were not chosen for metric development for a variety of reasons. They often depend on factors other than pollution stress, such as temporal and seasonal factors (Green and Vascotto 1978). Spieles and Mitsch (2000) found that diversity and biotic indices were not particularly useful in differentiating among invertebrate communities in constructed and natural wetlands in Ohio [Spieles, 2000 #182]. Green and Vascotto (1978) suggested that the use of number of species per sample (richness) is a simple, biologically meaningful measure that is less ambiguous than more complex indices (Green 1977 cited in (Green and Vascotto 1978).

In the United States, multimetric indexes are scored using one of several techniques (See (Barbour *et al.* 1999b) page 9-10, Fig. 9-7). The multimetric index value is a summation of the scores of the metrics from reference locations and has a finite range within each water body type (Barbour *et al.* 1999b). This range is sub-divided into any

number of categories corresponding to various levels of impairment that reflects the distribution of the scores for the reference sites (Barbour *et al.* 1999b).

Core samples were not considered useful because there were no taxa that could distinguish young from mature reference wetlands (Table 3.4).

For artificial substrate samples, young reference wetlands had metric scores less than zero and fell within the 95% confidence bands (Figure 3.10, Table 3.15). The calculation of the metric score based on two artificial substrate samples is shown in Appendix A.3. If the total metric score for a wetland fell below zero, the zoobenthic community would be considered equivalent to a young wetland (even for a 30 yr. old wetland). The relative abundance of each taxon in the metric can be found in Table 3.1B. A wetland with a maximum of nine physid snails, one caenid mayfly, four dytiscid beetles, no amphipods, and less than 14 families would be considered "young" (Appendix A.1.B).

Wetlands with a metric score greater than zero would be considered equivalent to "mature", with respect to the benthic community (Figure 3.10, Table3.11). The relative abundance of each taxon in the metric can be found in Table 3.1C. A "mature" zoobenthic community would have less than nine physid snails, two to eight caenid mayflies, two to eleven dytiscid beetles, up to 37 amphipods, and greater than nine families (Appendix A.1.B).

For sweep samples, young wetlands had metric scores less than zero and fell within the 95% confidence bands (Figure 3.11, Table 3.15). The expected range of taxa that would be needed to consider it equivalent to a young reference wetland would be up to 37 Tanytarsini midges, 17 physid snails, 21 planorbid snails, two baetid mayflies, up to 50-100 dytiscid beetles, less than eight notonectids, and no sphaeriid clams or caenid mayflies, based on a single composite sweep sample (Appendix A.1.C).

**Table 3.15.** Metric scores for each collection method. Metric scores were calculated from the metric equation derived using DFA on selected benthic taxa (listed at bottom of table). Components for each metric are listed in appendices A.3 A, B, respectively. Note that the values for 'Metric Artificial Substrate' have the sign reversed for ease of comparison to sweep samples.

| Site | Class | Metric Artificial Substrates | Metric Sweep | Age Class (0 = Young 1= Mature) | Wetland Age |
|---|---|---|---|---|---|
| CTW2000 | OSPM | 0.694 | | 0 | 2 |
| CTW2001 | OSPM | -4.431 | -5.201 | 0 | 3 |
| CTP2000 | OSPM | -5.244 | -1.078 | 0 | 4 |
| CTP2001 | OSPM | 0.395 | 1.859 | 0 | 5 |
| DP | OSPM | 1.078 | 5.580 | 1 | 8 |
| TP9 | OSPM | -0.437 | 3.263 | 1 | 8 |
| MFTN | OSPM | -5.736 | -3.271 | 1 | 10 |
| TP5 | OSPM | -2.865 | 4.918 | 1 | 12 |
| TP2 | OSPM | 0.893 | 2.130 | 1 | 12 |
| NW | OSPM | 1.072 | 4.738 | 1 | 15 |
| SCP | OSPM | 0.279 | -1.344 | 1 | 26 |
| PP | High Cond. Ref. | -2.585 | -1.749 | 0 | 0 |
| BL | High Cond. Ref. | -3.242 | -4.460 | 0 | 5 |
| CRM | High Cond. Ref. | -0.986 | -2.596 | 0 | 5 |
| SM | High Cond. Ref. | -0.161 | 2.930 | 1 | 10 |
| SB | High Cond. Ref. | 0.318 | 3.309 | 1 | 16 |
| HS | High Cond. Ref. | 0.571 | 3.864 | 1 | 16 |
| SBD | High Cond. Ref. | -0.764 | | 1 | 26 |
| SPit | High Cond. Ref. | 1.423 | 2.044 | 1 | 26 |
| CL | High Cond. Ref. | 1.234 | 0.779 | 1 | 29 |
| SBBP | Low Cond. Ref. | -2.867 | -0.998 | 0 | 1 |
| MID | Low Cond. Ref. | -1.107 | -3.456 | 0 | 1 |
| LP | Low Cond. Ref. | -3.482 | -1.706 | 0 | 2 |
| SW | Low Cond. Ref. | 0.498 | 3.695 | 1 | 8 |
| NWID | Low Cond. Ref. | 0.979 | 2.002 | 1 | 23 |
| TP1 | Low Cond. Ref. | 0.789 | 3.092 | 1 | 12 |
| BM | Low Cond. Ref. | 2.322 | 5.157 | 1 | 23 |
| H63W | Low Cond. Ref. | 2.087 | 1.735 | 1 | 23 |
| WID | Low Cond. Ref. | 0.897 | 2.488 | 1 | 26 |
| LLW | Low Cond. Ref. | 0.455 | 1.344 | 1 | 28 |
| H63I | Low Cond. Ref. | 0.750 | 1.859 | 1 | 30 |
| TR6.8R | Low Cond. Ref. | -0.157 | 4.805 | 1 | 30 |
| TR0.8R | Low Cond. Ref. | 3.027 | 1.245 | 1 | 30 |

Metric equation for artificial substrate samples:

- (Talitridae*1.478) + (Physidae*3.255) - (Dytiscidae*3.242) + (Caenidae*5.298) - (Richness*0.093) -0.256

Metric equation for sweep samples:

- (Dytiscidae*0.631) + (Notonectidae*2.220) - (%Orthocladiinae*0.094) - (Sphaeriidae*5.757) + (Planorbidae*1.755)- (Physidae*1.682) + (Caenidae*5.045) - (Baetidae*0.654) - (Tanytarsini*0.488) + 1.859

Mature wetlands had metric scores ranging greater than zero, and fell within or above the 95% confidence bands (Figure 3.11, Table 3.15). If one were to sample another OSPM-affected wetland, of known age, say 13 yrs., the expected range of taxa that would be needed to consider it equivalent to a mature reference wetland would be up to 140 Tanytarsini midges, 9-15 physid snails, up to 60 planorbid snails, up to 75 sphaeriid clams, up to 13 caenid mayflies, 40 baetid mayflies, less than 23 dytiscid beetles, and up to 24 notonectids (Appendix A.1.C).

For both artificial substrates and sweep samples, it is proposed that wetlands within the 95% confidence bands could be considered the minimum benchmark, against which to measure the state of the benthic community in all other wetlands. Wetlands located below the confidence limits are considered to have a zoobenthic community that is 'poorer than expected" for their age.

One must exercise some caution with the interpretation of these results. It is important to examine each component of the metric equation to determine if a site is heavily influenced by a few taxa. For example, one OSPM-affected wetland (SCP) falls in among "mature" reference wetlands for both artificial substrate and sweep samples (Figure 3.11, Figure 3.12). This wetland has a benthic community dominated by very high % Tanypodinae and relative abundance of Dytiscidae characteristic of young wetlands, and should not be taken as an indication of convergence with reference (Appendix A.5).

**Factors Limiting Colonisation and Succession**

In Chapter 2, different physicochemical aspects of a wetland were reported to significantly influence the richness (water pH, naphthenic acid concentration, detritus, and sediment ORP), and abundance (detritus, water conductivity, and macrophyte development)

of benthic invertebrates. In this chapter, an attempt had been made to explore which taxa characterised the differences in the biological (zoobenthic) community.

In the early stages of their development, the water chemistry in OSPM-affected wetlands will be the biggest limitation to colonisation. Naphthenic acids, released during the extraction process, act as surfactants on biological membranes and have been identified as accounting for most of the acute toxicity to aquatic biota in fresh OSPM waters (Fine Tailings Fundamentals Consortium 1995b). Once the toxic constituents in the OSPM have been allowed to degrade through UV and microbial activity (expected to be < 1 yr.) (Herman *et al.* 1994; Lai *et al.* 1996, Holowenko *et al.* 2002), water would become more comparable with respect to water quality and biological impacts in high conductivity reference wetlands. Consequently, zoobenthic communities in older OSPM-affected wetlands should be expected to succeed similarly to those in high conductivity reference wetlands.

The taxa seen in young wetlands are not surprising when one considers the development of the wetland community as a whole; flora, fauna, chemical and physical changes in the soil, and in the case of these wetlands, chemical changes to the water. Presuming that wetland waters (surface, pore, recharge) are not toxic, and thus not a barrier to colonisation, an initial lack of wetland vegetation may be a limiting factor. Without extensive vegetation cover, there will be little structural heterogeneity in the wetland and little available surface area, other than the sediment, to allow for the development of a microbial community (Nix and Martin 1992; Herman *et al.* 1994).

The taxa that would initially colonise a new area would need to be mobile and capable of exploiting this undeveloped area. Tube-building chironomids that feed on microorganisms and phytoplankton are well suited to such an environment. Once potential

prey species have established, richness and abundance of predators should increase. Thus, the presence of larval chironomids and larval corixids in young wetlands is not surprising.

Initially wetlands will be colonised by taxa that are strong fliers (corixid adults and dytiscid beetle adults ovipositing in new wetlands), followed by taxa that are weak fliers (chironomid taxa) that may require wind to disperse any great distance (Barnes 1983). Finally, wetlands will be colonised by taxa that lack a mobile adult forms capable of dispersal (worms, leeches, amphipods) (Barnes 1983).

Orthocladiinae larvae dominate young wetlands. This group of chironomids is capable of tolerating relatively high conductivity (488-741μS/cm) in saline lakes in central British Columbia (Cannings and Scudder 1978), and are generally tube-builders (Merritt and Cummins 1996), so their dominance in young wetlands could be expected.

In addition, young wetlands also have relatively high numbers of corixids and dytiscids. These are highly mobile (adults are strong fliers) taxa and are often colonisers of new areas (Merritt and Cummins 1996). Corixids are often herbivorous, but some are predacious on chironomid larvae (see Lovvorn *et al.* 1999). Thus, the co-occurrence of these taxa in young wetlands could be expected. Most of the corixids and dytiscids collected in these wetlands were immature specimens, indicating that they originated in these wetlands, and were not simply the adult dispersal stage. In older wetlands, corixids and dytiscids tend to have lower relative abundance, and their dominance in the community is lessened by the higher relative abundance of other taxa (Table 3.1).

In older, larger wetlands, emergent and submergent vegetation has become established, contributing three important things to the wetland: increased habitat heterogeneity (Oertli and Lachavanne 1995; de Szalay and Resh 2000), increased surface

area for microbial (Herman *et al.* 1994) and periphyton communities (Barnes 1983), and increased detrital inputs through the senescence of plants (Sistani *et al.* 1999). These factors support increases in the number of taxa (richness increases, Chapter 2), diverse feeding types (more grazers are now present), and an increase in predators with increasing prey base.

The oldest wetlands (>30 years) appeared to have fewer numbers or lower richness of zoobenthic taxa (See Chapter 2). Species diversity has been found to be highest at intermediate levels of disturbance along a gradient, with a decrease or increase in diversity along disturbance gradients (see Pollock *et al.* (1998)). Older, shallow wetlands may have anoxic sediments (due to large amount of organic matter and shallow water) and low dissolved oxygen at the sediment-water interface (Murkin and Kaldec 1985). This may limit the diversity of invertebrates in older wetlands, as only those able to withstand periods of anoxia, such as individuals of tribe Chironomini, would persist. The data collected from the current group of wetlands suggests that this is only the case for one site, Crane Lake. However, as D.O. measures were not collected at depth (due to meter malfunction) as a physicochemical measure, this conjecture requires further study.

Extinction of colonists may also have occurred. A number of chance events could lead to the loss of diversity: chance extermination of small founder populations, competitive or predatory interactions, or changes in the habitat (Barnes 1983). Given the proximity and connectivity of the wetlands in the area, new colonists are able to arrive easily. However, changes in the physical structure (e.g., macrophytes) of a wetland may be what determines the taxa in mature wetlands.

It may also be that older wetlands have dominant mono-cultures of *Typha* in their later years, which may decrease the diversity of habitat available to benthic invertebrates

(Cyr and Downing 1988). Again, in the present study, the correlation between macrophyte community composition (or relative abundance of different macrophytes) and zoobenthic community composition was not determined. Further research should be conducted to support or refute this conjecture.

An important factor that has not been examined in relation to the development of this metric, is the importance of organic matter (coarse particulate organic matter, or sediment organic matter (measured through loss on ignition (LOI)) to the development of the zoobenthic community. Richness and abundance of the zoobenthic community are influenced by the quantity of organic matter found in these wetlands (See Chapter 2). Many constructed wetlands in this study have very small drainage basins (<0.2 ha in size), and lack surrounding upland vegetation. The input of allochthonous organic matter (leaves, senescent shore vegetation, etc.) would be less than for more "natural" wetlands that form in meadows or on the forest floor (such as BM, TR0.8R, TR6.8R). The results from Chapter 2 suggest that this is the case for OSPM-affected wetlands that have lower amounts of organic material (CPOM and/or macrophytes) compared to reference wetlands of similar age.

**Which Metric and Sampling Type to Use?**

When considering which metric to use and what sampling type to recommend, the percent of correct classification of the metric (from the DFA results) was examined. This took into consideration the length of time it takes to collect and process each type of sample (personal experience).

Generally, core samples are easiest to collect and sort. However, because fauna tend to be dominated by chironomids and worms, taxonomic identification below the

family level is time consuming, labour intensive and potentially costly to a project. A metric was not developed for core samples, as no taxa separated young from mature reference wetlands, either effectively or accurately (Table3.4).

Artificial substrate samples require more time to collect as two trips are needed - one to set them in place and one to collect them. The length of time that they are left in the waterbody may also influence the taxa found on them (see DeShon 1995, Barbour *et al.* 1999b, and Benoit *et al.* 1998 for a summary of advantages and disadvantages of artificial substrates). Artificial substrate samplers are not routinely used in most U.S. Rapid Biological Protocols (RPBs) because it is thought that up to eight weeks are required for colonisation in streams (Barbour *et al.* 1999b). However, Benoit *et al.* (1998) reported that substrates, in lake littoral zones, had reached saturation density and abundance by eight days after placement.

Based on the current study, it appeared that five samplers, left for eight days, would be adequate for an estimate of the zoobenthic community in these wetlands. (Benthic invertebrates were collected from five artificial substrates and two artificial substrate samples (40% of total samples collected) were processed for each wetland, and with this method, significant differences between young and mature reference wetlands were found).

Artificial substrate samples require slightly more time to sort than cores. However, because a wider range of taxa is found in these samples, and because they tend to be larger-bodied taxa, identification to family and lower taxonomic resolution is relatively quick.

The proposed metric for artificial substrate samples performed adequately to separate (95%) of young from mature reference wetlands (Table 3.11). The taxa that were identified as being most important for distinguishing young and mature reference wetland

were amphipods (Talitridae), physid snails, dytisid beetles, caenid mayflies, and richness, (Table 3.11).

Sweep samples require the least time to collect of all the sampling methods used. Sweep samples are also the recommended method of sampling for RBPs, mainly due to the ease of collection. Sweep samples require the most time to sort because of the presence of large quantities of detritus collected. However, because a wider range of taxa is found in these samples, and they tend to be larger bodied taxa, identification to family and lower taxonomic resolution is relatively quick. Worms and chironomids are still collected when the net is passed through the surface of the substrate, and these taxa are easily identified to order (Oligochaeta) or subfamily/tribe (Chironomidae). The proposed metric for sweep samples separated, correctly, all of the young from mature reference wetlands (Table 3.12). The taxa that were identified as being most important for distinguishing young from mature reference wetlands, were predacious diving beetles (Dytiscidae), back swimmers (Notonectidae), snails, mayflies, Tanytarsini midges and the percent composition of Orthocladiinae within the chironomids (Table 3.12).

In future studies, use of sweep samples for assessment of the zoobenthic community in these wetlands is recommended, with the caveat that sweep samples are qualitative measures of the benthic community. The present study did not collect replicate sweep samples in each wetland. Collection of a single composite, sweep sample did not permit an estimation of the variability within each wetland. Future work may include the use of multiple sweep samples within a wetland to assess zoobenthic variability. If the number of taxa per wetland area is desired in the monitoring of these wetlands, then a quantitative measure, such as artificial substrates, should to be used.

To summarise, artificial substrate and sweep samples are recommended as sampling methods for these wetlands, along with the metric developed from each method.

# Chapter 4

## General Discussion

In this thesis, the development of the zoobenthic community in "natural" (reference) and industry affected (OSPM-affected) wetlands in the Fort McMurray oil sands region has been examined using various sampling and biostatistical methods. Wetlands of differing ages were simultaneously sampled, allowing for an inference into the chrono-sequence of change that accompanies wetland succession. Three wetland classes were evaluated, OSPM-affected wetlands, high conductivity reference wetlands, and low conductivity wetlands.

The objective was to determine whether the zoobenthic community composition of OSPM-affected wetlands became similar to that of local reference wetlands, and to develop an index by which to measure convergence. Wetlands were sampled using three methods. Core samples provided information primarily on the fauna in the sediment. Artificial substrates provided information on sediment-surface dwelling organisms and epiphytic-dwelling organisms. Sweep samples provided a measure of larger, rare and mobile taxa from a variety of habitats.

In Chapter 2, it was reported that zoobenthic richness and abundance reached asymptotes in 5-year old reference wetlands. Richness was significantly lower in young OSPM-affected wetlands than in similar aged reference wetlands. While the diversity was affected, the abundance in young OSPM-affected wetlands was not significantly lower than similar aged reference wetlands. Water pH, naphthenic acid concentration, detrital abundance, conductivity, salinity and sediment ORP were all significantly associated with

the richness of benthic invertebrates found in a wetland. The extent of macrophyte development, water conductivity, and detrital abundance were associated with the abundance of benthic invertebrates in a wetland. Water toxicity, sediment characteristics, and development of macrophytes may initially limit the zoobenthic community establishing in OSPM-affected wetlands.

In Chapter 3, principal components analysis and discriminant function analysis were used to classify each OSPM-affected wetland as being "equivalent to young" or "equivalent to mature". Midges and dytiscid beetles characterised "young" wetlands (OSPM-affected and reference). Gastropods, Chironomini midges, mayflies, and amphipods characterised "mature" reference wetlands.

Metrics developed for artificial substrate and sweep samples best separated "young" from "mature" reference wetlands. The metric for sweep samples is recommended as it provides the most robust description of the benthic community. However, it does not provide a quantitative measure of the zoobenthic community.

**Importance of Reference Wetlands**

The study of reference wetlands is desirable, as they permit one to identify reference standards that are representative of current natural conditions in a given region (Brinson and Rheinhardt 1996). Reference wetlands provide templates to which restored and created wetlands can be designed, and establish a framework against which a change in function resulting from anthropogenic impacts can be estimated (Brinson and Rheinhardt 1996). Data from reference wetlands provide benchmarks for directing restoration or creation of wetlands (Brinson and Rheinhardt 1996). Fundamental requirements for achieving success

of wetland creation and restoration projects are understanding wetland function, giving the system adequate time to develop ecosystem function, and allowing for the self-designing capacity of nature (Mitsch and Wilson 1996).

All of the OSPM-affected and most of the reference wetlands would be classed as "constructed" wetlands, since for all intents and purposes they were created by relatively recent modifications of the topography or drainage pattern. The most "natural" of the wetlands studied consisted of beaver ponds of indeterminate age, or old (20-30 yr.) roadside borrow pits. They are considered to be reference wetlands as they were not deliberately constructed for reclamation purposes nor do they contain OSPM. The oldest of these reference wetlands (those >7 yr. old) were considered to be the benchmark against which the development of the zoobenthic community in young reference and OSPM-affected wetlands is measured.

Reference wetlands in this study were divided into two groups, low conductivity reference wetlands and high conductivity reference wetlands (Chapter 2, Appendix A.2). Conductivity reflects the total ionic composition of the water. Inorganic ions contributed to high conductivity readings in wetlands used in this study.

The division into low or high conductivity reference wetlands may cause some difficulties when attempting to determine the benthic community in mature reference wetlands. Several studies have reported that the benthic community varies as a function of salinity in western North American wetlands (see Rawson and Moore 1944; Cannings and Scudder 1978; Lovvorn *et al.* 1999) and in southern Spain (Casas and Vilchez-Quero 1996). However, the zoobenthic communities in the wetlands that have been examined were generally similar, with mature reference wetlands having communities dominated by chironomini midges, worms, amphipods, mayflies and snails (Chapter 3).

## Factors Limiting Zoobenthic Colonisation and Succession

### Oil Sands Process Material (OSPM) Toxicity

The acute toxicity of oil sands process materials (OSPM), including tailings pore water, mature fine tailings, and consolidated/composite tailings, presents an initial and significant barrier to zoobenthic community development of OSPM-affected wetlands in the oil sands mining region. Fresh OSPM is acutely toxic to aquatic organisms ranging from bacteria to fish (Fine Tailings Fundamentals Consortium 1995b).

The toxicity of OSPM decreases rapidly during its first year of aging under natural conditions, free of the input from fresh OSPM, however. Survival of trout and *Daphnia magna* in tailings pond water was 60-80% after the water had aged for 10 months (Fine Tailings Fundamentals Consortium 1995b). Bench scale studies have indicated that detoxification of OPSM in wetlands is an aerobic process that may be nutrient (specifically phosphorus) limited (Fine Tailings Fundamentals Consortium 1995b). Addition of phosphate to Suncor pond top water resulted in detoxification within 7 - 10 weeks, in a bench top study, while field-scale trials showed enhanced ability to detoxify Suncor dyke drainage water (Fine Tailings Fundamentals Consortium 1995b).

It is unlikely that the reduced abundance and low richness characteristic of young reference and OSPM-affected wetlands are due mainly to a lack of propagules. In a 3-year study of restored wetlands in New York, Brown *et al.* (1997) reported that most taxa found at natural sites could also be found in similar numbers at restored sites. They reported that insects with aerial dispersal capability colonised the restored habitats rapidly, but some less mobile forms either colonised more slowly or not at all. Whelly (1999) reported no consistent evidence that chironomid adults avoided trays filled with OSPM water in a study

to estimate the colonisation potential of OSPM-affected wetlands. He reported that patterns of chironomid oviposition were determined by the different peaks of emergence at different wetlands.

Richness and abundance in high conductivity reference wetlands varied little as a function of wetland age. A richness of six to 20 families, for young wetlands, and 12-18 families for mature wetlands, and an abundance of 141-1100 individuals for young wetlands, 105-744 individuals for mature wetlands were recovered from artificial substrate samples (Appendix A.1). Young low conductivity reference wetlands generally contained slightly fewer wetland taxa and individuals than mature low conductivity reference wetlands. (8-13 families for young wetlands, 10-18 families for mature wetlands, and 182-482 individuals for young wetlands, 109-1247 individuals for mature wetlands in artificial substrate samples) (Appendix A.1).

Previous studies comparing OPSM-affected and reference wetlands have reported secondary production to be approximately four times greater at reference wetlands than at OSPM-affected wetlands (McDonald 1998; Hum 2000). Ganshorn (2002) reported lower densities and annual production for chironomids in OSPM-affected wetlands compared to reference wetlands. However, none of these studies compared production between low and high conductivity reference wetlands.

High conductivity reference wetlands may have higher productivity than low conductivity reference wetlands. Lovvorn *et al.* (1999) reported that mesosaline (800-3000 µS/cm, 5-18 g/L TDS) wetlands had more insect predators, and greater zooplankton and phytoplankton production than oligosaline wetlands in Wyoming. In the current study, productivity between different reference wetland classes was not measured. If one uses

abundance as a surrogate for productivity (assuming there is no difference in growth between low and high conductivity reference wetlands) then high conductivity reference wetlands have slightly higher productivity (459 individuals per artificial substrate sample; n=9) than low conductivity reference wetlands (364 individuals per artificial substrate sample; n=13).

Richness was most affected by water pH, naphthenic acid concentration, conductivity, salinity, detritus, and sediment ORP (see Table 2.7). This indicates that the wetland water and substrate in OSPM-affected wetlands are less suitable than those of reference wetlands for sediment dwelling organisms such as oligochaetes and chironomids. Water quality, (e.g., alkaline or saline conditions) might also impede the development of the macrophyte community (Figure 4.1).

Low invertebrate richness in OSPM-affected wetlands does not prevent these wetlands from producing similar quantities of individuals as more taxonomically rich wetlands. Generally, abundance (total number of individuals per unit area in a single wetland) was not significantly different from reference wetlands for any age of wetland (Chapter 2). Whelly (1999) and Bendell-Young *et al.* (2000) also reported lower richness and equal abundance in OSPM-affected wetlands compared to reference wetlands.

Abundance of invertebrates, measured using artificial substrates, was higher in high conductivity reference wetlands (662 -2000 µS/cm, 0.3 - 1.7 ‰) than in low conductivity reference wetlands (440-1050 µS/cm, 0-0.03 ‰) (Fig.2.22). Rawson and Moore (1944) reported a trend of increasing zoobenthic abundance with increasing TDS (a measure of salinity) up to 2250 ppm (~2700 µS/cm) in Saskatchewan saline lakes. Lovvorn *et al.*. (1999) reported an increased abundance in chironomid larvae, crustacean zooplankton and

insect predators in mesosaline (800-3000 μS/cm) lakes compared to more oligosaline lakes (<800 μS/cm) in southeast Wyoming. In contrast, Streever *et al.* 1996 reported that densities of common larval dipterans, in 10 natural and 10 created wetlands in central Florida (dominated by *Pontederia cordata*), were not significantly different. They also reported that pH, conductivity and sediment quality were only weakly related to dipteran community composition, and concluded that there was no convincing evidence of zoobenthic community differences between natural and created wetland dipteran communities.

In the Fort McMurray wetlands, abundance among wetlands was positively associated with quantity of detritus, amount of macrophyte development, and conductivity (Chapter 2). This suggests that zoobenthic abundance may be related to macrophyte and detrital biomass rather than direct effects of the chemical parameters. Chemical parameters, such as conductivity, may impede the establishment of macrophytes and slow the accumulation of detritus resulting from macrophyte production, thereby indirectly influencing the zoobenthic community (Figure 4.1).

**OSPM Toxicity and Potential Vegetation Effects**

Several potential barriers to zoobenthic community development exist beyond the toxicity of the constituents in the OSPM. Initially, chemical toxicity is the limiting factor in colonisation of young OSPM-affected wetlands. Once ultraviolet and microbial breakdown of the PAHs and naphthenic acids decrease toxicity sufficiently in the water column and

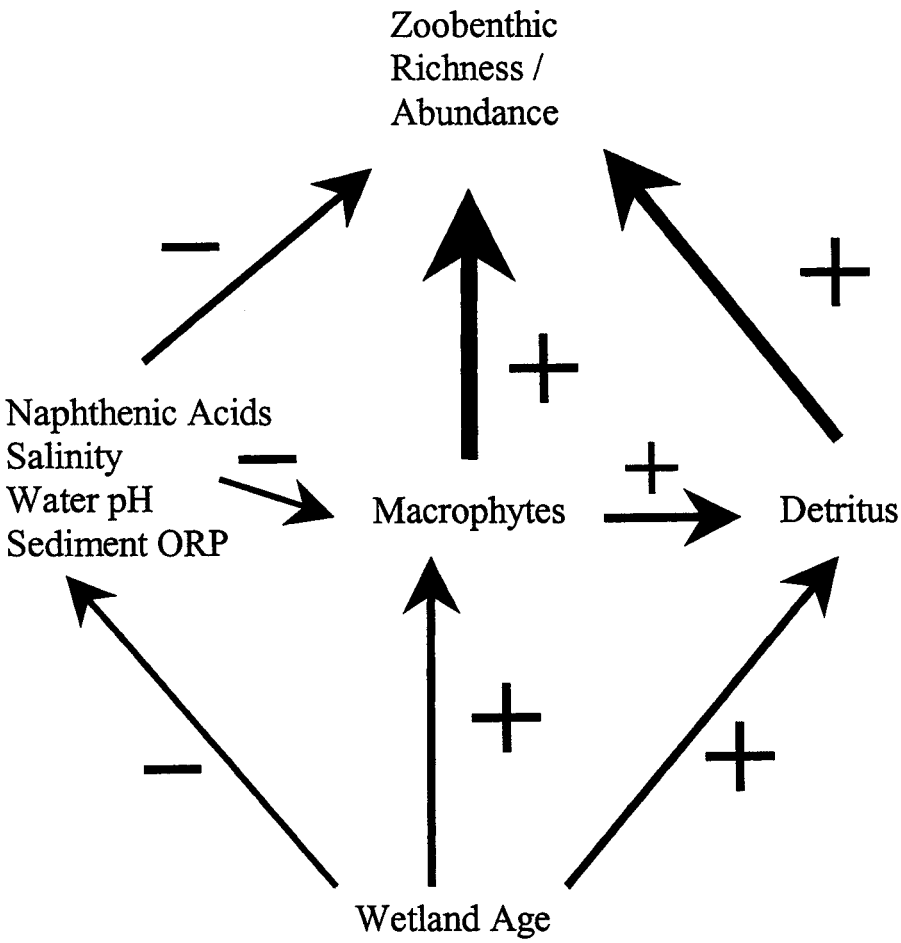



**Figure 4.1**. Hypothetical path analysis for interactions between physico-chemical factors and zoobenthic richness and abundance. Width of arrows indicates degree of importance. + indicates positive effect, - indicates negative effect.

sediment (Madill *et al.* 2001) the colonisation by mobile, salt-tolerant species can begin. The species that initially colonise can survive in wetlands that lack vegetation, have little habitat heterogeneity, and few food sources. Initial colonising species can include chironomids (Tribes Orthocladiinae and Tanytarsini), and other organisms that are capable of feeding on the biofilm that develops over the surface of the sediment and the small number of predators that feed on them. de Szalay & Resh (1996) reported that detritivores were early colonists in a seasonal saline marsh, followed by predators and algivores (de Szalay and Resh 1996).

Wetlands in this study appeared to be impaired by OSPM for at least the first seven years after construction (Chapter 2). OSPM-affected wetlands had reduced zoobenthic richness and abundance compared to reference wetlands of similar age, up to seven years after construction (Chapter 2). This may be the result of residual OSPM toxicity, but it is more likely the result of slowed rates of macrophyte development and organic matter accumulation in OSPM-affected wetlands (Chapter 2). Sistani *et al.* (1999) reported that wetlands constructed on top of coal mine spoils had less organic matter accumulation than occurred in reference wetlands, even after 3-4 yr., probably due to incomplete incorporation of dead plant residues into the substrates. Those wetlands however, possessed botanical and biogeochemical characteristics equivalent to natural wetlands within 3-4 years (Sistani *et al.* 1999). It is possible that lower amounts of coarse and fine organic material in OSPM-affected wetlands with extensive macrophyte development (MFTN, TP5, NW), is due to a lack of detritovores or a lack of microbial flora capable of breaking down organic, non-hydrocarbon-based material.

Wetlands that are being constructed for reclamation (detoxification and/or habitat) are not equivalent to naturally-formed wetlands in the area. Newly constructed wetlands

lack the quantity of organic material, or peat (>40 cm peat), required to consider them to be peatlands (Oil Sands Wetlands Working Group 2000). Thus, the zoobenthic community that develops in the wetlands is lacking a substantial organic matter base and is expected to be different from the zoobenthic community found in regional peatlands. Though one would expect soil organic matter to increase with time, there are no studies reporting how long it takes organic soils to develop (Oil Sands Wetlands Working Group 2000). Since the biological activity within a wetland extends only 15 cm into the substrate, and this activity will remove a few centimetres (approx. 2 cm) of organic material per year, if the organic material in a wetland is not replaced, then the benefits of additional organic material (habitat, energy base) may be lost within 20 years (L.Foote, University of Alberta, pers. comm.).

In most cases, young reference wetlands containing significant quantities of coarse, organic material (PP, LP, MID, BL) had more families of invertebrates (higher richness) than similar-aged OSPM-affected wetlands (CTW 2000, CTW 2001, CTP2000, CTP2001) (Chapter 2). However, these young reference wetlands had equal numbers of animals (abundance) as similar-aged OSPM-affected wetlands (Chapter 2 Fig. 2.10), suggesting that organic material in these wetlands is important for the zoobenthic community.

Decreased toxicity of OSPM constituents will also allow the establishment of wetland vegetation. Establishment of submergent and emergent macrophytes, such as *Chara* and *Typha*, provide increased surface area for biofilm development, food for phytophilous invertebrates, and habitat for predators and other invertebrates requiring vegetation for oviposition (see Lovvorn *et al.* 1999; de Szalay and Resh 2000; Hart and Lovvorn 2000). The presence of vegetation increases the autochthonous inputs of organic material through the senescence of macrophytes (Barko and Smart 1983). This increase in

organic material provides increased surface area for microbial fauna, food for detritus feeders, and organic material that can bind some of the toxic constituents to the sediment (Fine Tailings Fundamentals Consortium 1995b).

**Zoobenthic Composition of Wetlands in the Fort McMurray Oil Sands Region**

The presence of mobile, predacious taxa, such as dytiscid beetles, Tanypodinae, Tanytarsini and Orthocladiinae chironomids, was a good indicator for characterising young wetlands in this study. Periphyton grazers (snails), and detritivorous taxa such as chironomids (subfamily Chironomini), mayflies (Caenidae and Baetidae), worms (Oligochaeta), leeches (Hirudinea), and amphipods (Talitridae) characterised mature wetlands.

The taxa that characterised mature reference wetlands begin to show up in reference wetlands between 7 and 15 years after wetland construction. OSPM-affected wetlands zoobenthic community composition could be characterised by "young" reference wetland taxa throughout the age range studied. Several OSPM-affected wetlands appeared to be in transition from a young wetland zoobenthic community to one comparable to a mature reference wetland (Chapter 3).

The delay in the development of a "mature" zoobenthic community in OSPM-affected wetlands may be due to continued inputs of OPSM in two older wetlands (NW (15 yr.), SCP (25 yr.)), lack of vegetation in the wetland (DP, TP9, TP2), the presence of hydrocarbon films on the surface of the water within the vegetation (MFTN), or from water turbidity (TP9, DP, TP2 contain MFT or other fine particles that remain in suspension for a long time).

## Other Factors That May Affect the Zoobenthic Community

### Vegetation Development

The estimated density and extensiveness of macrophytes are important in determining zoobenthic community richness (Figure 4.1). In a study of 21 lentic water bodies in northeastern Finland, Heino (2000) reported that zoobenthic species richness was lowest in small bog lakes with simple bottom structure and few aquatic plants (Heino 2000). A suite of plant factors including morphology, surface texture, epiphytic algal growth and community composition, nutrient content of plant tissues, and presence of defensive chemicals, influences the abundance of epiphytic invertebrates (Cyr and Downing 1988).

### Wetland Surface Area

Wetland area may also influence invertebrate abundance and richness. Larger wetland surface area increases the potential amount of sunlight reaching wetland plants. Greater plant biomass and annual senescence of plants contribute to detritus. Reinartz and Warne (1993) reported that diversity and richness of native wetland macrophyte species increased with wetland age, wetland size, and with proximity to nearest established wetlands. Wetland size had a positive relationship to zoobenthic richness (for artificial substrate samples only). Larger wetlands tended to have more invertebrate families than smaller wetlands (Chapter 2).

**Salinity**

Change in salinity can result in a shift of the richness of the benthic community. Lovvorn *et al.* (1999) found that differing taxonomic composition of saline wetlands in southeast Wyoming resulted from salt toxicity to three competitive dominants - *Chara*, snails, and amphipods. The macroalgae *Chara*, epiphytic snails, and amphipods dominated low-salinity wetlands (80-800 μS/cm). More saline wetlands (800 - 3000 μS/cm) were dominated by *Potamogeton* (submergent macrophyte), chironomid larvae, and insect predators (odonates and hemipterans) and had greater densities of crustacean zooplankton (Lovvorn *et al.* 1999).

Examination of the biological attributes of "mature" reference wetlands in the Fort McMurray oil sands area showed a similar pattern in the benthic community as seen by Lovvorn *et al.* (1999). Generally, mature, low conductivity (salinity) reference wetlands had higher relative abundance values of gastropod taxa, than were found in mature high conductivity reference wetlands (Table3.5 A, Appendix A.1). Overall, these wetlands were fairly similar with respect to physico-chemical attributes (Figure 2.4, Appendix A.1).

In the present study, it was apparent that any change in the benthic community results largely from the concentration of naphthenic acids in the water (which was relatively low (1.0-12.2 mg/L) for "mature" reference wetlands), or possibly from a change in the dominant ions within the water. Scudder (1969) and several other authors have pointed out that the salinity tolerance of invertebrates can be altered by the ionic composition of the water, especially by the predominance of $Mg^{2+}$, $SO_4^{2-}$, or both, relative to $Na^+$ and $Cl^-$ (Lovvorn *et al.* 1999).

The influence of individual ions on the zoobenthic community was not examined in this study. However, a change in relative distribution of dominant ions was seen in the wetlands of this region (Appendix A.2). The saline-sodic overburden used in reclamation on oil sands leases is dominated by sodium salts (NaCl, $Na_2SO_4$, $CaCO_3$, $MgCO_3$ and $CaSO_4$), while OSPM-affected areas are dominated by chloride salts (NaCl, $Na_2SO_4$ and $NaHCO_3$) (Appendix A.2) (C. Qualizza and M. MacKinnon, Syncrude Ltd., pers. comm.).

Although OSPM-affected and reference wetlands may become biologically similar over time, it will be important to examine their ability to function in a hydrologically similar manner to local natural wetlands. Stolt *et al.* (2000) compared three constructed wetlands (4-7 yr. old) to adjacent palustrine forested and scrub-shrub reference wetlands in order to examine differences in topography, hydrology, soil properties and other environmental conditions. They reported that micro-relief was greater in reference wetlands, while seasonal fluctuations in water table levels were similar between reference and constructed wetlands, as were redox potential. However, they also reported that the observed differences in soil and other environmental conditions between reference and constructed wetlands suggest that constructed wetlands may not function in the same capacity as adjacent reference wetlands. (Stolt *et al.* 2000).

## Recommended Zoobenthic Assessment

### Metric Development

A measure of the benthic community in reference wetland can be developed using a multimetric index, multivariate techniques, or by a combination of the two. Multivariate

techniques have been used in this study to develop a single composite score, similar to a multimetric index.

Metrics developed for wetlands in this study should include relative abundance ($log_2$) of pioneer taxa, such as dytiscid beetles, Tanypodinae and Orthocladiinae chironomids, as well as "mature wetland" taxa such as, snails, mayflies, backswimmers (Notonectidae), and amphipods (Talitridae). The richness of taxa in wetlands, as well as the relative composition of the different tribes/subfamilies within the Chironomidae were important diagnostic factors useful in the development of an index of wetland recovery (Chapter 3).

Other metrics that have been developed for wetlands have also used richness, snails, (J.Helgen, Minnesota Pollution Control Agency, pers.comm., Burton *et al.* 1999) and the number of chironomid genera (J.Helgen, pers.comm.). Metrics developed in the United States for wetlands also proposed the use of odonate richness and relative abundance (Burton *et al.* 1999), abundance of Corixidae, abundance of Dytiscidae (Galatowitsch *et al.* 1999), and the proportion of Ephemeroptera and Trichoptera (Galatowitsch *et al.* 1999). The current metrics did not find Odonata to be useful in distinguishing among mature reference wetlands, probably because some odonate taxa, e.g. Lestidae, were found in wetlands of differing ages and classes (OSPM-affected and reference).

**Recommended Sampling Method**

Of the three sampling methods I used, the metric developed for artificial substrate and/or sweep samples is recommended. Artificial substrate samples yielded zoobenthic composition that classified correctly (95%) young and mature reference wetlands (Table

3.11). Zoobenthos from sweep samples gave correct classification of all young and mature reference wetlands (Table 3.12). Core sample zoobenthos did not classify young from mature reference wetlands, as no young reference wetlands were correctly classified (Table 3.4).

In general, young reference wetlands were characterised by predominance of chironomid midges (Tribe Tanytarsini, Subfamily Orthocladiinae and Tanypodinae), dytiscid beetles, and backswimmers (Corixidae). These taxa were ubiquitous among young OSPM-affected and reference wetlands (Chapter 3). Mature reference wetlands were characterised by Chironomini midges, snails, amphipods, worms and mayflies (Chapter 3). Mature OSPM-affected wetlands often had low relative abundance of the taxa that characterised mature reference wetlands. Most mature OSPM-affected wetlands had a zoobenthic community with taxa characteristic of both young and mature reference wetlands (Chapter 3). Two OSPM-affected wetlands receiving on-going OSPM inputs were often classified as being "mature" due to high abundances of a single taxon (e.g., worms for artificial substrates) or due to the constant in the DFA equation (Chapter 3).

Mature reference wetlands, used as the standard for the benthic community, have metric equation values of -1 to +4 (Chapter 3). Based on the samples collected by artificial substrate method, reference wetlands, were characterised by high relative abundance of amphipods, caenid mayflies, physid snails, and higher richness (Figure 3.10). The expected range of taxa would be up to nine physid snails, two to eight caenid mayflies, two to eleven dytiscid beetles, up to 37 amphipods and greater than nine families, based on two artificial substrate samples.

Mature reference wetlands, measured with a single composite sweep sampling protocol, had high relative abundance of Notonectidae, planorbid and physid snails, caenid

and baetid mayflies, and finger nail clams compared to young reference wetlands (Figure 3.11). The expected range of taxa that would be needed to consider it equivalent to a mature reference wetland would be up to 60 planorbid snails, nine - 15 physid snails, less than 23 dytiscid beetles, up to 24 notonectids, 13 caenid, and 40 baetid mayflies, and less than 34% Orthocladiinae within the Chironomidae.

If one sampling method were to be selected, the sweep sample method is recommended. Sweep samples are used most commonly for rapid bioassessment protocols, and for routine surveys of aquatic biota. Sweep samples are easy to collect, require no specialised equipment, and little special training. The biggest drawback that I see for sweep samples is that they are qualitative measures of the wetland biota, and the potential for collector bias. If a project's goal is to determine the number of taxa per unit area of wetland, then a quantitative method, such as artificial substrates or area delineated sweeps, should be used.

**Future Research Considerations**

It is expected that, based on the information of the current study, OSPM-affected wetlands should develop a biota that is similar to high conductivity reference wetlands in a finite time period. However, because the two oldest OSPM-affected wetlands sampled (Natural Wetland and Seepage Control Pond, 15 and 25 yr. old, respectively) are not isolated and receive periodic or continuous OSPM input, it is difficult to anticipate the ultimate composition of these sites. While they are the oldest test sites, they do not provide definitive data on evolution of areas that will receive one-time input of OSPM and then be isolated from OSPM input, and how it would develop its biota of such constructed wetlands

and the fate of toxic constituents. The next-oldest OSPM-affected wetlands are 13 yr. old (TP2 and TP5) and have zoobenthic community composition that is intermediate between young reference and mature reference wetlands (Chapter 3). These wetlands are isolated from surface input of OSPM water, but are in constant contact with soft fine tailings material, as is expected in water-capped options where slow diffusion and release water processes will occur.

Zoobenthic richness and abundance were also generally comparable to reference wetlands of similar age (Chapter 2). These results suggest that OPSM-affected wetlands may take at least 13 years to develop a zoobenthic community that is equivalent to mature reference wetlands. The youngest OPSM-affected wetland (CTW) had richness and abundance that were generally equivalent to young reference wetlands (Chapter 2), as well as having a taxonomic composition that is best characterised as young/intermediate. That is, even though the wetland may be only 3 years old, the results of the metric equation place it near the transition between young and mature reference wetland (Chapter 3).

It is believed that wetlands will act as passive bioreactors to detoxify the constituents in OSPM, leaving only saline water, and concentrations of naphthenic acids and PAHs within the normal background range for this region (Fine Tailings Fundamentals Consortium 1995a; Lai *et al.* 1996; Oil Sands Wetlands Working Group 2000). Periodic re-sampling of wetlands that received one-time OSPM inputs (Suncor CT Wetland, Syncrude CT Pond, Test Pond 9, Test Pond 2, Demo Pond, MFT-North, and Test Pond 5) is recommended to permit one to follow the trajectory for the development of the zoobenthic community and to measure changes in the water chemistry of the wetland for the next 10 years.

Macrophytes clearly influence the presence, number and type of benthic invertebrates found in a wetland (Hart and Lovvorn 2000). Macrophyte recruitment may also be influenced by the quality of the substrate (Barko and Smart 1983). Examination of the effect of substrate quality (particle size, toxicity, and organic content) on plant recruitment would be an important avenue to explore.

For example, Demo Pond is a 9-year-old OSPM-affected wetland (demonstration lake system) with elevated salinity and naphthenic acid concentrations, and a fine tailings (OSPM) textured sediment that lacks macrophytes except at its periphery. Demo Pond can support populations of fathead minnows (van den Heuvel *et al.* 1999; Gould 2000; Siwik *et al.* 2000), indicating that it is not acutely toxic, and is surrounded by other wetlands with abundant stands of *Typha* and *Scirpus*, which could serve as a seed bank or colonisation source. Yet, zoobenthic abundance and production are relatively low (Gould 2000).

In contrast, MFT-N is also an OSPM-affected wetland (demonstration lake system), 10 years old, also surrounded by other wetlands, with abundant emergent and submergent macrophytes, and higher invertebrate abundance and richness than Demo Pond. Factors that could explain the difference in the development of the plant community might include limited nutrients (P or N), low organic carbon content, or fine particle size. (Barko and Smart 1983) report that sediment texture may be important for plant rooting success and resistance to erosion.

In a study of natural colonisation of vascular plants in 11 created wetlands (1-3 yr. old) Reinartz and Warne (1993) predicted that naturally colonised wetlands would develop monocultures of *Typha*. They reported that wetlands seeded with native plants species had much higher diversity and richness of native wetland plant species than unseeded wetlands after 2 yr. (Reinartz and Warne 1993). They recommended early introduction of a variety

of wetland plants to enhance long-term diversity of vegetation in created wetlands (Reinartz and Warne 1993).

In another study, Galatowitsch and van der Valk (1996) reported that three years after restoration, natural wetlands had higher mean number of species (46) than restored wetlands (27). They also reported that emergent vegetation species richness in restored wetlands was similar to that in natural wetlands, although there were fewer seeds than those of natural wetlands (Galatowitsch and van der Valk 1996).

Examination of addition of amendments (peat, sand, or gravel) to OSPM for use in wetland substrates may be useful. Barko and Smart (1983) reported that very organic sediments might slow the growth of some aquatic plants. The inhibition of emergent and submergent plant growth was associated with high concentrations of soluble organic compounds and phytotoxins in sediment interstitial water (Barko and Smart 1983). This contrasts to the suggested function of sediment organic matter in wetlands of the oil sands region. It is assumed that organic matter accumulating in OSPM-affected wetlands may adsorb PAHs and naphthenic acids in the water, resulting in a decrease in the toxicity of the constituents in the wetland (Herman *et al.* 1994; Fine Tailings Fundamentals Consortium 1995b; Lai *et al.* 1996; Oil Sands Wetlands Working Group 2000; Leung 2001). It may be necessary to determine if sediment organic content, as hydrocarbon, has an influence on the establishment of macrophytes.

It could also be useful to determine the direct effect of macrophytes on the zoobenthic community. Simultaneous sampling of wetlands of differing ages could be used, as in the present study. A quantitative measure of macrophyte density and diversity should be correlated with invertebrate density and diversity. This would determine if the development of the zoobenthic community followed the same trajectory as for

macrophytes, and if macrophyte presence is one barrier to the development of reference condition zoobenthic community. The effects of macrophytes on the zoobenthic community could be evaluated by examining the zoobenthic development in wetlands with transplanted and volunteer macrophytes.

**Selecting the Most Appropriate Sampling Method**

When selecting a sampling method, it is important to have a clear definition of the objective of the sampling effort, as well as an understanding of the shortcomings of the method chosen (DeShon 1995). Rosenberg & Resh (1982) list several disadvantages of artificial substrates, including incomplete knowledge of colonisation dynamics, long exposure times needed to obtain a sample, loss of fauna on retrieval, and inconvenient use and awkward logistics (cited in DeShon 1995). Benoit *et al.* (1998) examined patterns of lentic macroinvertebrate colonisation on artificial substrates and reported that short time periods (<8 days) were required for sampling to best represent relative abundance. However, no standard exists for the time needed to leave artificial substrates in a wetland. It may be necessary to determine when the maximum diversity of invertebrates is reached on artificial substrates placed in these wetlands and use this as the standard length of time for that sampling method. However, Benoit *et al.* (1998) suggested that their artificial substrate design should give accurate estimates of the relative abundance of benthic invertebrates in the littoral region of lakes. This time frame is acceptable for wetlands. In addition, they suggested that the colonisation period (8 days) is short enough to minimise the effects of succession.

The metric proposed in Chapter 3 is preliminary and should be tested on other wetlands in the region, as well as wetlands with similar environmental stresses, e.g., saline wetlands in north-eastern Alberta, Saskatchewan (Rawson and Moore 1944) or British Columbia (Cannings 1973; Cannings and Scudder 1978). Wetlands located outside of this region will not be subject to naphthenic acids found in the wetlands tested. However, it would be useful to determine if high and low salinity wetlands outside of the oil sands region classified similarly to high and low saline reference wetlands within the region.

**Conclusions**

The restoration of mined areas to similar pre-mining conditions of diversity, abundance of habitat types, and qualities using wetlands is a viable option. Initially, the zoobenthic communities in newly created OSPM-affected wetlands lacked the number of families present in reference wetlands, but comparable richness developed in these wetlands within 7-13 yr. The total number of individuals (abundance) in OSPM-affected wetlands is comparable to reference wetlands after 5-13 years. However, 13-year old wetlands still do not have all of the taxa that characterise older reference wetlands (snails, amphipods, back swimmers, worms). Re-sampling of older "one-time-addition" OSPM-affected wetlands will be necessary to determine if these wetlands will develop as diverse a zoobenthic community as local reference wetlands. Mitigation (or reclamation) projects may require 15 - 20 yr., to judge the success or lack thereof in such projects (Mitsch and Wilson 1996). Alternatively, amending the substrates of OSPM-affected wetlands by adding peat to the substrate, or seeding with macrophytes may speed the rate of community development.

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Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | CTW 2000 | CTW 2001 | CTP 2000 | CTP2001 | SCP |
|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 3 | 15 | 1 | 0 | 282 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 0 | 0 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 0 | 0 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 0 | 0 | 0 | 0 | 0 |
| Valvatidae | MoGaPuVa | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 0 | 0 | 0 | 0 | 0 |
| Daphnia | ArCrClDaDa | 28 | 0 | 0 | 0 | 10 |
| Scapholeberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 1 | 2 | 0 | 7 | 11 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 0 | 0 | 0 |
| Chydoridae | ArCrClDaCh | 0 | 0 | 0 | 0 | 0 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 260 |
| Moinidae | ArCrClMo | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrClSiSi | 0 | 1 | 0 | 0 | 0 |
| Polyphemidae-Polyphemus | ArCrClPoPo | 0 | 0 | 0 | 0 | 0 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 12 | 9 | 3 | 1 | 58 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 0 | 0 | 5 | 30 | 1 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 0 | 1 | 0 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 0 | 2 | 0 | 2 | 0 |
| Agabus | ArInCoDyAg | 0 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCo | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 0 | 2 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 0 | 2 | 0 |
| Potamonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 0 | 0 | 0 | 0 | 1 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 0 | 0 | 0 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 0 | 1 |
| Hydrophilidae-Berosus | ArInCoHyBe | 0 | 0 | 0 | 0 | 0 |
| Gyrindae | ArInCoGy | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 73 | 106 | 45 | 20 | 102 |
| Chironomini | ArInDiChi | 0 | 0 | 6 | 1 | 0 |
| Tanytarsini | ArInDiTa | 3 | 47 | 28 | 12 | 13 |
| Orthocladiinae | ArInDiOr | 43 | 50 | 11 | 4 | 8 |
| Tanypodinae | ArInDiTn | 27 | 9 | 0 | 3 | 81 |
| Anthomyiidae | ArInDiAn | 0 | 0 | 0 | 0 | 0 |
| Ceratopogonidae | ArInDiCe | 1 | 1 | 6 | 7 | 6 |
| Chaoboridae | ArInDiCh | 0 | 0 | 1 | 4 | 0 |
| Dixidae | ArInDiDi | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 0 | 0 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | CTW 2000 | CTW 2001 | CTP 2000 | CTP2001 | SCP |
|---|---|---|---|---|---|---|
| Syrphidae | ArInDiSy | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 |
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 0 | 0 | 0 | 0 |
| Baetidae | ArInEpBa | 0 | 0 | 0 | 0 | 0 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 0 | 0 |
| Cordulidae | ArInOdCo | 0 | 0 | 0 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 0 | 0 | 0 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 0 | 0 | 0 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 0 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 0 | 3 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 3 | 0 |
| Lepidostomatidae | ArInTrLp | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 0 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 0 | 0 | 4 | 4 | 0 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 0 | 0 | 0 | 0 |
| Hydrazoa | | 0 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 1 | 3 | 0 | 1 | 2 |
| Other | | 0 | 0 | 2 | 0 | 4 |
| Indiv/Core | | 119 | 139 | 67 | 80 | 737 |
| Est. Indiv/Core | | 71 | 0 | 0 | 0 | 229 |
| Family/Core | | 5 | 7 | 4 | 10 | 8 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | TP9 | MFTN | TP5 | NW | DP | PP | CRM | SM |
|---|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 15 | 1 | 3 | 0 | 246 | 1 | 48 | 0 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Lymnaeidae | MoGaPuLy | 0 | 0 | 0 | 0 | 7 | 0 | 22 | 0 |
| Physidae | MoGaPuPa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 0 | 0 | 0 | 0 | 2 | 0 | 109 | 0 |
| Valvatidae | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 1 | 0 | 13 | 1 | 15 | 2 | 3 | 0 |
| Daphnia | ArCrClDaDa | 3 | 0 | 0 | 539 | 26 | 22 | 1 | 66 |
| Scapholeberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 1 | 6 | 276 | 203 | 0 | 0 | 903 | 223 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 118 | 0 | 2 | 0 | 107 | 0 |
| Chydoridae | ArCrClDaCh | 0 | 0 | 0 | 14 | 0 | 1 | 59 | 28 |
| Macrothricidae | ArCrClMa | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 |
| Moinidae | ArCrClMo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polyphemidae-Polyphemus | ArCrClPoPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 4 | 5 | 41 | 37 | 144 | 369 | 571 | 105 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 87 | 0 | 56 | 235 | 95 | 0 | 916 | 47 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 2 | 1 | 1 | 1 | 1 | 6 | 1 | 8 |
| Agabus | ArInCoDyAg | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Coptotomus | ArInCoDyCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hygrotus | ArInCoDyHy | 0 | 1 | 1 | 0 | 0 | 4 | 0 | 6 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| Potamonectes | ArInCoDyPo | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Haliplidae | ArInCoHa | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrophilidae-Berosus | ArInCoHyBe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gyrindae | ArInCoGy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 204 | 68 | 234 | 581 | 179 | 54 | 268 | 332 |
| Chironomini | ArInDiChi | 0 | 2 | 0 | 26 | 17 | 3 | 197 | 1 |
| Tanytarsini | ArInDiTa | 55 | 19 | 230 | 410 | 136 | 36 | 0 | 278 |
| Orthocladiinae | ArInDiOr | 149 | 4 | 4 | 33 | 17 | 12 | 55 | 43 |
| Tanypodinae | ArInDiTn | 0 | 43 | 0 | 112 | 9 | 3 | 16 | 10 |
| Anthomyiidae | ArInDiAn | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |
| Ceratopogonidae | ArInDiCe | 0 | 0 | 0 | 0 | 2 | 10 | 0 | 0 |
| Chaoboridae | ArInDiCh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dixidae | ArInDiDi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | TP9 | MFTN | TP5 | NW | DP | PP | CRM | SM |
|---|---|---|---|---|---|---|---|---|---|
| Syrphidae | ArInDiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 1 | 0 | 0 | 0 | 3 | 1 | 0 | 0 |
| Baetidae | ArInEpBa | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cordulidae | ArInOdCo | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 3 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Libellula | ArInOdLiLi | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lepidostomatidae | ArInTrLp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Corixidae | ArInHeCo | 3 | 0 | 1 | 2 | 0 | 0 | 0 | 6 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 0 | 0 | 0 | 2 | 1 | 54 | 0 |
| Hydrazoa | | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 |
| Diptera pupa | | 1 | 1 | 3 | 6 | 0 | 0 | 4 | 5 |
| Other | | 5 | 0 | 1 | 2 | 1 | 0 | 5 | 0 |
| Indiv/Core | | 329 | 77 | 803 | 1621 | 772 | 482 | 3072 | 829 |
| Est. Indiv/Core | | 0 | 0 | 1891.5 | 2043.5 | 1440 | 0 | 1864.25 | 0 |
| Family/Core | | 11 | 5 | 13 | 8 | 18 | 13 | 13 | 12 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | TP2 | SB | HS | SBD | S-Pit | CL | SBBP |
|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 114 | 222 | 57 | 82 | 0 | 31 | 3 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 0 | 2 | 0 | 1 | 3 | 0 | 0 |
| Physidae | MoGaPuPa | 2 | 0 | 0 | 0 | 0 | 0 | 4 |
| Planorbidae | MoGaPuPl | 6 | 0 | 8 | 0 | 1 | 7 | 4 |
| Valvatidae | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 61 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 4 | 0 | 23.75 | 0 | 0 | 0 | 1 |
| Daphnia | ArCrClDaDa | 0 | 8 | 2.75 | 0 | 44 | 63 | 298 |
| Scapholeberis | ArCrClDaSc | 0 | 0 | 0 | 112 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 1 | 0 | 344 | 0 | 9 | 7 | 17 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 451.25 | 0 | 2 | 18 | 0 |
| Chydoridae | ArCrClDaCh | 10 | 0 | 286.25 | 0 | 9 | 1 | 11 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 5 | 2 | 0 |
| Moinidae | ArCrClMo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polyphemidae-Polyphemus | ArCrClPoPo | 0 | 0 | 0 | 22 | 0 | 0 | 0 |
| Calanoida | ArCrCoCa | 4 | 1 | 0 | 11 | 0 | 1 | 0 |
| Cyclopoida | ArCrCoCy | 81 | 18 | 486.25 | 0 | 128 | 35 | 34 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 1 | 52 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 36 | 5 | 333.75 | 164 | 51 | 62 | 30 |
| Hyallela azteca | ArCrAmTaHy | 0 | 50 | 0 | 0 | 0 | 0 | 1 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| Agabus | ArInCoDyAg | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| Potamonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 8 | 2 | 2 | 2 | 0 | 0 | 0 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 4 | 1 | 2 | 0 | 0 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 4 | 1 | 0 | 2 | 0 | 0 | 0 |
| Hydrophilidae-Berosus | ArInCoHyBe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gyrindae | ArInCoGy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 402 | 30 | 233 | 174 | 161 | 46 | 51 |
| Chironomini | ArInDiChi | 78 | 1 | 51 | 124 | 55 | 26 | 6 |
| Tanytarsini | ArInDiTa | 290 | 24 | 29 | 19 | 0 | 2 | 35 |
| Orthocladiinae | ArInDiOr | 12 | 1 | 103 | 10 | 107 | 3 | 0 |
| Tanypodinae | ArInDiTn | 22 | 4 | 50 | 21 | 0 | 2 | 10 |
| Anthomyiidae | ArInDiAn | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceratopogonidae | ArInDiCe | 3 | 5 | 5 | 0 | 0 | 0 | 0 |
| Chaoboridae | ArInDiCh | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Dixidae | ArInDiDi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | | | | | |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | TP2 | SB | HS | SBD | S-Pit | CL | SBBP |
|---|---|---|---|---|---|---|---|---|
| Syrphidae | ArInDiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Baetidae | ArInEpBa | 7 | 0 | 0 | 0 | 3 | 0 | 0 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cordulidae | ArInOdCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Coenagrionidae | ArInOdCe | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mystacides | ArInTrLeMy | 0 | 8 | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lepidostomatidae | ArInTrLp | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phryganeidae | ArInTrPh | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Notonecta | ArInHeNo | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 5 | 0 | 1 | 0 | 0 | 0 | 1 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| Nematoda | | 30 | 2 | 24 | 0 | 0 | 0 | 0 |
| Hydrazoa | | 111 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 25 | 0 | 1 | 0 | 1 | 0 | 0 |
| Other | | 5 | 0 | 3 | 0 | 2 | 0 | 0 |
| Indiv/Core | | 947 | 356 | 2264.75 | 146 | 434 | 273 | 455 |
| Est. Indiv/Core | | 1292.55 | 0 | 4886.25 | 1326.75 | 544.85 | 375.25 | 0 |
| Family/Core | | 19 | 14 | 14 | 13 | 12 | 9 | 11 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | MID | LP | BL | SW | NWID | TP1 | BM | H63W |
|---|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 18 | 120 | 188 | 55 | 19 | 28 | 17 | 15 |
| Hirudinea | AnOlHi | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Lymnaeidae | MoGaPuLy | 7 | 1 | 0 | 0 | 1 | 3 | 0 | 0 |
| Physidae | MoGaPuPa | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 2 |
| Planorbidae | MoGaPuPl | 9 | 0 | 4 | 0 | 2 | 15 | 0 | 13 |
| Valvatidae | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 4 |
| Hydrachnida | ArArAcHy | 0 | 1 | 1 | 1 | 2 | 2 | 0 | 0 |
| Daphnia | ArCrClDaDa | 42 | 0 | 59 | 93 | 0 | 0 | 5 | 64 |
| Scapholeberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 7 | 1 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 15 | 0 | 29 | 33 | 0 | 8 | 2 | 53 |
| Ceriodaphnia | ArCrClDaCe | 0 | 3 | 0 | 62 | 0 | 0 | 0 | 0 |
| Chydoridae | ArCrClDaCh | 6 | 0 | 23 | 38 | 0 | 20 | 1 | 2 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Moinidae | ArCrClMo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 21 |
| Polyphemidae-Polyphemus | ArCrClPoPo | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 10 | 12 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 21 | 46 | 34 | 281 | 0 | 34 | 18 | 62 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 0 | 0 | 54 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 0 | 2 | 5 | 84 | 0 | 33 | 0 | 0 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 64 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 1 | 43 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 0 | 3 | 5 | 7 | 4 | 0 | 1 | 0 |
| Agabus | ArInCoDyAg | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCo | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 0 | 0 | 1 | 6 | 0 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| Potamonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 0 | 0 | 2 | 1 | 1 | 0 | 2 | 1 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 |
| Haliplus | ArInCoHaHa | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Hydrophilidae-Berosus | ArInCoHyBe | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gyrindae | ArInCoGy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 175 | 128 | 125 | 441 | 81 | 91 | 62 | 35 |
| Chironomini | ArInDiChi | 60 | 16 | 56 | 123 | 13 | 17 | 5 | 5 |
| Tanytarsini | ArInDiTa | 106 | 50 | 19 | 162 | 28 | 74 | 24 | 8 |
| Orthocladiinae | ArInDiOr | 3 | 38 | 45 | 70 | 28 | 0 | 20 | 12 |
| Tanypodinae | ArInDiTn | 6 | 24 | 5 | 86 | 12 | 0 | 13 | 10 |
| Anthomyiidae | ArInDiAn | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceratopogonidae | ArInDiCe | 0 | 3 | 0 | 4 | 4 | 1 | 0 | 0 |
| Chaoboridae | ArInDiCh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dixidae | ArInDiDi | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | MID | LP | BL | SW | NWID | TP1 | BM | H63W |
|---|---|---|---|---|---|---|---|---|---|
| Syrphidae | ArInDiSy | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 0 | 0 | 2 | 0 | 0 | 3 | 5 |
| Baetidae | ArInEpBa | 0 | 0 | 0 | 11 | 14 | 2 | 0 | 1 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cordulidae | ArInOdCo | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 0 | 1 | 1 | 8 | 0 | 0 | 2 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 1 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 3 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 3 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lepidostomatidae | ArInTrLp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Corixidae | ArInHeCo | 5 | 2 | 6 | 1 | 0 | 1 | 2 | 1 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 3 | 12 | 1 | 1 | 6 | 0 | 2 |
| Hydrazoa | | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 0 |
| Diptera pupa | | 5 | 5 | 1 | 5 | 0 | 1 | 4 | 1 |
| Other | | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 |
| Indiv/Core | | 304 | 318 | 502 | 1154 | 274 | 263 | 126 | 357 |
| Est. Indiv/Core | | 0 | 515.75 | 0 | 2011.75 | 0 | 91.75 | 0 | 0 |
| Family/Core | | 9 | 12 | 16 | 20 | 24 | 16 | 14 | 23 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | WID | LLW | H63I | TR0.8R | TR6.8R |
|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 38 | 97 | 31 | 5 | 24 |
| Hirudinea | AnOlHi | 0 | 2 | 1 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 0 | 15 | 2 | 0 | 0 |
| Physidae | MoGaPuPa | 0 | 0 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 4 | 1 | 0 | 0 | 0 |
| Valvatidae | MoGaPuVa | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 26 | 18 | 0 | 1 | 4 |
| Hydrachnida | ArArAcHy | 0 | 0 | 5 | 5 | 3 |
| Daphnia | ArCrClDaDa | 19 | 7 | 0 | 4 | 245 |
| Scapholeberis | ArCrClDaSc | 0 | 3 | 19 | 0 | 11 |
| Simocephalus | ArCrClDaSi | 0 | 44 | 0 | 0 | 0 |
| Ceriodaphnia | ArCrClDaCe | 4 | 0 | 0 | 0 | 0 |
| Chydoridae | ArCrClDaCh | 7 | 11 | 0 | 3 | 95 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 |
| Moinidae | ArCrClMo | 0 | 3 | 0 | 0 | 0 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 1 | 0 |
| Polyphemidae-Polyphemus | ArCrClPoPo | 0 | 0 | 24 | 0 | 11 |
| Calanoida | ArCrCoCa | 0 | 0 | 129 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 1 | 19 | 73 | 27 | 139 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 23 | 0 | 0 |
| Ostracoda | ArCrOs | 207 | 7 | 96 | 0 | 4 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 0 | 0 | 5 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 5 | 0 | 0 |
| Dytiscidae | ArInCoDy | 2 | 3 | 0 | 2 | 0 |
| Agabus | ArInCoDyAg | 0 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCo | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 2 | 1 | 0 | 2 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 2 | 0 | 0 | 0 |
| Potamonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 0 | 1 | 2 | 0 | 0 |
| Brychius | ArInCoHaBr | 0 | 0 | 1 | 0 | 0 |
| Haliplus | ArInCoHaHa | 0 | 0 | 0 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 1 | 1 | 0 | 0 |
| Hydrophilidae-Berosus | ArInCoHyBe | 0 | 0 | 0 | 0 | 0 |
| Gyrindae | ArInCoGy | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 17 | 270 | 66 | 35 | 38 |
| Chironomini | ArInDiChi | 0 | 73 | 10 | 2 | 13 |
| Tanytarsini | ArInDiTa | 0 | 187 | 32 | 28 | 2 |
| Orthocladiinae | ArInDiOr | 17 | 0 | 9 | 4 | 15 |
| Tanypodinae | ArInDiTn | 0 | 10 | 15 | 1 | 8 |
| Anthomyiidae | ArInDiAn | 0 | 0 | 0 | 0 | 0 |
| Ceratopogonidae | ArInDiCe | 1 | 0 | 0 | 0 | 0 |
| Chaoboridae | ArInDiCh | 0 | 0 | 0 | 0 | 0 |
| Dixidae | ArInDiDi | 0 | 0 | 0 | 3 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 0 | 0 |

Appenidx A.1.A. Raw zoobenthic data collected from 33 wetlands using core samples.

| Taxon | Code | WID | LLW | H63I | TR0.8R | TR6.8R |
|---|---|---|---|---|---|---|
| Syrphidae | ArInDiSy | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 2 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 1 | 0 |
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 0 | 0 | 0 | 4 |
| Baetidae | ArInEpBa | 0 | 0 | 1 | 1 | 1 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 0 | 0 |
| Cordulidae | ArInOdCo | 2 | 0 | 0 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 2 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 1 | 2 | 0 | 1 | 0 |
| Leucorrhinia | ArInOdLiLe | 1 | 2 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 0 | 1 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 0 | 0 | 0 |
| Coenagrionidae | ArInOdCe | 0 | 1 | 2 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 1 | 0 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 2 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 1 | 0 | 0 | 2 |
| Ceraclea | ArInTrLeCe | 0 | 1 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 2 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 0 | 0 |
| Lepidostomatidae | ArInTrLp | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 1 | 0 |
| Phryganeidae | ArInTrPh | 0 | 0 | 1 | 0 | 0 |
| Notonecta | ArInHeNo | 0 | 1 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 0 | 1 | 0 | 0 | 0 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 84 | 2 | 0 | 3 | 9 |
| Hydrazoa | | 0 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 1 | 10 | 1 | 8 | 11 |
| Other | | 3 | 2 | 1 | 1 | 1 |
| Indiv/Core | | 417 | 506 | 482 | 102 | 607 |
| Est. Indiv/Core | | 264.6875 | 735.25 | 774.125 | 0 | 0 |
| Family/Core | | 12 | 20 | 16 | 15 | 13 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | CTW 2000 | CTW2001 | CTP 2000 | CTP 2001 | DP |
|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 173 | 0 | 0 | 0 | 289 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 0 | 54 |
| Valvatidae -V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 0 | 0 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 0 | 0 | 0 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 0 | 1 |
| Hydrachnida | ArArAcHy | 0 | 0 | 0 | 0 | 41 |
| Daphnidae | ArCrClDa | 174 | 16 | 0 | 0 | 11 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 0 | 0 | 0 |
| Daphnia | ArCrClDaDa | 169 | 16 | 0 | 0 | 0 |
| Scaphloberus | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 5 | 0 | 0 | 0 | 11 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 0 | 0 |
| Chydoridae | ArCrClDaCh | 0 | 0 | 0 | 0 | 1 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 |
| Polyphemidae | ArCrClPo | 0 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 37 | 3 | 4 | 0 | 96 |
| Calanoidaa | ArCrCoCa | 0 | 0 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 47 | 3 | 8 | 0 | 125 |
| Ostracoda | ArCrOs | 0 | 0 | 18 | 14 | 252 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 0 | 0 | 73 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 4 |
| Dytiscidae | ArInCoDy | 2 | 5 | 3 | 0 | 10 |
| Agabus | ArInCoDyAg | 0 | 2 | 0 | 0 | 0 |
| Colymbetes | ArInCoDyCo | 0 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCp | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 |
| Hydroporus | ArInCoDyHd | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 0 | 0 | 0 | 0 | 1 |
| Ilybius | ArInCoDyIl | 0 | 2 | 0 | 0 | 0 |
| Liodessus | ArInCoDyLi | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 3 | 0 | 2 |
| Potomonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 5 |
| Rhantus | ArInCoDyRh | 2 | 1 | 0 | 0 | 1 |
| Haliplidae | ArInCoHa | 0 | 0 | 0 | 0 | 4 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 0 | 0 | 0 | 0 | 2 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 0 | 2 |
| Gyrinidae-Gyrinus | ArInCoGy | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 115 | 460 | 49 | 11 | 90 |
| Chironomini | ArInDiChi | 0 | 22 | 3 | 0 | 15 |
| Tanytarsini | ArInDiTa | 4 | 140 | 26 | 8 | 61 |
| Orthocladiinae | ArInDiOr | 82 | 236 | 11 | 2 | 5 |
| Tanypodinae | ArInDiTn | 29 | 62 | 9 | 1 | 9 |
| Ceratopoginidae | ArInDiCe | 8 | 0 | 17 | 0 | 16 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 2 | 0 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 0 | 0 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | CTW 2000 | CTW2001 | CTP 2000 | CTP 2001 | DP |
|---|---|---|---|---|---|---|
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 1 | 0 | 0 | 23 |
| Baetidae | ArInEpBa | 0 | 0 | 0 | 0 | 6 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 0 | 0 | 1 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 0 | 0 |
| Aeshna | ArInOdAeAe | 0 | 0 | 0 | 0 | 0 |
| Anax | ArInOdAeAn | 0 | 0 | 0 | 0 | 0 |
| Corduliidae | ArInOdCo | 0 | 0 | 0 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 |
| Gomphidae | ArInOdGo | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 1 | 0 | 0 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 1 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 3 | 8 | 0 | 0 | 0 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 0 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 0 | 15 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae | ArInTrHy | 0 | 0 | 0 | 0 | 0 |
| Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 |
| Agraylea | ArInTrHyAg | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 2 | 2 | 51 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 2 | 32 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 1 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 2 | 0 | 18 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 0 | 0 | 0 | 5 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 0 | 2 | 2 | 0 | 1 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 |
| Hydrozoa | | 0 | 0 | 0 | 0 | 8 |
| Other | | 0 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 7 | 6 | 3 | 0 | 3 |
| Trichoptera pupa | | 0 | 0 | 0 | 0 | 0 |
| Total Indiv | | 791 | 502 | 157 | 27 | 1612 |
| Total Famil | | 8 | 7 | 7 | 3 | 20 |
| Est. Total Ind. | | 681 | 0 | 0 | 0 | 1386 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | TP9 | MFTN | TP5 | TP2 | NW | SCP | PP | CRM |
|---|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 27 | 0 | 4 | 89 | 0 | 455 | 2 | 11 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 6 |
| Valvatidae -V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 0 | 4 | 0 | 1 | 0 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 |
| Lymnaeidae | MoGaPuLy | 34 | 6 | 0 | 0 | 0 | 0 | 0 | 40 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 1317 | 0 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 0 | 0 | 5 | 19 | 6 | 0 | 0 | 39 |
| Daphnidae | ArCrClDa | 11 | 70 | 18 | 1 | 377 | 66 | 16 | 106 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 10 | 0 | 1 | 0 | 0 | 35 |
| Daphnia | ArCrClDaDa | 9 | 0 | 0 | 1 | 125 | 66 | 16 | 0 |
| Scapholeberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 2 | 70 | 8 | 0 | 251 | 0 | 0 | 71 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chydoridae | ArCrClDaCh | 0 | 0 | 0 | 3 | 41 | 0 | 0 | 591 |
| Macrothricidae | ArCrClMa | 0 | 0 | 17 | 1 | 0 | 275 | 0 | 0 |
| Polyphemidae | ArCrClPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 8 | 6 | 12 | 112 | 48 | 185 | 0 | 161 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 8 | 6 | 12 | 87 | 36 | 185 | 183 | 364 |
| Ostracoda | ArCrOs | 129 | 0 | 205 | 32 | 379 | 0 | 0 | 471 |
| Hyallela azteca | ArCrAmTaHy | 3 | 0 | 2 | 23 | 0 | 0 | 0 | 0 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 3 | 5 | 3 | 37 | 3 | 2 | 3 | 18 |
| Agabus | ArInCoDyAg | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| Colymbetes | ArInCoDyCo | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydroporus | ArInCoDyHd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 1 | 1 | 1 | 13 | 1 | 1 | 3 | 4 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Liodessus | ArInCoDyLi | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| Potomonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 4 | 1 | 1 | 27 | 0 | 1 | 0 | 9 |
| Brychius | ArInCoHaBr | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 3 | 0 | 1 | 7 | 0 | 1 | 0 | 1 |
| Peltodytes | ArInCoHaPe | 1 | 0 | 0 | 20 | 0 | 0 | 0 | 8 |
| Gyrinidae-Gyrinus | ArInCoGy | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Chironomidae | ArInDiCh | 59 | 403 | 31 | 62 | 72 | 111 | 74 | 149 |
| Chironomini | ArInDiChi | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 35 |
| Tanytarsini | ArInDiTa | 29 | 295 | 28 | 41 | 34 | 10 | 36 | 33 |
| Orthocladiinae | ArInDiOr | 1 | 60 | 2 | 13 | 14 | 10 | 31 | 31 |
| Tanypodinae | ArInDiTn | 29 | 48 | 1 | 3 | 23 | 91 | 7 | 50 |
| Ceratopoginidae | ArInDiCe | 5 | 0 | 8 | 40 | 0 | 6 | 0 | 31 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 13 | 0 | 0 | 1 | 0 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | TP9 | MFTN | TP5 | TP2 | NW | SCP | PP | CRM |
|---|---|---|---|---|---|---|---|---|---|
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 |
| Tipulidae | ArInDiTi | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| Baetidae | ArInEpBa | 0 | 0 | 6 | 8 | 0 | 0 | 0 | 0 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Aeshna | ArInOdAeAe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Anax | ArInOdAeAn | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Corduliidae | ArInOdCo | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 3 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gomphidae | ArInOdGo | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 4 |
| Coenagrionidae | ArInOdCe | 0 | 15 | 0 | 8 | 2 | 0 | 0 | 2 |
| Enallagma | ArInOdCeEn | 0 | 15 | 1 | 8 | 5 | 1 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae | ArInTrHy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Agraylea | ArInTrHyAg | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 0 | 0 | 147 | 0 | 4 | 0 | 0 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 0 | 1 | 0 | 2 | 1 | 10 | 0 | 0 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrozoa | | 0 | 0 | 0 | 117 | 0 | 0 | 0 | 0 |
| Other | | 0 | 1 | 4 | 3 | 1 | 6 | 0 | 2 |
| Diptera pupa | | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 2 |
| Trichoptera pupa | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total Indiv | | 711 | 521 | 543 | 3488 | 2110 | 1578 | 281 | 2042 |
| Total Famil | | 14 | 11 | 11 | 17 | 20 | 15 | 8 | 18 |
| Est. Total Ind. | | 1018 | 0 | 1311 | 2305 | 3015 | 2479 | 0 | 3996 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | SM | SB | HS | SBD | S-PIT | CL | SBBP |
|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 0 | 18 | 143 | 26 | 0 | 107 | 86 |
| Hirudinea | AnOlHi | 0 | 1 | 6 | 1 | 0 | 2 | 0 |
| Valvatidae -V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 0 | 2 | 0 | 2 | 0 | 0 | 18 |
| Planorbidae | MoGaPuPl | 0 | 0 | 14 | 23 | 1 | 1 | 1 |
| Lymnaeidae | MoGaPuLy | 0 | 4 | 16 | 0 | 8 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 0 | 16 | 0 | 0 |
| Hydrachnida | ArArAcHy | 0 | 5 | 2 | 1 | 10 | 0 | 2 |
| Daphnidae | ArCrClDa | 48 | 20 | 407 | 56 | 35 | 34 | 214 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 252 | 0 | 0 | 12 | 0 |
| Daphnia | ArCrClDaDa | 30 | 20 | 1 | 44 | 27 | 19 | 213 |
| Scapholeberis | ArCrClDaSc | 0 | 0 | 58 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 18 | 0 | 96 | 12 | 8 | 3 | 1 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chydoridae | ArCrClDaCh | 33 | 0 | 79 | 18 | 14 | 0 | 0 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Polyphemidae | ArCrClPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 29 | 24 | 350 | 50 | 82 | 110 | 54 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Cyclopoida | ArCrCoCy | 29 | 24 | 350 | 50 | 82 | 109 | 54 |
| Ostracoda | ArCrOs | 112 | 3 | 609 | 103 | 140 | 443 | 161 |
| Hyallela azteca | ArCrAmTaHy | 0 | 74 | 0 | 10 | 47 | 2 | 3 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Dytiscidae | ArInCoDy | 1 | 0 | 6 | 3 | 6 | 2 | 3 |
| Agabus | ArInCoDyAg | 0 | 0 | 3 | 1 | 0 | 0 | 1 |
| Colymbetes | ArInCoDyCo | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCp | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hydroporus | ArInCoDyHd | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 0 | 0 | 2 | 0 | 5 | 2 | 1 |
| Ilybius | ArInCoDyIl | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Liodessus | ArInCoDyLi | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Potomonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 0 | 0 | 12 | 0 | 0 | 0 | 7 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 0 | 0 | 11 | 0 | 0 | 0 | 6 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Gyrinidae-Gyrinus | ArInCoGy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Chironomidae | ArInDiCh | 285 | 21 | 62 | 42 | 53 | 111 | 402 |
| Chironomini | ArInDiChi | 4 | 8 | 8 | 13 | 18 | 79 | 131 |
| Tanytarsini | ArInDiTa | 205 | 3 | 17 | 16 | 20 | 8 | 231 |
| Orthocladiinae | ArInDiOr | 71 | 4 | 24 | 7 | 13 | 20 | 19 |
| Tanypodinae | ArInDiTn | 5 | 6 | 13 | 6 | 2 | 4 | 21 |
| Ceratopoginidae | ArInDiCe | 0 | 5 | 27 | 0 | 0 | 1 | 0 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | SM | SB | HS | SBD | S-PIT | CL | SBBP |
|---|---|---|---|---|---|---|---|---|
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 4 | 0 | 0 | 2 | 0 | 2 |
| Baetidae | ArInEpBa | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aeshnidae | ArInOdAe | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Aeshna | ArInOdAeAe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anax | ArInOdAeAn | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Corduliidae | ArInOdCo | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gomphidae | ArInOdGo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| Coenagrionidae | ArInOdCe | 0 | 18 | 0 | 0 | 1 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 18 | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae | ArInTrHy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Agraylea | ArInTrHyAg | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 11 | 0 | 0 | 1 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mystacides | ArInTrLeMy | 0 | 11 | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Nematoda | | 0 | 0 | 70 | 0 | 0 | 14 | 0 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 0 | 0 | 3 | 4 | 4 | 0 | 4 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hydrozoa | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 3 | 0 | 0 | 0 | 0 | 0 | 9 |
| Trichoptera pupa | | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Total Indiv | | 512 | 210 | 2363 | 344 | 992 | 1480 | 422 |
| Total Famil | | 13 | 13 | 21 | 16 | 24 | 11 | 10 |
| Est. Total Ind. | | 0 | 0 | 5677 | 0 | 1285 | 2023 | 0 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | MID | LP | BL | SW | NWID | TP1 | BM | H63W |
|---|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 12 | 230 | 0 | 153 | 37 | 0 | 87 | 29 |
| Hirudinea | AnOlHi | 1 | 0 | 0 | 4 | 3 | 5 | 0 | 2 |
| Valvatidae -V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 |
| Physidae | MoGaPuPh | 2 | 0 | 14 | 1 | 1 | 0 | 1 | 1 |
| Planorbidae | MoGaPuPl | 28 | 0 | 0 | 2 | 0 | 16 | 2 | 0 |
| Lymnaeidae | MoGaPuLy | 6 | 14 | 49 | 4 | 17 | 1 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 0 | 0 | 7 | 2 | 9 | 27 | 12 | 7 |
| Daphnidae | ArCrClDa | 8 | 21 | 135 | 247 | 6 | 30 | 37 | 258 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 0 | 87 | 0 | 0 | 29 | 0 |
| Daphnia | ArCrClDaDa | 3 | 0 | 0 | 89 | 0 | 0 | 0 | 258 |
| Scapholeberis | ArCrClDaSc | 0 | 11 | 0 | 0 | 0 | 27 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 5 | 10 | 135 | 71 | 6 | 3 | 8 | 0 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Chydoridae | ArCrClDaCh | 0 | 2 | 0 | 105 | 2 | 104 | 15 | 4 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polyphemidae | ArCrClPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 4 | 69 | 114 | 271 | 25 | 52 | 13 | 17 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 21 | 0 | 2 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 4 | 46 | 114 | 250 | 25 | 50 | 13 | 17 |
| Ostracoda | ArCrOs | 0 | 2 | 28 | 479 | 7 | 74 | 0 | 0 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 0 | 19 | 10 | 2 | 43 | 39 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 |
| Dytiscidae | ArInCoDy | 0 | 11 | 3 | 9 | 0 | 5 | 1 | 0 |
| Agabus | ArInCoDyAg | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 |
| Colymbetes | ArInCoDyCo | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydroporus | ArInCoDyHd | 0 | 1 | 0 | 0 | 0 | 3 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 0 | 3 | 0 | 6 | 0 | 2 | 1 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Liodessus | ArInCoDyLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Potomonectes | ArInCoDyPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 1 | 0 | 3 | 8 | 3 | 4 | 1 | 2 |
| Brychius | ArInCoHaBr | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 0 | 0 | 3 | 4 | 0 | 1 | 1 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 4 | 3 | 3 | 0 | 2 |
| Gyrinidae-Gyrinus | ArInCoGy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 133 | 38 | 135 | 59 | 175 | 47 | 231 | 128 |
| Chironomini | ArInDiChi | 46 | 1 | 19 | 16 | 25 | 1 | 29 | 44 |
| Tanytarsini | ArInDiTa | 69 | 25 | 20 | 29 | 77 | 39 | 106 | 28 |
| Orthocladiinae | ArInDiOr | 7 | 11 | 93 | 3 | 61 | 6 | 72 | 41 |
| Tanypodinae | ArInDiTn | 11 | 1 | 3 | 11 | 12 | 1 | 24 | 15 |
| Ceratopoginidae | ArInDiCe | 0 | 5 | 0 | 26 | 0 | 75 | 0 | 1 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | MID | LP | BL | SW | NWID | TP1 | BM | H63W |
|---|---|---|---|---|---|---|---|---|---|
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 1 | 0 | 7 | 15 | 0 | 1 | 5 |
| Baetidae | ArInEpBa | 0 | 0 | 0 | 6 | 0 | 3 | 1 | 0 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 0 | 2 | 0 | 3 | 1 | 0 |
| Aeshnidae | ArInOdAe | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aeshna | ArInOdAeAe | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 |
| Anax | ArInOdAeAn | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Corduliidae | ArInOdCo | 0 | 0 | 0 | 2 | 0 | 10 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 1 | 0 | 10 | 0 | 0 |
| Gomphidae | ArInOdGo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 0 | 4 | 6 | 2 | 3 | 8 | 12 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 1 | 5 | 0 | 0 | 8 | 12 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 3 | 0 | 2 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 9 |
| Coenagrionidae | ArInOdCe | 7 | 0 | 3 | 0 | 10 | 0 | 9 | 1 |
| Enallagma | ArInOdCeEn | 7 | 0 | 3 | 3 | 4 | 2 | 9 | 1 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| Hydroptilidae | ArInTrHy | 0 | 0 | 0 | 6 | 1 | 0 | 0 | 0 |
| Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Agraylea | ArInTrHyAg | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 0 | 6 | 1 | 0 | 12 | 1 | 1 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Corixidae | ArInHeCo | 0 | 5 | 0 | 8 | 0 | 0 | 1 | 1 |
| Gerridae | ArInHeGe | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hydrozoa | | 0 | 2 | 0 | 0 | 0 | 15 | 1 | 0 |
| Other | | 0 | 28 | 1 | 2 | 0 | 12 | 1 | 1 |
| Diptera pupa | | 2 | 0 | 2 | 0 | 7 | 0 | 9 | 2 |
| Trichoptera pupa | | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Total Indiv | | 204 | 821 | 505 | 2358 | 332 | 927 | 477 | 393 |
| Total Famil | | 10 | 13 | 13 | 31 | 18 | 19 | 21 | 20 |
| Est. Total Ind. | | 0 | 1131 | 0 | 3759 | 0 | 1375 | 0 | 0 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | WID | LLW | H63I | TR6.8R | TR0.8R |
|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 275 | 199 | 0 | 17 | 4 |
| Hirudinea | AnOlHi | 1 | 1 | 5 | 0 | 0 |
| Valvatidae -V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 1 | 0 | 1 | 0 | 0 |
| Planorbidae | MoGaPuPl | 34 | 0 | 31 | 2 | 4 |
| Lymnaeidae | MoGaPuLy | 1 | 11 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 220 | 5 | 8 | 0 | 10 |
| Hydrachnida | ArArAcHy | 0 | 1 | 0 | 1 | 0 |
| Daphnidae | ArCrClDa | 3 | 23 | 2 | 1 | 135 |
| Ceriodaphnia | ArCrClDaCe | 1 | 0 | 0 | 0 | 0 |
| Daphnia | ArCrClDaDa | 0 | 0 | 2 | 1 | 113 |
| Scapholeberis | ArCrClDaSc | 0 | 11 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 2 | 12 | 0 | 0 | 22 |
| Sididae-Sida | ArCrClSiSi | 0 | 0 | 0 | 34 | 0 |
| Chydoridae | ArCrClDaCh | 6 | 11 | 5 | 1 | 4 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 |
| Polyphemidae | ArCrClPo | 1 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 1 | 11 | 6 | 7 | 7 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 1 | 4 | 6 | 7 | 7 |
| Ostracoda | ArCrOs | 963 | 50 | 10 | 0 | 9 |
| Hyallela azteca | ArCrAmTaHy | 0 | 7 | 2 | 1 | 12 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 11 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 6 | 4 | 0 | 0 | 0 |
| Agabus | ArInCoDyAg | 1 | 0 | 0 | 0 | 0 |
| Colymbetes | ArInCoDyCo | 0 | 0 | 0 | 0 | 0 |
| Coptotomus | ArInCoDyCp | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 |
| Hydroporus | ArInCoDyHd | 0 | 0 | 0 | 0 | 0 |
| Hygrotus | ArInCoDyHy | 4 | 4 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 |
| Liodessus | ArInCoDyLi | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 0 | 0 | 0 |
| Potomonectes | ArInCoDyPo | 1 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 3 | 1 | 1 | 0 | 1 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 1 |
| Haliplus | ArInCoHaHa | 0 | 0 | 0 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 3 | 1 | 1 | 0 | 0 |
| Gyrinidae-Gyrinus | ArInCoGy | 0 | 0 | 0 | 1 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 37 | 42 | 141 | 64 | 16 |
| Chironomini | ArInDiChi | 5 | 14 | 9 | 6 | 6 |
| Tanytarsini | ArInDiTa | 13 | 23 | 73 | 48 | 3 |
| Orthocladiinae | ArInDiOr | 5 | 4 | 52 | 5 | 5 |
| Tanypodinae | ArInDiTn | 14 | 1 | 7 | 5 | 2 |
| Ceratopoginidae | ArInDiCe | 21 | 3 | 0 | 0 | 1 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 0 | 0 | 0 | 1 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 |
| Empididae | ArInDiEm | 3 | 0 | 0 | 0 | 0 |

Appendix A.1.B. Raw zoobenthic data collected from 33 wetlands using artificial substrates.

| Taxon | Code | WID | LLW | H63I | TR6.8R | TR0.8R |
|---|---|---|---|---|---|---|
| Psychodidae | ArInDiPs | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 6 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 1 | 1 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 1 | 0 | 2 | 5 |
| Baetidae | ArInEpBa | 0 | 2 | 0 | 2 | 3 |
| Callibaetis | ArInEpBaCa | 0 | 1 | 0 | 2 | 3 |
| Cloeon | ArInEpBaCl | 0 | 1 | 0 | 0 | 0 |
| Aeshnidae | ArInOdAe | 0 | 0 | 0 | 0 | 0 |
| Aeshna | ArInOdAeAe | 0 | 0 | 0 | 0 | 0 |
| Anax | ArInOdAeAn | 0 | 0 | 0 | 0 | 0 |
| Corduliidae | ArInOdCo | 1 | 0 | 0 | 0 | 0 |
| Cordulia | ArInOdCoCo | 0 | 0 | 0 | 0 | 0 |
| Somatochlora | ArInOdCoSo | 1 | 0 | 0 | 0 | 0 |
| Gomphidae | ArInOdGo | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 2 | 0 | 0 | 0 | 1 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 0 | 0 | 1 |
| Sympetrum | ArInOdLiSy | 2 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 0 | 0 | 0 | 1 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 2 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 2 | 2 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae | ArInTrHy | 0 | 0 | 0 | 0 | 0 |
| Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 |
| Agraylea | ArInTrHyAg | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 2 | 0 | 0 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 |
| Mystacides | ArInTrLeMy | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 2 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrLeTr | 0 | 0 | 0 | 0 | 0 |
| Phryganeidae | ArInTrPh | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 1 | 0 | 0 |
| Nematoda | | 221 | 3 | 0 | 0 | 0 |
| Notonecta | ArInHeNo | 0 | 0 | 0 | 0 | 1 |
| Corixidae | ArInHeCo | 0 | 0 | 0 | 7 | 4 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 |
| Hydrozoa | | 0 | 12 | 0 | 0 | 0 |
| Other | | 0 | 14 | 0 | 4 | 3 |
| Diptera pupa | | 0 | 0 | 1 | 4 | 0 |
| Trichoptera pupa | | 0 | 0 | 0 | 0 | 0 |
| Total Indiv | | 1818 | 767 | 217 | 148 | 222 |
| Total Famil | | 18 | 21 | 14 | 12 | 18 |
| Est. Total Ind. | | 2863 | 1640 | 0 | 0 | 0 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | CTW2000* | CTW2001 | CTP2000 | CTP2001 | SCP |
|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 0 | 0 | 0 | 0 | 111 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 0 | 0 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 0 | 0 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 0 | 1 | 0 | 0 | 0 |
| Valvatidae-V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 0 | 0 | 0 | 0 | 0 |
| Daphnidae | ArCrClDa | 0 | 21 | 0 | 1 | 0 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 0 | 0 | 0 |
| Daphnia | ArCrClDaDa | 0 | 21 | 0 | 0 | 0 |
| Scaphloberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 0 | 0 | 0 | 1 | 0 |
| Chydoridae | ArCrClCh | 0 | 0 | 0 | 0 | 0 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 38 |
| Sididae-Sida | ArCrCSiSi | 0 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 0 | 0 | 0 | 0 | 6 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 0 | 0 | 0 | 0 | 6 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 0 | 0 | 0 | 0 | 0 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 0 | 0 | 0 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 0 | 410 | 320 | 0 | 62 |
| Chironomini | ArInDiChi | 0 | 12 | 5 | 0 | 0 |
| Tanytarsini | ArInDiTa | 0 | 30 | 251 | 0 | 9 |
| Orthocladiinae | ArInDiOr | 0 | 284 | 49 | 0 | 15 |
| Tanypodinae | ArInDiTn | 0 | 84 | 15 | 0 | 38 |
| Ceratopoginidae | ArInDiCe | 0 | 0 | 15 | 0 | 33 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 4 | 0 | 0 |
| Dixidae-Dixella | ArInDiDiDi | 0 | 0 | 0 | 0 | 0 |
| Ephydridae-Ephydra | ArInDiEpEp | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 0 | 0 | 0 | 0 |
| Baetidae | ArInEpBa | 0 | 0 | 0 | 0 | 0 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 0 | 0 |
| Centroptilum | ArInEpBaCe | 0 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 0 | 0 | 0 |
| Siphlonurus | ArInEpBaSi | 0 | 0 | 0 | 0 | 0 |
| Aeshnidae-Aeshna | ArInOdAeAe | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 0 | 3 | 0 | 0 | 0 |
| Libellula | ArInOdLiLi | 0 | 3 | 0 | 0 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 18 | 0 | 0 | 0 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 0 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | CTW2000 | CTW2001 | CTP2000 | CTP2001 | SCP |
|---|---|---|---|---|---|---|
| Corduliidae-Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 |
| Hydropsychidae | ArInTrHd | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae-Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 0 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrTr | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 |
| Polycentropodidae | ArInTrPoPo | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 0 | 8 | 14 | 0 | 4 |
| Acilius | ArInCoDyAc | 0 | 0 | 0 | 0 | 0 |
| Agabus | ArInCoDyAg | 0 | 0 | 0 | 0 | 0 |
| Colymbetes | ArInCoDyCo | 0 | 0 | 2 | 0 | 0 |
| Desmopachria | ArInCoDyDe | 0 | 0 | 0 | 0 | 0 |
| Dystiscus | ArInCoDyDy | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 0 | 0 |
| Hygrotus/Hyrdoporus | ArInCoDyHd/Hy | 0 | 1 | 0 | 0 | 0 |
| Hydaticus | ArInCoDyHa | 0 | 1 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 |
| Lacophilus | ArInCoDyLa | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 12 | 0 | 3 |
| Rhantus | ArInCoDyRh | 0 | 6 | 0 | 0 | 1 |
| Haliplidae | ArInCoHa | 0 | 0 | 0 | 0 | 0 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 0 | 0 | 0 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 0 | 0 |
| Hydrophilidae | ArInCoHaHy | 0 | 0 | 0 | 0 | 0 |
| Ametor | ArInCoHaAm | 0 | 0 | 0 | 0 | 0 |
| Cymbiodyta | ArInCoHaCy | 0 | 0 | 0 | 0 | 0 |
| Laccobius | ArInCoHaLa | 0 | 0 | 0 | 0 | 0 |
| Paracymus | ArInCoHaPa | 0 | 0 | 0 | 0 | 0 |
| Gyrinidae-Gyrinus | ArInCoGyGy | 0 | 0 | 0 | 0 | 0 |
| Scirtidae-Scirtes | ArInCoScSc | 0 | 0 | 0 | 0 | 0 |
| Chrysomelidae | ArInCoCh | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 1 |
| Nematoda | | 0 | 0 | 0 | 0 | 3 |
| Notonectidae | ArInHeNo | 0 | 0 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 0 | 0 | 131 | 3 | 3 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 |
| Hydrazoa | | 0 | 0 | 0 | 0 | 0 |
| Other | | 0 | 1 | 1 | 0 | 0 |
| Trich.pupae | | 0 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 0 | 16 | 12 | 0 | 9 |
| Ttl Est sample | | 0 | 0 | 560 | 0 | 0 |
| Taxa(fam)/Sweep | | 0 | 6 | 9 | 2 | 9 |
| Indiv/Sweep | | Sample missing | 477 | 496 | 4 | 270 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | TP 9 | MFTN | TP5 | TP2 | NW | DP | PP | CRM |
|---|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 7 | 1 | 2 | 130 | 0 | 24 | 4 | 148 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 0 | 2 | 0 | 5 | 0 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 0 | 0 | 0 | 14 | 0 | 7 | 0 | 21 |
| Valvatidae-V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 31 | 0 | 2 | 0 | 0 |
| Hydrachnida | ArArAcHy | 0 | 0 | 107 | 12 | 2 | 8 | 0 | 0 |
| Daphnidae | ArCrClDa | 72 | 130 | 340 | 236 | 411 | 15 | 22 | 669 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Daphnia | ArCrClDaDa | 67 | 0 | 25 | 1 | 58 | 9 | 22 | 37 |
| Scaphloberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 5 | 130 | 315 | 235 | 353 | 5 | 0 | 632 |
| Chydoridae | ArCrClCh | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrCSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 0 | 1 | 28 | 0 | 0 | 15 | 31 | 200 |
| Calanoida | ArCrCoCa | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 0 | 0 | 28 | 0 | 0 | 14 | 31 | 200 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 0 | 0 | 19 | 48 | 35 | 5 | 0 | 296 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 5 | 334 | 0 | 23 | 0 | 0 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Chironomidae | ArInDiCh | 39 | 34 | 426 | 419 | 191 | 21 | 442 | 175 |
| Chironomini | ArInDiChi | 0 | 0 | 7 | 66 | 0 | 2 | 18 | 34 |
| Tanytarsini | ArInDiTa | 26 | 17 | 323 | 265 | 173 | 13 | 345 | 15 |
| Orthocladiinae | ArInDiOr | 3 | 13 | 34 | 66 | 18 | 4 | 58 | 115 |
| Tanypodinae | ArInDiTn | 10 | 4 | 62 | 22 | 0 | 2 | 21 | 11 |
| Ceratopoginidae | ArInDiCe | 1 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chaoboridae-Chaoborus | ArInDiCa | 2 | 0 | 0 | 0 | 0 | 0 | 10 | 4 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 |
| Dixidae-Dixella | ArInDiDiDi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ephydridae-Ephydra | ArInDiEpEp | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 5 | 0 | 3 | 7 | 0 | 26 | 0 | 0 |
| Baetidae | ArInEpBa | 1 | 0 | 0 | 140 | 1 | 1 | 0 | 0 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Centroptilum | ArInEpBaCe | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 0 | 139 | 0 | 0 | 0 | 0 |
| Siphlonurus | ArInEpBaSi | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Aeshnidae-Aeshna | ArInOdAeAe | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 1 |
| Libellulidae | ArInOdLi | 0 | 0 | 9 | 36 | 28 | 0 | 2 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 5 | 0 | 28 | 0 | 2 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 4 | 36 | 0 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 0 | 1 | 5 | 0 | 57 | 0 | 0 | 9 |
| Coenagrionidae | ArInOdCe | 0 | 6 | 11 | 26 | 8 | 8 | 0 | 0 |
| Enallagma | ArInOdCeEn | 0 | 6 | 11 | 1 | 8 | 7 | 0 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 25 | 0 | 1 | 0 | 0 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | TP 9 | MFTN | TP5 | TP2 | NW | DP | PP | CRM |
|---|---|---|---|---|---|---|---|---|---|
| Corduliidae-Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Hydropsychidae | ArInTrHd | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae-Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 9 | 0 | 5 | 7 | 0 | 17 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Oecetis | ArInTrLeOe | 9 | 0 | 5 | 7 | 0 | 2 | 0 | 0 |
| Triaenodes | ArInTrTr | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polycentropodidae | ArInTrPoPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 20 | 4 | 1 | 29 | 0 | 9 | 54 | 14 |
| Acilius | ArInCoDyAc | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Agabus | ArInCoDyAg | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 2 |
| Colymbetes | ArInCoDyCo | 0 | 3 | 1 | 4 | 0 | 2 | 0 | 0 |
| Desmopachria | ArInCoDyDe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dystiscus | ArInCoDyDy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Graphoderus | ArInCoDyGr | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 |
| Hygrotus/Hyrdoporus | ArInCoDyHd/Hy | 0 | 1 | 0 | 0 | 0 | 1 | 42 | 7 |
| Hydaticus | ArInCoDyHa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 |
| Lacophilus | ArInCoDyLa | 1 | 0 | 0 | 3 | 0 | 0 | 1 | 1 |
| Oreodytes | ArInCoDyOr | 19 | 0 | 0 | 0 | 0 | 6 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 4 | 1 | 2 | 11 | 0 | 1 | 0 | 11 |
| Brychius | ArInCoHaBr | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 4 | 0 | 2 | 4 | 0 | 1 | 0 | 11 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| Hydrophilidae | ArInCoHaHy | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Ametor | ArInCoHaAm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cymbiodyta | ArInCoHaCy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Laccobius | ArInCoHaLa | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Paracymus | ArInCoHaPa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gyrinidae-Gyrinus | ArInCoGyGy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Scirtidae-Scirtes | ArInCoScSc | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chrysomelidae | ArInCoCh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 0 |
| Notonectidae | ArInHeNo | 0 | 1 | 10 | 21 | 49 | 0 | 0 | 1 |
| Corixidae | ArInHeCo | 36 | 10 | 18 | 66 | 148 | 4 | 25 | 13 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrazoa | | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 5 |
| Other | | 14 | 0 | 0 | 0 | 9 | 0 | 0 | 6 |
| Trich.pupae | | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 0 | 10 | 5 | 16 | 55 | 0 | 3 | 7 |
| Ttl Est sample | | 0 | 0 | 0 | 0 | 5494 | 0 | 0 | 0 |
| Taxa(fam)/Sweep | | 17 | 11 | 18 | 25 | 12 | 19 | 10 | 17 |
| Indiv/Sweep | | 206 | 201 | 996 | 1650 | 997 | 187 | 606 | 1575 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | SM | SB | HS | SBD* | S-Pit | CL | SBBP |
|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 0 | 11 | 1 | 0 | 1 | 38 | 3 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 0 | 1 | 2 | 1 |
| Lymnaeidae | MoGaPuLy | 0 | 13 | 48 | 0 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 0 | 0 | 18 | 0 | 0 | 0 | 17 |
| Planorbidae | MoGaPuPl | 0 | 1 | 118 | 0 | 0 | 0 | 4 |
| Valvatidae-V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 1 | 0 | 13 | 0 | 3 | 10 | 1 |
| Daphnidae | ArCrClDa | 236 | 10 | 61 | 0 | 269 | 775 | 164 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 12 | 0 | 0 | 605 | 0 |
| Daphnia | ArCrClDaDa | 0 | 0 | 0 | 0 | 234 | 140 | 164 |
| Scaphloberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 236 | 10 | 49 | 0 | 35 | 30 | 0 |
| Chydoridae | ArCrClCh | 0 | 0 | 28 | 0 | 0 | 42 | 0 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrCSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Copepoda | ArCrCo | 1 | 22 | 58 | 0 | 8 | 928 | 0 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 0 | 0 | 13 | 0 |
| Cyclopoida | ArCrCoCy | 1 | 22 | 58 | 0 | 8 | 915 | 0 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 46 | 2 | 167 | 0 | 0 | 4 | 0 |
| Hyallela azteca | ArCrAmTaHy | 0 | 94 | 0 | 0 | 0 | 43 | 0 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 | 10 | 0 |
| Chironomidae | ArInDiCh | 167 | 15 | 92 | 0 | 354 | 405 | 66 |
| Chironomini | ArInDiChi | 6 | 0 | 46 | 0 | 24 | 325 | 31 |
| Tanytarsini | ArInDiTa | 114 | 9 | 15 | 0 | 95 | 18 | 31 |
| Orthocladiinae | ArInDiOr | 41 | 2 | 17 | 0 | 120 | 44 | 2 |
| Tanypodinae | ArInDiTn | 6 | 4 | 13 | 0 | 115 | 18 | 2 |
| Ceratopoginidae | ArInDiCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 0 | 1 | 0 | 22 | 0 | 2 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dixidae-Dixella | ArInDiDiDi | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Ephydridae-Ephydra | ArInDiEpEp | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 1 | 0 | 0 | 3 | 1 | 0 |
| Baetidae | ArInEpBa | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Centroptilum | ArInEpBaCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Siphlonurus | ArInEpBaSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aeshnidae-Aeshna | ArInOdAeAe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Libellulidae | ArInOdLi | 19 | 0 | 0 | 0 | 0 | 1 | 0 |
| Libellula | ArInOdLiLi | 19 | 0 | 0 | 0 | 0 | 1 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 16 | 0 | 8 | 0 | 0 | 0 | 1 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 0 | 0 | 4 | 2 | 0 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 0 | 4 | 2 | 0 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | SM | SB | HS | SBD* | S-Pit | CL | SBBP |
|---|---|---|---|---|---|---|---|---|
| Corduliidae-Somatochlora | ArInOdCoSo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydropsychidae | ArInTrHd | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae-Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Triaenodes | ArInTrTr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polycentropodidae | ArInTrPoPo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 23 | 1 | 4 | 0 | 10 | 10 | 2 |
| Acilius | ArInCoDyAc | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Agabus | ArInCoDyAg | 0 | 1 | 2 | 0 | 0 | 2 | 0 |
| Colymbetes | ArInCoDyCo | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Desmopachria | ArInCoDyDe | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Dystiscus | ArInCoDyDy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Graphoderus | ArInCoDyGr | 1 | 0 | 0 | 0 | 0 | 3 | 0 |
| Hygrotus/Hyrdoporus | ArInCoDyHd/Hy | 5 | 0 | 2 | 0 | 3 | 4 | 0 |
| Hydaticus | ArInCoDyHa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 1 | 0 | 0 | 0 | 5 | 0 | 0 |
| Lacophilus | ArInCoDyLa | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 16 | 0 | 0 | 0 | 1 | 1 | 0 |
| Haliplidae | ArInCoHa | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Hydrophilidae | ArInCoHaHy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ametor | ArInCoHaAm | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cymbiodyta | ArInCoHaCy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Laccobius | ArInCoHaLa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Paracymus | ArInCoHaPa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gyrinidae-Gyrinus | ArInCoGyGy | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Scirtidae-Scirtes | ArInCoScSc | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chrysomelidae | ArInCoCh | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Notonectidae | ArInHeNo | 23 | 0 | 0 | 0 | 6 | 0 | 0 |
| Corixidae | ArInHeCo | 69 | 3 | 0 | 0 | 42 | 17 | 17 |
| Gerridae | ArInHeGe | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydrazoa | | 0 | 0 | 1 | 0 | 0 | 4 | 0 |
| Other | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trich.pupae | | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| Diptera pupa | | 6 | 0 | 5 | 0 | 25 | 7 | 2 |
| Ttl Est sample | | 0 | 0 | 886 | 0 | 0 | 0 | 0 |
| Taxa(fam)/Sweep | | 11 | 13 | 16 | 0 | 14 | 20 | 13 |
| Indiv/Sweep | | 608 | 179 | 624 | 0 | 752 | 2300 | 281 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | MID | LP | BL | SW | NWID | TP1 | BM | H63W |
|---|---|---|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 13 | 34 | 0 | 44 | 15 | 86 | 0 | 0 |
| Hirudinea | AnOlHi | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 19 | 39 | 0 | 18 | 354 | 0 | 0 | 3 |
| Physidae | MoGaPuPh | 0 | 0 | 13 | 9 | 0 | 0 | 1 | 4 |
| Planorbidae | MoGaPuPl | 4 | 0 | 21 | 26 | 2 | 116 | 0 | 0 |
| Valvatidae-V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 |
| Sphaeriidae | MoBiSp | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 0 |
| Hydrachnida | ArArAcHy | 3 | 1 | 0 | 5 | 10 | 24 | 0 | 0 |
| Daphnidae | ArCrClDa | 336 | 3 | 29 | 36 | 8 | 16 | 97 | 85 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Daphnia | ArCrClDaDa | 324 | 3 | 0 | 6 | 8 | 0 | 7 | 0 |
| Scaphloberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 12 | 0 | 29 | 29 | 0 | 15 | 90 | 85 |
| Chydoridae | ArCrClCh | 40 | 0 | 0 | 12 | 0 | 2 | 0 | 3 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrCSiSi | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 26 |
| Copepoda | ArCrCo | 29 | 0 | 0 | 17 | 21 | 5 | 1 | 30 |
| Calanoida | ArCrCoCa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 29 | 0 | 0 | 17 | 21 | 5 | 1 | 30 |
| Harpacticoida | ArCrCoHa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 0 | 0 | 1 | 29 | 0 | 2 | 0 | 0 |
| Hyallela azteca | ArCrAmTaHy | 0 | 0 | 4 | 37 | 90 | 2 | 53 | 0 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 |
| Chironomidae | ArInDiCh | 35 | 101 | 33 | 219 | 167 | 204 | 291 | 18 |
| Chironomini | ArInDiChi | 6 | 17 | 2 | 38 | 10 | 0 | 2 | 6 |
| Tanytarsini | ArInDiTa | 6 | 37 | 4 | 98 | 53 | 138 | 188 | 2 |
| Orthocladiinae | ArInDiOr | 22 | 28 | 24 | 64 | 86 | 60 | 89 | 9 |
| Tanypodinae | ArInDiTn | 1 | 20 | 3 | 19 | 18 | 6 | 12 | 1 |
| Ceratopoginidae | ArInDiCe | 0 | 7 | 1 | 25 | 11 | 0 | 0 | 0 |
| Tipulidae | ArInDiTi | 0 | 2 | 3 | 2 | 0 | 0 | 0 | 0 |
| Chaoboridae-Chaoborus | ArInDiCa | 8 | 12 | 15 | 0 | 0 | 0 | 0 | 0 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dixidae-Dixella | ArInDiDiDi | 0 | 3 | 1 | 0 | 2 | 0 | 0 | 0 |
| Ephydridae-Ephydra | ArInDiEpEp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 0 | 0 | 1 | 7 | 0 | 9 | 2 |
| Baetidae | ArInEpBa | 0 | 51 | 2 | 1 | 7 | 93 | 12 | 0 |
| Callibaetis | ArInEpBaCa | 0 | 51 | 2 | 0 | 0 | 0 | 12 | 0 |
| Centroptilum | ArInEpBaCe | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 0 | 0 | 0 | 1 | 0 | 93 | 0 | 0 |
| Siphlonurus | ArInEpBaSi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Aeshnidae-Aeshna | ArInOdAeAe | 0 | 0 | 0 | 0 | 1 | 5 | 2 | 0 |
| Libellulidae | ArInOdLi | 0 | 0 | 2 | 0 | 6 | 0 | 13 | 0 |
| Libellula | ArInOdLiLi | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 3 | 0 | 13 | 0 |
| Lestidae-Lestes | ArInOdLeLe | 2 | 0 | 3 | 22 | 1 | 0 | 10 | 21 |
| Coenagrionidae | ArInOdCe | 1 | 0 | 1 | 2 | 0 | 0 | 9 | 8 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Ischnura | ArInOdCeIs | 1 | 0 | 1 | 0 | 0 | 0 | 9 | 8 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | MID | LP | BL | SW | NWID | TP1 | BM | H63W |
|---|---|---|---|---|---|---|---|---|---|
| Corduliidae-Somatochlora | ArInOdCoSo | 0 | 1 | 0 | 11 | 0 | 0 | 0 | 0 |
| Hydropsychidae | ArInTrHd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae-Oxyethira | ArInTrHyOx | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 0 | 0 | 0 | 2 | 0 | 3 | 1 | 0 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Triaenodes | ArInTrTr | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 |
| Limnephilidae | ArInTrLi | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Polycentropodidae | ArInTrPoPo | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 6 | 105 | 7 | 37 | 5 | 2 | 0 | 5 |
| Acilius | ArInCoDyAc | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Agabus | ArInCoDyAg | 1 | 0 | 2 | 0 | 0 | 1 | 0 | 2 |
| Colymbetes | ArInCoDyCo | 2 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |
| Desmopachria | ArInCoDyDe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dystiscus | ArInCoDyDy | 0 | 10 | 2 | 0 | 0 | 0 | 0 | 1 |
| Graphoderus | ArInCoDyGr | 1 | 10 | 0 | 0 | 0 | 0 | 0 | 2 |
| Hygrotus/Hyrdoporus | ArInCoDyHd/Hy | 0 | 24 | 1 | 8 | 1 | 1 | 0 | 0 |
| Hydaticus | ArInCoDyHa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 2 | 3 | 0 | 0 | 4 | 0 | 0 | 0 |
| Lacophilus | ArInCoDyLa | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 33 | 2 | 29 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 0 | 0 | 0 | 7 | 5 | 0 | 0 | 2 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 0 | 0 | 0 | 5 | 2 | 0 | 0 | 2 |
| Peltodytes | ArInCoHaPe | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| Hydrophilidae | ArInCoHaHy | 0 | 3 | 4 | 0 | 0 | 0 | 0 | 1 |
| Ametor | ArInCoHaAm | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| Cymbiodyta | ArInCoHaCy | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Laccobius | ArInCoHaLa | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Paracymus | ArInCoHaPa | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gyrinidae-Gyrinus | ArInCoGyGy | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Scirtidae-Scirtes | ArInCoScSc | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chrysomelidae | ArInCoCh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 3 | 0 | 0 | 0 | 2 | 1 | 0 |
| Notonectidae | ArInHeNo | 0 | 8 | 0 | 24 | 6 | 0 | 9 | 6 |
| Corixidae | ArInHeCo | 121 | 15 | 7 | 57 | 6 | 365 | 28 | 4 |
| Gerridae | ArInHeGe | 0 | 2 | 2 | 1 | 3 | 0 | 0 | 1 |
| Hydrazoa | | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 |
| Other | | 0 | 5 | 0 | 15 | 7 | 74 | 1 | 1 |
| Trich.pupae | | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Diptera pupa | | 13 | 0 | 3 | 44 | 10 | 4 | 5 | 2 |
| Ttl Est sample | | 0 | 412 | 0 | 913 | 0 | 774 | 0 | 0 |
| Taxa(fam)/Sweep | | 15 | 30 | 19 | 27 | 24 | 18 | 17 | 19 |
| Indiv/Sweep | | 630 | 401 | 152 | 692 | 741 | 959 | 569 | 271 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | WID | LLW | H63I | TR6.8R | TR0.8R |
|---|---|---|---|---|---|---|
| Oligochaeta | AnOl | 41 | 78 | 0 | 2 | 3 |
| Hirudinea | AnOlHi | 1 | 0 | 0 | 0 | 0 |
| Lymnaeidae | MoGaPuLy | 39 | 14 | 0 | 0 | 0 |
| Physidae | MoGaPuPh | 11 | 15 | 0 | 0 | 0 |
| Planorbidae | MoGaPuPl | 55 | 22 | 0 | 2 | 13 |
| Valvatidae-V. tricarinata | MoGaPuVa | 0 | 0 | 0 | 0 | 0 |
| Sphaeriidae | MoBiSp | 108 | 0 | 0 | 2 | 74 |
| Hydrachnida | ArArAcHy | 4 | 5 | 0 | 2 | 1 |
| Daphnidae | ArCrClDa | 1 | 107 | 0 | 6 | 123 |
| Ceriodaphnia | ArCrClDaCe | 0 | 0 | 0 | 0 | 0 |
| Daphnia | ArCrClDaDa | 0 | 107 | 0 | 0 | 31 |
| Scaphloberis | ArCrClDaSc | 0 | 0 | 0 | 0 | 0 |
| Simocephalus | ArCrClDaSi | 1 | 0 | 0 | 6 | 92 |
| Chydoridae | ArCrClCh | 7 | 0 | 0 | 0 | 49 |
| Macrothricidae | ArCrClMa | 0 | 0 | 0 | 0 | 0 |
| Sididae-Sida | ArCrCSiSi | 0 | 0 | 0 | 3 | 1 |
| Copepoda | ArCrCo | 0 | 3 | 0 | 0 | 4 |
| Calanoida | ArCrCoCa | 0 | 2 | 0 | 0 | 0 |
| Cyclopoida | ArCrCoCy | 0 | 0 | 0 | 0 | 4 |
| Harpacticoida | ArCrCoHa | 0 | 1 | 0 | 0 | 0 |
| Ostracoda | ArCrOs | 104 | 0 | 0 | 0 | 96 |
| Hyallela azteca | ArCrAmTaHy | 0 | 6 | 0 | 0 | 17 |
| Gammarus lacustrus | ArCrAmGaGa | 0 | 5 | 0 | 0 | 0 |
| Chironomidae | ArInDiCh | 6 | 81 | 0 | 100 | 2 |
| Chironomini | ArInDiChi | 2 | 8 | 0 | 5 | 1 |
| Tanytarsini | ArInDiTa | 4 | 73 | 0 | 87 | 1 |
| Orthocladiinae | ArInDiOr | 0 | 0 | 0 | 8 | 0 |
| Tanypodinae | ArInDiTn | 0 | 0 | 0 | 0 | 0 |
| Ceratopoginidae | ArInDiCe | 2 | 0 | 0 | 3 | 3 |
| Tipulidae | ArInDiTi | 0 | 1 | 0 | 0 | 0 |
| Chaoboridae-Chaoborus | ArInDiCa | 0 | 0 | 0 | 0 | 1 |
| Dolichopodidae | ArInDiDo | 0 | 0 | 0 | 0 | 0 |
| Dixidae-Dixella | ArInDiDiDi | 7 | 0 | 0 | 0 | 0 |
| Ephydridae-Ephydra | ArInDiEpEp | 0 | 0 | 0 | 0 | 0 |
| Tabanidae | ArInDiTb | 2 | 0 | 0 | 0 | 0 |
| Caenidae-Caenis | ArInEpCaCa | 0 | 0 | 0 | 13 | 0 |
| Baetidae | ArInEpBa | 17 | 6 | 0 | 5 | 42 |
| Callibaetis | ArInEpBaCa | 0 | 0 | 0 | 5 | 42 |
| Centroptilum | ArInEpBaCe | 0 | 0 | 0 | 0 | 0 |
| Cloeon | ArInEpBaCl | 1 | 6 | 0 | 0 | 0 |
| Siphlonurus | ArInEpBaSi | 16 | 0 | 0 | 0 | 0 |
| Aeshnidae-Aeshna | ArInOdAeAe | 10 | 0 | 0 | 1 | 0 |
| Libellulidae | ArInOdLi | 0 | 1 | 0 | 0 | 7 |
| Libellula | ArInOdLiLi | 0 | 1 | 0 | 0 | 0 |
| Leucorrhinia | ArInOdLiLe | 0 | 0 | 0 | 0 | 0 |
| Sympetrum | ArInOdLiSy | 0 | 0 | 0 | 0 | 7 |
| Lestidae-Lestes | ArInOdLeLe | 39 | 0 | 0 | 0 | 7 |
| Coenagrionidae | ArInOdCe | 0 | 0 | 0 | 1 | 19 |
| Enallagma | ArInOdCeEn | 0 | 0 | 0 | 1 | 14 |
| Ischnura | ArInOdCeIs | 0 | 0 | 0 | 0 | 5 |

Appendix A.1.C. Raw zoobenthic data collected from 31 wetlands using sweep samples.

| Taxon | Code | WID | LLW | H63I | TR6.8R | TR0.8R |
|---|---|---|---|---|---|---|
| Corduliidae-Somatochlora | ArInOdCoSo | 37 | 1 | 0 | 0 | 0 |
| Hydropsychidae | ArInTrHd | 0 | 0 | 0 | 0 | 0 |
| Hydroptilidae-Oxyethira | ArInTrHyOx | 0 | 10 | 0 | 0 | 0 |
| Leptoceridae | ArInTrLe | 2 | 0 | 0 | 3 | 4 |
| Ceraclea | ArInTrLeCe | 0 | 0 | 0 | 0 | 0 |
| Oecetis | ArInTrLeOe | 2 | 0 | 0 | 3 | 4 |
| Triaenodes | ArInTrTr | 0 | 0 | 0 | 0 | 0 |
| Limnephilidae | ArInTrLi | 2 | 0 | 0 | 0 | 1 |
| Polycentropodidae | ArInTrPoPo | 0 | 0 | 0 | 0 | 0 |
| Dytiscidae | ArInCoDy | 8 | 2 | 0 | 1 | 5 |
| Acilius | ArInCoDyAc | 0 | 0 | 0 | 0 | 0 |
| Agabus | ArInCoDyAg | 0 | 1 | 0 | 0 | 0 |
| Colymbetes | ArInCoDyCo | 1 | 0 | 0 | 0 | 3 |
| Desmopachria | ArInCoDyDe | 0 | 0 | 0 | 0 | 0 |
| Dystiscus | ArInCoDyDy | 0 | 0 | 0 | 0 | 2 |
| Graphoderus | ArInCoDyGr | 2 | 0 | 0 | 0 | 0 |
| Hygrotus/Hyrdoporus | ArInCoDyHd/Hy | 5 | 1 | 0 | 1 | 0 |
| Hydaticus | ArInCoDyHa | 0 | 0 | 0 | 0 | 0 |
| Ilybius | ArInCoDyIl | 0 | 0 | 0 | 0 | 0 |
| Lacophilus | ArInCoDyLa | 0 | 0 | 0 | 0 | 0 |
| Oreodytes | ArInCoDyOr | 0 | 0 | 0 | 0 | 0 |
| Rhantus | ArInCoDyRh | 0 | 0 | 0 | 0 | 0 |
| Haliplidae | ArInCoHa | 10 | 1 | 1 | 0 | 0 |
| Brychius | ArInCoHaBr | 0 | 0 | 0 | 0 | 0 |
| Haliplus | ArInCoHaHa | 10 | 0 | 1 | 0 | 0 |
| Peltodytes | ArInCoHaPe | 0 | 1 | 0 | 0 | 0 |
| Hydrophilidae | ArInCoHaHy | 0 | 0 | 0 | 0 | 0 |
| Ametor | ArInCoHaAm | 0 | 0 | 0 | 0 | 0 |
| Cymbiodyta | ArInCoHaCy | 0 | 0 | 0 | 0 | 0 |
| Laccobius | ArInCoHaLa | 0 | 0 | 0 | 0 | 0 |
| Paracymus | ArInCoHaPa | 0 | 0 | 0 | 0 | 0 |
| Gyrinidae-Gyrinus | ArInCoGyGy | 0 | 0 | 0 | 0 | 0 |
| Scirtidae-Scirtes | ArInCoScSc | 0 | 0 | 0 | 0 | 0 |
| Chrysomelidae | ArInCoCh | 1 | 0 | 0 | 0 | 0 |
| Curculionidae | ArInCoCu | 2 | 0 | 0 | 0 | 0 |
| Nematoda | | 0 | 3 | 0 | 13 | 0 |
| Notonectidae | ArInHeNo | 1 | 0 | 0 | 0 | 0 |
| Corixidae | ArInHeCo | 18 | 23 | 1 | 6 | 6 |
| Gerridae | ArInHeGe | 1 | 0 | 0 | 0 | 0 |
| Hydrazoa | | 0 | 0 | 0 | 0 | 0 |
| Other | | 24 | 0 | 0 | 3 | 0 |
| Trich.pupae | | 0 | 0 | 0 | 0 | 0 |
| Diptera pupa | | 6 | 0 | 0 | 6 | 0 |
| Ttl Est sample | | 0 | 0 | 0 | 0 | 0 |
| Taxa(fam)/Sweep | | 31 | 20 | 2 | 16 | 21 |
| Indiv/Sweep | | 542 | 384 | 2 | 169 | 478 |

**Appendix A.2.** Detailed water and sediment chemistry values. Data on naphthenic acids concentration, $NH_3$, $NH_4$, ions, and metals provided by M. MacKinnon. Water and sediment samples processed at Syncrude Canada Ltd. Analytical Research Lab, Edmonton, AB.

| Code | Class | Age in 2001 | Date Const. | Latitude | Longitude | Date Sampled | pH | Cond (uS /cm) | $NH_3$ | N ($NH_4$) | Naphth. Acids | Na | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CTW 2000 | 1 | | 1998 | 56o59.534'N | 111o31.914'W | 000715 | 9.00 | 2500.00 | | <0.01 | 65.24 | 524.00 | 17.10 |
| CTW 2001 | | 3 | | | | 010719 | 8.53 | 2240.00 | BDL | BDL | 61.10 | 489.00 | 15.50 |
| CTP 2000 | 1 | | 1997 | 57o06.734'N | 111o40.874'W | 000720 | 8.63 | 4850.00 | | <0.01 | 45.50 | 1020.00 | 18.90 |
| CTP 2001 | | 5 | | | | 010724 | 8.47 | 5050.00 | | | 58.60 | 1110.00 | 19.40 |
| TP9 | 1 | 9 | 1993 | 57o05.05'N | 111o41.505'W | 010724 | 9.11 | 2550.00 | BDL | BDL | 42.10 | 623.00 | 6.09 |
| DP | 1 | 9 | 1993 | 57o04.917'N | 111o41.322'W | 000720 | 8.98 | 1050.00 | BDL | BDL | 7.20 | 225.00 | 4.40 |
| MFTN | 1 | 10 | 1991 | 56o 59.499'N | 111o 32.052' W | 010718 | 8.62 | 1323.00 | BDL | BDL | 56.90 | 465.00 | 11.80 |
| TP5 | 1 | 13 | 1989 | 57o05.071'N | 111o41.623'W | 010724 | 9.21 | 2720.00 | BDL | BDL | 24.90 | 624.00 | 7.90 |
| TP2 | 1 | 13 | 1989 | 57o05.071'N | 111o41.623'W | 010724 | 8.89 | 695.00 | BDL | BDL | 4.10 | 137.00 | BDL |
| NW | 1 | 15 | 1987 | 56o58.892'N | 111o30.642'W | 010719 | 8.39 | 1779.00 | 0.30 | 0.20 | 57.80 | 470.00 | 14.90 |
| SCP | 1 | 26 | 1975 | 57o06.008'N | 111o38.253'W | 000715 | 8.40 | 2100.00 | | 1.09 | 50.04 | 461.00 | 7.15 |
| PP | 2 | 0 | 2001 | 56o59.611'N | 111o37.49'W | 010719 | 7.01 | 3750.00 | BDL | BDL | 8.70 | 684.00 | 11.50 |
| BL | 2 | 4 | 1996 | 56o59.925'N | 111o56.697'W | 010719 | 7.86 | 768.00 | BDL | BDL | 1.40 | 87.10 | 9.85 |
| CRM | 2 | 6 | 1996 | 56o59.927'N | 111o33.488'W | 000715 | 8.10 | 2000.00 | | <0.01 | 14.88 | 198.00 | 3.00 |
| SM | 2 | 10 | 1992 | 56o59.625'N | 111o32.191'W | 010719 | 8.92 | 1836.00 | BDL | BDL | 7.30 | 156.00 | 17.50 |
| SB | 2 | 14 | 1985 | 56o59.769'N | 111o36.162'W | 010719 | 8.30 | 1970.00 | BDL | BDL | 7.60 | 309.00 | 14.30 |
| HS | 2 | 17 | 1985 | 56o59.837'N | 111o33.291'W | 010717 | 7.92 | 1517.00 | | | 3.80 | 248.00 | 13.60 |
| S-Pit | 2 | 26 | 1975 | 57o06.374'N | 111o38.378'W | 000715 | 10.10 | 1480.00 | | | 12.18 | 321.00 | 3.70 |
| SBD | 2 | 26 | 1975 | 56o59.749'N | 111o34.942'W | 010719 | 7.75 | 1059.00 | BDL | BDL | 3.20 | 136.00 | 7.50 |
| CL | 2 | 29 | 1972 | 56o59.873'N | 111o33.140'W | 010717 | 8.10 | 881.00 | | | 3.60 | 202.00 | 11.60 |
| MID | 3 | 1 | 2000 | 55o44.377'N | 109o28.576'W | 010719 | 7.47 | 281.00 | BDL | BDL | 1.00 | 10.60 | BDL |
| SBBP | 3 | 1 | 2000 | 56o43.997'N | 111o31.446'W | 010719 | 7.96 | 933.00 | BDL | BDL | 1.90 | 55.30 | BDL |
| LP | 3 | 2 | 1999 | * | * | 000715 | 8.20 | 1050.00 | | <0.01 | 2.46 | 46.20 | 3.10 |
| SW | 3 | 8 | 1993 | 57o04.899'N | 111o41.427'W | 000715 | 10.00 | 440.00 | | | 1.43 | 99.00 | 3.10 |
| BM | 3 | 10 | 1991 | 57o11.349'N | 111o35.945'W | 010719 | 8.22 | 285.00 | BDL | BDL | 1.10 | 12.70 | BDL |
| TP1 | 3 | 13 | 1989 | 57o05.102'N | 111o41.623'W | 010724 | 9.04 | 740.00 | BDL | BDL | 1.40 | 74.00 | BDL |
| H63W | 3 | 23 | 1970 | 56o57.246'N | 111o28.373'W | 010719 | 8.3 | 675.0 | BDL | BDL | 1.10 | 72.60 | BDL |
| NWID | 3 | 23 | 1970 | 57o06.705'N | 111o41.467'W | 010719 | 9.1 | 378.0 | BDL | BDL | 1.40 | 52.50 | BDL |
| WID | 3 | 26 | 1975 | 57o05.18'N | 111o41.751'W | 000715 | 8.2 | 600.0 | | | 1.89 | 69.70 | 1.60 |
| LLW | 3 | 28 | 1973 | 56o58.38'N | 111o27.49'W | 000715 | 9.1 | 452.0 | | <0.01 | 1.65 | 56.90 | 2.30 |
| H63I | 3 | 30 | 1971 | 57o07.381'N | 111o37.714'W | 010719 | 7.3 | 565.0 | BDL | BDL | 1.70 | 54.60 | BDL |
| TR6.8R | 3 | 30 | 1971 | 56o45.601'N | 111o34.915'W | 010719 | 7.5 | 176.0 | 1.2 | 0.8 | 1.00 | 13.90 | BDL |
| TR0.8R | 3 | 30 | 1971 | 56o44.747'N | 111o29.717'W | 010719 | 6.8 | 223.0 | BDL | BDL | 1.40 | 21.60 | BDL |
| SL | 2 | ? | Natural | 57o04.263'N | 111o31.193'W | 010723 | 9.0 | 5530 | | | <0.5 | 1090 | BDL |

BDL = below detectable level, blank areas = no measurements taken, * = no latitude/longitude reading available. $CO_3$, $HCO_3$ measured as wt. ppm, naphthenic acids as mg $L^{-1}$.

**Appendix A.2.** Detailed water and sediment chemistry values. Data on naphthenic acids concentration, $NH_3$, $NH_4$, ions, and metals provided by M. MacKinnon. Water and sediment samples processed at Syncrude Canada Ltd. Analytical Research Lab, Edmonton, AB.

| Code | Mg | Ca | F | Cl | $SO_4$ | $CO_3$ | $HCO_3$ | $NO_2$ | $NO_3$ | $PO_4$ | Al | B | Ba | Cd | Co | Cr | Cu | Fe | Li | Mn | Mo | Ni | Pb | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CTW00 | 64.90 | 41.60 | 0.40 | 60.00 | 930.00 | | 588.30 | BDL | BDL | BDL | BDL | 2.36 | 0.04 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 0.40 | BDL | BDL | BDL |
| CTW01 | 56.70 | 44.00 | 1.30 | 51.00 | 760.00 | 40.20 | 667.00 | BDL | BDL | BDL | BDL | 2.79 | BDL | BDL | BDL | BDL | BDL | 0.05 | BDL | BDL | 0.18 | BDL | BDL | 240.0 |
| CTP00 | 28.50 | 40.90 | BDL | 720.0 | 1200.0 | | 242.22 | BDL | BDL | BDL | BDL | 4.47 | 0.06 | BDL | BDL | BDL | BDL | BDL | BDL | 0.39 | 0.14 | BDL | BDL | |
| CTP01 | 31.50 | 44.00 | 0.10 | 650.0 | 1200.0 | 13.80 | 485.00 | BDL | BDL | BDL | BDL | 3.50 | BDL | 44.0 | BDL | BDL | BDL | BDL | BDL | 19.40 | BDL | 31.5 | BDL | BDL |
| TP9 | 11.90 | 11.00 | 2.40 | 270.0 | 230.00 | 78.60 | 727.00 | BDL | BDL | BDL | 0.95 | 2.29 | BDL | BDL | BDL | BDL | BDL | 0.40 | BDL | BDL | BDL | BDL | BDL | 90.60 |
| DP | 17.30 | 18.60 | BDL | 73.00 | 120.00 | 61.80 | 470.36 | BDL | BDL | BDL | 0.15 | 0.91 | 0.02 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | |
| MFTN | 16.20 | 16.40 | 1.80 | 31.00 | 320.00 | 61.80 | 683.00 | BDL | BDL | BDL | 0.71 | 3.23 | 0.03 | BDL | BDL | BDL | BDL | 0.19 | BDL | BDL | 0.33 | BDL | BDL | 140.0 |
| TP5 | 29.90 | 17.90 | 2.00 | 150.0 | 760.00 | 160.00 | 748.00 | BDL | BDL | BDL | 0.10 | 2.13 | 0.01 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | |
| TP2 | 14.40 | 14.70 | 0.37 | 23.00 | 21.00 | 25.80 | 339.00 | BDL | BDL | BDL | BDL | 0.47 | 0.08 | BDL | BDL | BDL | BDL | 1.33 | BDL | 2.13 | BDL | BDL | BDL | 889.0 |
| NW | 14.70 | 24.70 | 2.30 | 65.00 | 210.00 | 37.80 | 914.00 | BDL | BDL | BDL | BDL | 2.93 | 0.09 | BDL | BDL | BDL | BDL | 0.31 | BDL | 0.04 | 0.28 | BDL | BDL | 81.90 |
| SCP | 24.80 | 42.00 | 0.39 | 260.0 | 240.00 | 60.00 | 736.24 | BDL | BDL | BDL | BDL | 1.57 | 0.14 | BDL | BDL | BDL | BDL | 0.14 | BDL | 0.55 | BDL | BDL | BDL | |
| PP | 114.0 | 125.0 | BDL | 80.00 | 2800.0 | 0.00 | 53.70 | BDL | BDL | BDL | BDL | 0.47 | 0.08 | BDL | BDL | BDL | BDL | 1.33 | BDL | 2.13 | BDL | BDL | BDL | 889.0 |
| BL | 31.00 | 73.00 | 0.15 | 6.20 | 240.00 | 0.00 | 281.00 | BDL | BDL | BDL | BDL | 0.29 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 88.70 |
| CRM | 95.50 | 203.0 | BDL | 190.0 | 530.00 | 634.41 | BDL | BDL | BDL | | BDL | 0.09 | BDL | BDL | BDL | BDL | BDL | 0.11 | BDL | 0.70 | BDL | BDL | BDL | |
| SM | 63.40 | 125.0 | BDL | BDL | 1300.0 | 0.00 | 60.40 | BDL | BDL | BDL | BDL | 0.79 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 412.0 |
| SB | 71.60 | 101.0 | BDL | 80.00 | 860.00 | 8.40 | 191.00 | BDL | BDL | BDL | BDL | 0.61 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 0.04 | BDL | BDL | BDL | 308.0 |
| HSW | 69.90 | 97.70 | BDL | BDL | 880.00 | 0.00 | 160.00 | BDL | BDL | BDL | BDL | 0.80 | 0.01 | BDL | BDL | BDL | BDL | 0.23 | BDL | 0.10 | BDL | BDL | BDL | 347.0 |
| S-Pit | 34.40 | 8.83 | BDL | 220.0 | 290.00 | | 312.36 | BDL | BDL | BDL | BDL | 0.55 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | |
| SBD | 56.70 | 77.00 | BDL | 64.00 | 63.00 | 22.20 | 577.00 | BDL | BDL | BDL | BDL | 0.14 | 0.05 | BDL | BDL | BDL | BDL | BDL | BDL | 0.27 | BDL | BDL | BDL | 24.40 |
| CL | 39.00 | 33.50 | 0.11 | 4.70 | 210.00 | 13.80 | 510.00 | | | | BDL | 0.18 | 0.03 | BDL | BDL | BDL | BDL | 0.08 | BDL | 0.07 | BDL | BDL | BDL | 77.50 |
| MID | 10.90 | 42.50 | BDL | 2.00 | BDL | 0.00 | 190.00 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 0.59 | BDL | 0.61 | BDL | BDL | BDL | BDL |
| SBBP | 48.40 | 107.0 | BDL | 16.00 | 35.00 | 0.00 | 598.00 | BDL | BDL | BDL | BDL | 0.07 | 0.09 | BDL | BDL | BDL | BDL | BDL | BDL | 0.29 | BDL | BDL | BDL | 15.10 |
| LP | 40.80 | 164.0 | BDL | 60.00 | 450.00 | | 160.01 | BDL | BDL | BDL | BDL | 0.14 | 0.04 | BDL | BDL | BDL | BDL | 0.02 | BDL | 0.06 | BDL | BDL | BDL | |
| SW | 12.10 | 12.50 | BDL | 19.00 | 19.00 | | 310.25 | BDL | BDL | BDL | BDL | 0.07 | BDL | BDL | BDL | BDL | BDL | 0.10 | BDL | 0.02 | BDL | BDL | BDL | |
| BM | 7.87 | 38.40 | BDL | 40.00 | 11.00 | 0.00 | 93.90 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 4.89 |
| TP1 | 29.10 | 40.70 | BDL | 3.90 | 240.00 | 4.80 | 94.70 | BDL | BDL | BDL | BDL | 0.24 | 0.02 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | |
| H63W | 38.40 | 41.60 | BDL | 94.00 | 33.00 | 17.40 | 261.00 | BDL | BDL | BDL | BDL | 0.128 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 13.1 |
| NWID | 15.90 | 19.40 | BDL | 34.00 | 21.00 | 10.20 | 148.00 | BDL | BDL | BDL | BDL | 0.077 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 9.53 |
| WID | 23.20 | 57.00 | BDL | 8.00 | 190.00 | | 223.94 | BDL | BDL | BDL | BDL | 0.247 | BDL | BDL | BDL | BDL | BDL | 0.068 | BDL | 0.048 | BDL | BDL | BDL | |
| LLW | 16.20 | 25.40 | BDL | 51.00 | 70.00 | | 137.73 | BDL | BDL | BDL | BDL | 0.18 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | |
| H63I | 17.80 | 56.00 | BDL | 68.00 | 26.00 | 0.00 | 214.00 | BDL | BDL | BDL | BDL | BDL | 0.021 | BDL | BDL | BDL | BDL | 0.692 | BDL | 0.078 | BDL | BDL | BDL | 11.6 |
| TR6.8R | 8.67 | 21.70 | BDL | 0.63 | 8.00 | 0.00 | 124.00 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 0.533 | BDL | BDL | BDL | BDL | BDL | 3.73 |
| TR0.8R | 9.71 | 22.50 | BDL | 1.20 | 14.00 | 0.00 | 135.00 | BDL | BDL | BDL | BDL | 0.081 | BDL | BDL | BDL | BDL | BDL | 0.766 | BDL | 0.235 | BDL | BDL | BDL | 6.7 |
| SL | 33 | 61.5 | BDL | 1700 | 140 | 9 | 83.9 | BDL | BDL | BDL | BDL | 0.273 | 0.082 | 61.5 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | 33 | 0.048 | BDL |

BDL = below detectable level, blank areas = no measurements taken, * = no latitude/longitude reading available. $CO_3$, $HCO_3$ measured as wt. ppm, naphthenic acids as mg $L^{-1}$.

**Appendix A.2.** Detailed water and sediment chemistry values. Data on naphthenic acids concentration, $NH_3$, $NH_4$, ions, and metals provided by M. MacKinnon. Water and sediment samples processed at Syncrude Canada Ltd. Analytical Research Lab, Edmonton, AB.

| Code | Sb | Se | Si | Sr | Ti | V | Zn | Zr | Salinity (‰) | Sed. pH | Sed. ORP | Sediment Organic Content (%)LOI | Detritus-Cores (Ln+1, g) | Detritus Art. Subs. (Ln+1, g) | Detritus Sweep (Ln+1, g) | Med. Part. Size (phi) | Macro. Dev. | Area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CTW00 | 163.0 | BDL | BDL | 1.24 | ENA | 0.67 | BDL | BDL | 1.20 | 7.50 | -150.00 | 6.20 | 2.04 | 1.60 | | 3.00 | 1.00 | 2.00 |
| CTW01 | BDL | BDL | 3.87 | 0.71 | BDL | BDL | BDL | BDL | 1.50 | 6.00 | -115.00 | 6.20 | 3.62 | 0.77 | 1.08 | 3.00 | 2.00 | 2.00 |
| CTP00 | BDL | BDL | 2.70 | 1.43 | 0.03 | BDL | BDL | 0.02 | 3.50 | 7.10 | -143.00 | 5.81 | 1.63 | 1.31 | 0.88 | 4.10 | 1.00 | 3.00 |
| CTP01 | 1110 | BDL | BDL | BDL | 428.0 | BDL | BDL | 2.34 | 3.80 | 7.10 | -95.00 | 5.81 | 2.97 | 0.08 | 0.31 | 4.10 | 1.00 | 3.00 |
| TP9 | BDL | BDL | 6.35 | 0.15 | BDL | BDL | BDL | BDL | 2.00 | 8.20 | -170.00 | 9.61 | 1.89 | 2.20 | 0.94 | 3.00 | 1.00 | 2.00 |
| DP | BDL | BDL | 1.20 | 0.28 | 0.03 | BDL | BDL | 0.02 | 0.80 | 7.80 | -84.00 | 3.80 | 2.72 | 2.98 | 1.57 | 4.00 | 1.00 | 4.00 |
| MFTN | BDL | BDL | 2.40 | 0.29 | 0.03 | BDL | BDL | BDL | 1.10 | 8.10 | -117.00 | 4.60 | 1.97 | 2.24 | 0.83 | 4.00 | 3.00 | 4.00 |
| TP5 | BDL | BDL | 1.37 | 0.36 | 0.03 | BDL | BDL | 0.02 | 2.00 | 8.40 | -294.00 | 7.22 | 2.24 | 1.42 | 1.76 | 4.00 | 3.00 | 2.00 |
| TP2 | BDL | BDL | 2.51 | 2.09 | BDL | BDL | BDL | BDL | 0.30 | 7.00 | -153.00 | 3.10 | 2.71 | 2.26 | 2.16 | 2.75 | 3.00 | 2.00 |
| NW | BDL | BDL | 5.35 | 0.53 | 0.03 | BDL | BDL | BDL | 1.20 | 8.10 | -174.00 | 5.30 | 2.30 | 2.95 | 1.76 | 3.50 | 3.00 | 5.00 |
| SCP | BDL | BDL | 5.78 | 0.28 | 0.03 | BDL | BDL | 0.03 | 1.80 | 7.40 | -136.00 | 4.62 | 2.18 | 2.57 | 2.06 | 3.70 | 1.00 | 2.00 |
| PP | BDL | BDL | 2.51 | 2.09 | BDL | BDL | BDL | BDL | 3.00 | 8.30 | 71.00 | 9.05 | 3.91 | 1.81 | 3.70 | 3.40 | 1.00 | 3.00 |
| BL | BDL | BDL | BDL | 0.77 | BDL | BDL | BDL | BDL | 0.30 | 9.60 | -107.00 | 4.45 | 3.09 | 1.56 | 2.18 | 3.70 | 5.00 | 2.00 |
| CRM | BDL | BDL | 8.69 | 0.77 | BDL | BDL | BDL | BDL | 1.70 | 7.80 | -293.00 | 8.99 | 2.64 | 2.04 | 2.06 | 4.00 | 5.00 | 3.00 |
| SM | BDL | BDL | 1.23 | 1.97 | BDL | BDL | BDL | BDL | 1.10 | 7.50 | -32.00 | 16.7 | 3.47 | 1.75 | 2.06 | 4.50 | 2.00 | 4.00 |
| SB | BDL | BDL | 0.85 | 1.23 | BDL | BDL | BDL | BDL | 1.50 | 7.60 | -122.00 | 28.4 | 2.75 | 1.95 | 0.96 | 4.00 | 2.00 | 4.00 |
| HSW | BDL | BDL | 1.77 | 1.41 | BDL | BDL | BDL | BDL | 1.70 | 7.90 | -309.00 | 18.5 | 2.78 | 2.45 | 2.95 | 3.00 | 5.00 | 3.00 |
| S-Pit | BDL | BDL | 1.51 | 0.03 | BDL | BDL | BDL | BDL | 1.30 | 7.90 | -110.00 | 4.59 | 2.90 | 3.35 | 2.57 | 2.00 | 1.00 | 6.00 |
| SBD | BDL | BDL | 4.53 | 0.44 | BDL | BDL | BDL | BDL | 0.90 | 7.50 | -91.00 | 16.3 | 3.19 | 2.79 | | 4.00 | 4.00 | 3.00 |
| CL | BDL | BDL | 1.62 | 0.21 | 0.03 | BDL | BDL | BDL | 0.90 | 7.30 | -289.00 | 12.1 | 2.66 | 3.34 | 2.78 | 4.00 | 3.00 | 6.00 |
| MID | BDL | BDL | 2.46 | 0.10 | BDL | BDL | BDL | BDL | 0.00 | 9.50 | -73.00 | 4.48 | 3.61 | 0.81 | 0.27 | 3.00 | 2.00 | 4.00 |
| SBBP | BDL | BDL | 2.71 | 0.42 | BDL | BDL | BDL | BDL | 0.10 | 8.30 | -129.00 | 8.70 | 3.89 | 1.75 | 0.91 | 2.80 | 2.00 | 3.00 |
| LP | BDL | BDL | 0.39 | 0.41 | BDL | BDL | BDL | BDL | 0.02 | 7.60 | -236.00 | 4.48 | 2.85 | 2.97 | 1.90 | 3.00 | 1.00 | 2.00 |
| SW | BDL | BDL | 0.40 | 0.10 | BDL | BDL | BDL | BDL | 0.00 | 7.70 | -95.00 | 6.80 | 2.90 | 2.53 | 2.03 | 3.00 | 4.00 | 4.00 |
| BMR | BDL | BDL | 0.62 | 0.12 | BDL | BDL | BDL | BDL | 0.00 | 7.50 | -107.00 | 6.10 | 4.10 | 0.42 | 1.23 | 3.00 | 4.00 | 4.00 |
| TP1 | BDL | BDL | 0.35 | 0.31 | 0.03 | BDL | BDL | 0.02 | 0.20 | 7.80 | -84.00 | 19.9 | 2.31 | 2.64 | 1.97 | 4.20 | 3.00 | 2.00 |
| H63W | BDL | BDL | 0.83 | 0.25 | BDL | BDL | BDL | BDL | 0.40 | 8.30 | -179.00 | 6.20 | 1.68 | 1.57 | 0.29 | 2.00 | 4.00 | 4.00 |
| NWID | BDL | BDL | 0.45 | 0.12 | BDL | BDL | BDL | BDL | 0.00 | 9.60 | -122.00 | 2.80 | 2.49 | 1.54 | 1.23 | 4.00 | 3.00 | 4.00 |
| WID | BDL | BDL | 1.65 | 0.28 | BDL | BDL | BDL | BDL | 0.03 | 7.90 | -121.00 | 5.60 | 1.67 | 2.68 | 2.28 | 4.00 | 4.00 | 2.00 |
| LLW | BDL | BDL | 0.96 | 0.15 | BDL | BDL | BDL | BDL | 0.00 | 7.40 | -110.00 | 1.81 | 2.97 | 0.00 | 1.27 | 2.50 | 2.00 | 4.00 |
| H63I | BDL | BDL | 1.12 | 0.27 | BDL | BDL | BDL | BDL | 0.00 | 7.70 | -8.00 | 27.3 | 3.78 | 1.11 | 0.95 | 4.00 | 2.00 | 2.00 |

BDL = below detectable level, blank areas = no measurements taken, * = no latitude/longitude reading available. $CO_3$, $HCO_3$ measured as wt. ppm, naphthenic acids as mg $L^{-1}$.

**Appendix A.2.** Detailed water and sediment chemistry values. Data on naphthenic acids concentration, $NH_3$, $NH_4$, ions, and metals provided by M. MacKinnon. Water and sediment samples processed at Syncrude Canada Ltd. Analytical Research Lab, Edmonton, AB.

| Code | Sb | Se | Si | Sr | Ti | V | Zn | Zr | Salinity (‰) | Sed. pH | Sed. ORP | Sediment Organic Content (%)LOI | Detritus-Cores (Ln+1, g) | Detritus Art. Subs. (Ln+1, g) | Detritus Sweep (Ln+1, g) | Med. Part. Size (phi) | Macro. Dev. | Area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TR6.8R | BDL | BDL | 1.14 | 0.12 | BDL | BDL | BDL | BDL | 0.00 | 7.10 | -40.00 | 3.30 | 2.83 | 1.22 | 0.76 | 2.00 | 2.00 | 5.00 |
| TR0.8R | BDL | BDL | 1.81 | 0.11 | BDL | BDL | BDL | BDL | 0.00 | 7.50 | -50.00 | 10.4 | 3.56 | 1.44 | 1.84 | 2.00 | 4.00 | 5.00 |
| SL | 1090 | BDL | BDL | BDL | 56.30 | BDL | BDL | 1.98 | 4.00 | 8.90 | -300.00 | 12.5 | 2.75 | | 0.96 | 4.50 | 3.00 | 6.00 |

| Site | Pore Water Oil (g/100g) | Pore Water Solids (g/100g) | Mean OWS |
|---|---|---|---|
| CTW00-01 | 0.92 | 59.0 | |
| Based on 1-m CT) | 1.17 | 58.1 | |
| | 0.85 | 44.7 | |
| | 0.06 | 39.8 | |
| n=4; Mean ± S.D. | 3.50±.58 | 50.41±9.62 | 0.01±0.002 |
| MFTN | 0.44 | 59.7 | |
| | 1.52 | 27.6 | |
| | 1.91 | 51.8 | |
| n=3 | 5.33±0.58 | 46.35±16.73 | 0.03±0.02 |
| TP2,5 | 3.75 | 69.0 | |
| (based on TP5) | 1.47 | 77.1 | |
| | 3.75 | 69.0 | |
| | 1.47 | 77.1 | |
| | 1.47 | 77.1 | |
| n=5; Mean ± S.D. | 3±1.15 | 73.88±4.43 | 0.03±0.02 |
| NW | 0.14 | 35.2 | |
| | 0.24 | 26.2 | |
| | 0.36 | 34.2 | |
| | 0.64 | 37.4 | |
| n=4; Mean ± S.D. | 2.5±0.58 | 33.2±4.91 | 0.01±0.004 |

| Site | Pore Water Oil (g/100g) | Pore Water Solids (g/100g) | Mean OWS |
|---|---|---|---|
| HS | 0.62 | 43.0 | |
| | 0.20 | 54.7 | |
| | 0.60 | 57.5 | |
| | 0.67 | 32.8 | |
| n=4; Mean ± S.D. | 3.75±1.71 | 47.03±11.36 | 0.01±0.007 |
| CL | 0.87 | 27.5 | |
| | 1.13 | 36.3 | |
| | 0.26 | 64.2 | |
| | 0.14 | 24.5 | |
| n=4; Mean ± S.D. | 2.75±1.50 | 38.13±18.08 | 0.02±0.016 |

BDL = below detectable level, blank areas = no measurements taken, * = no latitude/longitude reading available. $CO_3$, $HCO_3$ measured as wt. ppm, naphthenic acids as mg $L^{-1}$.

Appendix A.3. Taxa used in the development of a metric for artificial substrate samples, A), and for sweep samples, B). An example of how to calculate a metric score for a given site is shown, C).

| **A)**Artificial Substrate | Talitridae | Metric Value | Physidae | Metric Value | Caenidae | Metric Value | Dytiscidae | Metric Value | Richness | Metric Value | Constant | Metric Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CTW2000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.15 | -0.49 | 10.00 | -0.93 | -0.26 | -0.69 |
| CTW2001 | 0.00 | 0.00 | 0.00 | 0.00 | 0.30 | -1.59 | 1.11 | -3.60 | 5.50 | -0.51 | -0.26 | 4.43 |
| CTP2000 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.97 | -6.38 | 9.50 | -0.88 | -0.26 | 5.24 |
| CTP2001 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.50 | -0.14 | -0.26 | -0.40 |
| DP | 2.40 | 3.55 | 0.00 | 0.00 | 0.45 | -2.39 | 0.82 | -2.65 | 25.00 | -2.32 | -0.26 | -1.08 |
| TP9 | 0.53 | 0.78 | 0.00 | 0.00 | 0.20 | -1.06 | 0.53 | -1.72 | 14.00 | -1.30 | -0.26 | 0.44 |
| MFTN | 0.00 | 0.00 | 0.66 | -2.15 | 0.25 | -1.34 | 0.97 | -3.15 | 7.00 | -0.65 | -0.26 | 5.74 |
| TP5 | 0.63 | 0.00 | 0.00 | 0.00 | 0.45 | -2.40 | 0.94 | -3.05 | 15.00 | -1.39 | -0.26 | 2.86 |
| TP2 | 1.08 | 0.00 | 0.07 | -0.22 | 0.07 | -0.36 | 0.90 | -2.92 | 27.50 | -2.55 | -0.26 | -0.89 |
| NW | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.19 | -0.62 | 15.50 | -1.44 | -0.26 | -1.07 |
| SCP | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | -0.63 | 0.23 | -0.74 | 15.00 | -1.39 | -0.26 | -0.28 |
| PP | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.05 | -3.40 | 6.00 | -0.56 | -0.26 | 2.59 |
| CRM | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.97 | -3.14 | 20.50 | -1.90 | -0.26 | 0.99 |
| SM | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.26 | -0.83 | 4.50 | -0.42 | -0.26 | 0.16 |
| SB | 2.68 | 0.00 | 0.66 | -2.15 | 0.45 | -2.39 | 0.00 | 0.00 | 7.00 | -0.65 | -0.26 | -0.32 |
| HS | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.38 | -1.23 | 16.67 | -1.55 | -0.26 | -0.57 |
| SBD | 1.97 | 0.00 | 0.66 | -2.15 | 0.00 | 0.00 | 0.90 | -2.93 | 12.50 | -1.16 | -0.26 | 0.76 |
| S-Pit | 2.52 | 0.00 | 0.00 | 0.00 | 0.38 | -2.02 | 0.68 | -2.21 | 18.00 | -1.67 | -0.26 | -1.42 |
| CL | 0.18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.18 | -0.59 | 14.00 | -1.30 | -0.26 | -1.23 |
| SBBP | 0.39 | 0.00 | 0.66 | -2.15 | 0.27 | -1.44 | 0.39 | -1.27 | 12.50 | -1.16 | -0.26 | 2.87 |
| MID | 0.00 | 0.00 | 0.66 | -2.15 | 0.00 | 0.00 | 0.00 | 0.00 | 8.50 | -0.79 | -0.26 | 1.11 |
| LP | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 | -0.90 | 1.25 | -4.05 | 13.00 | -1.21 | -0.26 | 3.48 |
| BL | 0.00 | 0.00 | 0.66 | -2.15 | 0.00 | 0.00 | 0.67 | -2.18 | 9.00 | -0.84 | -0.26 | 3.24 |
| SW | 0.82 | 0.00 | 0.06 | -0.18 | 0.36 | -1.89 | 0.44 | -1.44 | 27.50 | -2.55 | -0.26 | -0.50 |
| NWID | 2.00 | 0.00 | 0.38 | -1.24 | 0.45 | -2.39 | 0.00 | 0.00 | 15.00 | -1.39 | -0.26 | -0.98 |
| TP1 | 0.28 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.62 | -2.02 | 23.00 | -2.13 | -0.26 | -0.79 |
| BM | 2.68 | 0.00 | 0.27 | -0.89 | 0.27 | -1.45 | 0.27 | -0.89 | 14.50 | -1.35 | -0.26 | -2.32 |
| H63W | 2.68 | 0.00 | 0.24 | -0.78 | 0.45 | -2.39 | 0.00 | 0.00 | 11.33 | -1.05 | -0.26 | -2.09 |
| WID | 0.00 | 0.00 | 0.08 | -0.25 | 0.00 | 0.00 | 0.41 | -1.33 | 24.00 | -2.23 | -0.26 | -0.90 |
| LLW | 0.94 | 0.00 | 0.00 | 0.00 | 0.18 | -0.94 | 0.61 | -1.96 | 18.50 | -1.72 | -0.26 | -0.46 |
| H63I | 0.94 | 0.00 | 0.55 | -1.78 | 0.00 | 0.00 | 0.00 | 0.00 | 9.50 | -0.88 | -0.26 | -0.75 |
| TR6.8R | 0.74 | 0.00 | 0.00 | 0.00 | 0.45 | -2.39 | 0.00 | 0.00 | 9.50 | -0.88 | -0.26 | 0.16 |
| TR0.8R | 2.68 | 0.00 | 0.00 | 0.00 | 0.45 | -2.39 | 0.00 | 0.00 | 13.00 | -1.21 | -0.26 | -3.03 |

Metric Equation: - (Talitridae*1.48) + (Physidae*3.26) + (Dytiscidae*3.24) + (Caenidae*5.30) - (Richness*0.09) - 0.26

Appendix A.3. Taxa used in the development of a metric for artificial substrate samples, A), and for sweep samples, B). An example of how to calculate a metric score for a given site is shown, C).

| **B)** Sweep Sample | Tanytarsini | Met. Val. | Physidae | Met. Val. | Planorbidae | Met. Val. | Sphaeriidae | Met. Val. | Caenidae | Met. Val. | Baetidae | Met. Val. | Dytiscidae | Met. Val. | Notonectidae | Met. Val. | % Orthocladiinae | Met. Val. | Metric Score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CTW2001 | 2.87 | -1.81 | 0.00 | 0.00 | 0.27 | 0.48 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.42 | -0.90 | 0.00 | 0.00 | 69.27 | -6.51 | -6.46 |
| CTP2000 | 5.69 | -3.59 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.93 | -1.22 | 0.00 | 0.00 | 15.31 | -1.44 | -3.58 |
| DP | 2.99 | -1.89 | 0.00 | 0.00 | 2.25 | 3.94 | 0.19 | -1.12 | 0.96 | 4.84 | 0.62 | -0.40 | 2.54 | -1.60 | 0.00 | 0.00 | 19.05 | -1.79 | 4.27 |
| TP9 | 3.77 | -2.38 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.96 | 4.84 | 0.57 | -0.37 | 3.42 | -2.16 | 0.00 | 0.00 | 7.69 | -0.72 | 1.61 |
| MFTN | 3.24 | -2.05 | 1.00 | -1.68 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.58 | -1.00 | 0.58 | 1.29 | 38.24 | -3.59 | -4.70 |
| TP5 | 5.06 | -3.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.38 | 1.92 | 0.00 | 0.00 | 0.14 | -0.09 | 1.00 | 2.23 | 7.98 | -0.75 | 2.69 |
| TP2 | 4.09 | -2.58 | 0.38 | -0.64 | 0.89 | 1.56 | 0.19 | -1.12 | 0.51 | 2.57 | 3.25 | -2.12 | 1.46 | -0.92 | 1.18 | 2.63 | 15.75 | -1.48 | 0.33 |
| NW | 3.27 | -2.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 | -0.05 | 0.00 | 0.00 | 1.79 | 3.97 | 9.42 | -0.89 | 3.30 |
| SCP | 2.12 | -1.33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.31 | -0.83 | 0.00 | 0.00 | 24.19 | -2.27 | -2.27 |
| PP | 5.86 | -3.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.31 | -2.09 | 0.00 | 0.00 | 13.12 | -1.23 | -4.32 |
| CRM | 0.97 | -0.61 | 0.00 | 0.00 | 1.22 | 2.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.92 | -0.58 | 0.09 | 0.20 | 65.71 | -6.18 | -3.02 |
| SM | 4.30 | -2.72 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.26 | -1.42 | 2.26 | 5.01 | 24.55 | -2.31 | 1.04 |
| SB | 2.59 | -1.64 | 0.00 | 0.00 | 0.64 | 1.12 | 0.19 | -1.12 | 0.64 | 3.23 | 0.00 | 0.00 | 0.64 | -0.40 | 0.00 | 0.00 | 13.33 | -1.25 | 2.17 |
| HS | 1.77 | -1.12 | 1.96 | -3.29 | 4.32 | 7.57 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.71 | -0.45 | 0.00 | 0.00 | 18.48 | -1.74 | 3.09 |
| S-Pit | 3.77 | -2.38 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.48 | 2.44 | 0.00 | 0.00 | 1.22 | -0.77 | 0.85 | 1.88 | 33.90 | -3.19 | 0.39 |
| CL | 0.83 | -0.53 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 | 0.31 | 0.00 | 0.00 | 0.52 | -0.33 | 0.00 | 0.00 | 10.86 | -1.02 | 0.41 |
| SBBP | 3.59 | -2.26 | 2.30 | -3.86 | 1.28 | 2.24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.44 | -0.29 | 0.78 | -0.49 | 0.00 | 0.00 | 3.03 | -0.28 | -2.58 |
| MID | 0.97 | -0.61 | 0.00 | 0.00 | 0.71 | 1.24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.97 | -0.61 | 0.00 | 0.00 | 62.86 | -5.91 | -3.88 |
| LP | 3.35 | -2.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.29 | -2.15 | 3.42 | -2.16 | 1.58 | 3.51 | 27.72 | -2.61 | -3.18 |
| BL | 1.86 | -1.17 | 2.30 | -3.86 | 3.89 | 6.83 | 0.00 | 0.00 | 0.00 | 0.00 | 1.21 | -0.79 | 2.49 | -1.57 | 0.00 | 0.00 | 72.73 | -6.84 | -5.28 |
| SW | 3.92 | -2.48 | 1.20 | -2.02 | 2.25 | 3.95 | 0.19 | -1.12 | 0.19 | 0.98 | 0.19 | -0.13 | 2.67 | -1.68 | 2.16 | 4.79 | 29.22 | -2.75 | 1.97 |
| NWID | 3.03 | -1.91 | 0.00 | 0.00 | 0.34 | 0.60 | 0.20 | -1.12 | 0.96 | 4.84 | 0.96 | -0.63 | 0.74 | -0.47 | 0.86 | 1.90 | 51.50 | -4.84 | 0.67 |
| TP1 | 3.94 | -2.49 | 0.00 | 0.00 | 3.71 | 6.51 | 0.00 | 0.00 | 0.00 | 0.00 | 3.29 | -2.15 | 0.27 | -0.17 | 0.00 | 0.00 | 29.41 | -2.76 | 1.36 |
| BM | 5.09 | -3.21 | 0.23 | -0.39 | 0.00 | 0.00 | 0.00 | 0.00 | 0.96 | 4.84 | 1.64 | -1.07 | 0.00 | 0.00 | 1.37 | 3.04 | 30.58 | -2.87 | 2.92 |
| H63W | 0.80 | -0.50 | 1.31 | -2.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.80 | 4.02 | 0.00 | 0.00 | 1.51 | -0.95 | 1.68 | 3.74 | 50.00 | -4.70 | 1.38 |
| WID | 0.80 | -0.50 | 1.60 | -2.69 | 3.48 | 6.11 | 0.20 | -1.12 | 0.00 | 0.00 | 2.05 | -1.34 | 1.31 | -0.83 | 0.24 | 0.54 | 0.00 | 0.00 | 2.14 |
| LLW | 4.32 | -2.73 | 2.29 | -3.86 | 2.75 | 4.83 | 0.00 | 0.00 | 0.00 | 0.00 | 1.36 | -0.89 | 0.60 | -0.38 | 0.00 | 0.00 | 0.00 | 0.00 | -0.56 |
| TR6.8R | 5.71 | -3.61 | 0.00 | 0.00 | 1.13 | 1.98 | 0.20 | -1.12 | 0.96 | 4.84 | 1.98 | -1.30 | 0.67 | -0.42 | 0.00 | 0.00 | 8.00 | -0.75 | 2.29 |
| TR0.8R | 0.27 | -0.17 | 0.00 | 0.00 | 1.90 | 3.33 | 0.20 | -1.12 | 0.00 | 0.00 | 3.29 | -2.15 | 1.03 | -0.65 | 0.00 | 0.00 | 0.00 | 0.00 | 1.12 |

Metric Equation: -(Dytiscidae*0.63) + (Notoncetidae*2.22) - (%Orthocladiinae*0.09) - (Spaheriidae*5.76) + (Planorbidae*1.76) - (Physidae*1.68) + (Caenidae*5.05) - (Baetidae*0.65) - (Tanytarsini*0.49) + 1.86

Appendix A.3. Taxa used in the development of a metric for artificial substrate samples, A), and for sweep samples, B). An example of how to calculate a metric score for a given site is shown, C).

**C)**

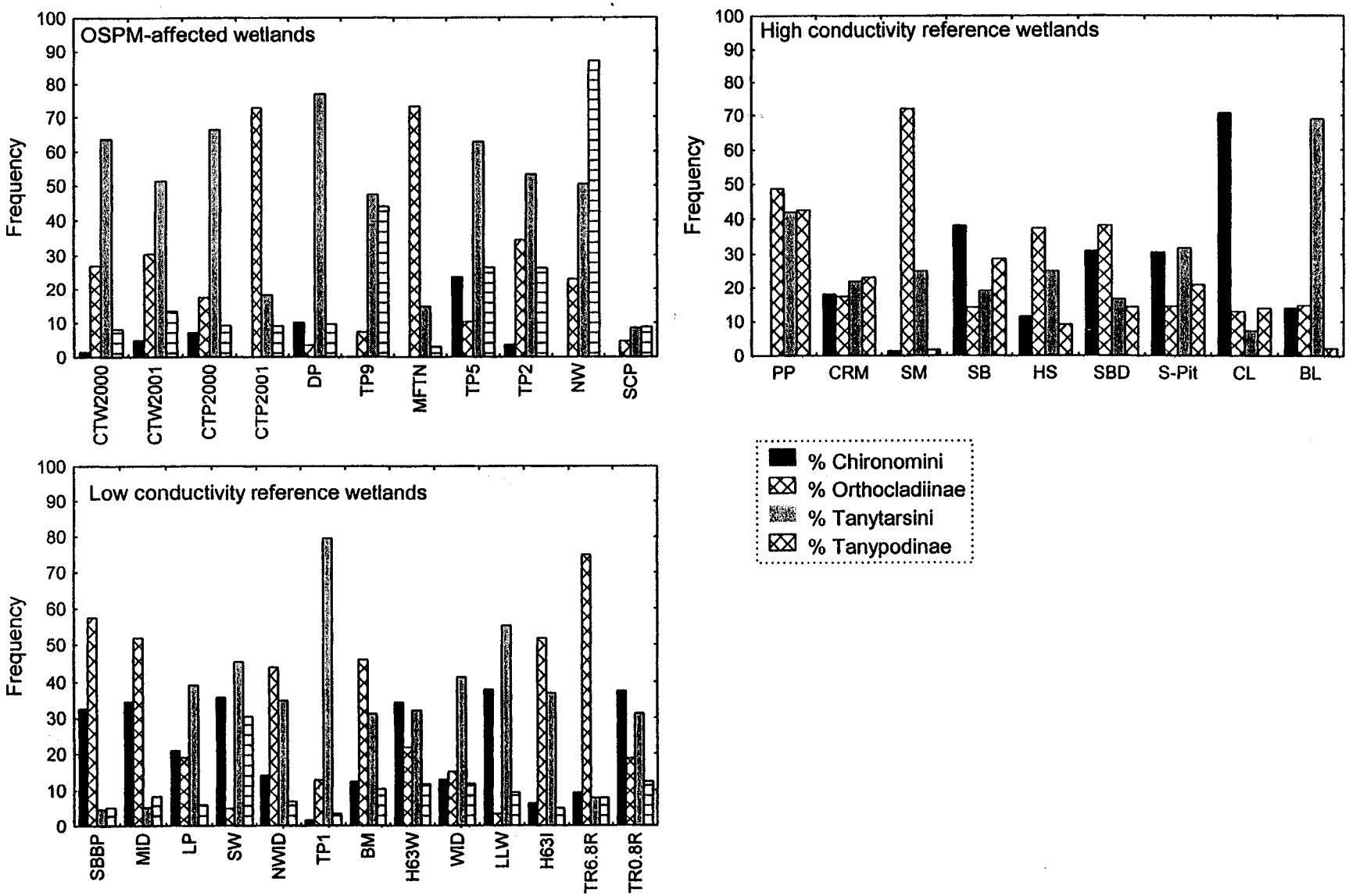
| Site | Artificial substrate | Taxon Rel. Abund., % | Metric Contrib. | Metric Value |
|---|---|---|---|---|
| CL | Talitridae | 0.18 | -1.48 | -0.27 |
| | Physidae | 0.00 | 3.26 | 0.00 |
| | Caenidae | 0.00 | 5.30 | 0.00 |
| | Dytiscidae | 0.18 | 3.24 | 0.59 |
| | Richness | 14.00 | -0.09 | -1.30 |
| | Constant | -0.26 | | |
| | Metric Score | | | -1.23 |

| Site | Sweep | Taxon Rel. Abund., % | Metric Contrib. | Metric Value |
|---|---|---|---|---|
| BM | Tanytarsini | 4.09 | -0.49 | -2.58 |
| | Physidae | 0.23 | -1.68 | -0.39 |
| | Planorbidae | 0.00 | 1.75 | 0.00 |
| | Sphaeriidae | 0.00 | -5.76 | 0.00 |
| | Caenidae | 0.96 | 5.05 | 4.84 |
| | Baetidae | 1.64 | -0.65 | -1.07 |
| | Dytiscidae | 0.00 | -0.63 | 0.00 |
| | Notonectidae | 1.37 | 2.22 | 3.04 |
| | %Orthocladiinae | 30.58 | -0.09 | -2.87 |
| | Constant | 1.86 | | |
| | Metric Score | | | 2.92 |

**Appendix A.4.** Overall mean relative distribution of sub-families and tribes within the family Chironomidae from 2000-2001

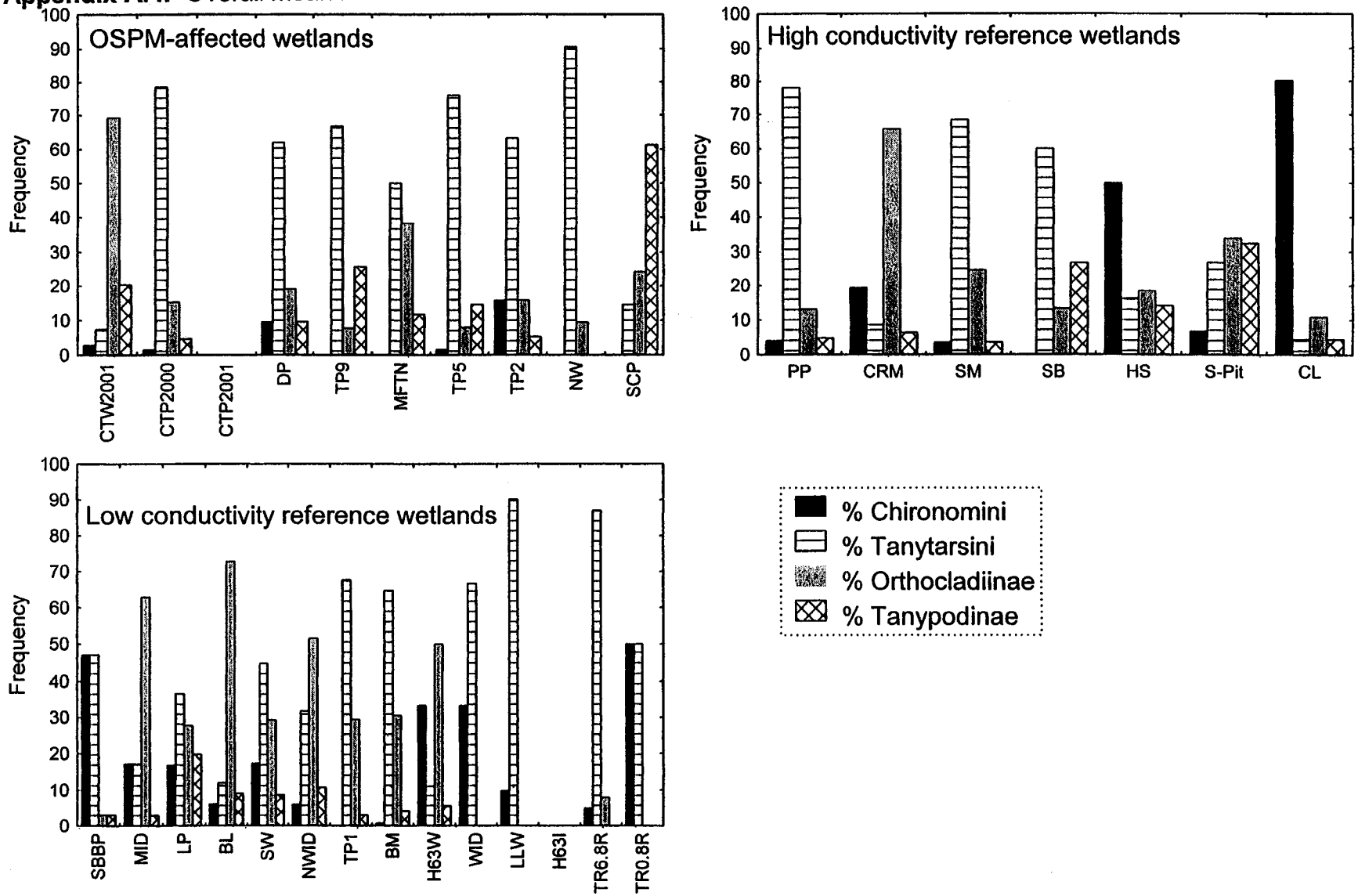
| | Artificial Substrate Samples | | | | Sweep Samples | | | |
|---|---|---|---|---|---|---|---|---|
| Site | Chironomini | Orthocladiinae | Tanytarsini | Tanypodinae | Chironomini | Orthocladiinae | Tanytarsini | Tanypodinae |
| CTW2000 | 1.54 | 26.85 | 63.58 | 8.02 | | | | |
| CTW2001 | 4.78 | 30.43 | 51.30 | 13.48 | 2.93 | 7.32 | 69.27 | 20.49 |
| CTP2000 | 7.14 | 17.35 | 66.33 | 9.18 | 1.56 | 78.44 | 15.31 | 4.69 |
| CTP2001 | 0.00 | 72.73 | 18.18 | 9.09 | 0.00 | 0.00 | 0.00 | 0.00 |
| DP | 10.12 | 3.48 | 76.78 | 9.62 | 9.52 | 61.91 | 19.05 | 9.52 |
| TP9 | 0.00 | 7.33 | 47.41 | 30.43 | 0.00 | 66.67 | 7.69 | 25.64 |
| MFTN | 0.00 | 73.20 | 14.89 | 3.02 | 0.00 | 50.00 | 38.24 | 11.77 |
| TP5 | 23.71 | 10.34 | 62.93 | 26.43 | 1.64 | 75.82 | 7.98 | 14.55 |
| TP2 | 3.61 | 34.37 | 53.30 | 26.26 | 15.75 | 63.25 | 15.75 | 5.25 |
| NW | 0.00 | 23.11 | 50.46 | 30.43 | 0.00 | 90.58 | 9.42 | 0.00 |
| SCP | 0.00 | 4.62 | 8.46 | 8.72 | 0.00 | 14.52 | 24.19 | 26.67 |
| PP | 0.00 | 48.65 | 41.89 | 30.43 | 4.07 | 78.05 | 13.12 | 4.75 |
| CRM | 18.12 | 17.42 | 21.95 | 23.14 | 19.43 | 8.57 | 65.71 | 6.29 |
| SM | 1.40 | 71.93 | 24.91 | 1.75 | 3.59 | 68.26 | 24.55 | 3.59 |
| SB | 38.10 | 14.29 | 19.05 | 28.57 | 0.00 | 60.00 | 13.33 | 26.67 |
| HS | 11.64 | 37.21 | 24.89 | 9.10 | 33.33 | 16.30 | 18.48 | 14.13 |
| SBD | 30.95 | 38.10 | 16.67 | 14.29 | | | | |
| S-Pit | 30.59 | 14.51 | 31.76 | 20.95 | 6.78 | 26.84 | 33.90 | 26.67 |
| CL | 70.68 | 12.96 | 7.25 | 13.96 | 33.33 | 4.44 | 10.86 | 4.44 |
| SBBP | 32.59 | 57.46 | 4.73 | 5.22 | 33.33 | 46.97 | 3.03 | 3.03 |
| MID | 34.59 | 51.88 | 5.26 | 8.27 | 17.14 | 17.14 | 62.86 | 2.86 |
| LP | 20.95 | 19.05 | 39.05 | 5.91 | 16.83 | 36.63 | 27.72 | 19.80 |
| BL | 14.07 | 14.81 | 68.89 | 2.22 | 6.06 | 12.12 | 72.73 | 9.09 |
| SW | 35.75 | 4.88 | 45.40 | 30.43 | 17.35 | 44.75 | 29.22 | 8.68 |
| NWID | 14.29 | 44.00 | 34.86 | 6.86 | 5.99 | 31.74 | 51.50 | 10.78 |
| TP1 | 1.61 | 12.90 | 79.57 | 3.40 | 0.00 | 67.65 | 29.41 | 2.94 |
| BM | 12.55 | 45.89 | 31.17 | 10.39 | 0.69 | 64.61 | 30.58 | 4.12 |
| H63W | 34.38 | 21.88 | 32.03 | 11.72 | 33.33 | 11.11 | 50.00 | 5.56 |
| WID | 13.04 | 15.22 | 41.30 | 11.91 | 33.33 | 66.67 | 0.00 | 0.00 |
| LLW | 37.96 | 3.40 | 55.25 | 9.46 | 9.88 | 90.12 | 0.00 | 0.00 |
| H63I | 6.38 | 51.77 | 36.88 | 4.96 | 0.00 | 0.00 | 0.00 | 0.00 |
| TR6.8R | 9.38 | 75.00 | 7.81 | 7.81 | 5.00 | 87.00 | 8.00 | 0.00 |
| TR0.8R | 37.50 | 18.75 | 31.25 | 12.50 | 33.33 | 50.00 | 0.00 | 0.00 |

**Appendix A.4.** Overall mean relative distribution of sub-families and tribes within the family Chironomidae from 2000-2001




Pattern of chironomid tribe and sub-families, within the family Chironomidae, for artificial substrate samples collected in 2000 and 2001.

**Appendix A.4.** Overall mean relative distribution of sub-families and tribes within the family Chironomidae from 2000-2001




Pattern of chironomid tribe and sub-families, within the family Chironomidae, for sweep samples collected in 2000 and 2001.