SECONDARY PRODUCTION, TROPHIC POSITION, AND POTENTIAL FOR ACCUMULATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN PREDATORY DIPTERA IN FOUR WETLANDS OF THE ATHABASCA OIL SANDS, ALBERTA, CANADA

by

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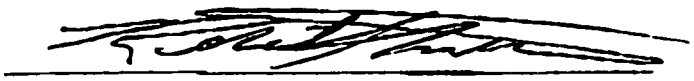

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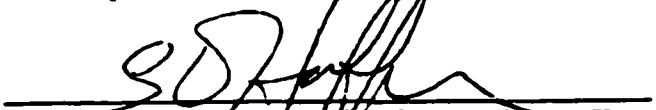

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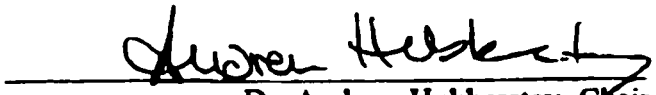

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ABSTRACT

Larvae of aquatic Diptera are important predators in fishless wetlands of northeast Alberta. Annual production was estimated for benthic (Chironomidae: Tanypodinae) and pelagic (Chaoboridae: *Chaoborus*) dipteran populations in 4 wetlands in surface-mined areas of the Athabasca oil sands, two of which received oil sands mine process material (OSPM; containing polycyclic aromatic hydrocarbons (PAHs)), and two of which were reference wetlands. The structure of benthic and pelagic food webs was estimated by measuring stable carbon and nitrogen isotopes. Stable nitrogen isotopes were also used to determine trophic levels of Tanypodinae and *Chaoborus* in order to determine their potential to bioaccumulate PAHs. Annual production was estimated along with total PAH body burdens to determine the potential for biomass and PAH export by the emergent adult insects.

Tanypodinae production (1.55 - 28.77 $g/m^2/y$) consistently exceeded *Chaoborus* production (0.009 - 0.372 $g/m^2/y$). Greater Tanypodinae densities at OSPM-affected wetlands than at reference wetlands resulted in greater production in the former. Stable carbon and nitrogen isotopes showed that food web structure differed with wetland type. Stable nitrogen isotopes indicated that benthic and pelagic taxa occupied higher trophic positions in OSPM-affected wetlands than reference wetlands.

Chaoborus trophic position estimates were consistently greater than estimates for Tanypodinae, suggesting greater PAH bioaccumulation potential for *Chaoborus*. However, greater PAH concentrations in Tanypodinae suggested that habitat and diet are more important determinants of PAH body burdens. Biota:sediment accumulation ratios of total

PAHs were not significantly different than 1.0, indicating that PAHs do not bioaccumulate in these organisms.

Tanypodinae had greater potential to export PAHs (1.86 - 37.1 $mg/m^2/y$) than *Chaoborus* ($1.1x10^{-2}$ - $4.5x10^{-1}$ $mg/m^2/y$) due to greater production and PAH body burdens. Tanypodinae at OSPM-affected wetlands had greater potential to export PAHs than their counterparts at reference wetlands due to higher production and likely greater PAH accumulation at OSPM-affected wetlands.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

GENERAL INTRODUCTION

This research deals with benthic and pelagic invertebrate biology in constructed saline reference wetlands and constructed or naturally formed saline oil sands process material (OSPM)-affected wetlands. Wetlands in general are among the most productive ecosystems in the world (Moss 2000). They are utilized by an estimated 25% of plants and 45% of animals that are listed as threatened or endangered (Mattingly 1992). Wetlands make up a significant portion of the landscape in Canada's north and provide a variety of important ecosystem functions such as critical wildlife habitat (e.g., Reinhold et al. 1999), and water storage, which can prevent flooding and improve water quality (Mattingly 1992). Until recently, wetlands have generally been viewed by society as waste areas which become useful only when drained for farming or development. As a result, relatively few studies of ecosystem structure and function have been conducted in these water bodies, (saline wetlands in particular; Lovvorn et al. 1999), compared to lakes and streams, which are home to long recognized economically important (and hence better-studied) organisms such as fish.

The goal of my study is to improve understanding of the bioenergetics and potential for bioaccumulation and transfer through aquatic biota of the constituents associated with the development and subsequent landscape reclamation of the oil sands surface mining areas in the Athabasca deposit of Fort McMurray, Alberta. The focus will be on wetland habitats that are expected to be a major component in reclaimed landscapes in this region, either as naturally formed waterbodies and/or as constructed waterbodies for the purpose of

wastewater treatment. The companies (Syncrude Canada Limited, Suncor Energy Incorporated) that mine the oil sands in this region have leased the land from the province of Alberta. A stipulation of these leases is that the land is left as a viable component of the surrounding ecosystem, with productivity equal to or greater than pre-disturbance conditions (Golder Associates Ltd. 2000). This thesis will contrast the magnitude of biomass (energy) flow through invertebrate (predatory dipterans) components of the benthic and pelagic food webs of wetlands by quantifying secondary production. Energy sources (and thus pathways of biomass or energy flow) will be inferred through the use of stable isotopes of carbon and nitrogen. Since the flow of biomass and hydrophobic organic compounds are closely paralleled in food webs, stable isotopes will also be used to trace the flow of polycyclic aromatic hydrocarbons (PAHs) in benthic and pelagic food webs. Stable isotopes of nitrogen will be used to determine the relative potential of benthic and pelagic predatory dipterans to bioaccumulate PAHs. Since invertebrates have a limited ability to metabolize PAHs, they may act as vectors transporting PAHs along with biomass to terrestrial vertebrate insectivores predators when the terrestrial adults emerge from wetlands. Vertebrates can rapidly convert PAHs to potentially toxic metabolites, and thus insects pose a potential threat to terrestrial vertebrate insectivores. As a result, an evaluation of the potential export of both biomass and PAHs via top dipteran predators of benthic and pelagic pathways to the terrestrial environment in general, and to insectivorous tree swallows in particular, will be assessed through the combination of larval secondary production estimates with larval PAH body burdens.

Researchers have studied oil sands locations to assess whether or not wetlands located on oil-sands mining sites can support productive and sustainable biological communities that are comparable with reference communities (Bendell-Young et al. 1997, van den Heuvel et al. 1999, van den Heuvel et al. 1999, Whelly 1999, Bendell-Young et al. 2000, Oil Sands Wetlands Working Group 2000, McCormick 2000, Smits et al. 2000, Harris 2001, Leung et al. 2001, Madill et al. 2001). The work presented in this thesis is a continuation of this research. It will complement future research on biological processes in wetlands that contain oil sands process material (OSPM).

There are fundamental differences in the energy bases of different aquatic ecosystems. One can take advantage of this fact to carry out unique studies in wetlands. In lakes, and especially large lakes with a low ratio of littoral surface area to volume, phytoplankton are thought to regulate production (Vadeboncoeur et al. 2002). Consequently, inputs of allochthonous carbon (e.g., tree leaves) are less important in regulating whole lake production than is autochthonously produced carbon. In streams, particularly those that are shaded by riparian vegetation, production is regulated by the relatively large inputs of allochthonous carbon (Junger and Planas 1994).

In wetlands, an intermediate condition potentially exists. Both autochthonous and allochthonous carbon may be important in regulating secondary productivity for the following reasons. First, productivity in wetlands is facilitated by the high littoral surface area to water volume ratio. Most of the surface area of a wetland is typically exposed to sunlight, and because wetlands are shallow (i.e., have a high surface area: water volume ratio), light penetrates throughout the water column thereby making primary production via

phytoplankton and particularly benthic and epiphytic algae important. This primary production fuels both benthic and pelagic secondary production. Secondly, wetlands, like streams, receive and store large quantities of allochthonous organic carbon relative to their area, and this material fuels benthic secondary production (Horne and Goldman 1994). These features provide a unique opportunity to study biomass (energy) flow in that these are systems where benthic and pelagic routes can be equally important pathways of secondary production.

Wetlands are increasingly being used to temporarily store and detoxify anthropogenic effluents such as stormwater runoff (e.g., Kennedy and Mayer 2002), agricultural drainage (e.g., Borin et al. 2001), and oil sands mining tailings (Golder Associates Ltd. 2000). When wetlands are used in such a way, the aquatic insect larvae that inhabit them can potentially accumulate certain anthropogenic constituents and export them to the terrestrial environment when they become winged adults (Menzie 1980, Larsson 1984, Reinhold et al. 1999).

My overall objective is to outline and quantify the relative importance of benthic (associated with wetland substrates) and pelagic (within the water column) pathways of biomass and PAH transfer through predatory aquatic Diptera (two-winged flies) of reclaimed wetlands to terrestrial insectivores. By studying these processes in both constructed reference and OSPM-affected wetlands, comparisons can be made that will assist in the assessment of wetlands' utility as part of a responsible, effective strategy for storing and detoxifying oil sands mining waste material (Oil Sands Wetlands Working Group 2000). It will also shed light on how these processes work in wetlands that form naturally in oil sands mining areas, e.g., from dyke seepage water.

This thesis is composed of 6 chapters. Chapter 1 reviews background information relevant to this study. To begin with, an overview of oil sands mining is given. Following this, background information concerning polycyclic aromatic hydrocarbons (PAHs) is given. The concepts of food webs and biomass (energy) flow are then reviewed and specifically related to stable isotopes of carbon and nitrogen. Next, an overview of the secondary production of aquatic macroinvertebrates is given and its relevance to this study is discussed.

Chapter 2 describes some of the features of wetlands in general and constructed wetlands in the Athabasca oil sands region in particular. General features of the focal study organisms are reviewed and a brief overview of the study sites, general timing of sampling, and the types of samples collected for this study are discussed. Following this, a detailed description of the four constructed wetlands sampled in this study is given. Finally, seasonal depth change at each wetland, water chemistry, and sediment chemistry are described.

The third chapter of this thesis deals with the secondary production of predatory midge larvae inhabiting constructed OSPM-affected and reference wetlands. The major objective of this chapter is to estimate potential biomass export from the wetlands via two different pathways - benthic (bottom dwelling) predatory midges (Diptera: Chironomidae) and pelagic (water column dwelling) predatory midges (Diptera: Chaoboridae). This knowledge is required in order to estimate the export of PAHs via these pathways and the relative importance of the two pathways in providing biomass and PAHs to the diet of terrestrial insectivores. The secondary production of all benthic midges that inhabit constructed OSPM-affected and reference wetlands will also be discussed.

Chapter 4 deals with the structure and function of benthic and pelagic food webs in constructed OSPM-affected and reference wetlands with a focus on predatory benthic and pelagic midges. The major goal of this chapter is to use stable isotopes to estimate trophic positions of the predatory taxa and their potential energy sources. This information can be used to assess the relative bioaccumulation potential of benthic and pelagic predatory midges at reference and OSPM-affected wetlands.

Chapter 5 focusses on PAHs in constructed wetlands of the Athabasca oil sands. This chapter includes a description of the PAH profile of sediments, water, and predatory midges at constructed wetlands. An assessment of PAH bioaccumulation in benthic and pelagic predatory midges will also be made. The primary focus of this chapter is to estimate the relative importance of predatory benthic and pelagic midges in exporting biomass and PAHs to the terrestrial environment in general, and to terrestrial insectivores in particular. Results from chapters 3 and 4 will be incorporated into the discussion section of this chapter.

Finally, Chapter 6 is a summary of the conclusions of this thesis.

OIL SANDS MINING

The Athabasca oil sands deposit, located in northeastern Alberta is the largest of 4 Alberta oil sands areas (Athabasca, Cold Lake, Wabasca, and Peace River (Fig. 1.1)). The deposits, with the oil sand typically located 30 to 90 m beneath the surface, contain an estimated 869 billion barrels of bitumen (Fine Tails Fundamentals Consortium 1995a). Bitumen is a heavy oil, which is refined to produce lighter oils following extraction from the sand. Bitumen is presently mined in this area by Syncrude Canada Ltd. and Suncor Inc., with

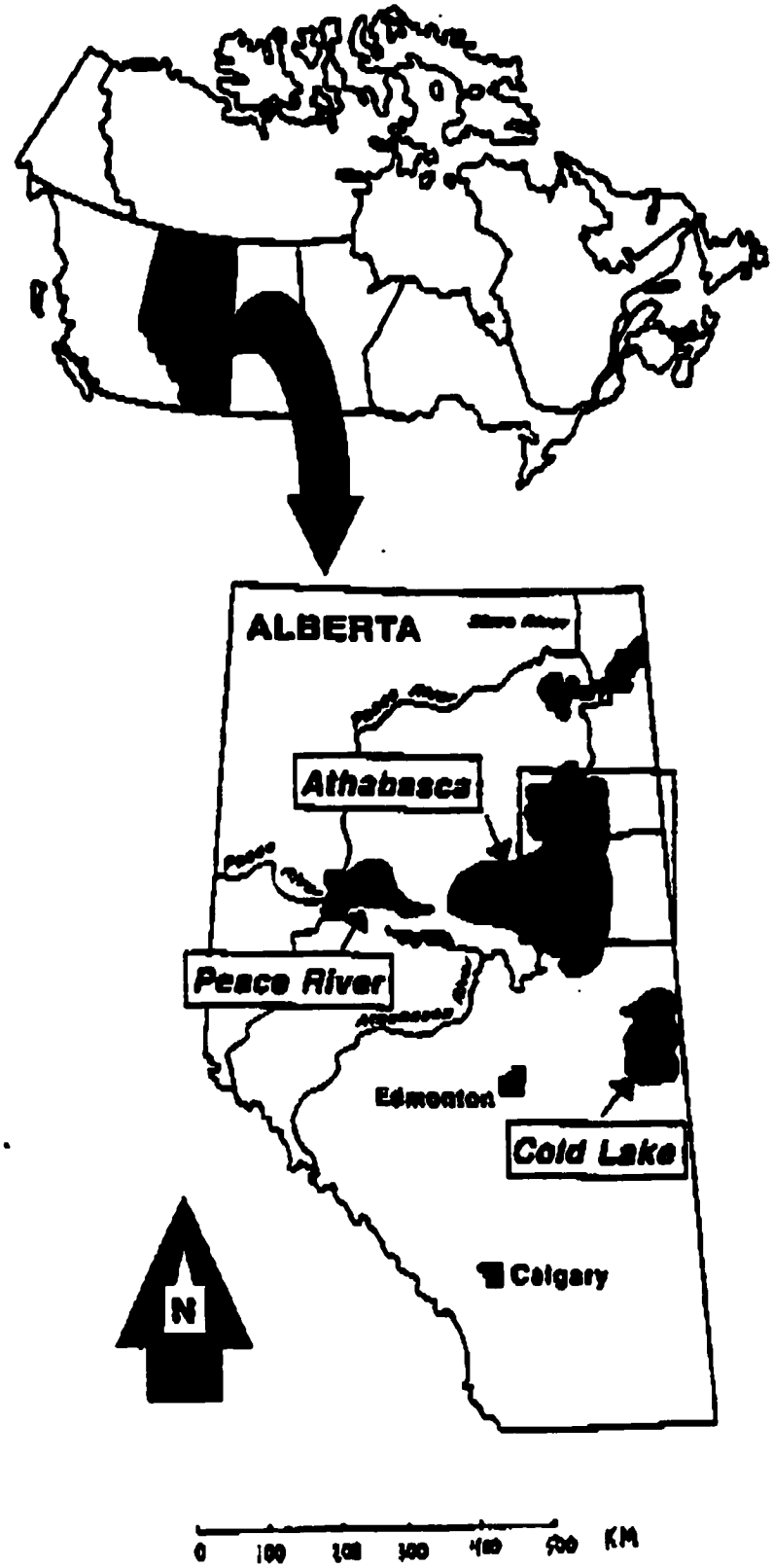


Fig. 1.1. Map of Alberta, with the Athabasca oil sands deposit indicated (modified from Whelly 1999).

other companies (Shell Canada Ltd. and Mobil Oil Canada Properties) planning to open new mines (Oil Sands Wetlands Working Group 2000). Current extraction accounts for about 20% of Canada's crude oil production with projections of 50% in the next few years (Leung et al. 2001). Although the existence of these deposits has been known for hundreds of years, technology has only recently made their mining economically feasible (Fine Tails Fundamentals Consortium 1995a). As technology to mine and extract the bitumen improves, and as the world's oil supply continues to decline, this deposit is expected to become more intensively mined.

1) The Mining Process

The bitumen deposits are close enough to the surface that surface mining is employed to recover them. Oil sands surface mining involves stripping off up to 100 m of overburden (vegetation, topsoil, clays) overlying the oil sands, which disrupts natural drainage and hydrology in the process. These materials are then stockpiled and may be used for future land reclamation.

2) Extraction

Once the oil sands ore reaches the extraction facility it undergoes a process known as Clark hot water extraction (Fine Tails Fundamentals Consortium 1995a). The ore is turned into a slurry by placing it in large tumblers with hot water, steam, and sometimes caustic soda (NaOH). This separates the bitumen from the sand and tailings slurry (silt, clay, water, and

unrecovered bitumen). The bitumen is then refined into a lighter oil and pumped 500 km southwest to Edmonton, Alberta, via a pipeline where it is further refined.

The tailings slurry is discharged into temporary ponds (tailings ponds) where the coarse sand quickly settles out. This sand is used to build retention dykes around the ponds. The remaining fine tailings material enters a tailings pond where it slowly dewaters and becomes known as mature fine tailings (MFT). This process results in a layer of relatively clear water approximately 3 m deep on top of the tailings pond (oil sands process water - OSPW). This water is pumped back to the extraction facilities where it is reused in the bitumen extraction process.

It is estimated that when MFT is completely consolidated (naturally), it will be unable to support construction traffic that would be necessary to reclaim the landscape (Fine Tails Fundamentals Consortium 1995a). This hinders efforts to restore mined landscapes so research has been conducted on techniques to dewater MFT more completely and rapidly. Currently, gypsum ($CaSO_4 \cdot 2H_2O$) is added to some fine tailings and MFT as an agglutinant to produce consolidated/composite tailings (CT). The CT in tailings ponds settles much more quickly than fine tailings or MFT (Fine Tails Fundamentals Consortium 1995c).

3) Reclamation

The extraction of bitumen from oil sands ore leads to the production of large volumes of fluid and saturated tailings materials that occupy landscapes which will require eventual reclamation. Fresh tailings material and OSPW are acutely toxic, killing fish within 96 h of exposure (LC_{50} < 10% - Fine Tails Fundamentals Consortium 1995b). The OSPW derived

from the fine tails material is saline, contains PAHs, naphthenic acids, and trace metals, and has a pH between 8 and 9 (Fine Tails Fundamentals Consortium 1995a).

Currently, the fluid mine tailings materials are stored in large tailings- or settling basins. One option for eventual reclamation of the aquatic landscapes containing water and soft materials is the use of freshwater-capped tailings lakes and the formation of a network of lakes and/or wetlands that receive the OSPM (Golder Associates Ltd. 2000). Other mined areas will be restored as terrestrial landscapes, as some waste materials such as bitumen-poor oil sands and tailings sand must also be reclaimed. Regardless of the type of reclamation, the biota will be exposed to OSPM either directly as in the aquatic strategies, or indirectly through leaching, runoff, and drainage from terrestrial reclamation structures (Golder Associates Ltd. 2000). Likewise, whether or not the oil sands mining companies choose to use wetlands to detoxify OSPM, there will be OSPM-affected wetlands present in the lease closure landscape.

4) Biological Significance of OSPM-affected Wetlands

Most of the acute toxicity of OSPM to aquatic biota is mitigated through natural biological degradation processes (Nix and Martin 1992, Madill et al. 2001, M. MacKinnon, 2002, Syncrude Canada Ltd., pers. comm.). Despite this, freshly derived wastewaters are acutely toxic to *Hyalella azteca* (10% survival in Suncor fine tails porewater - EVS 1992) and rainbow trout (96-h LC_{50} at 3.2-28% tailings pond surface water content - Hackbarth and Nastasa 1979, MacKinnon 1990, MacKinnon and Sethi 1993, Sheeran 1993, and Mikula, et al. 1995), and less so to *Daphnia magna* (96-h LC_{50} at 76% Syncrude tailings pond surface

water content - MacKinnon and Sethi 1993; 96-h LC_{50} at 98-100% Suncor tailings pond surface water content - MacKinnon and Sethi 1993, Boyd and Montgomery 1963; 96-h LC_{50} at >100% Suncor fine tails porewater - Boyd and Montgomery 1963). The Microtox assay also indicates that the same freshly collected wastewaters are acutely toxic to bacteria based on the inhibition of luminescence (IC_{50} at 16-50% of tailings pond surface water content - MacKinnon and Retallack 1982, Zenon Environmental Inc. 1986, EVS 1992, Mackay and Verbeek 1993, Nelson et al. 1993, and Enviro-Test Laboratories and HydroQual Laboratories 1994; IC_{50} at 20-80% fine tails porewater content - Boyd and Montgomery 1963, Mackay and Verbeek 1993, and Nelson et al. 1993). Despite this knowledge, the impacts of OSPM on the biota in landscape components, such as wetlands, are poorly understood as are the rates at which these impacts operate (Fine Tails Fundamentals Consortium 1995b, Ciborowski 1999). To better understand these aspects, studies addressing the chronic effects of OSPM exposure have been carried out.

Bioaccumulation potential and chronic toxicity are concerns in these systems due to the presence of dissolved organic compounds (naphthenic acids), polycyclic aromatic hydrocarbons (PAHs), salts (e.g., NaCl), and trace metals (e.g., Al, Fe, Sr, Ti) in the OSPM, and because the substrates used to construct lake or wetland basins may in some cases contain significant concentrations of residual hydrocarbons (e.g., 'lean oil sands').

The effects of chronic exposure of biota to OSPM have been studied in a variety of organisms, with results indicating both sublethal and no effects of OSPM exposure. Whelly (1999) found no difference in the incidences of mentum deformities in the mouthparts of chironomid larvae collected from reference and OSPM-affected wetlands (Whelly 1999).

However, the composition of wetland benthic invertebrate communities in general, and chironomid communities in particular are different in OSPM-affected wetlands compared to reference wetlands. Both chironomid density and biomass were significantly greater at OSPM-affected wetlands compared to reference wetlands (Bendell-Young et al. 2000). Preliminary data of Hum (2000) and McDonald (2001) indicate that chironomid production rates are slower in OSPM-affected wetlands than in environmentally similar matched pairs. The benthic invertebrate community of saline reference wetlands had higher abundances but lower richness than low salinity wetlands, whereas OSPM-affected (saline) wetlands exhibited reduced abundance and richness compared to saline reference wetlands (Whelly 1999). These results suggest that oil sands process materials such as OSPW and/or tailings in constructed lakes and wetlands may affect the community composition of these aquatic systems over the long term.

Studies of the effects of OSPM-affected wetlands on terrestrial species have also been done. Ecologically relevant endpoints in nestling tree swallows (*Tachycineta bicolor*) adjacent to OSPM-affected and reference wetlands were examined by Smits et al. (2000). Tree swallows forage mainly on aerial insects whose larval life stages are completed within wetlands (Smits et al. 2000). Consequently, tree swallows nesting adjacent to OSPM-affected wetlands may be exposed to OSPM via their prey. Despite this, nestling tree swallows showed no difference between OSPM-affected and reference wetlands with respect to ecologically relevant endpoints such as growth, reproduction (clutch size or mass), and immune response (Smits et al. 2000).

Saline wetlands which lack the elevated concentrations of PAHs characteristic of OSPM-affected wetlands have formed in various parts of the current oil sands operations on Syncrude Canada's and Suncor's lease sites. These wetlands receive saline runoff from the sodic overburden storage areas (which may contain lean oil sands) or are formed along the edges of tailings ponds as result of water seeping through the dyke walls. These wetlands are useful as reference areas against which to contrast bioaccumulation in OSPM-affected wetlands because both types are 1) saline, which markedly alters wetland food web structures (Lovvorn et al. 1999), and 2) contain predatory, benthic chironomids (e.g., Tanypodinae) and predatory, pelagic phantom midges (*Chaoborus*). These insects are important food items and potential sources of PAH transfer to insectivorous vertebrates (amphibians and birds), especially swallows, in boreal habitats (Smits et al. 2000). The divergent feeding behaviour of these two related but ecologically distinct taxa permits evaluation of the bioaccumulation potential and routes of biomass flow through these insects to insectivorous terrestrial predators. It is unclear which trophic pathway (benthic or pelagic) is more important in terms of accumulating and exporting PAHs to the terrestrial environment from either OSPM-affected or reference wetlands (Ciborowski 1999). It is possible that since the majority of PAHs in a wetland are located in the sediment (Neff 1979) that the benthic route may be the major pathway of PAH accumulation and export. However, differences in PAH accumulation and export via these two pathways will also depend on the secondary production of these pathways.

BACKGROUND

1) Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are aromatic hydrocarbons with two or more fused carbon rings. The PAHs of biological interest are small enough to move across cell membranes. This is a characteristic of many PAHs, ranging from naphthalene ($C_{10}H_8$, MW 128.16) to coronene ($C_{24}H_{12}$, MW 300.36) (Neff 1979). PAHs are naturally present in the bitumen fraction of the oil sands. The PAH 'fingerprint' of bitumen is different from that of thermally-derived or refinery-produced PAHs in that it is dominated by the 2-4 ring components, with many C1 to C4 alkyl substitutions (Smits et al. 2000, Headley et al. 2001). Little of the parent PAHs is observed in either the fresh oil sands or in oil sands tailings (M. MacKinnon 2002, Syncrude Canada Ltd., pers. comm.). Concentrations of PAHs in the sediments and water of OSPM-affected wetlands exceed those in the natural ponds in the area due to residual particle-associated PAHs present in the mining effluent. The PAHs are present at 1.5-150 fold greater concentrations in sediments of OSPM-affected wetlands compared to reference sediments, and at 4-6 fold greater concentrations in water of OSPM-affected wetlands compared to water of reference wetlands (Smits et al. 2000).

PAHs are cause for concern because the metabolites of parent priority PAHs may be carcinogenic in fish (Baumann 1989) and in humans (Research Triangle Institute 1995) and have genotoxic effects (Neff 1979). However, little is known about the bioaccumulation effects or relative toxicity of the metabolites of alkylated PAHs (Dutta and Harayama 2001). Metabolites of PAHs are produced from the mixed function oxidase (MFO) enzyme system (Van Veld 1990). PAHs extracted from pore water of tailings showed low levels of binding

to the Ah receptor and induced minimal ethoxyresorufin-*0*-deethylase (EROD) activity in primary rat hepatocytes (Madill et al. 2001). This suggests that PAHs present in the Athabasca oil sands may have a low potential to induce MFO activity and hence to produce potentially toxic metabolites.

Production of metabolites by the MFO system is slower in invertebrates than vertebrates (James 1989, McElroy et al. 1989), and the ability of invertebrates to take up PAHs from aquatic environments has been well documented (Leversee et al. 1982, Clements et al. 1994, Harkey et al. 1994, Harkey et al. 1995, Borchert et al. 1997, Kaag 1998, Hatch and Burton 1999, Lamoureux and Brownawell 1999, Thomann and Komlos 1999, Gewurtz et al. 2000, Bell 1995, Baussant et al. 2001). Thus, invertebrates having aquatic larval stages and terrestrial adult stages act as potential transfer agents of potentially toxic unmetabolized PAHs to terrestrial insectivores.

In general, PAHs are relatively insoluble in water. Solubilities are expressed as log K_{ow} (octanol-water partition coefficient). This is a biologically relevant measure because octanol is assumed to act as a surrogate for lipid which is the major biological storage tissue for hydrophobic compounds. The higher a chemical's log K_{ow} value, the more hydrophobic the chemical is. This indicates how the chemical will partition between different phases in the environment and how the chemical will behave inside of an organism.

Neff (1979) described the cycling of PAHs in the aquatic environment which is summarized in the following section. Any PAHs entering an aquatic system rapidly adsorb onto both organic and inorganic particulate matter. In aquatic ecosystems, the amount of PAHs is generally highest in the sediments and suspended particulate matter, intermediate

in aquatic biota, and lowest freely dissolved in the water column. Compounds with low log K_{ow} values are expected to exhibit higher concentrations freely dissolved in the water column than compounds with higher log K_{ow} values. It is also possible for a small fraction of sediment-associated PAHs to be returned to the water column via leaching or biological activity. This freely dissolved fraction of PAHs is small and not easily quantified without a passive sampler or analysis of large quantities of water. In the present study, freely dissolved PAHs in the water column were quantified with semi-permeable membrane devices (SPMDs) (Metcalfe et al. 2000). These passive samplers absorb freely dissolved PAHs from the water column, eventually reaching equilibrium, thereby providing relative concentrations of the PAH congeners in the water column (Metcalfe et al. 2000).

There are many pathways by which PAHs may be removed from an aquatic system, including volatilization from the water surface (mainly restricted to more volatile, low log K_{ow} compounds) (Sage and Sage 2000), photooxidation (Neff 1979, Duxbury et al. 1994, Oris 1994, Bell 1995, Dutta and Harayama 2001), chemical oxidation (Neff 1979), microbial mineralization (Neff 1979, Fine Tails Fundamentals Consortium 1995b), metabolism by higher metazoans (Neff 1979, Varanasi et al. 1989, van den Heuvel et al. 1999), and emigration of organisms from the system (e.g., aquatic larval insects pupate and become terrestrial - Menzie 1980, Larsson 1984, Reinhold et al. 1999, Smits et al. 2000). However, PAHs may persist in the sediment for long, even geologically-significant, times because photochemical and biological oxidation are limited in sediments (Neff 1979).

PAHs can be taken up by biota through their food (biomagnification) and/or via water (bioconcentration) (Gobas and Morrison 2000). Biomagnification is a dynamic process

whereby a chemical is taken up through food and eliminated via respiration, fecal egestion, and metabolism. Biomagnification of a chemical is thus only observed when the rate of uptake exceeds the combined rate of elimination. The process of bioconcentration involves the uptake of freely dissolved chemicals from water via diffusion across respiratory surfaces as well as loss of chemicals via respiratory surfaces, metabolic transformation, and fecal egestion. Bioconcentration is thus observed when the rate of uptake exceeds the combined rate of chemical elimination. Food web bioaccumulation, or simply bioaccumulation, is a combination of both biomagnification and bioconcentration. However, biomagnification is the driving process behind bioaccumulation (Gobas et al. 1993). As the biomagnification process is repeated from one trophic level to the next, a progressive increase is observed in the body burden or chemical concentration in the organism (lipid-normalized), provided that the chemical is susceptible to bioaccumulation (i.e., it has a log K_{ow} greater than 5), and is not susceptible to metabolism (Thomann and Komlos 1999).

Regardless of the mode of uptake, the capacity of an organism to hold a chemical is determined by its lipid content (Gobas et al. 1993, Gobas and Morrison 2000). More hydrophobic (higher log K_{ow}) compounds have a greater capacity to bioconcentrate because the rate of elimination from lipids to water decreases with increasing hydrophobicity (Gobas and Morrison 2000). This same principle can be applied to the biomagnification process. Biomagnification is only observed for compounds with a log K_{ow} greater than 5 (Thomann et al. 1992, Gobas and Morrison 2000) because high log K_{ow} compounds have relatively low elimination rates as a consequence of their insolubility in water (i.e., once they are in the lipid phase of an organism it is hard to remove them). Since most of the parent PAH compounds

in the Fort McMurray area are alkylated, most of them will have a log K_{ow} greater than 5. The lower log K_{ow} compounds are eliminated more quickly than the higher log K_{ow} compounds (Hendriks et al. 2001), causing them to reach a steady-state within the organism faster. Consequently, they should be present at a lower lipid-normalized concentration than the high log K_{ow} compounds (assuming that no metabolism occurs).

As already noted, metabolism of PAHs is limited in invertebrates and varies from species to species (Neff 1979). If an aquatic invertebrate possesses only a limited ability to metabolize a given PAH congener, then biomagnification, and hence bioaccumulation of that congener will be possible. The potential for PAHs in wetland ecosystems to ultimately cause carcinogenic or genotoxic effects in swallows or other insectivorous predators will depend on the amount of chemical accumulation in the invertebrates upon which the birds feed (e.g., Tanypodinae and *Chaoborus*).

Water chemistry features can also influence the bioaccumulation potential of PAHs. As the amount of dissolved organic matter (DOM) in the water increases, the bioavailability of PAHs decreases because the PAHs become increasingly bound to this material (Black and McCarthy 1988, Servos et al. 1989, Weinstein and Oris 1999). High salinity can increase the freely dissolved PAH concentration by enhancing the destabilisation of PAHs bound to particles in the water (Johnson et al. 1994), thereby increasing the amount of bioavailable PAHs.

PAH distribution among biota in a food web depends on a number of factors including the log K_{ow} of individual PAH congeners, the susceptibility of individual PAH congeners to metabolism (which will vary among taxa), as well as the concentrations of

individual PAH congeners in dietary items. The PAH body burden of an organism will also depend on its lipid content, which varies from one taxon to another and seasonally within a single taxon (Gardner et al. 1985, Goedkoop et al. 1998).

As previously discussed, studies of PAHs in aquatic environments suggest that they accumulate in sediments, are rapidly broken down in the water column, and accumulate in aquatic invertebrates. This allows the simple prediction that PAH bioaccumulation and export is greatest via benthic invertebrates that spend the majority of their lives in contact with the sediment unless secondary production of the pelagic food web greatly exceeds that of the benthic food web. However, expectations are difficult to formulate with respect to PAH bioaccumulation and export via benthic invertebrates in reference vs. OSPM-affected wetlands as secondary production and trophic position may or may not be higher for benthic predators inhabiting OSPM-affected wetlands. These issues will be addressed in subsequent sections.

2) Food Webs, Energy Flow, and Stable Isotopes

Food chains are useful in illustrating the concept of the trophic level but are otherwise unrealistic because organisms typically have more than one predator, more than one source of food, and can exhibit ontogenetic shifts in diet. Food webs reflect the complexity of these dynamics, and trophic levels become represented in a continuous instead of a discrete manner (Cabana and Rasmussen 1994).

Energy enters food webs as light energy from the sun, which primary producers convert into chemical energy. This primary production may occur within the wetland,

(autochthonous production; Kvet et al. 1998), or it may occur on land and the carbon may enter the aquatic system with run-off (allochthonous production; Day et al. 1998). Energy then flows through the food web in one direction only as it is transferred from one trophic level to the next. It may later be recycled through the system by detritivores, which feed on decomposing plant and animal matter. At each transfer, energy is lost (as heat) due to inefficiencies in energy conversion from one organism to another. Thus, the total available energy decreases as trophic level increases, thereby placing an upper limit on the number of possible trophic levels in a food web (Smith 1996). Similarly, total production declines with each successive trophic level.

When studying chemical transfer in food webs it is important to know the trophodynamics of the ecosystem(s) of interest because biomass (energy) and potentially toxic hydrophobic chemicals flow in parallel paths. Associated with the flow of biomass is the flow of elements essential to life such as carbon and nitrogen. Both of these elements are important constituents of tissues (lipid and protein) that are considered to represent dietary energy (Tibaldi et al. 1996). Lipid, which is also the primary storage tissue for hydrophobic organic chemicals, is assembled with carbon backbones. Nitrogen is an important component of cellular molecules, amino acids, which are the building blocks of proteins. Therefore, the flow of carbon and nitrogen can be useful in describing trophic relationships in a food web and hence the transfer of matter (biomass and hydrophobic organic chemicals) from one trophic level to the next.

The isotopes of an element differ in the number of neutrons present in the nucleus (Hart 1990). Some of these isotopes are stable while others radioactively decay. The features

of stables isotopes of biologically relevant elements and their behaviour within organisms can be exploited to gain insight into trophic relationships and thus the pathways of biomass and hydrophobic chemical flow in a food web (Fry 1991). Both carbon and nitrogen have a heavy stable isotope (^{13}C and ^{15}N) and a light stable isotope (^{12}C and ^{14}N), and the stable isotope signature of an organism for either element is the ratio of the heavy to light stable isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). When stable isotopes are subjected to biological reactions, the ratio of the heavy to the light stable isotope may be altered or fractionated (Peterson and Fry 1987). Rates of isotopic fractionation vary depending on the physical and biological processes to which the elements are subjected (e.g., Hecky and Hesslein 1995). This leads to differences in the isotopic signature of the source pools of carbon and nitrogen for different organisms at the base of a food web. Therefore, in order to make comparisons among samples, we must express the ratio of stable isotopes to a standard. For carbon, the standard used is the ratio of $^{13}C/^{12}C$ in a Cretaceous marine fossil of *Belemnitella americana* from the PeeDee formation in South Carolina (Peterson and Fry 1987). The standard for nitrogen is the ratio of $^{15}N/^{14}N$ in the air, which is assumed to be constant (Peterson and Fry 1987). The isotope signature of a sample is then calculated using the following equations for C and N:

$$\delta^{13}C = \frac{^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{standard}}{^{13}C/^{12}C_{standard}} \times 1000$$

$$\delta^{15}N = \frac{^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard}}{^{15}N/^{14}N_{standard}} \times 1000$$

A positive δ value indicates that a sample is enriched in the heavier isotope relative to the

standard material and a negative δ value indicates that a sample is depleted in the heavier isotope relative to the standard material.

Stable isotopes are a continuous integrator of trophic dynamics. Thus, their use takes into account both spatial and temporal variation in the feeding habits of the study organisms. The $\delta^{13}C$ signature reflects the general diet of organisms and can be used to describe predator-prey relationships in complex food webs (Kiriluk et al. 1994) primarily because the carbon signature of a predator is nearly identical to that of its prey (< +1‰ - DeNiro and Epstein 1978, Fry and Sherr 1984). This means that the $\delta^{13}C$ signature of an organism should be nearly identical to that of the primary carbon source in the food web. Thus, one can use $\delta^{13}C$ to confirm whether any group of organisms belong to the same food web. For example, one should be able to discriminate benthic from pelagic organisms due to differences in the $\delta^{13}C$ signatures of the benthic and pelagic primary producers, benthic algae and phytoplankton (Hecky and Hesslein 1995).

A higher degree of isotopic fractionation in organisms is observed with stable nitrogen isotopes. In a wide variety of animals, an average change in $\delta^{15}N$ of +3.4‰ occurs from prey to predator because organisms preferentially incorporate the heavier nitrogen isotope during protein synthesis (Minagawa and Wada 1984). The $\delta^{15}N$ signature of an organism can thus be used to indicate its trophic position (Minagawa and Wada 1984, Kiriluk et al. 1994). Because $\delta^{15}N$ increases proportionately with each level up the food web, it is important to know the baseline food web value and variation in $\delta^{15}N$ when comparing trophic positions among different sites (Vander Zanden et al. 1997). This can be done by expressing $\delta^{15}N$ values of different organisms relative to those of a primary consumer common to all

study sites such as zooplankton or unionid mussels (Cabana and Rasmussen 1994). For example, trophic position of fish = $2 + (\delta^{15}N_{fish} - \delta^{15}N_{mussel})/3.4$ where 2 is the trophic position of the mussels and 3.4 is the average enrichment in $\delta^{15}N$ per trophic level.

The goal of my thesis is to contrast biomass and PAH flow through the invertebrate components of the benthic and pelagic food webs of wetlands (Fig. 1.2). Stable carbon and nitrogen isotope signatures of each sample collected will be measured to take into account complex trophic interactions. Stable carbon isotopes will be used to determine food sources of a variety of taxa, while stable nitrogen isotopes will be used to estimate the trophic positions, and hence the potential to bioaccumlate PAHs, of the same taxa. The concentration of PAH congeners in predatory Tanypodinae and *Chaoborus* will also be measured to assess bioaccumulation in each of the two food webs.

The benthic communities of reference and OSPM-affected wetlands of the Fort McMurray area are dominated by Chironomidae, Oligochaeta, and Ostracoda (Whelly 1999). Whelly (1999) found that the relative abundance of chironomids was always higher at OSPM-affected wetlands than at environmentally most similar reference wetlands, meaning that Chironomidae dominate these wetlands to a greater extent than they do in reference wetlands. Chironomidae densities and biomasses were as much four- and 26-fold greater respectively in OSPM-affected wetlands compared to reference wetlands (Bendell-Young et al. 2000). Within the Chironomidae, *Chironomus* and Tanypodinae constitute the dominant benthic taxa in both reference and OSPM-affected wetlands (Whelly 1999). Both taxa are often among the most abundant chironomids of saline wetlands (Rawson and Moore 1944, Cannings and Scudder 1978).

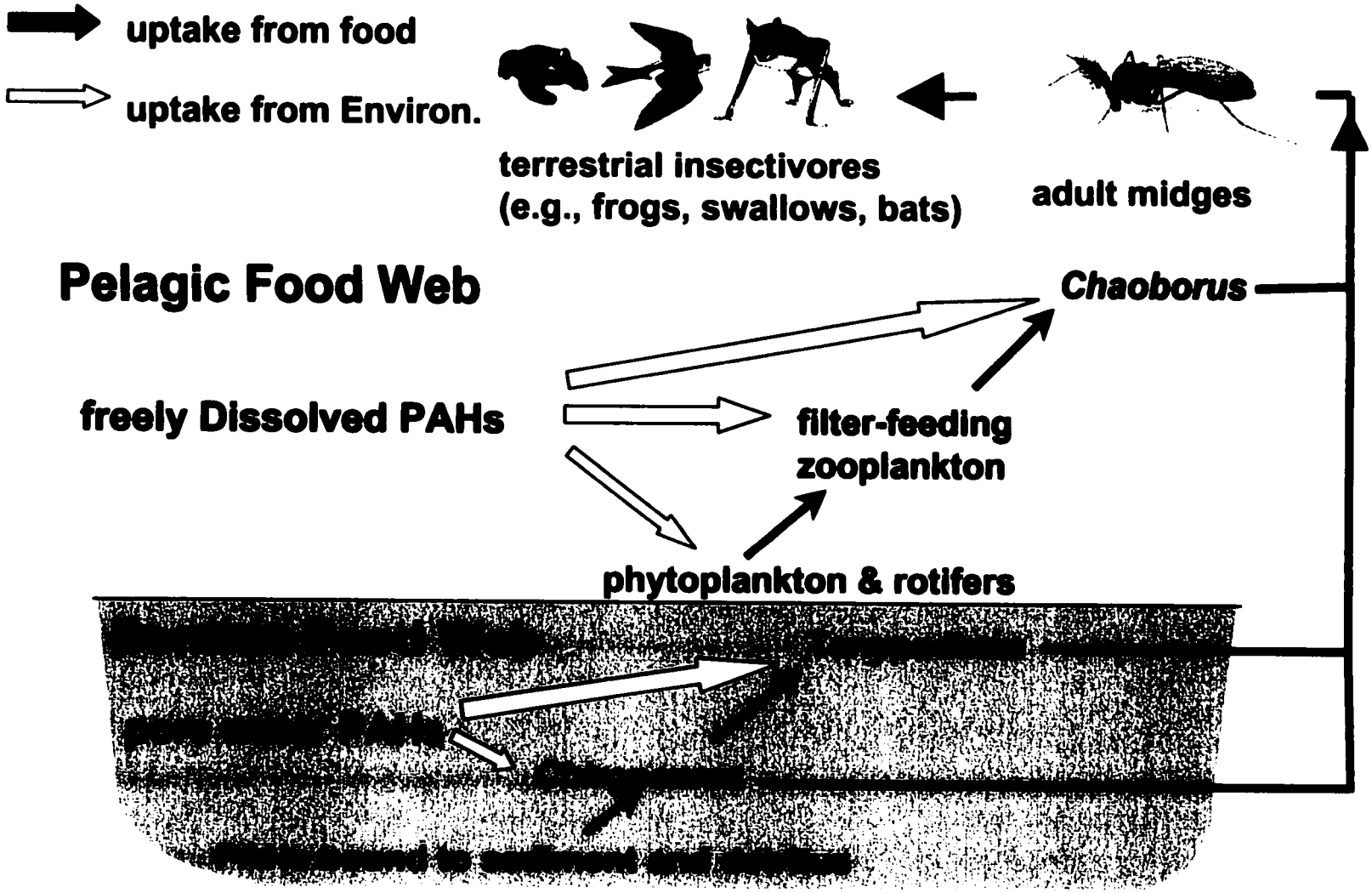


Fig. 1.2. Schematic diagram of the generalized food webs and routes of PAH exposure at constructed reference and OSPM-affected wetlands.

In the wetlands of the Athabasca oil sands area, *Chaoborus* is often the most abundant pelagic predator (pers. obs.). Its prey is primarily zooplankton (Fedorenko 1975).

Pelagic food webs subject to chemical stress generally exhibit reduced phytoplankton and zooplankton species diversity, an increase or decrease in algal biomass, a shift to large phytoplankton dominating the phytoplankton community, and a decrease in total zooplankton biomass (large phytoplankton are too large to be eaten by most zooplankton) relative to reference food webs (Xu et al. 1999). In the Athabasca oil sands, microcosm experiments with a natural phytoplankton community exposed to OSPM of varying ages showed no significant effects on phytoplankton communities when the OSPM was greater than 5 y old (Leung et al. 2001).

The decrease in phytoplankton or zooplankton biomass caused by chemical stress may limit biomass flow to top predators in the pelagic food web because the production of a predator depends upon the production of its prey, which is related to the prey's biomass (Benke 1996). Therefore, if chemical stress limits biomass flow, then it would ultimately have the effect of reducing the potential amount of PAHs that can be exported out of OSPM-affected wetlands.

The above scenario illustrates that a chemical stress can cause food web dynamics and structure to differ between reference and OSPM-affected wetlands, potentially resulting in the same study organism occupying different trophic positions at different wetlands. Because the concentration of a hydrophobic chemical in an organism is a function of trophic position (Rasmussen et al. 1990), a given organism's concentration of PAHs will likely vary from

reference to OSPM-affected wetlands even if the concentrations of PAHs in the two wetlands are identical. This makes it important to accurately quantify trophic positions.

The differences in invertebrate feeding relationships in OSPM-affected versus reference wetlands are poorly understood. If the observed trophic position of a given taxon is increased in OSPM-affected wetlands relative to reference constructed wetlands, then the potential for PAH bioaccumulation and export is greater in OSPM-affected wetlands. Conversely, if the observed trophic position of a taxon is less at OSPM-affected wetlands, then the potential to bioaccumulate and export PAHs is less at these water bodies.

The use of stable isotopes will allow me to detect differences in community structure and function (i.e., differences in the trophic position of a given organism) between OSPM-affected and reference wetlands. The use of stable isotopes in this situation is desirable because, 1) the feeding relationships in the rest of the invertebrate community do not need to be observed, and 2) it is a simple method that provides critical information to potentially account for among-wetland variation in the concentration of various PAH congeners in the same organism due to differences in diet ($\delta^{13}C$) and observed trophic level ($\delta^{15}N$).

3) Secondary Production of Aquatic Macroinvertebrates

Difference in the secondary production of aquatic insects inhabiting OSPM-affected and reference wetlands must be understood for responsible mining lease closures as OSPM-affected wetlands should be biologically productive, non-hazardous, and sustainable (Oil Sands Wetlands Working Group 2000). The terrestrial adult forms of insects with aquatic larval stages are important prey items of terrestrial insectivores such as swallows and

amphibians (Bendell-Young et al. 1997, Smits et al. 2000). If their production is greatly reduced in OSPM-affected wetlands, then there may be ecological and toxicological consequences at higher trophic levels which depend on wetlands for food (e.g., tree swallows). On the other hand, if their production is greater in OSPM-affected wetlands, then the study organisms may become relatively more important vectors of biomass and PAHs to swallows and other predators.

Preliminary work on chironomid secondary production in constructed wetlands of the Athabasca oil sands suggests that chironomid production is approximately four times greater at reference wetlands compared to OSPM-affected wetlands (Hum 2000, McDonald 2001). Since PAHs are present (although at low levels) in reference wetlands, PAH export could potentially be greater at reference sites than at OSPM-affected wetlands. The relative amount of PAH export will depend on the extent to which secondary production differs among OSPM-affected and reference wetlands.

In addition to using secondary production data to address the issue of PAH export, my study will answer a basic ecological question: What is the relative importance of benthic and pelagic pathways of biomass (energy) flow within a wetland as represented by the secondary production of predatory benthic and pelagic dipterans?

EXPECTATIONS

In terms of potential biomass export to the terrestrial environment within a given wetland, it is expected that predatory benthic dipterans (Tanypodinae) will export more biomass to the terrestrial environment than predatory pelagic dipterans (*Chaoborus*). This is

based on work done on annual production estimates for these two different taxa in independent studies (Chironomids: Ruzickova 1987, Drake and Arias 1995, Tokeshi 1995a, and Wolfram 1996; *Chaoborus*: Dermott et al. 1977 and Iwakuma et al. 1989). It follows that Tanypodinae should also comprise a greater portion of the diet of terrestrial insectivores such as swallows.

The bioaccumulation potential of PAHs in benthic and pelagic predatory dipterans is expected to be related to both trophic position and habitat. The trophic positions of Tanypodinae and *Chaoborus* are expected to be similar as they are both predatory dipterans. Thus, the bioaccumulation potential of PAHs is expected to be related more to the predominant habitat and diet of the organisms. Since the majority of PAHs in wetlands are sequestered in the sediment which is inhabited by Tanypodinae, it is expected that Tanypodinae will have a greater potential to bioaccumulate PAHs by virtue of its habitat. Tanypodinae also consume sediment material (where the majority of PAHs are) as well as other benthic invertebrates that consume sediment material (Berg 1995), whereas *Chaoborus* consume mainly planktonic organisms (Fedorenko 1977), which have a relatively low exposure to PAHs. Thus, Tanypodinae should also accumulate more PAHs by virtue of its diet. Taking into account both the relative expected annual production estimates of these predatory benthic and pelagic taxa and the expected bioaccumulation potentials, it is anticipated that Tanypodinae will export more biomass and PAHs to the terrestrial environment as well as contribute more biomass and PAHs to diet of terrestrial insectivores.

Tanypodinae are more predominant at OSPM-affected wetlands than they are at reference wetlands (Whelly 1999). This suggests that they may attain higher levels of annual

production at OSPM-affected wetlands. Thus, higher levels of PAHs in the sediments of OSPM-affected wetlands combined with potentially higher annual production of Tanypodinae will likely result in the largest estimates of PAH export coming from Tanypodinae inhabiting OSPM-affected wetlands.

CHAPTER 2

Environmental Characteristics of Four Constructed Wetlands in the Athabasca Oil Sands

INTRODUCTION

The purpose of this chapter is to describe general characteristics and specific physico-chemical characteristics of the water and sediment of wetlands sampled in this study and to outline major differences between OSPM-affected and reference wetlands. It begins with a definition of what a wetland is and a brief overview of the types of wetlands typically present in northern Alberta including which category constructed wetlands of the Athabasca oil sands fit into. Following this, an overview is given of the benthic invertebrate communities, the phytoplankton communities, and the zooplankton communities present in constructed OSPM-affected and reference wetlands. This chapter also introduces the focal study organisms (Tanypodinae and *Chaoborus*), the general types of sampling conducted, and the timing of sampling. Following this, a detailed description of each study site sampled is given. Finally, data related to the water and sediment chemistry are presented.

A number of different wetland types exist, making it difficult to define reference wetland characteristics against which OSPM-affected wetlands can be compared to assess their productivity, sustainability, and potential hazard to organisms that inhabit these waterbodies and/or depend on them for food or habitat. As a result, the Oil Sands Wetlands Working Group was formed to develop a guideline for appropriate wetland establishment on oil sands leases (Oil Sands Wetlands Working Group 2000).

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" (Oil Sands Wetlands Working Group 1988). Most of Alberta's 114,000 km^2 of wetlands are located in the boreal forest region of the province (Oil Sands Wetlands Working Group 2000), where the Athabasca oil sands are situated. Due to the cool, moist climate, the dominant wetland types are peat-forming fens and bogs. Non-peat forming wetlands (which can be subdivided into shallow open waters, marshes, and swamps) have <40 cm of accumulated organic material. Peat-forming wetlands have >40 cm of accumulated organic material. These can be subdivided into a) fens (pH>4.5) and b) bogs (pH<4.5) (Oil Sands Wetlands Working Group 2000).

Wetlands constructed on oil sands mining leases in the Athabasca oil sands area typically fit into the non-peat forming class of wetlands, unless they have been lined with a base of >40 cm of organic material. Non-peat forming wetlands are relatively common in the boreal forest region, making up approximately 7% of total wetland area of Alberta. However, peat-forming wetlands are much more dominant (93% of total wetland area) (Oil Sands Wetlands Working Group 2000). In this sense, constructed wetlands are atypical of local natural wetlands. They are also atypical in that they are often saline due to the presence of OSPM or runoff from sodic overburden storage areas which may contain lean oil sands (it is uncommon to find natural wetlands in the oil sands region that are saline - Oil Sands Wetlands Working Group 2000).

1) Planktonic Community of Constructed Wetlands - OSPM-Affected vs. High Salinity Reference

Phytoplankton

The presence of naphthenic acids and salts in OSPM-affected wetlands changes the size structure of the phytoplankton community as well as the species present and their relative abundances (Leung et al. 2001). Nevertheless, OSPM-affected and reference saline environments, much like other natural shallow saline waterbodies (Rawson and Moore 1944), are capable of supporting a productive and diverse phytoplankton community (Leung et al. 2001). Leung et al. inoculated microcosms filled with filtered water collected from OSPM-affected wetlands of varying age (0 to 8 y) and history with phytoplankton from a reference water body (Mildred Lake Reservoir) and compared the development of the phytoplankton community in the different microcosms. The phytoplankton community of Mildred Lake reservoir could not be distinguished from the community that developed in water collected from OSPM-affected wetlands greater than 4 y old. On the other hand, a difference in phytoplankton community composition and size structure was observed in waters collected from OSPM-affected wetlands that were less than 4 y old (Leung et al. 2001). The lack of differences in the phytoplankton community of waters collected from older OSPM-affected wetlands was primarily correlated with lower concentrations of naphthenic acids and salts in the water.

Zooplankton

The zooplankton community composition has been examined under the same conditions as phytoplankton community composition (McCormick 2000, Harris 2001). In

microcosm experiments conducted in different types of constructed waterbodies, 80 % of the variation in zooplankton community size (total biomass) and composition could be explained by differences in salt and naphthenic acid concentrations (i.e., the type of OSPM, which varied by age and origin).

However, direct field sampling appears to contradict results from the microcosm studies of the zooplankton communities in the water bodies under varying OSPM exposures. Compared to inoculated microcosm samples, field samples from different OSPM-affected wetlands were less different in zooplankton community composition relative to the reference Mildred Lake zooplankton community. However, Harris (2001) pointed out that marked seasonal variation in zooplankton community composition reduced the power of statistical tests to detect significant differences in community composition among OSPM treatments. Despite this fact, field samples showed the same trends as the microcosm experiments with respect to community size (total biomass). In both experiments the community size decreased as the OSPM characteristics changed from MFT (mature fine tailings) capped with clean water to MFT capped with settling basin water, to CT (consolidated tailings) water (Harris 2001).

The mechanism by which OSPM affects zooplankton communities remains unclear, and more research needs to be done. Regardless of the effects of OSPM exposure, saline environments in the Athabasca oil sands (reference and OSPM-affected waterbodies) are capable of supporting diverse zooplankton communities (Harris 2001) just as are natural shallow saline waterbodies (Rawson and Moore 1944).

2) Benthic Community of Constructed Wetlands - OSPM-affected vs. High Salinity Reference

Past work has shown that chironomid generic richness is reduced as the OSPM concentration increases (Whelly 1999). Whelly (1999) also found that the benthic macroinvertebrate (especially chironomid) community composition of OSPM-affected wetlands differed from the composition of environmentally similar reference wetlands. Species of Chironominae and to a lesser extent, Orthocladiinae, dominate the chironomid community at reference wetlands. Larvae of Tanypodinae (e.g., *Derotanypus*) are also found at reference wetlands but are not considered to be among the dominant taxa (Table 2.1; Whelly 1999).

At OSPM-affected wetlands, species of Tanypodinae dominate the chironomid community in addition to species of Chironominae and Orthocladiinae (Table 2.1). Shallow saline waterbodies in Saskatchewan contain chironomid communities that are similar those in the saline OSPM-affected wetlands (Rawson and Moore 1944). Saline Saskatchewan wetlands were dominated by Chironominae (*Chironomus*, *Cryptochironomus*, and *Tanytarsus*) and Tanypodinae (*Procladius*). Similar communities also occur in saline wetlands of interior British Columbia (Cannings and Scudder 1978).

Although reference and OSPM-affected wetlands differ with respect to the relative dominance of chironomid taxa and taxa richness, similar taxa are present in both types of wetlands. This allows researchers to compare ecological processes in the same organisms inhabiting different habitats.

In addition to finding differences in the relative composition of the chironomid community with respect to OSPM exposure under field conditions, Whelly (1999) showed

Table 2.1. Predominant chironomid taxa (in terms of abundance) in dip net samples at 3 reference and 3 OSPM-affected wetlands (modified from Whelly1999).

	Predominant Taxa	
Wetland Type	**Dominant**	**Subdominant**
reference	CHIRONOMINAE (*Chironomus*, *Tanytarsus*)	CHIRONOMINAE (*Chironomus*, *Paratanytarsus*) & ORTHOCLADIINAE (*Cricotopus*, *Psectrocladius*)
OSPM-affected	CHIRONOMINAE (*Tanytarsus*) & ORTHOCLADIINAE (*Psectrocladius*)	TANYPODINAE (*Derotanypus*), CHIRONOMINAE (*Chironomus*), & ORTHOCLADIINAE (*Cricotopus*,*Psectrocladius*)

that survival, growth, and development time of both lab and field-derived cultures of *Chironomus tentans* were negatively influenced when larvae were grown in high concentrations of OSPW in the laboratory.

METHODS

1) Study Sites

Four wetlands were sampled during 2000 and 2001. Two sites were located on the Syncrude Canada Ltd. lease area and two were situated on Suncor Energy Inc. lease area (Fig. 2.1). One reference wetland and one OSPM-affected wetland was identified on each lease area. The reference wetlands sampled are locally called Shallow Wetland South Ditch (Syncrude) and High Sulphate Wetland (just east of Hwy 63 near Ruth Lake). The OSPM-affected wetlands sampled were Test Pond 7 (Syncrude) and Natural Wetland (Suncor).

2) Study Organisms

The focal study organisms are closely related insects, belonging to the suborder Nematocera (Diptera). Chironomids (midges, including Tanypodinae) and *Chaoborus* (the phantom midge) pass through 4 larval instars and a pupal stage, after which they emerge from the aquatic habitat and become terrestrial adults similar in appearance to adult mosquitoes. Tanypodinae and most other chironomids are benthic (sediment-dwelling), whereas *Chaoborus* larvae are mostly pelagic in fishless wetlands. Many species of *Chaoborus* spend daytime periods at the bottom of water bodies (Teraguchi and Northcote 1966, Roth 1968, Goldspink and Scott 1971), especially in the presence of fish (Dawidowicz et al. 1990,

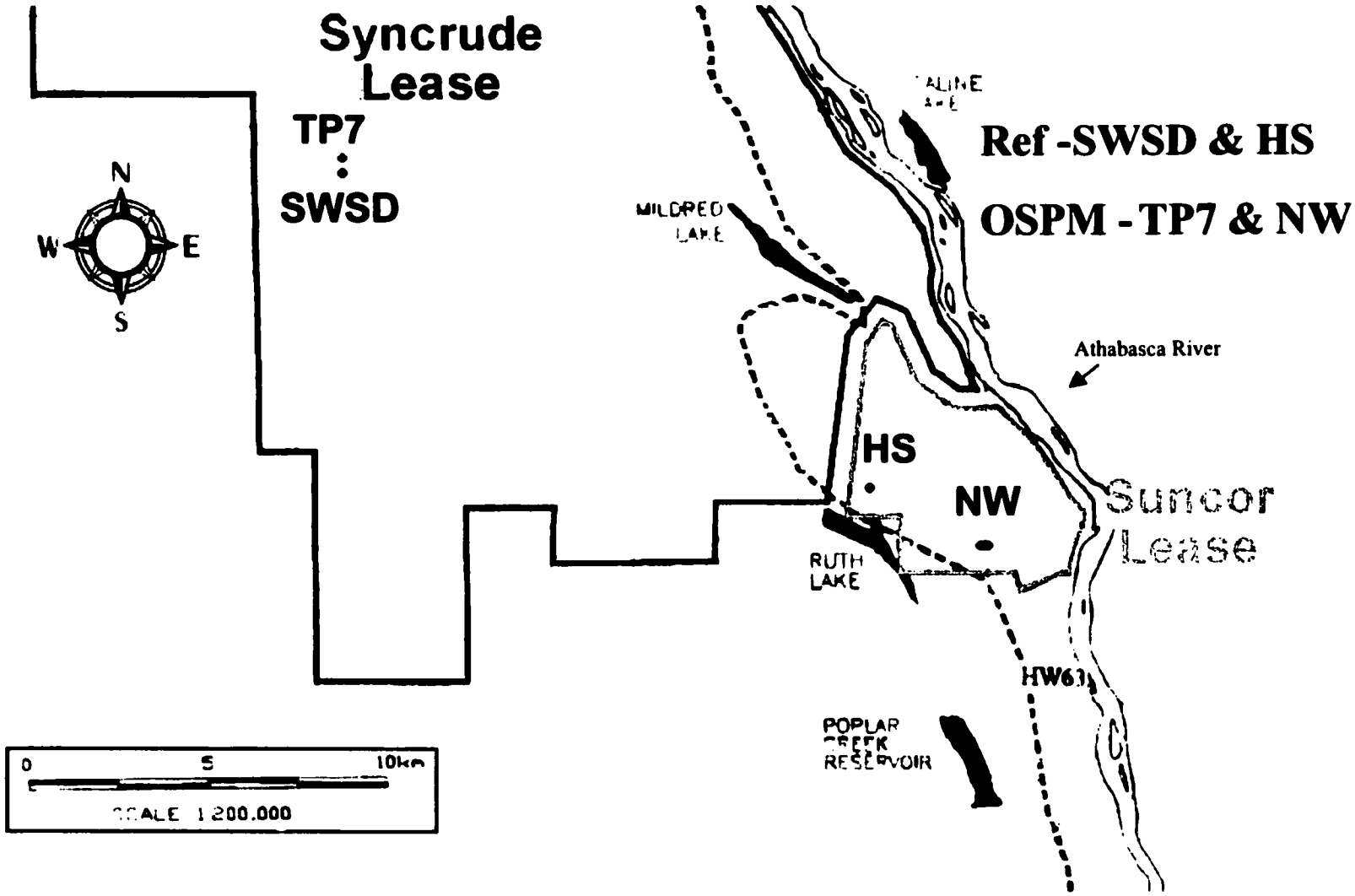


Fig. 2.1. Partial map of oil sands mining leases (modified from Whelly 1999) illustrating the location of study wetlands (SWSD = Shallow Wetland South Ditch, HS = High Sulphate, TP7 = Test Pond 7, and NW = Natural Wetland).

Jossem 1990). Since fish are absent from the study systems, *Chaoborus* larvae spend most of their time in the water column, where they forage on plankton. Tanypodinae and *Chaoborus* were chosen as the focal study organisms because they are abundant in wetlands of the Fort McMurray area, whether the wetlands are natural or contain oil sands process materials (Whelly 1999; pers. obs.). They were also chosen because they are top predators in their respective food webs, Tanypodinae in the benthic food web (Berg 1995) and *Chaoborus* in the pelagic food web (Fedorenko 1975) (Table 2.2). Therefore, they should be good indicators of differences in food web structure and function between OSPM-affected and reference wetlands.

To broaden understanding of food web dynamics at the study sites, other taxa were also collected from both the benthic and pelagic food webs (Table 2.2). These taxa were collected because they are typical and abundant components of wetland ecosystems in general (Lovvorn et al. 1999), and of constructed OSPM-affected and reference wetlands in particular (Whelly 1999). The choice to collect the particular size fractions of plankton in Table 2.2 was based on the size of planktonic organisms and size spectrum theory (Jennings et al. 2002). A 500-μm sieve was expected to allow planktonic organisms to pass through while retaining debris, and a 180-μm sieve was expected to retain large zooplankton and allow smaller zoo- and phytoplankton to pass through (MacIsaac 2001, University of Windsor, pers. comm.). Size spectrum theory states that the greater the biomass of an organism, the greater its trophic position (Jennings et al. 2002). In other words, larger organisms are assumed to eat smaller organisms and this principle was used to assume different size classes of plankton represent different trophic guilds.

Table 2.2. Operationally defined trophic guilds in the pelagic and benthic food webs at constructed reference and OSPM-affected wetlands.

Trophic Guild (or size class)	**Pelagic Food Web**	**Benthic Food Web**
0.5 - 20-μm	nanoplankton	detritus
20 - 180-μm	small phyto- and zooplankton	detritus
180 - 500-μm	large phyto- and zooplankton	detritus
detritivore/herbivore	*Daphnia*	*Chironomus*, miscellaneous chironomids, Gastropoda
predator	*Chaoborus*, Zygoptera, Anisoptera, Notonectidae, Dytiscidae	Tanypodinae

Parallel size fractions of detritus were collected to represent the base of the benthic food web (Table 2.2).

From mid-spring to early fall, larvae of the focal study organisms Tanypodinae and *Chaoborus* emerge from the wetlands as flying adults (Whelly 1999). As they enter the terrestrial food web they bring with them a portion of the PAHs that they accumulated living in the wetlands as larvae (Reinhold et al. 1999). These PAHs can then be transferred to the terrestrial predators that feed on these insects (Reinhold et al. 1999, Smits et al. 2000).

Tanypodinae and *Chaoborus* adults do not feed, live for only a few days, and have limited dispersal ability (Armitage 1995). The longest oviposition flight observed for a chironomid was 850 m, and most dispersal is passively mediated by wind (Armitage 1995). Even wind dispersal will be limited because the lifespan of most adult Chironomidae is only a few days (Armitage 1995). Chaoboridae have oviposition flights of up to 1.2 km (Berendonk and Bonsall 2002), illustrating that their dispersal is limited in the same manner as it is for Chironomidae.

The limited dispersal of chaoborids and chironomids is the basis for two assumptions that I have made: 1) any PAHs transferred to terrestrial insectivores via midges originated in the wetland in which the larvae lived; and 2) adults in a certain area lived out the larval stages of their life in waterbodies in that area.

3) Timing of Sampling

Almost all samples processed in this study were collected in 2001 and thus this section pertains to collections made in 2001. Food web collections for PAH and stable

isotope analysis were made one wetland at a time to minimize the potential effect of temporal variation in community composition on observed trophic relationships. Natural Wetland was sampled in early May, followed by sampling of Test Pond 7 beginning in early June. High Sulphate Wetland was sampled at the beginning of July followed by collections at Shallow Wetland South Ditch at the end of July.

4) Types of Sampling

a) physico-chemical sampling

b) sampling of food web components (Table 2.2)

c) sampling to estimate secondary production of top invertebrate predators (*Chaoborus* and Tanypodinae) and non-predatory chironomid taxa. Collections for chironomid and *Chaoborus* productivity were done twice weekly at each wetland (see Chapter 3).

5) Physico-Chemical Data

Wetland Dimensions

Measurements of wetland dimensions were taken at each wetland in order to estimate surface area and water volume. These measurements were necessary to generate comparable estimates of chironomid and *Chaoborus* annual production within each wetland. Annual production of chironomids was expressed on a per m^2 basis whereas *Chaoborus* production was calculated on a per m^3 basis. In order to convert *Chaoborus* annual production to an areal

basis at each wetland, the estimate at a wetland was multiplied by the volume of water in that wetland and divided by the surface area.

To begin with, the maximum length and width of each wetland were measured. Each wetland was then divided into a grid by demarcating 5 or 6 parallel lines across the length and width of each water body. Line positions were marked along the margin of the wetland with flagging tape tied to riparian vegetation. The distance between lines was measured to the nearest cm with a 100-m tape measure. The water depth at the points of intersection of the lines was then measured with a meter stick to the nearest cm. This was accomplished by wading through the wetlands except for at Shallow Wetland South Ditch which required the use of a canoe. Each wetland was surveyed in its entirety in a single day (between Aug. 2-8) so that all depth measurements were comparable. The depth of Test Pond 7 could not be measured with a meter stick since it was not possible to discern the precise point of the MFT-water interface. Instead, a mean depth of 40 cm was assumed, based on the wetland construction blueprints (M. MacKinnon 2002, Syncrude Canada Ltd., pers. comm.).

A depth contour map of each wetland was generated by plotting the water depth measured along transects at each wetland and the length of each transect. The wetland shoreline, and depth contours were then sketched by hand on the map.

An image of each wetland map was captured with an image scanner and digitized. The area of each depth contour was then measured using the image analysis software SigmaScan® Pro 5.0 (Jandel Scientific; San Rafael, CA). The volume within a contour boundary was calculated by multiplying the mean depth of that contour by the area circumscribed by the contour. Total surface area and volume were then calculated by adding

up the surface areas and volumes within each contour. This was done for all wetlands except Natural Wetland since a more comprehensive bathymetric survey has been done on this water body (Bishay 1992). Thus, the surface area and volume reported for Natural Wetland are based on the work of Bishay (1992) rather than the map presented in this study.

General Environmental Features

Qualitative field notes were made on each visit to a wetland, consisting of prevailing weather conditions (wind direction and speed, cloud cover, air temperature) and start and finish times, as well as any unusual wetland features at the time of sampling. Shoreline vegetation type and the relative amount of wetland bottom covered by submergent macrophytes were recorded on each visit.

Physico-chemical Characteristics of Constructed Study Wetlands

Seasonal Depth Change

The water level was measured twice weekly until early August by reference to meter sticks fixed in place in each wetland at the beginning of the summer.

General Sampling Schedule for Water and Sediment Chemistry

The water and sediment chemistry of the four wetlands was sampled regularly (every Monday and Friday) between May and mid-August 2001. The specific dates and times at which measurements were recorded at each wetland are included in Appendix 2.1A-D. All of the measurements in this appendix were taken in the field.

Additional water chemistry data were measured monthly at each wetland from samples collected at approximately one-half of the maximum wetland depth (analysis performed by M. MacKinnon, Syncrude Canada Ltd., Edmonton, Appendix 2.2).

Water Chemistry

At each wetland, pH, oxidative-reductive potential (ORP), dissolved oxygen concentration, salinity, conductivity, and temperature were recorded. Water was collected in a 4-L plastic jug held 20 cm beneath the water surface along the margin of a wetland. The pH of this water was measured with an Orion QuiKchecK™ model 106 pH•ATC pocket meter. The ORP was measured with an Orion QuiKchecK™ model 108 ORP pocket meter. Salinity, conductivity, and temperature were measured using a YSI Model 33 - S-C-T Meter. Dissolved oxygen was measured using a YSI Model 51B oxygen meter.

Sediment Chemistry

A small trowel was used to scoop up approximately 250 mL of surficial sediment (top 5 cm) from each wetland. The sediment was placed in a plastic tray, and pH and ORP were measured. Sediment pH and ORP were measured with the same meters used to measure water pH and ORP. The total organic carbon content and PAH concentrations of the sediments were also measured (see Chapter 5 for detailed methodology).

RESULTS AND DISCUSSION

1) Wetland Dimensions and General Environmental Features

The data for the average depths, areas, and volumes of each of the contours at each wetland are located in Appendix 2.3. Calculations of total surface area and volume were made for the open water zones of each wetland only.

"Natural" Wetland

Natural Wetland (NW - Figs. 2.2 and 2.3) is a saline, 21-y old experimental wetland located adjacent to a tailings pond on the Suncor lease (56°58'892"N, 111°30'642"W). It is roughly rectangular in shape and has a relatively uniform depth. It is the largest of the 4 study wetlands (Table 2.3), with a surface area of 1.27×10^4 m^2 and water volume of 3.30×10^3 m^3 (Bishay 1992).

Natural Wetland was naturally formed in a depression from tailings pond water seeping beneath a retaining dyke. It was subsequently modified to facilitate its use as a study site. This wetland has a visible inflow at its northern end and outflow at its southern end. The discharge rate of water from the outflow is estimated to be 2 L/s for a residence time of about 19-d (Bishay 1992). Natural Wetland continually receives inputs of dyke seepage water from the adjacent tailings pond to the north. Past researchers have conducted experiments during which time water from consolidated tailings (CT) was pumped into Natural Wetland during the open water season via a 25-cm diameter pipe discharging upstream of the inflow (Smits et al. 2000). This pump was operated intermittently over the course of sampling for this study (early May 2001 to early August 2001).

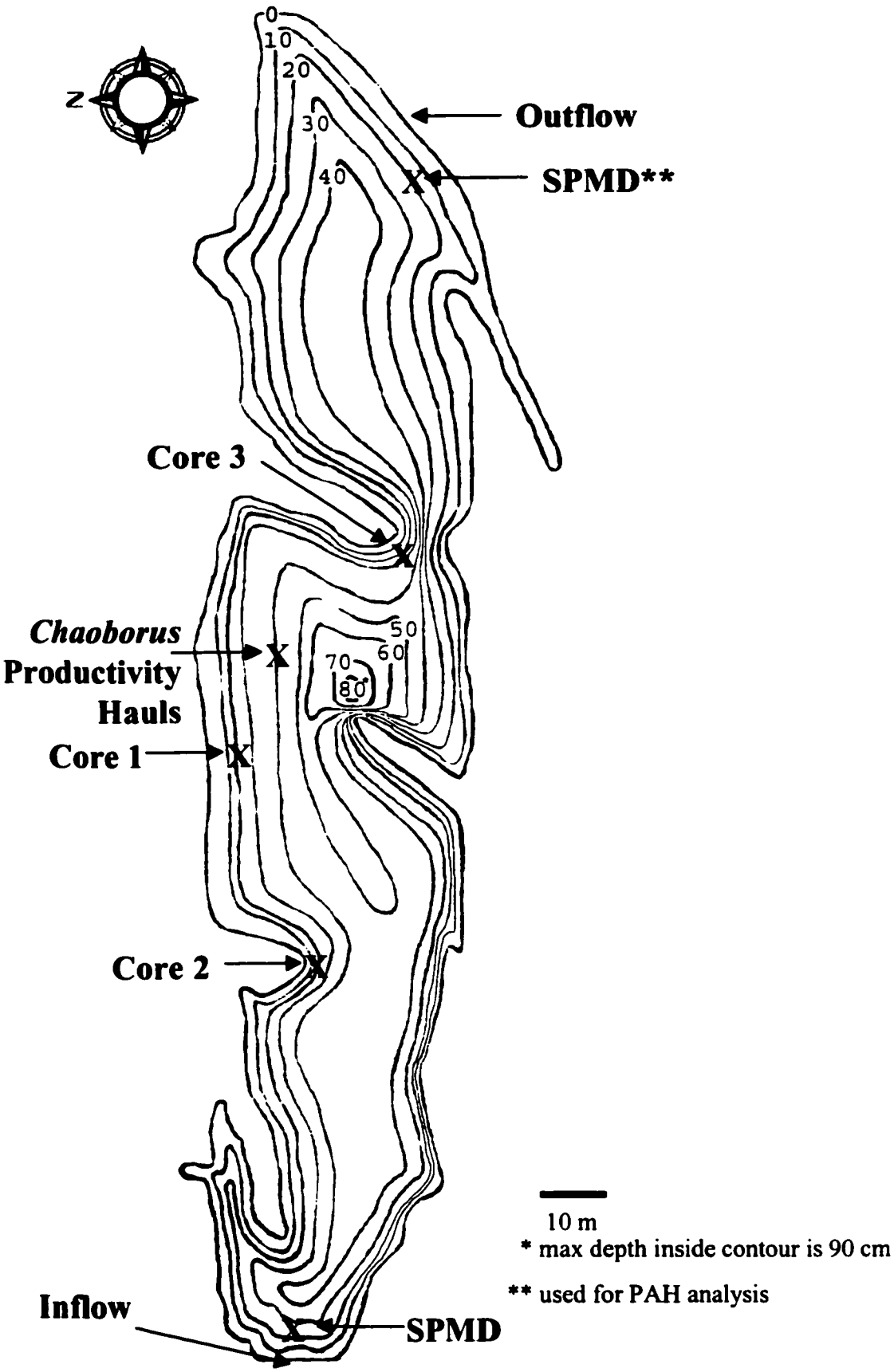


Fig. 2.2. Depth profile of Natural Wetland. (All depths in cm)

Fig. 2.3. Photograph of Natural Wetland (OSPM).

Table 2.3. Surface area and volume of study sites.

Site	Surface Area open water (m^2)	Volume open water (m^3)
Natural Wetland*	12700	3300
Test Pond 7	797	319
High Sulphate	1208	669
Shallow Wetland South Ditch	722	418

*from Bishay (1992)

The sediment of NW consists of an ~10-cm deep layer of organic substrate over a base of sand and organic material. It contains the second highest levels of PAHs among the study sites (Chapter 5). The total organic carbon content of the 180 - 500-μm detritus is 27.3% (Chapter 5). The bitumen content of sediment (oil : water solids of sediment pore water) collected from the water-sediment interface is 0.017% (n = 5, S.D. = 0.0045%; Appendix 2.4). The sediment particle size distribution of mineral solids as determined by laser diffraction after bitumen was removed from the sediment is given in Appendix 2.4. The median particle size of the sediment at this wetland is 44-μm (Appendix 2.4).

The riparian vegetation is dominated by sedges (*Carex*) and to a much lesser extent cattails (*Typha*), with grasses and sparsely distributed poplar stands surrounding the wetland beyond 2 m from the shoreline (personal observations). From early May until June the submergent macrophyte *Chara* appeared to cover a large portion (> ~ 75%) of the bottom of the wetland as observed from the shoreline. As the summer progressed the *Chara* beds grew very dense and tall and then suddenly all died off on July 14 or 15, 2001. The die-off corresponded to an increase in water turbidity and a visible change in the crustacean zooplankton community.

Syncrude Test Pond 7

Test Pond 7 (TP7 - Figs. 2.4 and 2.5) is a saline experimental wetland located on the Syncrude lease approximately 2.5 km west of the plant (57°05'070"N, 111°41'625"W). It was one of 7 experimental ponds constructed in 1989, each of which received a unique combination of fill material and source water. The pond is roughly 17 m wide and 43 m long

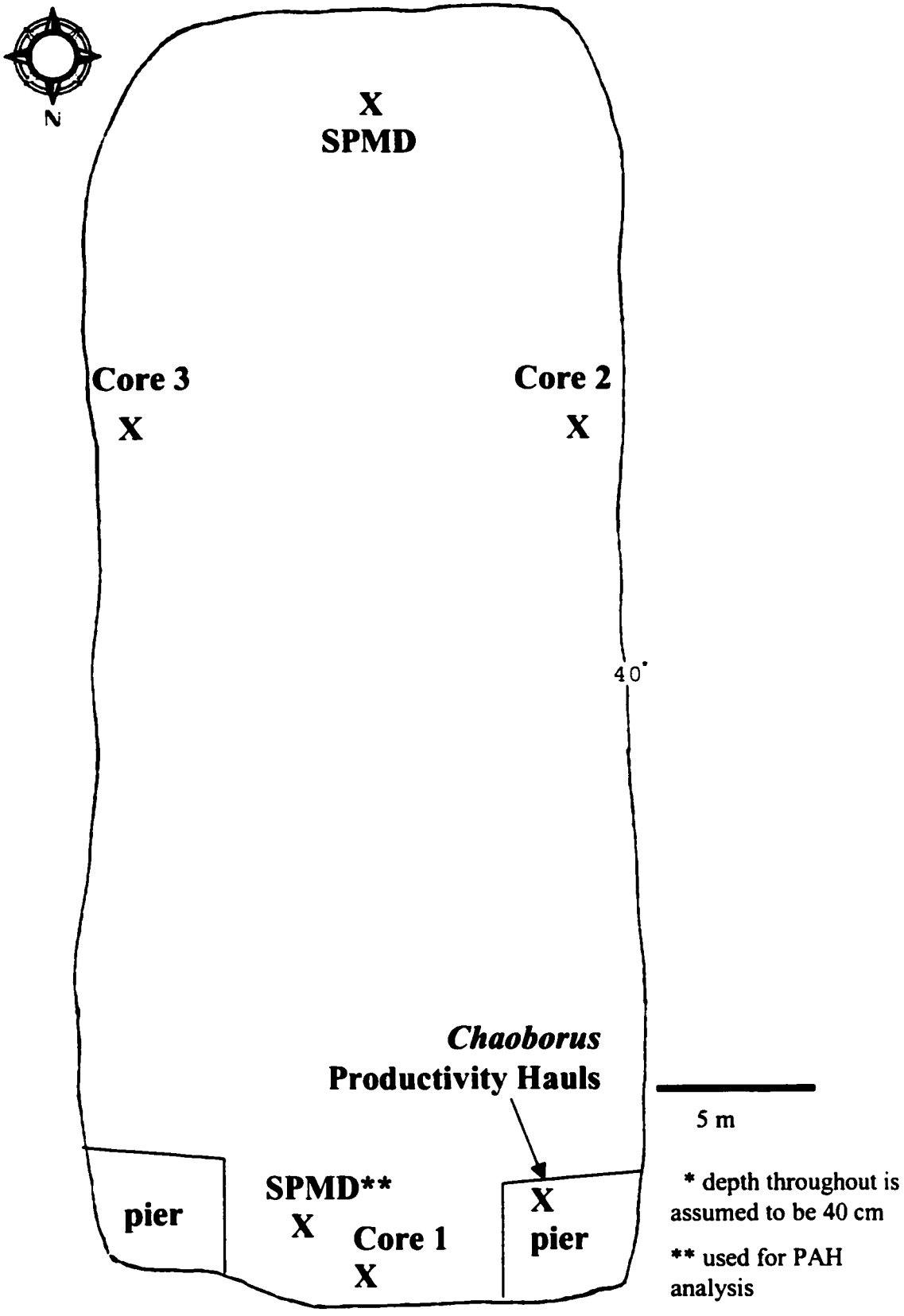


Fig. 2.3. Depth profile of Test Pond 7.

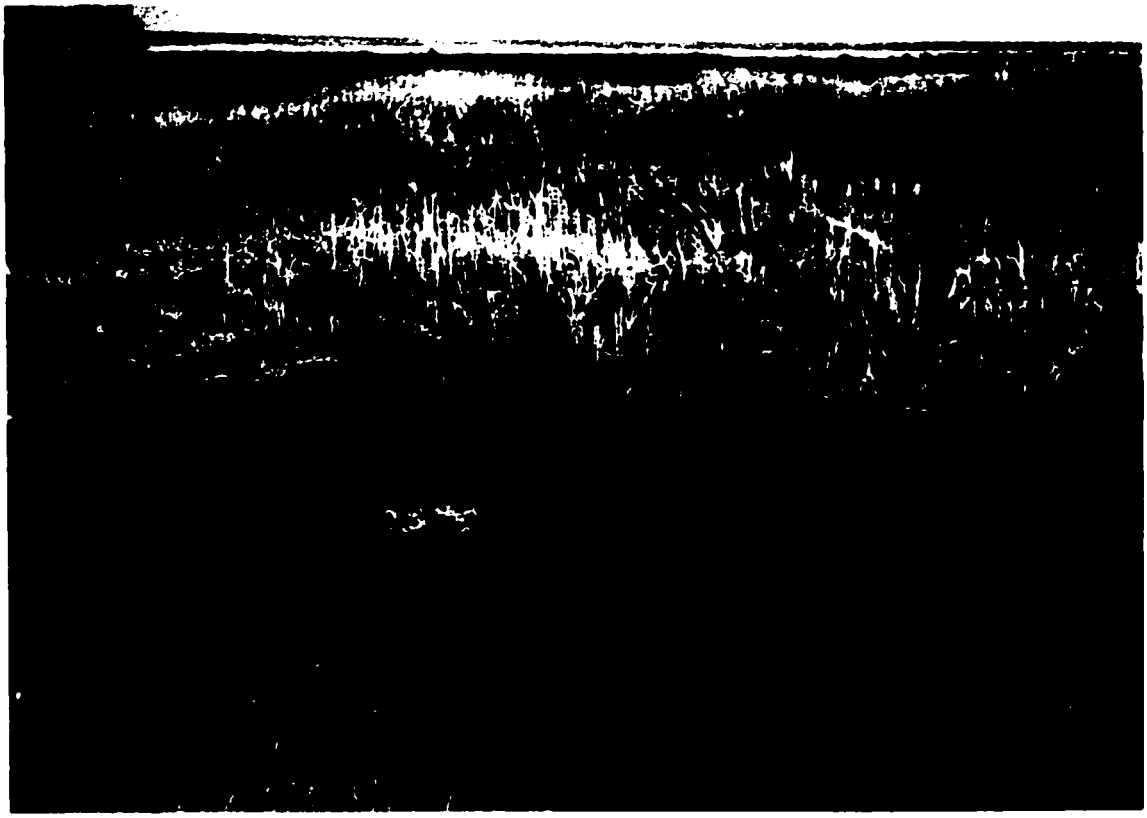


Fig. 2.5. Photograph of Test Pond 7 (OSPM).

(Fig. 2.4). It was built by digging out a pit and filling it with 1000 m^3 of MFT. The surface water layer is approximately 40 cm deep and is composed largely of MFT release water since the MFT compacts slowly over a long period of time and has been continually contributing release water to the pond. The area of this wetland is 797 m^2 and the volume of water is 319 m^3 (Table 2.3).

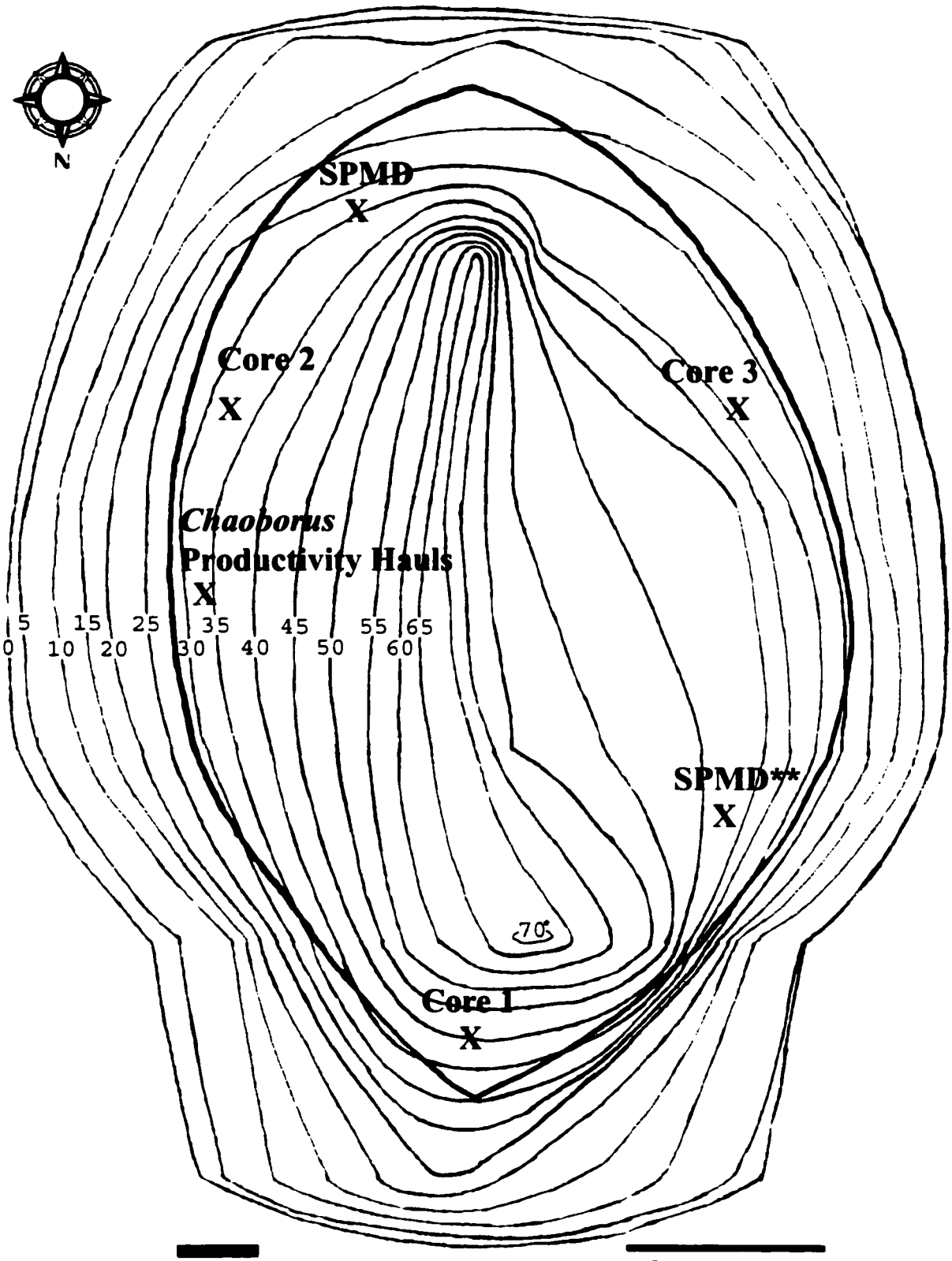
The walls of TP7 are steep, and the terrestrial vegetation surrounding it consists mainly of sedge (*Carex*) and grasses. The riparian vegetation consists of sedge (*Carex*) and cattail (*Typha*). The sedge is restricted to the wetted margin, and the cattails extend up to approximately 1 m into the water from the margin. *Chara* is abundant and dense on the wetland bottom within 2 m of the wetland margin. However, the water is so turbid that is difficult to estimate the extent to which *Chara* covers the bottom of the wetland farther from shore. The wetland substrate for the most part consists of MFT, except for a strip approximately 0.5 m wide around the edges where the pit was dug out. This substrate contains the highest levels of PAHs (Chapter 5) and likely has an organic carbon content of approximately 4.8% which is the average of the organic carbons contents at the environmentally similar Test Ponds 1, 2, and 5 (4.7%, 5.7%, and 4.1% organic carbon, respectively, Appendix 5.4). The bitumen content of sediment (oil : water solids of sediment pore water) collected from the water-sediment interface from the environmentally similar wetland, Pond 5 (near Waste Area 11), is 0.035% (n = 6, S.D. = 0.0019%; Appendix 2.4). The sediment particle size distribution of mineral solids at Pond 5 as determined by laser diffraction after bitumen was removed from the sediment is given in Appendix 2.4. The median particle size of the sediment at Pond 5 is 125-μm (Appendix 2.4).

High Sulphate Wetland

High Sulphate (HS - Figs. 2.6 and 2.7) is a 16-y old high conductivity reference wetland located west of the Suncor lease area and the tailings pond beside which NW is located (56°59'837"N, 111°33'291"W). The substrate is composed of lean oil sands mixed with overburden materials (e.g., stockpiled peat) and is saline. The area of this wetland is 1208 m^2 and the volume of water is 669 m^3 (Table 2.3).

High Sulphate is a small wetland whose surrounding terrestrial vegetation consists of sedges (*Carex*), bushes, willow, and poplar. Within the pond itself, there is a margin of cattails (*Typha*) and horsetails (*Equisetum*) ranging from 1 - 5 m in width. Unlike the other sites, cattails grow in a large portion of the entire High Sulphate wetland. Therefore, the wetted area of HS is approximately 27% larger than the open water area estimated from the grid sampling procedure. This potentially alters the available habitat for pelagic organisms such as *Chaoborus* as well as benthic organisms such as chironomids.

Within the submergent zone, *Chara* completely covers the wetland bottom. The total organic carbon content of the 180 - 500-μm detritus is 25.6% (Chapter 5) and the sediment at this wetland contains the lowest levels of total PAHs (Chapter 5). The bitumen content of sediment (oil : water solids of sediment pore water) collected from the water-sediment interface is 0.011% (n = 5, S.D. = 0.0038%; Appendix 2.4). The sediment particle size distribution of mineral solids as determined by laser diffraction after bitumen was removed from the sediment is given in Appendix 2.4. The median particle size of the sediment at this wetland is 125-μm (Appendix 2.4).



Cattail boundary

* max depth inside contour is 75 cm

** used for PAH analysis

Fig. 2.4. Depth profile of High Sulphate. (All depths in cm)

Fig. 2.7. Photograph of High Sulphate (Reference).

High Sulphate nearly dried up in the summer of 1999. This event may have affected the community composition to some extent in the summer of 2001, and hence food web collections and feeding relationships. This drying up would have led to a transient increase in the degree of salinisation, which affects both benthic (Lovvorn et al. 1999) and pelagic invertebrate community structure (Leung et al. 2001, Harris 2001).

Shallow Wetland South Ditch

Shallow Wetland South Ditch (SWSD - Figs. 2.8 and 2.9) is a low salinity reference wetland (57°04'899"N, 111°41'427"W) that was constructed in 1993. It has steep banks and is about 10 m wide and 165 m long. The area of this wetland is 722 m^2 and the volume of water is 418 m^3 (Table 2.3).

The vegetation surrounding SWSD consists mainly of grasses and shrubs. The riparian vegetation consists of sedge (*Carex*) along the margins of the wetland and sparsely distributed cattails (*Typha*) extending up to approximately 2-m from the margin. *Chara* covered approximately 50% of the bottom of the wetland in early spring, and by midsummer it had grown to very dense beds covering approximately 75% of the wetland bottom. The sediment of the adjacent Shallow Wetland has a relatively high clay content (Smits et al. 2000). The similarity in appearance of the sediment at Shallow Wetland with that of SWSD suggests that the sediment at SWSD also has a high clay content. The 180 - 500-μm detritus collected from SWSD has a total organic carbon content of 5.2% (see Chapter 5 for determination). The sediment at SWSD contains the second lowest levels of PAHs (Chapter 5), which were higher than that at High Sulphate solely due to high levels of the single

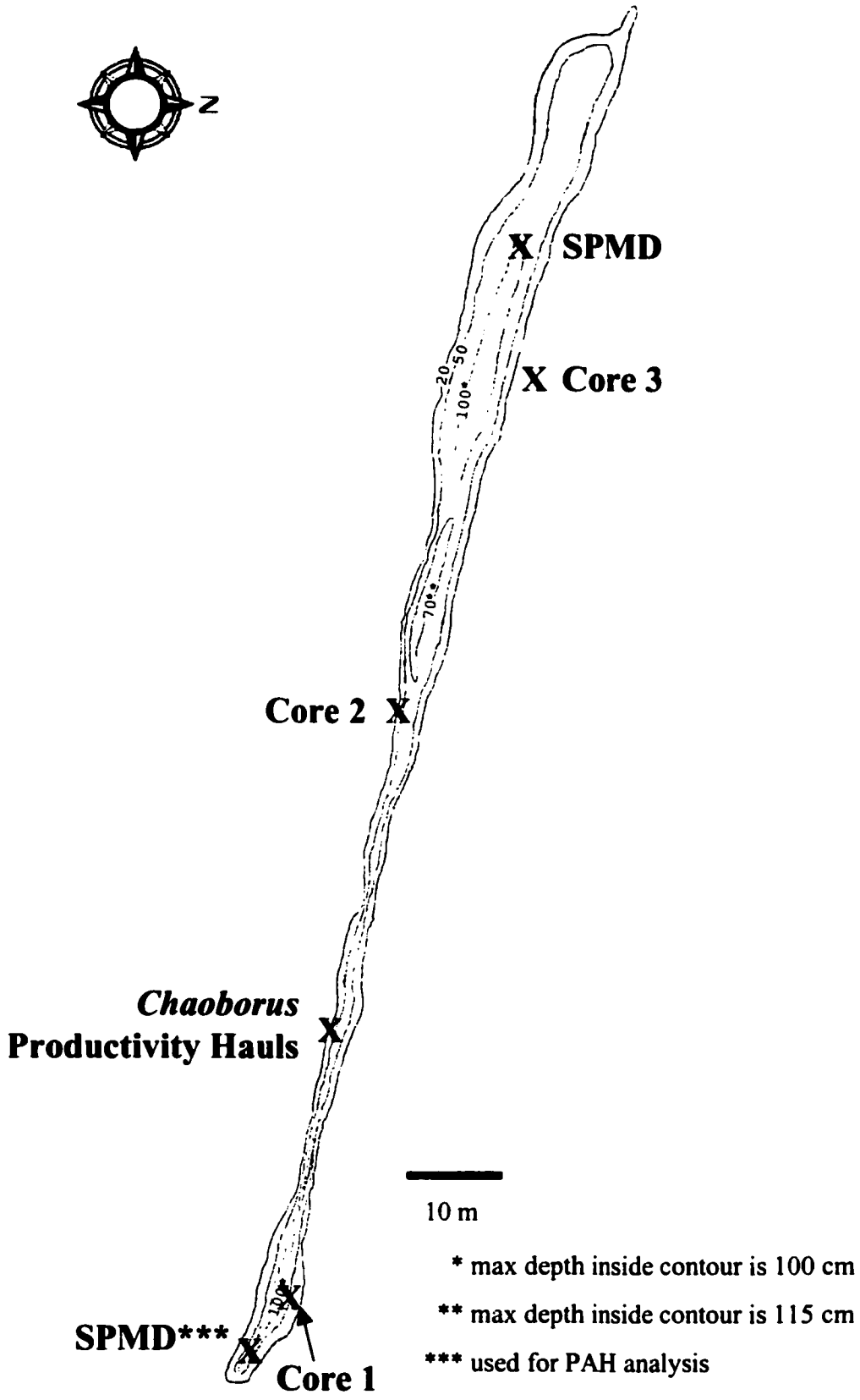


Fig. 2.5. Depth profile of Shallow Wetland South Ditch. (All depths in cm)

Fig. 2.9. Photograph of Shallow Wetland South Ditch (Reference).

congener C2 naphthalene. The bitumen content of sediment (oil : water solids of sediment pore water) collected from the water-sediment interface from the environmentally similar Shallow Wetland is 0.0013% (n = 11, S.D. = 0.0013%; Appendix 2.4). The sediment particle size distribution of mineral solids at Shallow Wetland as determined by laser diffraction after bitumen was removed from the sediment is given in Appendix 2.4. The median particle size of the sediment at Shallow Wetland is 2.8-μm (Appendix 2.4).

2) Physico-chemical Characteristics of Constructed Study Wetlands

Seasonal Variation In Depth Change

The raw data on seasonal variation in depth change are summarized in Appendix 2.1A-B. The seasonal pattern of depth change was similar at 3 (TP7, HS, and SWSD) of the 4 study sites, consisting of an overall decline in the water level most likely due to evaporative losses (Fig. 2.10). The summer of 2001 was particularly dry in northern Alberta, which may have contributed to the seasonal decline in water depth. Occasionally, small increases were observed in water level throughout the summer coinciding with rainfall events. These increases may be more typical in years with more precipitation, and if they occur often enough, a seasonal decrease in depth may not be observed.

The exception to this general pattern was Natural Wetland where there was a steady increase in the water level in the early summer followed by a few sharp decreases and increases. This pattern is due to active pumping of CT water into the wetland through most of the summer and because Natural Wetland receives dyke seepage water from the adjacent tailings pond.

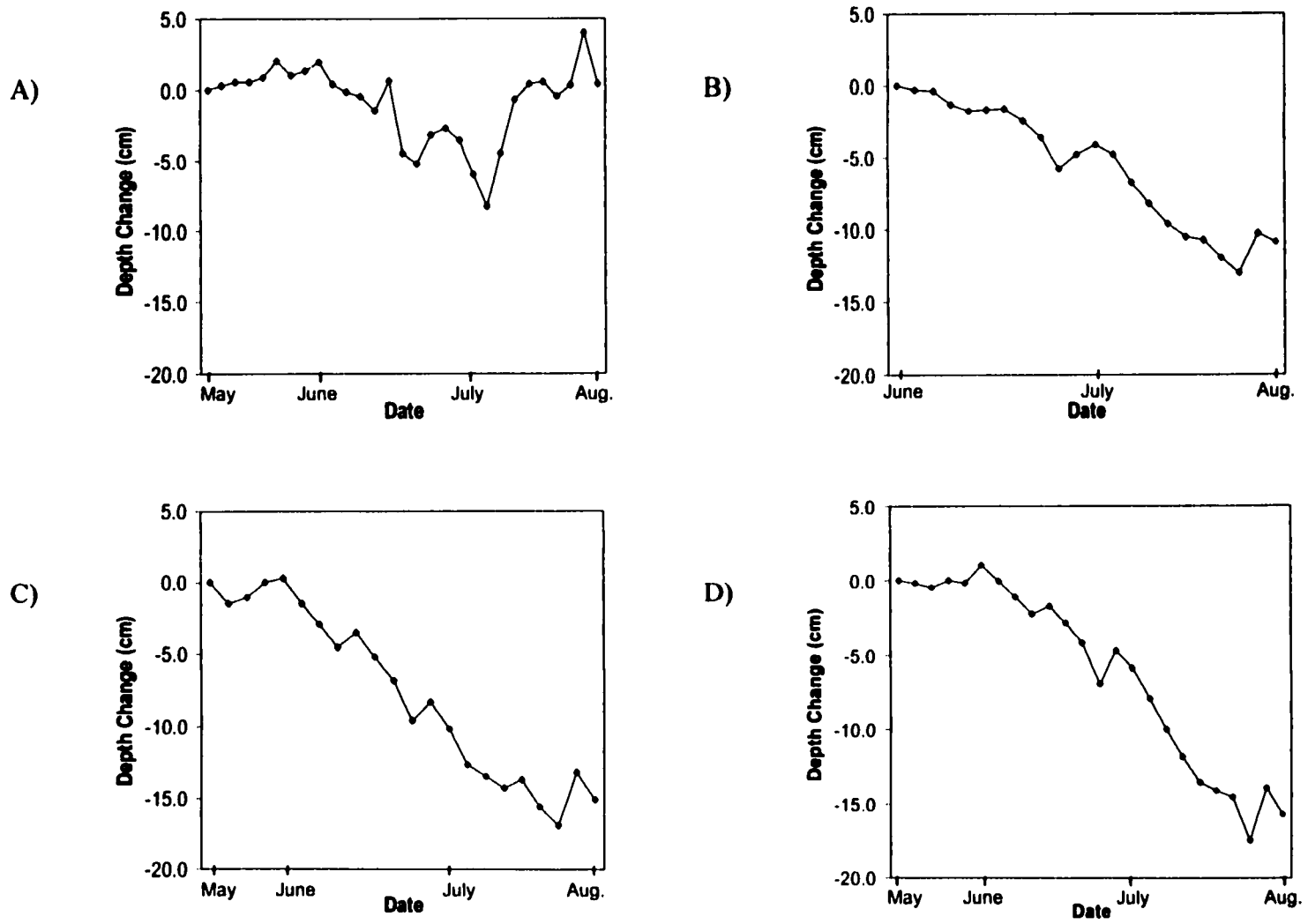


Fig. 2.10. Water depth change, summer 2001 – A) Natural Wetland, B) Test Pond 7, C) High Sulphate, D) Shallow Wetland South Ditch.

Water and Sediment Chemistry

The raw data for water and sediment chemistry characteristics measured on regular visits to the study sites are located in Appendix 2.1A-D and a summary is presented in Table 2.4. Water chemistry data for the study sites from the summers of 2000 and 2001, which were provided by Syncrude Canada Ltd., are included in Appendix 2.2. The latter analyses report on a wide variety of water chemistry characteristics, including metals, sulphates, nitrates, nitrites, ammonia, ammonium, carbonates, phosphates, naphthenic acids, pH, DO, and conductivity.

Water Chemistry

The following overview of trace metals, non-ionic elements and compounds, and ionic compounds and elements pertains to water chemistry data from the summers of 2000 and 2001, courtesy of Syncrude Canada Ltd. (Appendix 2.2).

i) Trace Metals and Non-ionic Elements and Compounds

The majority of the metals (Cd, Co, Cr, Cu, Li, Ni, Pb, Sb, Se, V, Zn, Zr) were present in water at levels below the detection limit (BDL) at all study sites (Appendix 2.2). However, trace quantities of Zn and Zr were detected in 2 of 9 measurements at Test Pond 7 (<0.04 mg/L and <0.03 mg/L, respectively). Chromium was also detected in only one of 9 measurements at Test Pond 7 (0.032 mg/L). The only element that was detected solely at the OSPM-affected sites (TP7 and NW) was Al (~ 6 times more at TP7 than at NW). Molybdenum was detected only at Natural Wetland. Strontium was detected at the highest

Table 2.4. Mean values for water and sediment chemistry of the study sites from early May 2001 to early August 2001* (see Appendix 2.1A-D for raw data and collection dates).

Site	Salinity (‰)	Conductivity (µS/cm)	DO Surface (mg/L)	DO Bottom (mg/L)	Water pH	Sed pH	Water ORP (mV)	Sed. ORP (mV)
NW (mean)	**1**	**1498**	**8.2**	**5**	**9.1**	**8.6**	**163.1**	**-268**
n	34	34	31	3	31	28	35	35
S.D.	0.4	416.7	1.9	0.5	0.8	0.8	66.6	85.9
TP7 (mean)	**1.1**	**1714**	**7.8**	**8**	**9.2**	**8.2**	**163.6**	**-160**
n	24	24	22	3	21	19	24	24
S.D.	0.2	250.8	0.63	0.5	1	1.1	43.2	44
HS (mean)	**1**	**1530**	**8.8**	**6.2**	**8.4**	**7.7**	**152.6**	**-179**
n	27	27	26	3	25	19	28	28
S.D.	0.2	388.9	1.1	0.8	1	0.9	49.7	52.3
SWSD (mean)	**0.2**	**612**	**9.4**	**7.3**	**8.9**	**7.7**	**157**	**-100**
n	29	29	26	2	27	25	30	30
S.D.	0.2	98.1	1.1	1.8	0.8	0.8	59.9	92.27

DO = Dissolved Oxygen
Sed. = Sediment
ORP = oxidative/reductive potential
* Sampling of Test Pond 7 did not begin until late May 2001

levels in High Sulphate followed by Natural Wetland, Shallow Wetland South Ditch, and Test Pond 7. The elements Ba, Mn, and Ti were detected in low amounts at all 4 wetlands. Iron was detected in low levels at both reference wetlands and relatively high levels at both OSPM-affected wetlands. Sulphur was detected at the highest levels in High Sulphate and relatively similar levels at the other three wetlands (3-6 times lower than High Sulphate). Levels of NH_3 were below detection limits at all wetlands. Naphthenic acids were present at much higher (~ 2-40 times) concentrations at OSPM-affected sites than at reference sites.

The above results indicate that there are background levels of numerous metals and compounds at reference sites and that their presence at OSPM-sites is not necessarily attributable to the presence of OSPM. However the presence of certain metals such as aluminum and iron characterized the two OSPM-affected wetlands studied. This is attributable to aluminum and iron cations associated with suspended fine clays in OSPM-affected wetlands undergoing cation exchange with Ca^{+2} present in the water (Fine Tails Fundamentals Consortium 1995b). Naphthenic acids are a constituent of OSPM, and the observation of high levels at OSPM-affected wetlands reflects this. The range of naphthenic acid concentrations at OSPM-affected wetlands relative to reference wetlands is large and reflects characteristics of the reference wetlands. The low end of the range reflects the presence of peat (and hence naphthenic acids) at High Sulphate while the high end of the range reflects the absence of peat in the substrate of Shallow Wetland South Ditch.

ii) Ionic Compounds

Ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{-3}) were all below detection limits at all wetlands with the exception of the occasional detection of very low amounts of NH_4^+ at Shallow Wetland South Ditch. This suggests that all wetlands are nutrient-poor systems with respect to freely dissolved nitrogen and phosphorous which can limit production in wetlands (Horne and Goldman 1994). Sulphate (SO_4^{-2}) was present in the highest levels at High Sulphate (~ 5 times higher than at NW and SWSD and ~ 10 times higher than at TP7). Levels of carbonate (CO_3^{-2}) were reported as 0.0 mg/L for the two reference sites and levels were about twice as high at Test Pond 7 compared to Natural Wetland. Bicarbonate (HCO_3^-) levels were similar at Test Pond 7 and Natural Wetland and were approximately 4 times higher than the levels at both High Sulphate and Shallow Wetland South Ditch. The higher levels of carbonate and bicarbonate in the water of OSPM-affected wetlands reflects the greater alkalinity (pH) of the water compared to reference wetlands.

iii) Ionic Elements

Magnesium was the only element that was present at much higher levels in both reference sites (HS and SWSD) than at OSPM-affected sites (TP7 and NW). Chlorine was the only element present in low levels at both reference wetlands and high levels at both OSPM-affected wetlands. Potassium was present in High Sulphate and Natural wetland at levels 2 and 7 times that at Shallow Wetland South Ditch and Test Pond 7 respectively. Sodium was present in high levels at all wetlands but consistently higher at OSPM-affected

wetlands. Calcium was present at the highest levels in High Sulphate and values reported were approximately 4-5 times greater than at Natural Wetland and Shallow Wetland South Ditch and 10 times greater than that at Test Pond 7. Fluorine was detected only at OSPM-affected wetlands and in similar amounts at both of them.

The relative concentrations of some ionic compounds and elements characterize OSPM-affected and reference wetlands. Relatively high levels of carbonate, bicarbonate, chlorine, sodium and fluorine characterize OSPM-affected wetlands and contribute to their relatively high salinities (Table 2.4). Salinity was also high at the reference wetland High Sulphate (Table 2.4) and this is attributable to relatively high levels of sulphate, calcium, magnesium, and potassium in this water body. At High Sulphate, the relatively high levels of calcium and sulphate are likely due to the presence of peat, whereas the relatively high levels of these ions at Natural Wetland are likely derived from gypsum ($CaSO_4 \cdot 2H_2O$) which has entered the wetland with consolidated/composite tailings water (of which gypsum is a constituent). The relatively low levels of ionic elements and compounds at Shallow Wetland South Ditch reflect low levels of salinity measured at this wetland.

iv) Other Water Quality Measures

The following results pertain to measures taken during regular visits to each study site, a summary of which is given in Table 2.4. The raw data is presented in Appendix 2.1A-D. The salinities and conductivities of the OSPM-affected wetlands were similar and greater than that of the reference wetland Shallow Wetland South Ditch. Shallow Wetland South Ditch can thus be classified as a low salinity reference wetland. The reference wetland High

Sulphate had salinity and conductivity measures comparable with the OSPM-affected wetlands thereby conferring the status of a saline reference wetland to this water body. The pH of the water was close to 9 at all sites, but slightly higher at OSPM-affected wetlands than at reference wetlands. The dissolved oxygen concentration in surface waters was greater at reference wetlands than at OSPM-affected wetlands whereas there was no pattern with respect to wetland type for dissolved oxygen concentrations at the water-sediment interface. The oxidative-reductive potential (ORP) of surface waters was similar at all wetlands sampled and reflect the small differences in the dissolved oxygen concentration in the surface waters of the wetlands.

Water quality measures suggest that OSPM-affected wetlands are characterized mainly by higher salinity and conductivity (attributable to ions in the water), slightly higher pH readings, softer water (relatively low levels of Ca^{+2} and Mg^{+2}), and slightly lower dissolved oxygen levels in the surface waters. The OSPM-affected wetlands are very saline, having salinities comparable with those of other small water bodies in western Canada (Rawson and Moore 1944). On the other hand, reference wetlands are characterized by their salinity, conductivity, water hardness (relatively high levels of Ca^{+2} and Mg^{+2}), relatively high surface water dissolved oxygen levels, and lower pH readings. High Sulphate is also considered a very saline wetland and Shallow Wetland South Ditch is considered moderately saline relative to other freshwater bodies in western Canada (based on Rawson and Moore 1944).

Sediment Chemistry

The following results pertain to measures taken on regular visits to each study site, a summary of which is given in Table 2.4. The raw data are presented in Appendix 2.1A-D. The pH of the sediments at OSPM-affected wetlands (8.2-8.6) was higher than at reference wetlands (7.7 at both). There was no pattern with respect to wetland type and the ORP of the sediments, although the most negative values were reported at the OSPM-affected Natural Wetland and the most positive values at the reference wetland Shallow Wetland South Ditch. The more negative ORP values suggest larger populations of reductive anaerobic bacteria in the sediment and thus the results indicate that sediments of OSPM-affected wetlands are capable of sustaining higher populations of these organisms. Larger bacterial populations in the sediments of OSPM-affected wetlands may confer greater productivity to the benthic community of these wetlands compared to reference wetlands.

CONCLUSION

The information presented in this chapter illustrates that there are defining characteristics which separate OSPM-affected wetlands from reference wetlands. These include biotic components in the benthic and pelagic environment, as well as the chemistry of the water and sediment. Other work showed that the benthic macroinvertebrate community composition and the chironomid community composition is different among OSPM-affected and reference wetlands (Whelly 1999). The relative abundances of taxa present in both types of wetlands are also different (Whelly 1999). The phytoplankton (Leung et al. 2001) and zooplankton (McCormick 2000) communities of OSPM-affected and reference wetlands are

different with respect to total biomass and species present, and differences appear to become smaller as the age of an OSPM-affected wetland increases.

With respect to water chemistry, OSPM-affected wetlands generally have higher salinities and conductivities (though not always), and typically have slightly higher pH readings and lower levels of dissolved oxygen in the surface waters. The sediments of OSPM-affected wetlands generally have a pH higher than that of reference wetlands and appear to be more reduced (anaerobic), suggesting that they may support larger populations of reductive anaerobic bacteria. The water levels at all wetlands (except at Natural Wetland for artificial reasons) in the summer of 2001 decreased which may or may not be typical as the majority of water which these wetlands receive is from rain run-off and the summer of 2001 was relatively dry.

CHAPTER 3

Secondary Production of Selected Aquatic Macroinvertebrates Inhabiting Tailings-affected and Reference Wetlands in the Athabasca Oil Sands

INTRODUCTION

Aquatic insects are important components of both constructed OSPM-affected wetlands and constructed reference wetlands in the Athabasca oil sands region (Whelly 1999, Bendell-Young et al. 2000, Gould 2000). The aquatic larvae of chironomids are among the predominant benthic macroinvertebrates inhabiting constructed wetlands on the oil sands leases (Whelly 1999, Bendell-Young et al. 2000) and *Chaoborus* are an important component of the pelagic system in these water bodies (Bendell-Young et al. 2000). Understanding how secondary production dynamics of these aquatic macroinvertebrates inhabiting OSPM-affected wetlands differs from that in reference wetlands is important because secondary production is a measure that is of great ecological significance. In terms of the population dynamics of aquatic macroinvertebrates, the magnitude of secondary production reflects the product of individual growth and population survivorship. In terms of community dynamics, secondary production estimates are important when quantifying and comparing the flow of biomass and hydrophobic organic chemicals (e.g., PAHs) in different pathways (e.g., benthic vs. pelagic).

Annual production estimates of the Chironomidae as a whole in still-water habitats vary widely ranging from less than 1.0 to nearly 100 g dry mass /m^2/y (Tokeshi 1995a). These estimates come primarily from lakes, where shallow areas are more productive for chironomids than deeper areas. Thus shallower water bodies are expected to support greater chironomid production per unit area. In a coastal lagoon, mean annual production of the

single species *Chironomus salinarius* was estimated to be 16.8 g dry mass/m^2/y (Drake and Arias 1995). In a carp pond, the production of the genus *Chironomus* was estimated to be 124 g dry mass /m^2/y in areas unprotected from carp and 297 g dry mass /m^2/y in areas protected from carp (Ruzickova 1987). In a stressed system (a shallow alkaline lake), production of the entire chironomid community was estimated to be 6.64 g dry mass /m^2/y in sheltered near-shore areas and 0.55 g dry mass /m^2/y in the open waters of the lake (Wolfram 1996).

Annual secondary production of *Chaoborus punctipennis* in Lake Memphremagog, Quebec-Vermont was reported to vary between 0.066 and 0.348 g dry mass /m^2/y (Dermott et al. 1977). In a constructed eutrophic pond in Japan, annual production of *Chaoborus flavicans* was reported to be 11.7-g dry mass/m^2/y (Iwakuma et al. 1989). These studies suggest that in general, production of benthic chironomids exceeds that of pelagic *Chaoborus*. However, annual production of *Chaoborus* in nutrient enriched water bodies has the potential to be greater than that of the entire chironomid community in stressed water bodies. In light of these comparisons it is likely that annual production of the chironomid community within OSPM-affected and reference wetlands exceeds that of the chaoborids. As a result, annual biomass export out of a given wetland (OSPM-affected or reference) via the benthic predatory dipteran, Tanypodinae, is likely greater than that via pelagic predatory dipteran, *Chaoborus*.

The goal of this chapter is to compare annual production of benthic (Tanypodinae) and pelagic (*Chaoborus*) predatory dipterans at two OSPM-affected and two reference wetlands in the Athabasca oil sands area. This measure is being used as a surrogate for

potential biomass export from the wetlands via these organisms. Production of major taxa in the chironomid community at OSPM-affected and reference wetlands will also be examined. Chironomids and chaoborids are important food items of terrestrial insectivores such as swallows (Smits et al. 2000), and they are capable of accumulating potentially toxic PAHs (e.g., Clements et al. 1994, Gewurtz et al. 2000). It is important to understand how biomass export differs in the benthic and pelagic environment because of the presence of potentially toxic PAHs in these systems, OSPM-affected wetlands in particular which have higher levels of PAHs present. The majority of PAHs in aquatic environments are located in the sediments (Neff 1979), suggesting that benthic predatory dipterans will have a greater potential to export PAHs than pelagic predatory dipterans given equivalent annual production values of benthic and pelagic predators. This would be even more true if the benthic Tanypodinae had greater annual production than the pelagic *Chaoborus*. If *Chaoborus* annual production is much greater than that of Tanypodinae then the potential exists for PAH export to be greater via the pelagic route.

1) Significance of Secondary Production Parameters

Secondary production is the amount of living organic matter (biomass) that is produced by an animal population over an interval of time. Thus, it is a measure of the rate at which biomass is produced (carbon flux) as opposed to the standing-stock biomass, which is the amount of biomass per unit area at a given moment in time. Production and standing-stock biomass can be calculated for a single age class (cohort) and an estimate of annual secondary production can also be calculated (Benke 1996). However, many aquatic insects

are multivoltine and exhibit overlapping cohorts rather than having synchronous development. In these situations a non-cohort technique such as the size-frequency method may be applied to estimate secondary production (Benke 1996).

Using the size-frequency method to estimate secondary production requires or allows the calculations of various other values useful in making comparisons among OSPM-affected and reference wetlands. To calculate production, estimates of density and mean standing stock biomass of each size class are required. The mean standing stock biomass of the average cohort is calculated based on densities and weights of individuals. The estimation of annual production requires that cohort production is estimated first. The ratio of cohort production to mean standing stock biomass and the ratio of annual production to mean standing stock biomass can also be calculated (cohort and annual P:B ratios, respectively). The details of these calculations are outlined in the Secondary Production Calculations of the Methods section. This section discusses the utility of these variables in assessing differences with respect to the secondary production dynamics of chironomids (Tanypodinae in particular) and *Chaoborus* inhabiting OSPM-affected and reference wetlands.

Density

Density reflects the number of organisms present per unit area and is subject to variation depending on physical and chemical characteristics of the water body and the intensity of interspecific and intraspecific competition and predation. It can influence the magnitude of the estimates of mean standing stock biomass of the average cohort, cohort production, annual production, cohort P/B, and annual P/B.

Mean Standing Stock Biomass of the Average Cohort

This is the amount of biomass per unit area at a given moment in time. This measure influences the magnitude of the estimates of cohort and annual production as well as cohort P/B and annual P/B.

Cohort Production

This is the amount of biomass produced per unit area during a specified interval of time (June 11 - July 23, 2001 in this case). Cohort production in part influences annual production. In order to compare these estimates among taxa and within a taxon among wetlands, the assumption that all populations are in the same phase of production must be made.

Annual Production

This is the amount of biomass produced per unit area during a year. Since chironomids and *Chaoborus* do not actively grow throughout the whole year in northern Alberta, "year" can be considered synonymous with growing season. Ice covering water bodies in the area melts in late April and air temperatures are cool (daily highs <15°C) until mid to late May. This suggests that it may also take until mid to late May until the temperature in the sediment of wetlands reaches the approximately 7°C which is required for chironomid growth rates to be greater than zero (Tokeshi 1995b). Since daytime highs begin to be <15°C again in August, it was assumed that chironomid growth rates would decrease

to zero sometime in September. Thus, the growing season was assumed to be 100 d in this study.

Cohort P/B

P/B ratios provide information pertaining to the production dynamics of animals. The cohort P/B is related to the shapes of the growth and survivorship curves of a population and is independent of the amount of time it takes a cohort to complete its aquatic life stage (Benke 1996). Cohort P/B ratios typically vary between about 2 and 8 under a variety of conditions (Waters 1969).

Annual P/B

Annual P/B ratios are directly related to individual growth rates and are roughly equivalent to the biomass turnover rate, the rate at which biomass is replaced (Benke 1996). The units are inverse time (y^{-1}) where a year represents a 100-d growing season for aquatic insects in northern Alberta. These measures can vary greatly from one taxon to another, and are dependent on environmental conditions (Benke 1996) thereby making them excellent indicators of the growth potential of taxa inhabiting OSPM-affected and reference wetlands.

Turnover Time

The reciprocal of annual P/B is the turnover time and this is a measure of the time it takes to replace the biomass of a population (Benke 1996). It can be expressed in days by

multiplying the reciprocal of annual P/B by the number of days in the growing season. Hence, turnover time is also a measure directly related to individual growth rate.

METHODS

1) Study Sites

Secondary production estimates were made at two OSPM-affected wetlands (Natural Wetland and Syncrude Test Pond 7), and two reference wetlands (High Sulphate and Shallow Wetland South Ditch). Detailed environmental characteristics of each wetland were reported in Chapter 2.

2) Field Collection Methods

Chironomid Secondary Production

At the beginning of May 2001, three sites were chosen within each wetland where cores of sediment would be taken (Figs. 2.2, 2.4, 2.6, and 2.8) every Monday and Friday until the beginning of August (it is recommended that for midges with life cycles of two to three weeks, samples be taken at least every three days - Cowell and Vodopich 1981). Core locations were roughly evenly spaced around the margins of each wetland.

Coring tubes consisted of ~ 30-cm long by 6.5-cm ID polycarbonate with a line demarking a distance of 5-cm from the top of the tube. Previous work has shown that the majority of benthic macroinvertebrates reside in the top 5-cm of the sediment in constructed wetlands of the Athabasca oil sands (Ciborowski and Whelly, University of Windsor, unpublished data). Sediment was extracted from tubes with the aid of a plunger. The plunger

consisted of a 5.7-cm OD length of PCV pipe. The plunger end was sealed with duct tape and that end of the tube was wrapped with more tape until the outer diameter was only slightly smaller than the inner diameter of the coring tube.

To take a sample, a coring tube was pushed 6 - 10 cm into the sediment. A cap was placed on top of the tube to form a seal. The tube (containing water and a plug of sediment) was then removed from the wetland with a hand placed underneath it to prevent loss of material from the bottom. The plunger was inserted into the bottom of the core tube and slid slowly upward to push the sediment to the top of the core tube. The line 5-cm from the top of the coring tube was used to help gauge the 5-cm mark of sediment core. The plunger was pushed just far enough into the coring tube to extrude a 5-cm thickness of sediment, which was separated from the sediment beneath with a spatula. This sediment was placed in a labelled plastic bag and enough formalin-ethanol solution (10:5:1 v/v/v water:95% ethanol:formalin) was added to cover the sample. The bag was then sealed with a twist tie. Before shipping the core samples back to the University of Windsor, each bag was checked to ensure that the sample had not begun to rot and topped off with more preservative if necessary.

Chaoborus Secondary Production

Plankton hauls were taken every Monday and Friday from June 11 to July 23, 2001 from the same location within each of the two OSPM-affected and two reference constructed wetlands (Figs. 2.2, 2.4, 2.6, and 2.8). A custom-built plankton net (Fig. 3.1; 45 x 15 cm mouth diameter, 150-cm long) with 500-μm Nitex mesh attached to a 9-m long rope was

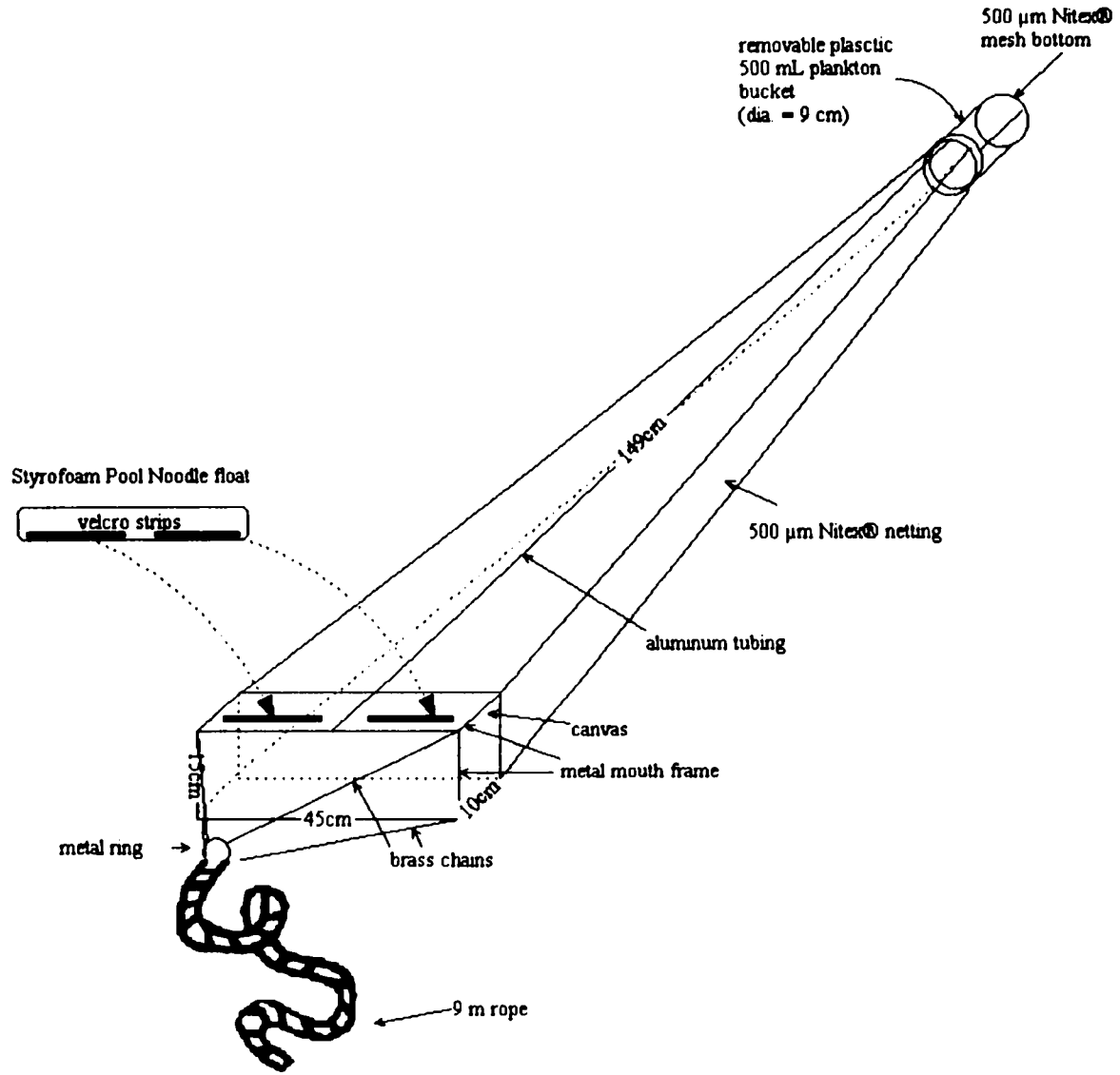


Fig. 3.1. Schematic diagram of custom-built 500-µm plankton net.

thrown 10 times (in a different direction each time) on each collection date. The tow speed of each haul was approximately 1 m/s, and each haul filtered approximately 0.4-m^3 of water. The contents of 10 consecutive hauls were placed in a plastic tray containing a depth of <1-cm of pond water. The samples were inspected to ensure that at least 10 *Chaoborus* larvae were present. Whole plankton samples (i.e., contents of plastic tray) were poured through a 180-μm brass testing sieve and placed into 20-mL glass scintillation vial(s) containing Carnoy's solution (3:1 v/v 95% ethanol:glacial acetic acid). The Carnoy's solution was changed periodically until the solution ceased to become discoloured.

3) Laboratory Methods

Chironomids

Sample Processing

Core #1 taken from each site (Figs. 2.2, 2.4, 2.6, and 2.8) on each sampling date was processed for secondary production estimates following previously developed protocols (Ciborowski 1991). The bag containing the sample was partially filled with water and the contents poured through a 180-μm sieve into a pail. Formalex® was added to the pail to neutralize the preservative after which the material was discarded. The sample was then rinsed through a stacked series of sieves (4-mm, 1-mm, 500-μm, and 250-μm) that was placed in a sink approximately 30 - 40 cm beneath the tap. After washing the sample through the 4-mm sieve, the organic fraction of the contents of that sieve were placed in a petri dish.

The remaining sample was then further rinsed. The 1-mm sieve was removed and its contents emptied into a enamelled pan. The organic material was decanted off into a 180-μm

sieve, leaving the inorganic material behind. The enamelled tray was repeatedly filled with tap water and poured off until all of the organic material had been decanted off. Tapwater was used to rinse the entire sample to one edge of the sieve. The water was gently squeezed out of the sample with a plastic spatula, and the fraction was removed from the sieve. The same procedure was repeated for material in the 500-μm and 250-μm sieves.

Zoobenthos and light detritus were separated from refractory detritus by flotation with Ludox® silica colloid (Burgess 2001). Each sample fraction was placed in a separatory funnel containing approximately 1-L of Ludox solution mixed to a specific gravity of 1.15, which is less than that of most of the organic debris in a sample but greater than that of invertebrates (s.g of meiofauna is near 1.15, Burgess 2001). The sample was gently stirred, allowed to sit for 30-min, stirred again, and allowed to sit for another 30-min. The contents of the bottom of the funnel were collected into a 1-L flask. Another 1-L flask was used to collect the remaining solution containing light detritus and invertebrates. Any material adhering to the sides of funnel was rinsed into this 1-L flask as well. The contents of the 1-L flask containing the invertebrate sample was poured through a 180-μm sieve resting in an enamelled tray. The sample was then rinsed with tap water to remove residual Ludox. The Ludox was kept for reuse.

Each size fraction of detritus+invertebrates was placed in a petri dish. All invertebrates were removed from each size fraction using a dissection microscope at 6.4X (4-mm and 1-mm size fractions) or 12X (500-μm and 250-μm size fractions) magnification. Each sample was completely sorted.

Identification

Each chironomid from each core processed was mounted ventral side up on a microscope slide in a drop of CMC-9AF® aqueous mounting medium (Master's Chemical Company, Des Plaines, Illinois). A number of chironomids were mounted on each slide and then a cover slip was applied with gentle pressure in order to separate the mouthparts and properly orient the head capsule after which the slides were left to clear for 5 d. Each chironomid was identified to subfamily (Orthocladiinae, Tanypodinae) or tribe (subfamily Chironominae - tribes Chironomini and Tanytarsini) using the key of Coffman and Ferrington (1984).

Taxa Used in Chironomid Secondary Production Calculations

Secondary production estimates were calculated for predatory chironomids, Tanypodinae. In addition to Tanypodinae, secondary production estimates were generated for other major chironomid taxa (subfamily Orthocladiinae; subfamily Chironominae -tribes Chironomini and Tanytarsini), which typically differ in feeding habits and hence position in the food web (Table 3.1).

General feeding habits of chironomids can be used to make generalizations about the diet of chironomids (Berg 1995) which in turn can later be used to interpret production estimates of the various chironomid taxa. Collector-gatherers feed on fine particulate organic matter (FPOM) including detritus and algae and associated microorganisms. Collector-filterers filter their food from the water column with catchnets or strands of salivary secretions. They feed on suspended algae, detritus, microorganisms, and invertebrates that

Table 3.1. Feeding habits and food web of taxa for which secondary production estimates were calculated.

Family or subfamily	Tribe or genus	Food Web	Dominant feeding habit(s)*
Tanypodinae	n/a	benthic	predator
Orthocladiinae	n/a	benthic	collector-gatherers, scrapers
Chironominae	Chironomini	benthic	collector-gatherers
	Tanytarsini	benthic	collector-gatherers, collector-filterers
Chaoboridae	*Chaoborus* sp.	pelagic	predator

* feeding habits taken from Coffman & Ferrington, 1984

may get caught in the catchnets. Scrapers can generally be considered to feed on algae growing on substrates such as macrophytes and rocks.

Length and Dry Weight Determination

Slides containing the mounted chironomids were placed under a Hitachi VK-C370 video camera equipped with an Olympus 28-mm wide angle lens attached to a computer containing a Targa 64® video digitizing card. The image of the chironomids was captured and the length of each chironomid was measured to the nearest 0.5 mm using Mocha® 1.2 image analysis software (Jandel Scientific; San Rafael, CA).

Lengths of individual chironomids (Tanypodinae, Orthocladiinae, Chironomini, Tanytarsini) were used to estimate the dry mass of each individual with the following equation:

$$W = a \times L^b$$

where W is the dry mass of an individual (mg), 'a' and 'b' are constants determined from a range of individuals that have been measured and weighed on a balance, and L is the measured length of an individual. The constants 'a' and 'b' are specific to a taxon and reflect taxonomic differences in the relationship between the length of an individual and its dry mass. The constants used and their sources are presented in Table 3.2. The dry mass of each individual was used to generate secondary production estimates of the different chironomid taxa for which lengths were measured (see: 4) Secondary Production Calculations).

Table 3.2.Constants (a and b) used to calculate chironomid and *Chaoborus* dry mass based on length and the degree of correlation between length and dry mass for the corresponding taxa.

Taxa	a	b	r	Source
Chironomini Tanytarsini Orthocladiinae	$e^{-5.279}$	2.32	0.94	(Smock 1980)
Tanypodinae	$e^{-5.573}$	2.41	0.92	(Smock 1980)
***Chaoborus* spp.**	0.1127	3.2345	0.97	(Dumont and Balvay 1979)

Chaoborus

Sample Processing

The Carnoy's solution from the vial(s) from each collection date was emptied into a waste container and the samples were sorted (i.e., *Chaoborus* removed) in a petri dish containing 70% ethanol beneath a dissecting microscope. In the rare case that there were fewer than 100 individual *Chaoborus* in a sample, the whole sample was sorted. In most cases, there were many hundred *Chaoborus* in each sample and subsampling was conducted to remove them. In these instances between 40 and 100 individuals were removed from the samples and their lengths measured. Such samples were evenly spread over the bottom of the Petri dish, and a one-quarter sampler was used to split the sample. The isolated quarter of the sample was placed in another Petri dish and, if necessary, a one-quarter subsample of this material was sorted. Single samples were split up to 4 times before sorting commenced. Regardless of whether or not the sample was subsampled, the *Chaoborus* were removed from the rest of the plankton and placed in another petri dish containing ethanol. Each sample was sorted twice - once at 6.4X and once at 12X magnification.

Identification

Chaoborus were identified to genus using the keys of Clifford (1991). Attempts were made to identify the larvae to species using the key of Saether (1972), but definitive identifications could not be made due to overlap in the features of the collected specimens that are used to discriminate among species in the taxonomic key.

Length and Dry Weight Determination

After sample sorting, the petri dish containing the removed *Chaoborus* was placed beneath a Hitachi VK-C370 video camera with an Olympus 28-mm wide angle lens attached to a computer. Images were digitized with a Targa 64® grabber card which was installed in the computer. Images were digitized of approximately 10 *Chaoborus* at a time. Mocha® 1.2 image analysis software (Jandel Scientific Ltd.; San Rafael, CA) was used to measure the length of each individual to the nearest 0.5-mm. The dry mass of each individual *Chaoborus* was calculated in the same manner as it was for the different chironomid taxa (Table 3.2).

4) Secondary Production Calculations

The Size-frequency Method

This method is based on the assumption that the average size-frequency distribution calculated from samples collected over a period of time approximates the average survivorship of a hypothetical average cohort (Hamilton 1969, Benke 1996). This average cohort does not actually represent the number of individuals reaching each size class defined in the size-frequency distribution, as would be observed if one were dealing with an actual cohort. However, assuming linear growth, it is possible to approximate the number of individuals that reach each size class over a period of time (Hamilton 1969, Benke 1996). The assumption of linear growth is probably not true (Benke 1996), however, Benke and Waide (1977) suggested that the assumption of linear growth will not usually lead to large errors in production estimates.

Individuals of a given taxon were grouped into size classes according to their measured lengths. Distinct chironomid (Tanypodinae, Orthocladiinae, Chironomini, and Tanytarsini) and *Chaoborus* cohorts could not be discerned from the field data, so a noncohort technique, the size-frequency method, was employed to calculate estimates of secondary production (Benke 1996). This method assumes that the combined size-frequency distribution of all individuals collected over a number of sampling dates approximates the mortality curve of an average cohort. In this sense it can still be considered a cohort method even though data may be collected simultaneously for two or more cohorts at different stages of completion.

General calculations and their rationale for use are explained in detail by Benke (1996). The methods are briefly outlined below and so are the ways in which issues specific to this study were handled. The same general methods were used to calculate chironomid (Tanypodinae, Orthocladiinae, Chironomini, and Tanytarsini) and *Chaoborus* production with the exception of the calculation of density (see below). A list of samples used in secondary production calculations is provided in Table 3.3.

Density and Biomass Determination

For each taxon at each wetland, the density of individuals (chironomids/m^2 or *Chaoborus*/m^2) was calculated for each of the 10 size classes. For example, if the size-frequency distribution of a chironomid taxon was based on 13 cores, then the total number of individuals in each size class was divided by the area sampled by 13 cores (which is π x (3.25 cm)^2 x 13). For *Chaoborus*, if the size-frequency distribution was based on12

Table 3.3. Collection dates of samples used to calculate estimates of chironomid and *Chaoborus* secondary production.

Date (2001)	Natural Wetland (OSPM)		Test Pond 7 (OSPM)		High Sulphate (Ref)		Shallow Wetland South Ditch (Ref)	
	Chiron. (Core 1)	*Chaob.*	Chiron. (Core 1)	*Chaob.*	Chiron. (Core 1)	*Chaob.*	Chiron. (Core 1)	*Chaob.*
June 11	✓	✓*	✓	✓	✓	✓	✓	✓
June 15	✓	✓*	✓	✓	✓	✓	✓	✓
June 18	✓	✓	✓	✓	✓	✓	✓	✓
June 22	✓	✓	✓	✓	✓	✖	✓	✓
June 25	✓	✓	✓	✓	✓	✖	✓	✓
June 29	✓	✓	✓	✓	✓	✓	✓	✓
July 2	✓	✓	✓	✓	✓	✓	✓	✓
July 6	✓	✓	✓	✓	✓	✓	✓	✓
July 9	✓	✓	✓	✓	✓	✓	✓	✓
July 13	✓	✖	✓	✓	✖	✓	✓	✓
July 16	✓	✓	✓	✓	✓	✓	✓	✓
July 20	✓	✓	✓	✓	✓	✓	✓	✓
July 23	✓	✓	✓	✓	✓	✓	✓	✓

✓ sample was processed
✖ sample not processed (misplaced)
* sample was field sorted thereby biasing it towards larger individuals

samples, the volumetric density of each size class (number/m^3) was calculated by dividing the number of individuals in that size class by the water volume filtered by the plankton net while collecting the sample (12 samples x 0.45-m wide x 0.15-m tall x 9-m/tow x 10 tows). To make estimates of *Chaoborus* production comparable with estimates of chironomid production, volumetric density was converted to areal density (number/m^2) by multiplying the volumetric density (number/m^3) by the volume of the wetland and then dividing by the surface area (see Chapter 2 for wetland dimensions).

The mean individual dry mass (W, mg) was calculated for each size class based on the calculated masses of each individual in a size class. The mean standing stock biomass (B) of each size class was then calculated by multiplying the areal density of each size class (N) by the mean individual mass of that size class (W). The mean standing stock biomass of the average cohort present from June 11 - July 23, 2001 was then estimated by summing the biomasses of the individual size classes.

Cohort Production

Secondary production as determined by the size-frequency method is based on calculations of changes in dry mass between different size classes. More specifically, it is based on calculations of production lost from one size class to the next as a product of the decrease in density (ΔN) and the mean of the average individual masses (W) of two successive size classes (see Appendices 3.1 and 3.2 where these calculations are illustrated in table form for chironomids and *Chaoborus*, respectively). The masses lost between size classes (W x ΔN) are then multiplied by the number of size classes (10 in all cases). This is

necessary because it is assumed that growth is linear and hence an insect will remain in each of the N size classes for 1/N of the sampling period. Since each size class lasts 1/N of the sampling period, the mean number of individuals in each size class is multiplied by the number of size classes (N) to estimate the number of individuals that grew to that size class during the sampling period. These estimates are summed over all size classes, giving a measure of the biomass lost, which is equivalent to biomass produced, or cohort production. Any negative values are excluded from the summation as they are likely artifacts of inefficient sampling of larvae of certain size classes, the small larvae in particular (Benke 1996). This cohort production value is representative of the production that occurred during the sampling period (June 11 - July 23, 2001).

Annual Production

Production (i.e., generation of new biomass) in the insects of interest is limited to the larval life stage because the pupae and adults do not feed. The time required to complete the aquatic life stage of the various taxa for which production was calculated is less than the length of the growing season in northern Alberta (assumed in this study to be 100 d for aquatic macroinvertebrates). As a result, a correction factor must be employed in order to estimate annual production (i.e., production over the 100-d growing season). The correction factor is called the cohort production interval (CPI) correction factor where the CPI is the amount of time required to complete the larval life stage and it is calculated as follows:

CPI correction factor = (days in growing season)/(CPI). (Benke 1996)

This correction factor is multiplied by cohort production to give an estimate of annual

production. In order to apply this correction to different taxa, an estimate of the length of the larval life stage of the different taxa was required. It was not possible to determine this from field data, so published data documenting chironomid (Mackey 1977a, Balci and Kennedy 2002) and *Chaoborus* (Hanazato and Yasuno 1989) larval development times at 15°C were used (Appendix 3.3). This temperature was chosen as water temperature in the wetlands was often close to air temperature (Appendix 3.3) and near to 20°C during the day. At night temperatures drop to less than 10°C. Thus 15°C was used as an estimate of the average temperature that these organisms are subjected to. Table 3.4 summarizes the CPIs and CPI correction factors used for each taxon.

Cohort P/B and Annual P/B

Cohort P/B was calculated for each taxon by dividing the cohort production by the mean standing stock biomass of the average cohort. Annual P/B was calculated by dividing the annual production estimate by the mean standing stock biomass of the average cohort. Alternatively, annual P/B can also be estimated by multiplying the cohort P/B by the CPI correction factor. These two methods give identical estimates of annual P/B.

Turnover Time

Turnover time was calculated for each taxon by taking the reciprocal of annual production and multiplying it by 100 because the number of days available during the year for production to occur was assumed to be 100. The turnover time is roughly the time it takes to replace the biomass in the population.

Table 3.4. Calculation of Cohort Production Interval (CPI) correction factors used in the calculation of annual secondary production.

Taxa	Growing Season (d)	CPI, 15°C(d)*	CPI Correction Factor
Tanytarsini	100	9.2	6.58
Chironomini	100	30.7	2.72
Tanypodinae	100	25.9	3.14
Orthocladiinae	100	12.9	5.3
Chaoborus	100	37.8	2.28

CPI Correction Factor = Days in Growing Season / CPI

* see Appendix 3.3 for determination

RESULTS

All comparisons are based on the observed estimates rather than statistical analyses. The nature of secondary production estimates is such that they depend on a number of multiplication factors and combined values thereby making it difficult to analytically envision a true measure of variance. Habitat heterogeneity of the entire wetland is also not reflected in the production estimates (since cores were consistently taken from one area within each wetland) thereby making it even more difficult to accurately calculate variance estimates. Summaries of the calculations can be found in tables in the following text. The secondary production tables that were used to calculate the values of all variables (except turnover time which is the reciprocal of annual P/B x 100 d) at each wetland are located in Appendix 3.1 (all chironomid taxa) and Appendix 3.2 (*Chaoborus*).

1) Secondary Production Variables: Benthic (All Chironomids) vs. Pelagic (*Chaoborus*) Food Web

Cohort Production and Annual Production

The dipteran component of the benthic food web (i.e., chironomids, Table 3.5; cohort and annual production of individual chironomid subfamilies and tribes ranged from 0.23-27.48 g/m^2 and 1.55-89.39 g/m^2/y, respectively) was far more productive than the dipteran component of the pelagic food web (i.e., *Chaoborus*, Table 3.6; cohort and annual production ranged from 0.0034-0.1407 g/m^2 and 0.0090-0.3721 g/m^2/y, respectively) in terms of both cohort production and annual production. This was true for all individual taxa of chironomids for which secondary production was estimated at both OSPM-affected and reference wetlands. It was also true with respect to production of the entire benthic

Table 3.5. Secondary production and associated values for chironomid subfamilies and tribes at OSPM-affected and reference wetlands.

Site	Taxon	Density (#/m^2)	Cohort P (g/m^2)*	Annual P (g/m^2/y)*	Cohort B (g/m^2)*	Cohort P/B	Annual P/B (y^{-1})	Turnover Time (d)
Natural Wetland (OSPM)	**Tanytarsini**	18452	3.77	41.02	0.62	6.07	65.99	1.52
	Chironomini	1229	0.56	1.81	0.12	4.47	14.53	6.88
	Tanypodinae	5540	7.44	28.77	1.33	5.58	21.57	4.64
	Orthocladiinae	1368	0.53	4.14	0.11	4.72	36.67	2.73
	Total:	26589	12.31	5.74	2.19	5.61	34.54	2.89
Test Pond 7 (OSPM)	**Tanytarsini**	8925	2.56	27.86	0.53	4.82	52.43	1.91
	Chironomini	385	1.06	3.46	0.23	4.72	15.34	6.52
	Tanypodinae	1275	2.65	10.23	0.53	4.95	19.15	5.22
	Orthocladiinae	185	0.23	1.76	0.05	4.2	32.67	3.06
	Total:	10770	6.5	43.31	1.35	4.83	32.2	3.11
High Sulphate (Reference)	**Tanytarsini**	6680	1.43	15.5	0.27	5.3	57.64	1.73
	Chironomini	16072	27.48	89.39	4.76	5.78	18.8	5.32
	Tanypodinae	1356	0.99	3.83	0.19	5.24	20.25	4.94
	Orthocladiinae	4420	0.84	6.56	0.14	5.92	46	2.17
	Total:	28528	30.74	115.28	5.36	5.74	21.52	4.65
Shallow Wetland South Ditch (Reference)	**Tanytarsini**	10826	3.18	34.55	0.35	9.11	99.04	1.01
	Chironomini	3871	1.69	5.49	0.28	5.99	19.5	5.13
	Tanypodinae	1576	0.4	1.55	0.1	4.19	16.18	6.18
	Orthocladiinae	1205	0.33	2.57	0.06	5.2	40.39	2.48
	Total:	17478	5.6	44.17	0.79	7.09	55.9	1.79

*dry weight

Table 3.6. Secondary production and associated values for *Chaoborus* at OSPM-affected and reference wetlands.

Site	Density (#/m^2)	Cohort P (g/m^2)*	Annual P ($g/m^2/y$)*	Cohort B (g/m^2)	Cohort P/B	Annual P/B (y^{-1})	Turnover Time (d)
Natural Wetland (OSPM)	1	0.00035	0.0009	0.0001	5.68	15.02	6.66
Test Pond 7 (OSPM)	453	0.1407	0.3721	0.0243	5.8	15.34	6.52
High Sulphate (Reference)	836	0.0706	0.1869	0.0147	4.82	12.75	7.84
Shallow Wetland South Ditch (Reference)	40	0.0127	0.0335	0.0021	5.9	15.61	6.41

*dry weight

chironomid community (Table 3.5; cohort and annual production of the entire chironomid community ranged from 5.60-30.71 g/m^2 and 43.31-75.74 $g/m^2/y$, respectively).

Density and Biomass

In most cases the density of individual chironomid taxa (Table 3.5) at a particular wetland exceeded that of *Chaoborus* (Table 3.6) in the same wetland. The exception to this was *Chaoborus* from Test Pond 7, which had higher densities than Chironomini and Orthocladiinae at Test Pond 7. At all wetlands, cohort biomass for each of the chironomid taxa was at least an order of magnitude higher than that of *Chaoborus* at the same wetland. The only exception to this was at Test Pond 7 where the cohort biomass of Orthocladiinae was only about twice that of *Chaoborus* from Test Pond 7.

Cohort P/B

The cohort P/B for chironomids (Table 3.5) and *Chaoborus* (Table 3.6) typically varied from 4 - 6 suggesting that growth and survivorship curves of chironomids and *Chaoborus* are similar at both OSPM-affected and reference wetlands.

Annual P/B and Turnover Time

Annual P/B and turnover time are essentially identical measures directly related to individual growth but are expressed with different units. Generally speaking, the annual P/B of chironomid taxa (Table 3.5) at a particular wetland was greater than that for *Chaoborus* (Table 3.6) at the same wetland. Likewise, the turnover time of chironomid taxa (Table 3.5)

was generally less than that of *Chaoborus* (Table 3.6) at any given wetland. Thus, for a given biomass, chironomids had a greater production efficiency than *Chaoborus*.

2) Secondary Production Variables in the Chironomid Community

A summary of the dominant chironomid taxa with respect to different secondary production related variables at each wetland is presented in Table 3.7. The following is a more detailed assessment of general trends with respect to these variables in the chironomid community of OSPM-affected and reference wetlands. All trends refer to values reported in Table 3.5.

Density

Trends between OSPM-affected and Reference Wetlands

There was no consistent pattern in the density of Tanytarsini at OSPM-affected wetlands compared to reference wetlands. The density of Chironomini was consistently higher at reference wetlands. The density of Tanypodinae was similar at all wetlands except Natural Wetland where it was 3 - 4 times greater than at the other sites. There were no consistent differences in the density of Orthocladiinae between OSPM-affected and reference wetlands.

Trends within Wetlands

At OSPM-affected wetlands, Tanytarsini was present at the highest densities followed by Tanypodinae. Densities of Chironomini and Orthocladiinae were similar within an OSPM-

Table 3.7. Dominant chironomid taxa at the 4 study wetlands with respect to selected secondary production parameters.

Site	Dominant Chironomid Taxa				
	Density ($\#/m^2$)	**Cohort B (g/m^2)**	**Cohort P (g/m^2)**	**Annual P ($g/m^2/y$)**	**Annual P/B (y^{-1})**
NW (OSPM)	Tanytarsini	Tanypodinae	Tanypodinae	Tanytarsini	Tanytarsini
TP7 (OSPM)	Tanytarsini	Tanypodinae	Tanypodinae	Tanytarsini	Tanytarsini
HS (Ref)	Chironomini	Chironomini	Chironomini	Chironomini	Tanytarsini
SWSD (Ref)	Tanytarsini	Tanytarsini	Tanytarsini	Tanytarsini	Tanytarsini

affected wetland. All taxa had higher densities at Natural Wetland compared to Test Pond 7. At reference wetlands Chironominae dominated in terms of density with Tanytarsini and Chironomini present at the two highest densities at each reference wetland followed by Tanypodinae and Orthocladiinae (in no consistent order).

Cohort Biomass

Trends between OSPM-affected and Reference Wetlands

Cohort biomass of Tanytarsini and Tanypodinae was consistently higher at OSPM-affected wetlands compared to reference wetlands. With respect to Chironomini, cohort biomass was consistently higher at reference wetlands. Cohort biomass of Orthocladiinae was similar at both OSPM-affected and reference wetlands.

Trends within Wetlands

At OSPM-affected wetlands, Tanypodinae had the highest cohort biomass followed by Tanytarsini (cohort biomass for these two taxa was equal at Test Pond 7), Chironomini, and Orthocladiinae. At reference wetlands, Tanytarsini and Chironomini (in no consistent order) had the highest cohort biomass followed by Tanypodinae and Orthocladiinae.

Cohort Production

Trends between OSPM-affected and Reference Wetlands

There was no consistent pattern in the differences in cohort production of Tanytarsini or Orthocladiinae between OSPM-affected and reference wetlands. Cohort production of

Chironomini was consistently higher at reference sites than at the OSPM-affected sites, and predatory Tanypodinae cohort production was consistently higher at OSPM-affected sites than at the reference wetlands.

Trends within Wetlands

At both OSPM-affected wetlands cohort production was highest for Tanypodinae followed by Tanytarsini. Substantially lower cohort production was observed for both Chironomini and Orthocladiinae, with Chironomini having consistently higher cohort production than Orthocladiinae within their respective OSPM-affected wetlands. At reference wetlands, cohort production was dominated by Chironomini and Tanytarsini (in no consistent order) followed by Tanypodinae. Orthocladiinae had the lowest cohort production at both reference wetlands.

Annual Production

Trends between OSPM-affected and Reference Wetlands

There was no consistent pattern in the differences in annual production of Tanytarsini or Orthocladiinae between OSPM-affected and reference wetlands. Annual production of Chironomini was consistently higher at reference sites than at the OSPM-affected sites, and predatory Tanypodinae annual production was consistently higher at OSPM-affected sites than at reference wetlands.

Trends within Wetlands

At both OSPM-affected wetlands, annual production of Tanytarsini was highest followed by Tanypodinae. Annual production of Chironomini and Orthocladiinae was similar within and between OSPM-affected wetlands and substantially lower than that of Tanytarsini and Tanypodinae. At both reference wetlands, the annual production of Chironominae (Tanytarsini and Chironomini) was substantially higher than annual production of either Tanypodinae or Orthocladiinae. Orthocladiinae annual production was slightly higher than that of Tanypodinae at both reference wetlands.

Annual P/B

Trends between OSPM-affected and Reference Wetlands

There was no consistent difference in the annual P/B of Tanytarsini and Tanypodinae between OSPM and reference sites. Chironomini and Orthocladiinae annual P/B were consistently higher at reference wetlands than at the OSPM-affected wetlands.

Trends within Wetlands

Within both OSPM wetlands, annual P/B of Tanytarsini was the highest, followed by Orthocladiinae, Tanypodinae, and Chironomini. Within both of the reference wetlands sampled, Tanytarsini had the highest annual P/B followed by Orthocladiinae. Annual P/B of Chironomini and Tanypodinae were similar at each reference wetland and about half that of Orthocladiinae. Annual P/B reflects individual growth and so does turnover time which is the reciprocal of annual P/B x 100 d.

3) Secondary Production Variables for *Chaoborus*

All trends discussed in the following sections are in reference to the data presented in Table 3.6.

Trends in Density between OSPM-affected and Reference Wetlands

Densities of *Chaoborus* varied widely from site to site with no consistent pattern between OSPM-affected wetlands and reference wetlands. High Sulphate had the greatest density of *Chaoborus* followed by Test Pond 7 where the density was approximately half that at High Sulphate. Shallow Wetland South Ditch had the next highest *Chaoborus* density, which was an order of magnitude smaller than that of High Sulphate. Finally, Natural Wetland had the lowest *Chaoborus* density. Densities at these wetlands in Summer 2000 appeared to be similar and in the same relative order as those observed in Summer 2001 based on visual inspection of plankton hauls from these wetlands.

Trends in Cohort Biomass between OSPM-affected and Reference Wetlands

There was no consistent pattern in the magnitude of cohort biomass between OSPM-affected and reference wetlands. Cohort biomass of *Chaoborus* was highest at Test Pond 7 followed by High Sulphate. Shallow Wetland South Ditch had the next highest cohort biomass followed by Natural Wetland, which were both substantially lower than cohort biomass at the other two wetlands.

Trends in Cohort Production between OSPM-affected and Reference Wetlands

Values of cohort production exhibited no pattern between OSPM-affected and reference wetlands. Cohort production was highest at Test Pond 7 followed by High Sulphate. *Chaoborus* from Shallow Wetland South Ditch had the next highest values of cohort production followed by Natural Wetland.

Trends in Annual Production between OSPM-affected and Reference Wetlands

As with the other measures discussed thus far, there was no consistent trend in annual production between OSPM-affected and reference wetlands. *Chaoborus* annual production was highest at Test Pond 7 followed by High Sulphate. Shallow Wetland South Ditch had the next highest values of *Chaoborus* annual production followed by Natural Wetland which had the lowest values.

Trends in Annual P/B between OSPM-affected and Reference Wetlands

Unlike the other measures related to the secondary production of *Chaoborus*, annual P/B values were relatively consistent between all wetlands. Values from Test Pond 7, Shallow Wetland South Ditch, and Natural Wetland were all very close ranging from 15.02 - 15.61 y^{-1} while the value reported at High Sulphate Wetland was 12.75 y^{-1} (Table 3.6).

4) Secondary Production Variables for Benthic and Pelagic Predatory Dipterans

As mentioned earlier in this chapter, density, cohort biomass, cohort production, and annual production were greater for the predatory benthic Tanypodinae than for predatory

pelagic *Chaoborus* within each wetland and between all wetlands. Compared to these measures, annual P/B of Tanypodinae and *Chaoborus* are relatively similar although values for Tanypodinae are still greater within each wetland and between all wetlands.

DISCUSSION

1) OSPM-affected Wetlands - Chironomid Community and Feeding Types

Taxa that are mainly collector-gatherers (Chironomini and Orthocladiinae) seemed to exhibit the largest differences between OSPM-affected wetlands and reference wetlands. Differences were manifested in the form of lower densities, generally lower annual production values, lower annual P/B values, and longer turnover times at OSPM-affected wetlands. These differences could be related to feeding behaviour and distribution of PAHs as these animals feed mainly on sediment material which contains the majority of potentially toxic PAHs present in wetlands. The levels of PAHs in the sediment are also higher at the OSPM-affected wetlands (see Chapter 5). Tanytarsini, which includes many genera that are collector-filterers, appeared to thrive at both OSPM-affected and reference wetlands, but more so at OSPM-affected wetlands. This may be due to their feeding behaviour as well. If these taxa filter their food from the water column, their diet would likely consist of markedly fewer PAHs than the diet of collector-gatherers. Estimates of the concentration of PAHs in suspended detrital/algal particles could confirm this conjecture but concentrations of PAHs in this material were not measured (although samples are frozen at -80°C). Lower dietary PAH exposure, and possibly reduced interspecific competition with chironomids in the subfamily Orthocladiinae and tribe Chironomini, may allow Tanytarsini to maintain higher

densities, higher annual production values, and faster individual growth rates (i.e. higher annual P/B) at OSPM-affected wetlands.

Like Tanytarsini, predatory Tanypodinae were more abundant at OSPM-affected wetlands than at reference wetlands. These observations are consistent with previous work on chironomids in OSPM-affected and reference wetlands in the Athabasca oil sands (Bendell-Young et al. 2000). At OSPM-affected wetlands, Tanypodinae dominated in terms of cohort production and cohort biomass. It may be easier for Tanypodinae to successfully attack filter-feeding chironomids than chironomids that feed on detritus and/or benthic algae. Tanytarsini tend to be more epibenthic than detritivores since their filtering strands must extend above the substrate to catch suspended particles, whereas detritivores may be buried directly within the sediment on which they are feeding. As such, encounter rates with Tanytarsini may be higher for Tanypodinae than encounter rates with other chironomid taxa. If this is the case, higher Tanytarsini (and lower Chironomini and Orthocladiinae) densities and production at OSPM-affected wetlands may play a part in determining the success of Tanypodinae at these wetlands. Most of the production (73%) of the tanypod genus *Thienemannimyia* has been attributed to the animal content of its diet (only 18-33%, Smith and Smock 1992), suggesting that animal prey plays an important role in regulating production in Tanypodinae. Similarly, other researchers have suggested that the higher quality of food from animal sources may be important for larval tanypod growth (Konstantinov 1971). The tanypod *Procladius culiciformis* (Linnaeus) did not moult beyond the third instar when fed only algae and detritus (Vodopich and Cowell 1984). Likewise, the

growth of the tanypod *Ablabesmyia monilis* (Linnaeus) decreased when denied animal food (Mackey 1977b).

2) OSPM-affected vs. Reference Wetlands: The Chironomid Community

The remainder of the discussion will focus on two variables that relate to key population features which may or may not be altered by the presence of OSPM. Annual production will be discussed because it is the rate at which populations accumulate biomass (Benke 1996) and is a reflection of density, cohort biomass, and the number of cohorts that can be completed in a growing season. Annual P/B will be discussed because it is a reflection of individual growth rate (Benke 1996) in that it is the amount of production that can be produced for a given amount of biomass present.

Chironomid Annual Production

Entire Benthic Chironomid Community

Production deals with the transfer of biomass (energy) from one organism to another, thus annual production results may be interpreted with respect to primary production potential of the study wetlands. In 1998, the chlorophyll a levels (which reflect primary production) at Natural Wetland (0.87 µg/L), Test Pond 7 (readings at environmentally similar Test Ponds 2 and 5 used a surrogate - 0.98 and 1.25 µg/L, respectively), High Sulphate (4.24 µg/L), and Shallow Wetland South Ditch (0.74 µg/L) were measured. If annual production of the entire benthic chironomid community is solely dependent on primary production potential in the water column, then the rank order of total chironomid annual

production at the study wetlands (HS > NW > SWSD > TP7) should parallel the rank order of chlorophyll a concentrations (HS > TP7 > NW > SWSD). Since the rank orders are not parallel, there must be energetic factors other than primary production potential of the water column that regulate chironomid production in these wetlands.

One such factor may be the detrital (organic carbon) content of the sediment which can be an important source of energy for chironomids (Muthukrishnan and Palavesam 1992, Berg 1995) and/or provide the preferred habitat type (Wolfram 1996). The level of total organic carbon in the 180 - 500-μm detrital fraction of sediments was similar and highest at Natural Wetland and High Sulphate whereas it was about 5 times lower at Shallow Wetland South Ditch and Test Pond 7 (Appendix 5.4). Thus, lower levels of organic carbon at Shallow Wetland South Ditch and Test Pond 7 may explain why production of the entire benthic chironomid community was lowest at these wetlands.

Variation in detrital content of the sediment within a wetland (Berg 1995) as well as variation in sediment characteristic within a wetland (Benke 1996) can result in variation within a wetland in terms of chironomid densities (Bendell-Young et al. 2000). The standard errors of densities of the entire benthic chironomid community are known to vary from 15.3 - 18.9% from the mean densities at constructed reference wetlands and from 6.5 - 15.2% of the mean densities at constructed OSPM-affected wetlands (Bendell-Young et al. 2000). Most of this variation is likely due to variation in the density of small larvae (1^{st} and 2^{nd} instars) as they are the most numerous in chironomid populations (Benke 1996). Small larvae contribute relatively little biomass to the overall magnitude of annual production estimates

(Benke 1996), so it is likely that variation in annual production estimates due to habitat heterogeneity is small.

Chironomid Subfamilies and Tribes

Annual production of Orthocladiinae was similar and low at all wetlands sampled. As such, trends with respect to OSPM-affected vs. reference wetlands will not be discussed for this taxon.

Tanytarsini populations seem to exhibit a generally, but not necessarily, higher annual production at OSPM-affected wetlands compared to reference wetlands. Tanytarsini annual production was highest among all chironomid taxa at OSPM-affected wetlands despite the fact that cohort production of Tanypodinae was higher than that of Tanytarsini in these wetlands. This reflects the short generation time of Tanytarsini (about 9 days to complete development of a cohort, (Appendix 3.3), relative to Tanypodinae, which take roughly 26 days to complete cohort development, (Appendix 3.3). About twice as many cohorts of Tanytarsini are able to develop in a 100-d growing season, thereby contributing to their high annual production estimates.

Annual production of Orthocladiinae and Chironomini at OSPM-affected wetlands was similar and lower than that of the other two taxa. This was primarily due to low cohort production rather than differences in cohort development time.

The rank order of annual production estimates of all taxa among reference wetlands was different only for Tanypodinae and Orthocladiinae. The relatively short time required for Tanytarsini to complete development (Appendix 3.3) did not allow it to achieve higher

production than Chironomini at High Sulphate due to greater cohort production of Chironomini at this wetland. Cohort production of Tanytarsini was already higher than that of Chironomini at the other reference wetland (Shallow Wetland South Ditch) and the shorter time to complete cohort development (Appendix 3.3) allowed Tanytarsini to sustain higher levels of annual production at this wetland. Tanypodinae annual production at reference wetlands was lower than that of all other taxa at reference wetlands whereas its cohort production was higher than that of Orthocladiinae. With respect to Tanytarsini, this is because Tanytarsini populations have greater cohort production than Tanypodinae and because they complete more cohorts in a year. With respect to Chironomini, it is solely due to greater cohort production, since they take only approximately 4 d longer to complete a cohort than Tanypodinae (Appendix 3.3). Orthocladiinae can complete development of a cohort in about half as many days as Tanypodinae can (Appendix 3.3) and this resulted their achieving higher values of annual production at reference wetlands.

Results of food web simulations indicate that insect predators inhabiting saline wetlands in Wyoming are strongly food-limited (Lovvorn et al. 1999). If this is also the case for insect predators inhabiting constructed saline wetlands of the oil sands mining region, then greater annual production by the generalist predatory Tanypodinae (Berg 1995) at OSPM-affected wetlands suggests one of two things:

There may be more food available to predatory Tanypodinae at OSPM-affected wetlands than at reference wetlands. If this is the case then their prey must also sustain higher levels of production at OSPM wetlands or the dominant available prey species may be easier to capture at OSPM-affected wetlands. Chironomids often comprise the majority of tanypod

diets (Berg 1995), so production of the chironomid community as a whole can be examined to assess this conjecture that there may be more food available to Tanypodinae at OSPM-affected wetlands. As discussed at the beginning of the "Chironomid Annual Production" section of the discussion, total chironomid production was not consistently higher at reference wetlands. This suggests that higher production of Tanypodinae at OSPM-affected wetlands is not due solely to increased production of their chironomid prey and that other food items must be important in regulating their production.

Another possible explanation for higher Tanypodinae production at OSPM-affected wetlands is related to the feeding habits of the chironomids that comprise the diet of Tanypodinae and the fact that tanypods are generalists capable of switching their diet depending on the availability of prey and the ease with which they are captured. Filter-feeding Tanytarsini have comparable densities at both OSPM-affected and reference wetlands. However, at OSPM-affected wetlands their abundance relative to other chironomid taxa is higher. Thus, when searching for prey at reference wetlands, Tanypodinae likely encounter other chironomid taxa which require greater handling time more often. On the other hand, the most likely prey item they will encounter at OSPM-affected wetlands in terms of chironomids is the epibenthic taxon Tanytarsini. Amongst chironomids, free-living species are more susceptible to predation than tube-dwelling species which spend significant amounts of time out of their tubes where they are more susceptible to predation (Tokeshi 1995c). Furthermore, Tanytarsini are relatively small and hence are more susceptible to predation than larger chironomids (Tokeshi 1995c). The combination of these two factors may allow

Tanypodinae to feed more often at OSPM-affected wetlands and spend less time and energy looking for food. The energy that they save can then be spent on production of new tissue.

Comparison With Other Studies

In a coastal lagoon (which can be considered a highly productive wetland - Horne and Goldman 1994), mean annual production of the single species *Chironomus salinarius* was estimated to be 16.8 g dry mass/m^2/y (Drake and Arias 1995). In a carp pond the production of the genus *Chironomus* was estimated to be 124 g dry mass /m^2/y in areas unprotected from carp, and 297 g dry mass /m^2/y in areas protected from carp (Ruzickova 1987). Production of the entire chironomid community in a sewage lagoon in Oregon was estimated to be 161 g dry mass /m^2/y (Menzie 1980). Estimates of chironomid production at constructed OSPM-affected and reference wetlands in this study were for subfamilies and tribes which encompass more than one genus or species. Thus it would appear that annual production of chironomids in unstressed and nutrient enriched shallow water bodies elsewhere in the world exceeds that in constructed wetlands of the Athabasca oil sands. In a stressed system (a shallow alkaline lake), production of the chironomid community as a whole was estimated to be 6.64 g dry mass /m^2/y in sheltered near-shore areas and 0.55 g dry mass /m^2/y in the open waters of the lake (Wolfram 1996). Total benthic chironomid annual production at constructed OSPM-affected and reference wetlands sampled in this study exceeds the estimates in this stressed shallow alkaline lake. This suggests that chironomid communities in constructed wetlands of the Athabasca oil sands are capable of being more productive than chironomid communities in other stressed water bodies.

Chironomid Annual P/B

Annual production to cohort biomass ratios are measures of individual growth rate (Benke 1996). Tanytarsini had the highest annual P/B at all wetlands, i.e., for a given amount of biomass, Tanytarsini were capable of generating the most production of all chironomid taxa. The magnitude of these values was sometimes higher and sometimes lower at OSPM-affected wetlands compared to reference wetlands, suggesting that the growth rate of Tanytarsini in these ponds was regulated by factors other than OSPM. Again, this may be related to the specific feeding habits of Tanytarsini (see section at the beginning of the Discussion on OSPM-affected wetlands and chironomid feeding types). Orthocladiinae exhibited the next highest annual P/B at all wetlands sampled, and the values at OSPM-affected wetlands were about 3/4 those at reference wetlands. This suggests the individual growth rate of Orthocladiinae may have been limited by a factor associated with OSPM-affected wetlands. The observation of higher annual P/B estimates for Tanytarsini and Orthocladiinae at all wetlands relative to other taxa are a reflection of the relatively small size at which these taxa reach maturity and hence the greater number of generations that they can complete during a growing season.

Annual P/B of Chironomini was lowest at all sites sampled except for Shallow Wetland South Ditch where it was close to, but a little higher than that of Tanypodinae. Chironomini annual P/B at OSPM-affected wetlands was about 3/4 that of Chironomini at reference wetlands, suggesting that individual growth rate of this taxon is reduced to 75% of its normal capacity at OSPM-affected wetlands.

The annual P/B ratio of Tanypodinae at all wetlands fell in between the values observed for Chironomini and Orthocladiinae with the exception of Shallow Wetland South Ditch where annual P/B ratio of Chironomini was marginally higher. There was little difference between annual P/B values estimated for Tanypodinae at OSPM-affected and reference wetlands, suggesting that the individual growth rate of Tanypodinae was not affected by the type of wetland in which they occurred.

3) OSPM-affected vs. Reference Wetlands: *Chaoborus*

As with the chironomid community, discussion of the differences and similarities between *Chaoborus* inhabiting OSPM-affected wetlands and reference wetlands will focus on annual production and annual P/B. It was assumed that all *Chaoborus* collected belong to the same species which may or may not be true as there is more than one species in Alberta (Borkent 1979), and different species of *Chaoborus* are capable of coexisting (Roth 1968).

Chaoborus Annual Production

There was no consistent pattern in the estimates of annual production between OSPM-affected and reference wetlands. This suggests that production dynamics of *Chaoborus* inhabiting OSPM-affected wetlands are no different than that of *Chaoborus* inhabiting reference wetlands.

Primary production in the water column, as represented by chlorophyll a concentrations, may provide a partial explanation for the observed results. Chlorophyll a concentrations in the water column at High Sulphate were 4 - 6 times greater than the

concentrations at the other wetlands and this wetland had the second highest estimates of *Chaoborus* annual production. The highest estimates of *Chaoborus* annual production were observed at Test Pond 7 where the chlorophyll a concentrations were estimated to be approximately 4 times less than that at High Sulphate. It may be that microbial degradation of bitumen in the sediment of Test Pond 7 is an important indirect source of energy for *Chaoborus*, conferring high levels of annual production at this wetland. Shallow Wetland South Ditch and Natural Wetland had the lowest chlorophyll a concentrations in the water column and correspondingly had the lowest estimates of *Chaoborus* annual production (although not in the same order). Levels of chlorophyll a will ultimately influence other ecological factors such as the availability of zooplankton prey which may play a more important part in determining the production of *Chaoborus* at these wetlands (Ramcharan et al. 2001).

Annual production estimates of *Chaoborus* at the 4 study sites ranged from 0.0009 (Natural Wetland) to 0.372 g dry mass/ m^2/y (Test Pond 7), with the upper and lower boundaries of this range coming from both of the OSPM-affected wetlands. In Lake Memphremagog, Quebec-Vermont, *Chaoborus* production was reported to vary between 0.066 and 0.348 g dry mass /m^2/y (Dermott et al. 1977). In a constructed pond in Japan, *Chaoborus* annual production was reported to be 11.7 g dry mass/m^2/y (Iwakuma et al. 1989). These chaoborids were able to complete development of 10 full cohorts from April to December, which in part explains why the estimate of annual production is much higher than what was observed in this study. Based on growth rates of *Chaoborus* at 15 °C (Hanazato and Yasuno 1989) and the assumption of a 100-d growing season, it was estimated that

Chaoborus in the study area are capable of completing less than 3 full cohorts in a year. However, the difference in the number of cohorts completed in a year is not enough to account for the large difference between annual production reported by Hanazato and Yasuno (1989) and this study. *Chaoborus* in OSPM-affected wetlands appear to be able to sustain annual production values that fall in the range reported for other natural waterbodies.

Chaoborus Annual P/B

Estimates of *Chaoborus* annual P/B ratios were approximately 15 at three of the four wetlands sampled. The reference wetland High Sulphate had a lower P/B estimate, which was close to 13. The fact that observed annual P/B estimates at OSPM-affected wetlands were greater than or equal to those at reference wetlands suggests that habitation of OSPM-affected wetlands does not affect the growth rate of individual chaoborids.

4) Secondary Production of Benthic and Pelagic Predatory Dipterans

Consequences for Potential Biomass and PAH Export

Annual production of taxa collected at OSPM-affected and reference wetlands may be viewed as potential biomass (energy) export from wetlands when the insects emerge and become terrestrial adults. A major goal of this thesis is to compare biomass export via benthic (Tanypodinae) and pelagic (*Chaoborus*) predatory dipterans within and between OSPM-affected and reference wetlands. These insects are important food items of terrestrial insectivores (Smits et al. 2000), and living in an OSPM-affected wetland could potentially alter the export of biomass to the insectivores via these benthic and pelagic routes. It is also

important to know the relative importance of these two pathways in terms of biomass export at both OSPM-affected and reference wetlands primarily due to the distribution of potentially toxic PAHs in the waterbodies. Most of these compounds are sequestered in wetland sediments (Neff 1979), and invertebrates are capable of accumulating them (Clements et al. 1994, Harkey et al. 1994, Bell 1995, Harkey et al. 1995) and ultimately transferring them to insectivores (e.g., Reinhold et al. 1999, Smits et al. 2000)). If the pelagic pathway (*Chaoborus*) dominates in terms of biomass export, then relatively small amounts of PAHs will likely be exported to the terrestrial environment. However, if the benthic pathway dominates biomass export then significant amounts of PAHs could be exported to the terrestrial environment as benthic organisms are likely more exposed to PAHs than pelagic organisms.

Benthic vs. Pelagic Predatory Dipterans - All Wetlands

The annual production of Tanypodinae at both OSPM-affected and reference wetlands was at least 2 orders magnitude higher than the annual production of *Chaoborus* at the same wetland. This confers potential biomass export via benthic predatory dipterans to be greater than that via pelagic predatory dipterans independent of wetland type. Therefore, adult Tanypodinae are potentially a more important component of the diet of terrestrial insectivores than adult *Chaoborus*, all other things being equal. The difference in potential biomass export between Tanypodinae and *Chaoborus* is even more pronounced at OSPM-affected wetlands as Tanypodinae production is greater at these wetlands than at reference wetlands (differences

in estimates of *Chaoborus* annual production are irrelevant in this situation as the absolute values are minimal compared to Tanypodinae).

Knowledge of the potential of these two different pathways to export biomass from wetlands provides information that is useful in predicting which pathway may be more important in exporting PAH from the system. Production differences dictate that benthic predatory dipterans are likely much more important PAH vectors than pelagic predatory dipterans regardless of the type of wetland. Furthermore, Tanypodinae live in the sediment where the majority of PAHs are sequestered, whereas *Chaoborus* spend most of their time in the water column where PAH concentrations are low.

Benthic OSPM Wetland-associated Predators vs. Benthic Reference Wetland-associated Predators

Annual production, and hence potential biomass export of predatory Tanypodinae was greater at OSPM-affected wetlands than at reference wetlands. The concentration of PAHs in the sediments of OSPM-affected wetlands is also higher than at reference wetlands (see Chapter 5). These two factors combined suggest that potential PAH export via Tanypodinae is likely greater at OSPM-affected wetlands than at reference wetlands. Thus, Tanypodinae at OSPM-affected wetlands have a greater potential to contribute both biomass and PAHs to the diet of terrestrial insectivores than Tanypodinae at reference wetlands.

Pelagic OSPM Wetland-associated Predators vs. Pelagic Reference Wetland-associated Predators

The highest and lowest estimates of *Chaoborus* annual production, and hence potential biomass export, came from the two OSPM-affected wetlands. Therefore, potential biomass and PAH export via *Chaoborus* may or may not be greater at OSPM-affected wetlands. PAH concentrations are lower in the sediment and water column at reference wetlands than at OSPM-affected wetlands (see Chapter 5). Therefore, PAH export via OSPM-affected *Chaoborus* is possibly greater than that via reference *Chaoborus* regardless of annual production estimates.

SUMMARY

The differences in annual production (i.e., potential biomass export) of the predatory benthic and pelagic dipterans, Tanypodinae and *Chaoborus*, reported in this chapter will be integrated with results from the following two chapters to fully assess the export of biomass and PAHs via benthic and pelagic predatory dipterans. Future work in the oil sands region on the export of biomass and PAHs, and on secondary production in general, should focus on the benthic component of the aquatic macroinvertebrate community as it is more productive than the pelagic community. Chironomid production values at the four constructed wetlands sampled in this study are likely less than those at natural wetlands but greater those of other stressed shallow water bodies. *Chaoborus* production estimates are similar to those in lakes but less than that of nutrient enriched ponds. Benthic dipteran secondary production exceeds planktonic dipteran secondary production at the study sites sampled, and this is typical of other water bodies.

Results indicate that there are characteristic differences in the production dynamics of chironomid communities inhabiting OSPM-affected and reference wetlands which may be related to feeding behaviour. The production of some of the chironomid taxa in OSPM-affected wetlands differed from that of the same taxa in reference wetlands suggesting that it may take longer than 12 y (the age of the youngest OSPM-affected wetland at the time of sampling) for the structure and function of chironomid communities of OSPM-affected wetlands to become similar to that of reference wetlands. This was observed despite the fact that the benthic invertebrate community richness at OSPM-affected wetlands appears to reach reference richness after the wetland has reached 7 y of age (C. Leonhardt 2002, University of Windsor, pers. comm., unpublished data). The lack of pattern between OSPM-affected wetlands and reference wetlands with respect to *Chaoborus* secondary production suggests that the structure and function of the planktonic community at OSPM-affected wetlands returns to reference conditions in less than 10 y. This conclusion is consistent with work on phytoplankton (Leung et al. 2001) and zooplankton (Harris 2001) communities in these water bodies, which suggest that reference conditions are reached in approximately 5 y.

Finally, the potential for biomass and PAH export from OSPM-affected and reference wetlands is greater via benthic predatory dipterans (Tanypodinae) than via pelagic predatory dipterans (*Chaoborus*). For Tanypodinae, the potential for both biomass and PAH export is highest at OSPM-affected wetlands, and for *Chaoborus* there were no clear patterns with respect to wetland type.

CHAPTER 4

Stable Isotopes: Food Web Structure, Energy Flow, and Bioaccumulation Potential in Tailings-affected and Reference Wetlands in the Athabasca Oil Sands

INTRODUCTION

The goal of this chapter is to ultimately interpret the stable isotope signatures of nitrogen and carbon in selected biota of wetlands to assess the bioaccumulation potential of PAHs in predatory benthic (Tanypodinae) and predatory pelagic (*Chaoborus*) dipterans at OSPM-affected and reference wetlands. This information can be combined with the results of secondary production estimates (Chapter 3) to make a detailed assessment of the relative potential for biomass (energy) and PAH export via benthic and pelagic predatory dipterans at OSPM-affected and reference wetlands.

Stable isotopes of carbon and nitrogen are increasingly being used by ecologists to delineate pathways of energy flow and identify trophic relationships. This is primarily due to similar rates of isotopic fractionation of the different sized atoms (i.e., stable isotopes) of carbon and nitrogen within a wide variety of organisms (Minagawa and Wada 1984, Peterson and Fry 1987). This facilitates their use a single powerful tool in determining food web relationships among a wide variety of taxa.

An organism's stable isotope signature reflects the signature of its diet (Peterson and Fry 1987), and integrates both spatial and temporal variation in feeding habits. This feature makes the use of stable isotopes in food web studies much more appealing than the use of gut content analysis, which offers only an instantaneous picture of feeding relationships, and

requires frequent, repeated samples to generate unbiased estimates (Vander Zanden et al. 1997).

Stable isotopes of nitrogen are used to determine the trophic position of organisms in a food web (Minagawa and Wada 1984), and stable isotopes of carbon are used to confirm food sources (e.g., Hart 1990, Gu et al. 1996). The trophic position of an organism reflects the pathways by which it acquires energy (i.e., the underlying food web structure). Thus, observed differences in trophic position of aquatic macroinvertebrates, or lack thereof, will give insights into how wetland ecosystem structure and function differ between OSPM-affected and reference wetlands and between benthic and pelagic food webs within each of these types of wetlands.

The ability of $\delta^{15}N$ signatures to characterize trophic positions is well documented (Minagawa and Wada 1984, Peterson and Fry 1987, Gu et al. 1994, Cabana and Rasmussen 1996, Gu et al. 1996, Vander Zanden and Rasmussen 1999, Vander Zanden et al. 1999, Post et al. 2000, Post 2002). The $^{15}N/^{14}N$ ratio typically becomes enriched by a factor of 3.4‰ from one trophic level to the next for a wide variety of food webs (Minagawa and Wada 1984). However, the $\delta^{15}N$ signature of an organism alone does not represent its trophic position, primarily because the signature of organisms at the base of the food web, which are capable of converting inorganic N to organic N, varies greatly among systems (Kling et al. 1992) and over time (Gu et al. 1994). Therefore, the $\delta^{15}N$ signature of an organism must be expressed relative to a site-specific baseline (i.e., base of the food web) $\delta^{15}N$ signature.

The ability of $\delta^{13}C$ to characterize food sources is also well documented (Peterson and Fry 1987, Hart 1990, Hesslein et al. 1991, Junger and Planas 1994, Hecky and Hesslein 1995,

Gu et al. 1996, Gu et al. 1997). There is relatively little change (< +1‰) of stable carbon isotope ratios from one trophic level to the next (DeNiro and Epstein 1978, Fry and Sherr 1984), and this property allows their use in tracing pathways of energy flow in food webs.

However, determining food sources in aquatic food webs remains difficult for a number of reasons. First, benthic and pelagic food webs are tightly linked, and primary producers at the base of each food web have different $\delta^{13}C$ signatures. For example, benthic algae in Lake Malawi had an average $\delta^{13}C$ signature of -11.66‰, whereas planktonic algae had a signature of -24.84‰ (Hecky and Hesslein 1995). This is due primarily to differences in the rate at which CO_2 diffuses into benthic and planktonic algal cells (Farquhar et al. 1989). The rate of CO_2 diffusion into planktonic algal cells is greater than that for benthic algal cells because the entire surface area of the planktonic cells is exposed to the water column. This results in less isotopic fractionation of CO_2 by benthic algae (atmospheric CO_2 has a $\delta^{13}C$ signature of -1 to 0‰ by the time it diffuses into the water - Hecky and Hesslein 1995) and hence a less negative $\delta^{13}C$ signature. Thus, if organisms rely on both benthic and pelagic food webs as energy sources as they typically do in wetlands, a two-source mixing model specific to the study system must be employed (e.g., Post et al. 2000).

Another factor that must be taken into account when choosing an appropriate baseline for the study sites is the bacterial degradation of bitumen which is present to varying degrees in the sediments (Chapter 2). Bitumen ($\delta^{13}C = -30.3 \pm 0.1$‰ - A. Farwell 2002, University of Waterloo, pers. comm.) can be a significant carbon source for bacteria, and if algal production is low, then microbial production fueled by bitumen in the sediments could be an important source of energy to both higher benthic and pelagic organisms.

If the trophic positions of aquatic macroinvertebrates are known, an assessment of the relative bioaccumulation potential of PAHs in predatory dipterans of benthic (Tanypodinae) and pelagic (*Chaoborus*) food webs of OSPM-affected and reference wetlands can be made. Trophic positions are a reflection of pathways of energy (biomass) flow. Lipids constitute more than twice as many calories per unit dry mass than either proteins or carbohydrates (Gardner et al. 1985). Thus, trophic positions are in part a reflection of the flow of lipids in a food web. Lipids are also the primary storage tissue and transfer medium of hydrophobic organic chemicals (e.g., PAHs) in biota. Consequently, trophic positions also reflect the flow of hydrophobic organic chemicals in a food web. Since metabolism of these compounds is limited in invertebrates (Neff 1979, James 1989), there is potential to see progressive increases in PAH concentration at increasingly high trophic positions (Gobas et al. 1993). Thus, the higher an organism's trophic position, the greater the potential to accumulate PAHs.

This chapter presents stable isotope signatures of benthic and pelagic biota and energy sources (detritus) collected at OSPM-affected and reference wetlands. These data will facilitate the evaluation of energy sources and trophic positions of organisms in these two food webs. This information will allow a comparison of trophic level and hence bioaccumulation potential among taxa (the predatory benthic and pelagic dipterans Tanypodinae and *Chaoborus* in particular) within and among wetland types. The trophic levels of these organisms will depend on the structure of the food webs at OSPM-affected and reference wetlands. I expect that trophic positions of both taxa should be greater at

OSPM-affected wetlands based on reductions in taxonomic diversity in the benthic and pelagic food webs of these water bodies (see Discussion).

METHODS

1) Study Sites

Samples for stable isotope analysis were collected from two OSPM-affected wetlands (Natural Wetland and Syncrude Test Pond 7), and two reference wetlands (High Sulphate and Shallow Wetland South Ditch). Detailed environmental characteristics of each wetland were reported in Chapter 2.

2) Field Collection Methods

Samples Collected

Collections of benthic and pelagic biota were made one wetland at a time (May-July 2002) to minimize effects of temporal variation in the stable isotope signatures. All samples were stored at -20°C.

The lower levels of the planktonic food web were collected by sampling each wetland with plankton nets of different mesh sizes. The size fractions of plankton collected were determined based on the size of plankton and the fact that zooplankton diets are in part determined by size of the prey (Fedorenko 1975, Pennak 1978). Hence, different types of plankton (functional feeding groups) are expected to be retained by different sieve sizes. The size fractions collected were nano- and picoplankton (0.5 - 20-μm; expected to be mainly phytoplankton (Appendix 4.1)), microplankton (particles 20 - 180-μm; expected to be a

mixture of large phytoplankton and small zooplankton), and large zooplankton (180 - 500-μm; H. MacIsaac, University of Windsor, pers. comm.). Filter-feeding *Daphnia* were also collected at wetlands as were the pelagic predatory macroinvertebrates Dysticidae, Notonectidae, and *Chaoborus*. A list of the samples collected for stable isotope analysis in the pelagic food web, their functional feeding group, and most likely food source is presented in Table 4.1.

Brass testing sieves were used to collect size fractions of detritus from the benthic food web that were parallel to the size fractions of plankton collected (Table 4.2). Herbivorous benthic taxa (miscellaneous chironomids, Chironomini, and Gastropoda) and predaceous benthic taxa (Tanypodinae, Anisoptera, and Zygoptera) were also collected. A list of the samples collected for stable isotope analysis in the benthic food web, their functional feeding group, and most likely food source is presented in Table 4.2.

Collections of Taxa in the Pelagic Food Web

Collections were made in each wetland of predaceous *Chaoborus*, a range of other invertebrates (e.g., Notonectidae, Dytiscidae, Zygoptera, Anisoptera, *Daphnia*), and of 3 size fractions of plankton - large crustaceans and phytoplankton (180 - 500-μm), smaller phytoplankton, and rotifers (20 - 180-μm), and nano- and picoplankton (0.5 - 20-μm).

Phytoplankton, Rotifers, and Crustaceans

A variety of sampling techniques was employed to collect different size fractions of planktonic material. Samples composed of smaller organisms required large amounts of water

Table 4.1. Taxa collected in the pelagic environment, their functional feeding groups, and most likely food sources.

Taxon	Functional Feeding Group	Most Likely Food Source
0.5 - 20-μm plankton -phytoplankton	primary producers	autochthonous producer
20 - 180-μm plankton -phytoplankton	primary producer	autochthonous producer
-rotifers	herbivores, predators	algae, detritus, bacteria, small Metazoa (Pennak 1978)
180 - 500-μm plankton -large zooplankton	herbivore	phytoplankton, protozoa and detritus (Pennak 1978)
Daphnia	herbivore	phytoplankton, protozoa and detritus (Pennak 1978)
Dytiscidae	predator	generalists (Pennak 1978)
Notonectidae	predator	crustaceans (entomostracans) & aquatic insects (Pennak 1978)
Chaoborus	predator	rotifers & crustaceans (Fedorenko 1975)

Table 4.2. Materials and taxa collected in the benthic environment, their functional feeding groups, and most likely food sources.

Material or Taxon	Functional Feeding Group	Most Likely Food Source
0.5 - 20-μm detritus	base of benthic food web	n/a
20 - 180-μm detritus	base of benthic food web	n/a
180 - 500-μm detritus	base of benthic food web	n/a
miscellaneous small chironomids	herbivore	phytoplankton, protozoa and detritus (Pennak 1978)
Chironomini	detritivore/herbivore	detritus, algae (suspended and benthic) (Berg 1995)
Gastropoda	herbivore	benthic and epiphytic algae (Pennak 1978)
Tanypodinae (includes *Derotanypus*)	predator	chironomids & crustaceans, detritus & algae too (Berg 1995)
Anisoptera	predator	aquatic insects, annelids, small crustaceans and mollusks (Pennak 1978)
Zygoptera	predator	aquatic insects, annelids, small crustaceans and mollusks (Pennak 1978)

to be filtered in order to obtain sufficient material, whereas samples composed of larger organisms required less effort to collect. Sampling techniques were modified accordingly and thus are not identical for plankton samples of different size classes.

i) 0.5 - 180-μm Planktonic Material (Nano- and Picoplankton, Smaller Phytoplankton, and Rotifers)

To collect the 20 - 180-μm plankton and the 0.5 - 20-μm plankton, a hand-bilge pump was used to pump whole pond water through a 180-μm brass testing sieve (to retain any large debris) into several 20-L plastic pails. The intake hose of the bilge pump was continuously moved throughout the water column while pumping in order to sample the plankton community in a representative manner.

The 20-L pails were transported back to the laboratory where a Millipore vacuum pump and Millipore filtration apparatus were used to filter the plankton from the water. Plankton in the pails were resuspended by stirring the water, and 1,000-mL aliquots were filtered under vacuum through pre-weighed 20-μm polycarbonate filters.

The fresh mass of 20 - 180-μm plankton was determined by re-weighing each filter. The filtrate was poured into another 20-L plastic pail and was itself filtered using pre-combusted, pre-weighed 0.5-μm glass fibre filters. Each filter was weighed after filtration to determine sample mass.

For taxonomic identification purposes, a single filter from all 0.5 - 20-μm and all 20 - 180-μm plankton samples collected was preserved in Lugol's solution following a 30-s anaesthetization period in carbonated water.

ii) 180 - 500-μm Planktonic Material (Large Crustaceans and Large Phytoplankton)

Collections were made using an 80-μm Wisconsin plankton net. Material collected with this net was washed from the collecting bucket into a stack of brass testing sieves - a 500-μm sieve first then a 180-μm sieve. The material was then rinsed through the sieves using a squirt bottle filled with 180-μm filtered native pond water. Contents of the 180-μm sieve were collected into 20-mL scintillation vials and filled with native 180-μm filtered pond water. The samples were drained of water at the Syncrude Environmental Complex and frozen at -20°C for stable isotope analysis.

A fraction of each 20 - 180-μm plankton sample collected was anaesthetized for 30-s in carbonated water and preserved in buffered formalin for identification purposes.

Chaoborus

Chaoborus larvae were collected using a large, 500-μm plankton net (Fig. 3.1), which was custom-designed to efficiently collect *Chaoborus* in shallow water bodies. The net was attached to a 9-m long rope. The net was thrown and retrieved several times, and then the contents of the collection bucket were emptied into a plastic sorting tray containing less than a cm of water. Forceps were used to pick individual *Chaoborus* out of the tray, which were placed in a 20-mL scintillation vial containing native pond water. Between hauls with the plankton net, the scintillation vials were kept in a cooler containing ice packs. Every effort was made to keep the vials out of the sun to minimize stress to the larvae.

To ensure that only *Chaoborus* larvae were retained, the collected specimens were emptied into a plastic sorting tray containing native pond water at the end of each collection

day. Larvae were hand-picked from the tray and placed back into 20-mL scintillation vials with native pond water. Upon return to the laboratory at Syncrude Canada Ltd each day, the water was drained from the vials, the live mass of the larvae was measured on an electronic balance, a collection label was placed in the vial, and the vial was placed in the freezer and stored at -20°C.

Daphnia, Dytiscidae, & Notonectidae

These three taxa were collected from the same plankton samples gathered to collect *Chaoborus*. Individuals of each taxon were picked from the plastic sorting tray with forceps and placed in a labelled 20-mL scintillation vial(s) containing native pond water. As with the *Chaoborus* samples, vials were kept in a cooler containing ice packs while the plankton net was being thrown, and out of the sun while organisms were being placed in the vials. Due to their absence at the time of collection, notonectids were not collected at Natural Wetland, and dytiscids were not collected from reference wetlands. Samples of all 3 taxa were processed for storage and subsequent analysis in the same manner as the *Chaoborus* samples upon return to the Syncrude laboratory at the end of each collection day.

Benthic Collections

Collections were made in each wetland of a variety of benthic macroinvertebrates - herbivorous/detritivorous and predaceous chironomids, gastropods (snails), odonates (Anisoptera - dragonflies, and Zygoptera - damselflies). Several size fractions of detritus (parallel to the plankton size fractions) were also collected, including fine particulate organic

matter (FPOM - 180 - 500μm), very fine particulate organic matter (VPOM - 20 -180-μm), and ultrafine particulate organic matter (UPOM - 0.5 - 20-μm).

Detritus

Detritus was collected by placing several scoops of approximately the top 5 cm of wetland sediment into the top unit of a stacked pair of brass testing sieves (500 and 250-μm aperture) held over a plastic sorting tray. Sediment that did not pass through the 500-μm sieve was discarded. All samples were stored frozen at -20°C for subsequent stable isotope analysis.

i) 180 - 500-μm Detritus (FPOM)

The material passing through the 500-μm sieve was washed through the 180-μm sieve into the plastic sorting tray with pond water from a squirt bottle. Material remaining in the 180-μm sieve was transferred into a plastic sorting tray and repeatedly flushed with water from the wetland. Detritus suspended by the flushing was poured with the water back into the 180-μm sieve. This process was repeated several times until all or most of the detritus was separated from the inorganic material. Detritus (180 - 500-μm) was then transferred from the sieve into a 20-mL glass scintillation vial. The vial was stored refrigerated until the end of the day, weighed in the laboratory, labelled, and frozen.

ii) 20 - 180-μm Detritus (VFPOM)

The tray containing material <180-μm was repeatedly flushed with water, and the suspended detritus and water decanted into a 20-L plastic pail. The pail and contents were taken back to laboratory. The fine detritus was resuspended by stirring the water, and 1,000-mL aliquots were filtered under vacuum through pre-weighed 20-μm polycarbonate filters. The fresh mass of 20 - 180-μm detritus was determined by re-weighing each filter. Only enough material (a few mg) was collected on these filters for stable isotope analysis.

iii) 0.5 - 20-μm Detritus (UPOM)

The filtrate was poured into another 20-L plastic pail and was itself filtered using pre-combusted, pre-weighed 0.5-μm glass fibre filters. Each filter was weighed after filtration and enough material (a few mg) was collected for stable isotope analysis.

Chironomidae, Gastropoda, and Odonata (Zygoptera and Anisoptera)

In order to collect these taxa, a D-shaped dip net was swept along the bottom of the wetlands, just penetrating the surface of the sediment. Sediment collected with the net was placed into a plastic sorting tray and the different taxa were removed with forceps and placed in labelled 20-mL scintillation vials containing native pond water. Between sweeps with the dip net the vials were kept in a cooler containing ice packs. Every effort was made to keep the vials out of the sun while organisms were being placed into them in order to minimize stress to the animals. Tanypodinae, *Derotanypus*, and Chironomini samples were emptied into a sorting tray at the end of each collection day and the larvae were placed back into 20-

mL scintillation vials filled with native pond water. This was done to ensure taxonomic purity of the samples. This was not necessary for gastropod or odonate samples as the organisms are large enough that relatively few individuals need to be collected and the individuals that were collected were rinsed of debris before they were placed collection vials.

Upon return to the laboratory each day, the water was drained from the vials, the live mass of the sample was measured on an electronic balance, a collection label was placed in the vial, and the vial was placed in the freezer.

Not all taxa could be collected in sufficient quantities from all 4 wetlands. *Derotanypus* could not be collected from reference wetlands, Tanypodinae (including *Derotanypus*) could not be collected from High Sulphate, Chironomini could not be collected from Test Pond 7, and miscellaneous chironomids were not collected from Shallow Wetland South Ditch. Gastropods could not be collected from Natural Wetland.

3) Laboratory Methods

Sample Storage, Mass Estimation, and Allocation

Upon return to Windsor in mid-August 2001, all samples were stored frozen at -80°C. Samples for stable isotope and PAH analyses (Chapter 5) were collected at the same time and in the same vials. Most samples were composites collected over a number of days, and hence a single sample usually consisted of more than one vial. To ensure temporal synchrony of stable isotope and PAH samples, the sample mass taken from each collection vial for stable isotope analysis was a fixed proportion of that vial's contribution to the total mass of the sample. Following determination of the correct mass, two or three samples at a time were

removed from the freezer and taken in an ice-filled Styrofoam cooler to a balance (Sartorius Research Type R 160 D). This was done in early December, 2001.

A scalpel and fine forceps were used to extract the appropriate sample biomass from each vial for stable isotope analysis. The forceps and scalpel had been rinsed with acetone, then hexane, and then soaked in a detergent solution for 5 min. Before weighing out each sample, the forceps and scalpel were thoroughly rinsed with de-ionized water. The extracted sample was placed into a scintillation vial that had been soaked for two h in a Sparkleen® and MilliRX® de-ionized water solution and rinsed 5 times with de-ionized water. Following this, the sample was placed in a Thelco® Model 15 desiccator (Precision Scientific Company, Chicago, IL) at 60°C for 24 h.

For most samples a total of 0.5 g wet mass (w.m.) was weighed out. However, if the total mass available was much greater than 5 g as it was for most of the 180 - 500-μm detritus, then 1.0 g w.m. was used. Some samples such as the *Daphnia* samples were comprised of less than 0.5 g w.m. of material. In such cases, the entire sample was used for stable isotope analysis. Dried samples were stored in their sealed vials at room temperature awaiting stable isotope analysis.

In December 2001, samples were taken to the University of Waterloo's Stable Isotope Analytical Laboratory. Each sample was ground to a fine powder. A ball grinder mill was used if the sample was coarse. A mortar and pestle were used if the material was already very fine. An aliquot of 1.0 ± 0.1 mg of the ground sample was weighed into a tin capsule. Each tin capsule was crushed into a ball with a pair of forceps and placed onto a sample tray for

subsequent analysis (early February 2002). Sample processing materials were wiped clean with Kimwipes® before preparation of each sample.

All samples were analysed using a Micromass VG Isochrom continuous-flow isotope ratio mass spectrometer and total carbon content, total nitrogen content, and the stable carbon and nitrogen isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) were reported.

Determination of Stable Isotope Signatures

Ratios of isotopic fractionation depend on the physical and biological processes to which the elements are subjected, so one must express the ratio of stable isotopes to a standard in order to make comparisons. The following equations were used to calculate the stable carbon and nitrogen isotope signatures of the samples:

$$\delta^{13}C = \frac{^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{standard}}{^{13}C/^{12}C_{standard}} \times 1000$$

$$\delta^{15}N = \frac{^{15}N/^{14}N_{sample} - ^{15}N/^{14}N_{standard}}{^{15}N/^{14}N_{standard}} \times 1000$$

where $^{13}C/^{12}C_{standard}$ is the ratio of $^{13}C/^{12}C$ in a Cretaceous marine fossil of *Belemnitella americana* from the PeeDee formation in South Carolina (Peterson and Fry 1987), and $^{15}N/^{14}N_{standard}$ is the ratio of $^{15}N/^{14}N$ in the air, which is assumed to be constant (Peterson and Fry 1987). A positive δ value indicates that a sample is enriched in the heavier isotope relative to the standard material and a negative δ value indicates that a sample is depleted in the heavier isotope relative to the standard material. The measured $\delta^{13}C$ signatures of *Daphnia* collected from all sites were increased by +0.5‰ due to the presence of inorganic

carbon in their highly calciferous carapace (Kiriluk et al. 1994). The inorganic carbon in *Daphnia* causes the $\delta^{13}C$ signature to become more depleted in the heavier carbon isotope, and as this is a food web study, only the $\delta^{13}C$ signature of organic carbon is desired.

A continuous (i.e., trophic positions are not expressed as integers) estimate of trophic position was calculated for each taxon collected using the formula:

$$\text{Trophic Position} = 1.0 + (\delta^{15}N_{organism} - \delta^{15}N_{baseline})/3.4$$

where 1.0 is the trophic position of the baseline (detritus) and 3.4 is a 1.0 trophic level increase in $\delta^{15}N$.

Sorting & Identification of Plankton Samples (0.5 - 20-μm, 20 - 180-μm, 180 - 500-μm)

Each plankton sample was placed in a round-bottomed volumetric flask, topped up to 100-mL volume with tapwater, and a Hensen-Stemple pipette was used to take 1-mL subsamples. Subsamples were placed into a Sedgewick-Rafter cell with a 1-mL capacity, and covered with a coverslip.

All organisms in the 1-mL sample were identified under an inverted microscope. Each sample was completely scanned twice. The 180 - 500-μm plankton were sorted at 10X and 20X, and the 0.5 - 20-μm and 20 - 180-μm size fractions were sorted at 20X and 40X. All zooplankton were identified using the keys of Pennak (1978). Most phytoplankton were identified to genus using the keys of Thompson (1959). Diatoms were identified using keys of Patrick (1959).

Only relative abundances of taxa within a sample are comparable from one sample to another because the total volume of water filtered to collect all plankton samples was different and not recorded.

RESULTS

1) Among-wetland Variation in Stable Isotope Signatures

In order to examine patterns among the wetlands and within wetlands with respect to stable isotope signatures, $\delta^{15}N$ was plotted against $\delta^{13}C$ for all materials and taxa collected at each wetland (Fig. 4.1A-D).

Carbon

There was considerable variability in the $\delta^{13}C$ signatures of taxa within a wetland (Fig. 4.1A-D). This variability seemed to be greater at reference wetlands (Fig. 4.1C and 4.1D) than at OSPM-affected wetlands (Fig. 4.1A and 4.1D). These same figures suggest that OSPM-affected wetlands are generally more depleted in ^{13}C compared to reference wetlands.

Nitrogen

Taxa collected at OSPM-affected wetlands were generally enriched in ^{15}N (i.e., had higher $\delta^{15}N$ signatures) compared to taxa at reference wetlands (Fig. 4.1).

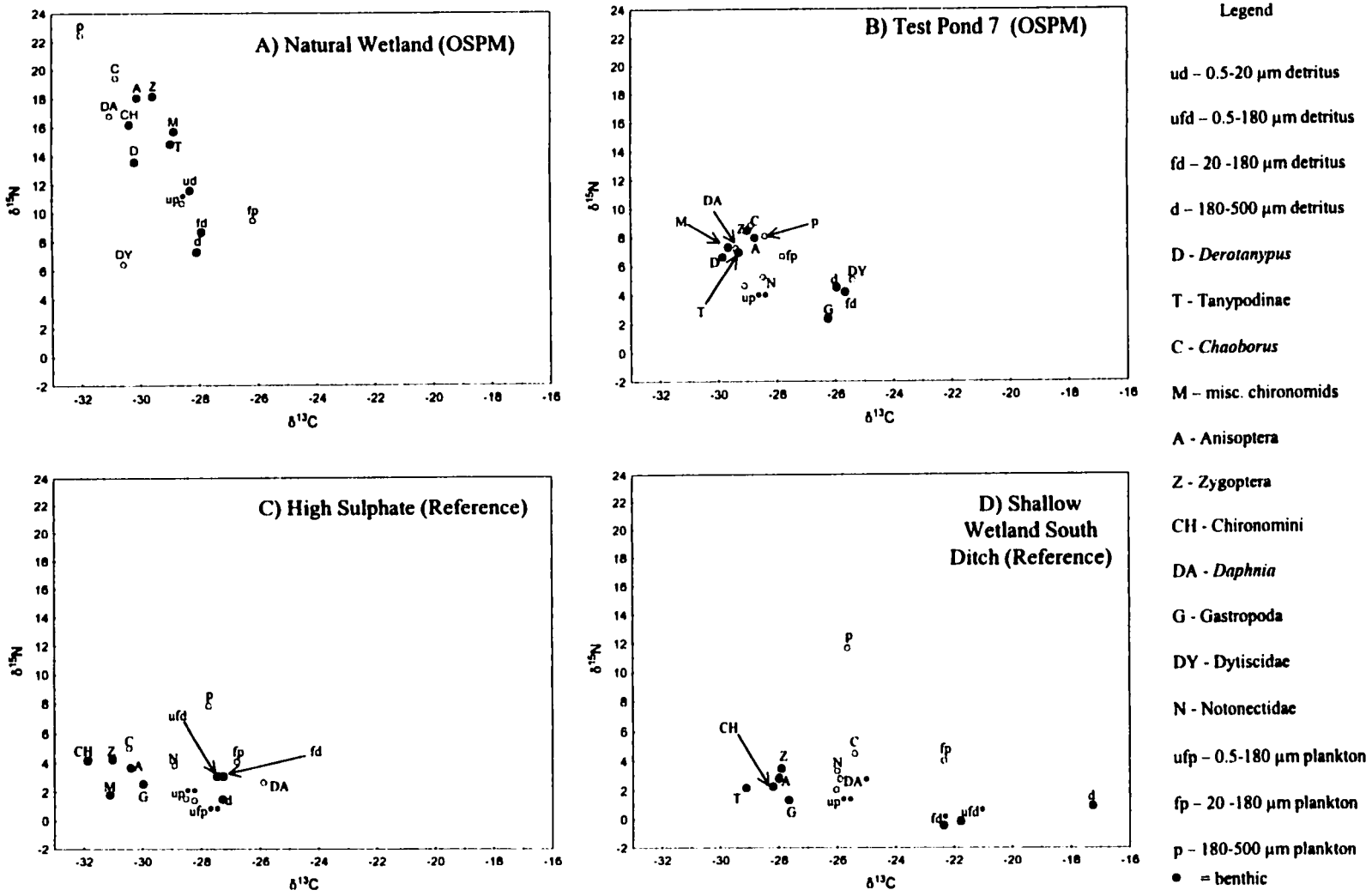


Fig. 4.1A-D. $\delta^{15}N$ vs. $\delta^{13}C$ for benthic and pelagic taxa in constructed wetlands.

* outside Carbon detection range

** outside Carbon and Nitrogen detection range

2) Composition of Plankton Samples

The raw data for taxa observed in all plankton samples (0.5 - 20-μm, 20 - 180-μm, 180 - 500-μm) are in Appendix 4.1A-C.

0.5 - 20-μm Plankton

These samples were composed entirely of phytoplankton at all study sites. Not all taxa were present at all sites and the relative abundances vary.

20 - 180-μm Plankton

In terms of relative abundances, these samples were composed mainly of phytoplankton at all sites with the presence and relative abundances varying from one site to another. Animals comprised a fraction of all samples and their presence and relative abundances also varied from one site to another.

180 - 500-μm Plankton

These samples were also dominated by phytoplankton in terms of relative abundances which were different from one site to another. Animals comprised a greater portion of these samples than in the 20 - 180-μm samples, and a wider variety of taxa were also present. The presence and relative abundance of animals in this size fraction also varied from one wetland to another.

3) $\delta^{15}N$ Trophic Position Calculations

The raw stable isotope data and trophic position calculations are presented in Appendix 4.2A-D. The trophic positions of all taxa collected from each wetland are presented in Table 4.3. The mean of detrital $\delta^{15}N$ signatures from all detrital samples collected at each wetland was used as the baseline $\delta^{15}N$ to calculate trophic positions. Trophic position (baseline corrected $\delta^{15}N$ signatures) of all taxa versus their $\delta^{13}C$ signatures was plotted for each wetland (Fig. 4.2).

The trophic position of most taxa collected at reference and OSPM wetlands was higher at the two OSPM-affected wetlands than at either of the reference wetlands (Table 4.3). Exceptions to this include Gastropoda (herbivores) and Notonectidae (predators of pelagic invertebrates), whose trophic positions were estimated to be higher in the reference wetlands than in the OSPM-affected wetlands. For the 3 different size fractions of plankton collected, the 180 - 500-μm fraction had higher trophic position estimates at all wetlands compared to the 20 - 180-μm fraction. The 20 - 180-μm plankton had higher trophic position estimates than the 0.5 - 180-μm fraction at all wetlands except Natural Wetland. This result should be interpreted with caution at the other three wetlands as the 0.5 - 20-μm plankton samples were outside of the detection range with respect to $\delta^{15}N$. Trophic positions of all plankton size fractions were neither consistently higher nor lower at OSPM-affected wetlands compared to reference wetlands.

Table 4.3. Trophic position of taxa at 4 constructed wetlands calculated using the average $\delta^{15}N$ signature of detrital samples as the baseline $\delta^{15}N$.

Taxon	Feeding Group	Trophic Position $(1 + (\delta^{15}N_{organism} - \delta^{15}N_{baseline})$			
		OSPM		Reference	
		Natural Wetland	Test Pond 7	High Sulphate	Shallow Wetland South Ditch
BENTHIC FOOD WEB					
***Derotanypus* (Tanypodinae)**	predator	2.32	1.67	n/a	n/a
Other Tanypodinae	predator	2.67	1.77	n/a	1.58
Zygoptera	predator	3.64	2.25	1.47	1.99
Anisoptera	predator	3.6	2.04	1.34	1.8
Chironomini	herbivore/ detritivore	3.06	n/a	1.49	1.62
Misc. Chiron.	herbivore	2.9	1.88	0.78	n/a
Gastropoda	herbivore	n/a	0.42	1	1.35
PELAGIC FOOD WEB					
0.5 - 20-µm plankton	autotroph	1.44	1.08*	0.70*	1.56*
20 - 180-µm plankton	autotroph, herbivore, predator	1.08	1.67	1.44	2.13
180 - 500-µm plankton	autotroph, herbivore, predator	4.92	2.09	2.55	4.37
Daphnia	herbivore	3.24	1.84	1.03	1.77
Notonectidae	predator	n/a	1.26	1.37	1.92
Dytiscidae	predator	0.2	1.19	n/a	n/a
Chaoborus	predator	4.01	2.31	1.72	2.28

* outside of detection range for both carbon and nitrogen stable isotopes

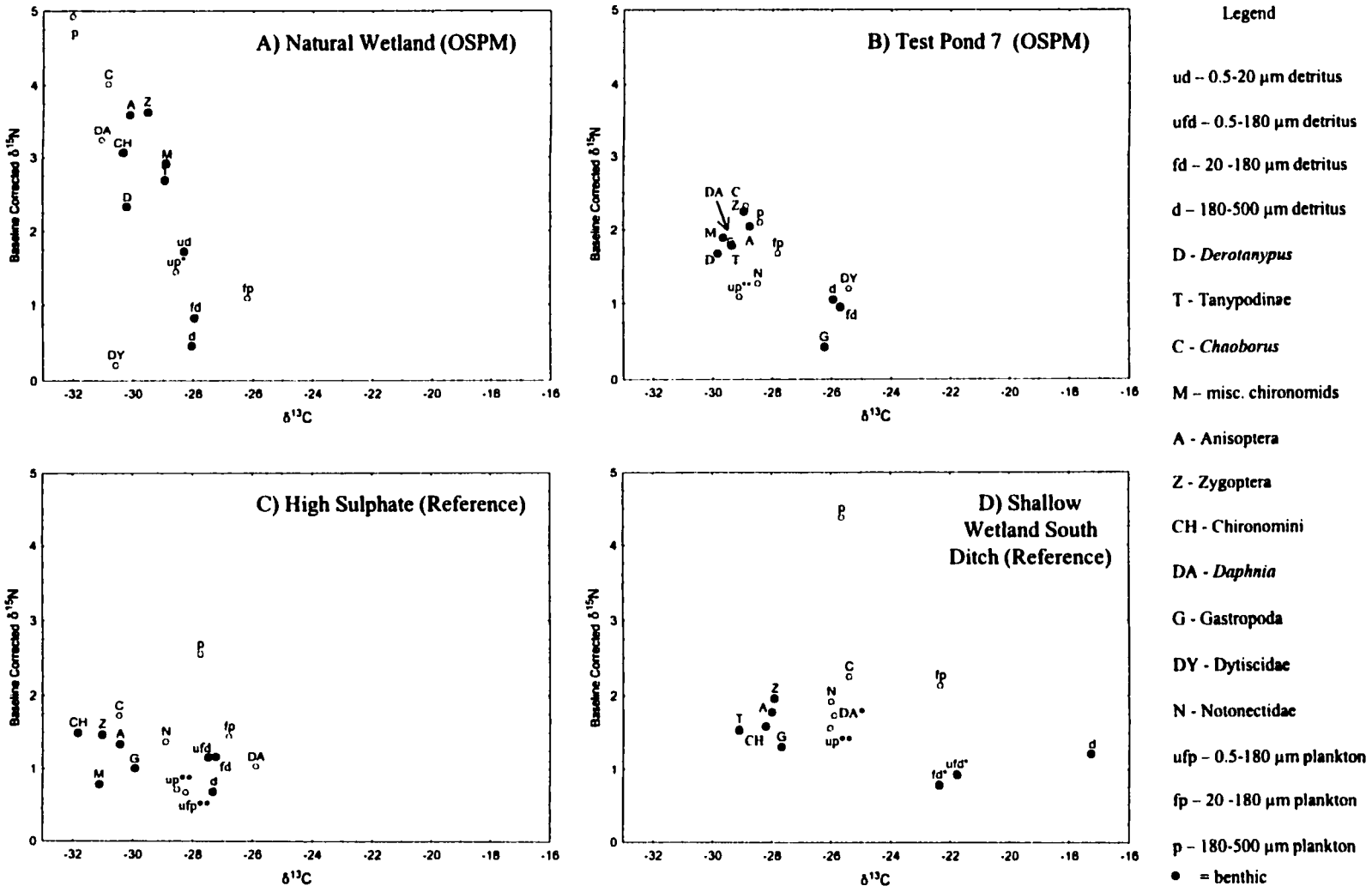


Fig. 4.2A-D. Trophic position (baseline corrected $\delta^{15}N$) vs. $\delta^{13}C$ of benthic and pelagic taxa at constructed wetlands.

* outside Carbon detection range

** outside Carbon and Nitrogen detection range

4) Trophic Positions - OSPM-affected vs. Reference Wetlands

To determine if the average trophic position of taxa at OSPM-affected wetlands was higher than the trophic position of the same taxa at reference wetlands, the trophic position of each taxon at OSPM-affected and reference wetlands was calculated. Only taxa collected from at least one OSPM-affected wetland and from at least one reference wetland were considered. If a taxon was collected at both OSPM-affected sites or both reference sites, a mean was calculated for the overall signature of that taxon at OSPM-affected or reference sites. If a taxon was only collected at one OSPM-affected or reference site, then the trophic position from that site was used to estimate the trophic position of the taxon at either OSPM-affected or reference wetlands.

The 0.5 - 20-μm, 20 - 180-μm, and 180 - 500-μm plankton samples were excluded from this comparison for a number of reasons. The taxonomic composition of plankton samples varied in both the species present and their relative abundances (Appendix 4.1A-C), thereby making direct comparisons of trophic positions of different size fractions of plankton among wetlands inappropriate. Some of the 20 - 180-μm and 180 - 500-μm samples also contained primary producers, primary consumers, and predators, but in different proportions. This wide variety of taxa could potentially have large effects on estimates of trophic position among wetlands, the degree of which depending on the variety of taxa in the sample. Furthermore, using the detrital $\delta^{15}N$ signature as a baseline to estimate trophic position may not be very appropriate for these samples as phytoplankton (autotrophs whose trophic position by definition is 1.0) comprise a large portion of them (Appendix 4.1). Notonectids

were also excluded from the analysis as they are capable of flying between wetlands (Pennak 1978) potentially invalidating estimates of their trophic position.

To determine if the average of the trophic positions of taxa collected from OSPM wetlands was higher than that of taxa collected from reference wetlands (Table 4.4), a Shapiro-Wilk's W test for normality was performed. The mean trophic positions at the OSPM-affected and reference wetlands were not normally distributed ($W = 0.85, p = 0.08$ and $W = 0.93, p = 0.44$, respectively) so a sign test was performed to compare the averages of the mean trophic position of each taxon at OSPM-affected and reference wetlands (Table 4.4).

H_o: trophic position of taxa at OSPM-affected wetlands =
trophic position of taxa at reference wetlands,

H_a: trophic position of taxa at OSPM-affected wetlands $\neq$
trophic position of taxa at reference wetlands

The results of the sign test indicate that trophic positions of taxa at OSPM-affected wetlands were significantly greater than trophic positions of equivalent taxa collected from reference wetlands ($n = 8$, $p = 0.035$). The mean ($\pm$ SD) of the mean trophic positions at OSPM-affected wetlands was 2.44 ± 0.88 while it was 1.48 ± 0.37 at reference wetlands.

Of particular interest are the trophic positions of Tanypodinae and *Chaoborus* at OSPM-affected and reference wetlands because they are top predators in the benthic and pelagic food webs, respectively. Tanypodinae had a trophic position of 2.17 at OSPM-affected wetlands and 1.58 at reference wetlands. Likewise, the trophic position of

Table 4.4. Sign test comparing trophic positions of taxa collected at constructed OSPM-affected and reference wetlands.

Taxa	Trophic Position (TP) $(1 + (\delta^{15}N_{organism} - \delta^{15}N_{baseline})$ OSPM	Reference	Sign $(TP_{OSPM} - TP_{Reference})$	p (2-tailed Sign Test) $p_{critical} = 0.05$
Tanypodinae	2.17	1.58	+	= Prob ($X \geq 7$)
Zygoptera	2.95	1.73	+	= 0.03125 + 0.00391
Anisoptera	2.82	1.57	+	= 0.03516
Chironomini	3.06	1.56	+	0.035 < 0.05
Misc. Chiron.	2.39	0.78	+	
Gastropoda	0.42	1.18	-	$\therefore$ reject H_o. The trophic position of taxa at OSPM-affected wetlands is significantly higher than at Reference wetlands.
Daphnia	2.54	1.4	+	
Chaoborus	3.16	2	+	
		Total:	"+" = 7 "-" = 1	

Chaoborus was higher at OSPM-affected wetlands (3.16) than at reference wetlands (2.00). This has important implications for the potential of top dipteran predators in these different food webs to bioaccumulate PAHs. For example, if biota-sediment accumulation factors (see Chapter 5) are 2 for both Tanypodinae and *Chaoborus* at OSPM-affected wetlands, then the concentrations of PAHs in the organisms can be estimated. For Tanypodinae in OSPM-affected wetlands, the estimated concentrations of PAHs would be $2^{2.17}$ (=4.5) times the PAH concentration in the sediment. For *Chaoborus* in OSPM-affected wetlands, the estimated concentrations of PAHs would be $2^{3.16}$ (= 8.9) times the concentration of PAHs in the sediment.

DISCUSSION

1) Among-wetland Variation in Stable Isotope Signatures

$\delta^{13}C$ Signatures - OSPM-affected and Reference Wetlands

The depletion of ^{13}C in organisms collected from OSPM-affected sites and the clustering of signatures close to $\delta^{13}C$ = -30‰ suggests that these organism rely almost completely on bitumen-derived carbon ($\delta^{13}C$ of bitumen = -30.3 ± 0.1‰, A. Farwell, University of Waterloo, pers. comm.) as a carbon source at the base of the food web. This same trend was seen in the reference wetland High Sulphate, which is built on lean oil sands and hence has some bitumen present (Chapter 2). The clustering of $\delta^{13}C$ signatures around the signature of bitumen at High Sulphate was however not as tight as it was at the OSPM-affected wetlands, suggesting another important source of carbon at this wetland. At the reference Shallow Wetland South Ditch, $\delta^{13}C$ signatures were enriched compared to the other

sites. This site is relatively free of bitumen (Chapter 2) and it appears that organisms rely, to some extent, on autochthonously produced carbon, which is richer in ^{13}C than bitumen (Hecky and Hesslein 1995).

Since bitumen is associated with the benthic environment, it was not expected that pelagic organisms would have $\delta^{13}C$ signatures similar to that of bitumen. However, naphthenic acids are a component of bitumen which are liberated during the caustic hot water extraction of bitumen from oil sands (Fine Tails Fundamentals Consortium 1995b). These compounds are carboxylic acids and are readily water-soluble. Naphthenic acids are present in the water of all of the study sites (Appendix 2.2), with the highest levels observed at Natural Wetland (approximately 59 mg/L), followed by Test Pond 7 (approximately 19 mg/L), High Sulphate (approximately 10 mg/L), and Shallow Wetland South Ditch (approximately 1.4 mg/L). It is likely (although not confirmed) that the $\delta^{13}C$ signature of the liberated naphthenic acids is similar, if not identical, to that of bitumen. If naphthenic acid concentrations of the water inside of organisms (water content of benthic and planktonic invertebrates in general is approximately 1/6 = 83%, Waters 1977) is in equilibrium with that of the water in the wetland, perhaps naphthenic acids are responsible for the bitumen-like $\delta^{13}C$ signatures observed in pelagic organisms. This is supported by the clustering of the $\delta^{13}C$ signatures of pelagic organisms near the $\delta^{13}C$ of -30.3‰ (the signature of bitumen and likely naphthenic acids). The degree of clustering seems to decrease (Fig. 4.2) as the concentration of naphthenic acids in the water decreases. The tightest clustering around $\delta^{13}C$ = -30.3‰ was observed at Natural Wetland (Fig. 4.2), which has the highest concentrations of naphthenic acids in the water (Appendix 2.2). This contrasts Shallow Wetland South Ditch which had

relatively low levels of naphthenic acids (Appendix 2.2) and $\delta^{13}C$ signatures of biota that were more enriched in ^{13}C than bitumen (and likely naphthenic acids). In addition, it is possible that microbial degradation of naphthenic acids in the water contribute to the bitumen-like $\delta^{13}C$ signatures observed in organisms via the microbial loop.

$\delta^{13}C$ Signatures - Carbon Source and the Selection of Baseline $\delta^{15}N$ Signatures for Trophic Position Calculations

The similarity of detrital $\delta^{13}C$ signatures to the $\delta^{13}C$ signatures of most taxa collected at all wetlands except Shallow Wetland South Ditch suggests that the $\delta^{15}N$ signature of detritus is a suitable baseline against which to compare the $\delta^{15}N$ signatures of other organisms. Regardless of the peculiar nature of the detrital $\delta^{13}C$ signatures observed at Shallow Wetland South Ditch, the detrital $\delta^{15}N$ signatures should still function as an appropriate baseline from which to calculate trophic positions as they are below the $\delta^{15}N$ signatures of all other samples collected at the wetland.

The highly enriched $\delta^{13}C$ values for the 0.5 - 180-μm (ufd) and 20 - 180-μm (fd) detritus at Shallow Wetland South Ditch should be ignored when trying to determine carbon sources of various taxa as these samples were outside of the carbon detection range. The $\delta^{13}C$ value for the 180 - 500-μm detritus (d) was also highly enriched in ^{13}C and this may be due to inorganic carbon comprising a large amount of the total carbon in the sample (Kiriluk et al. 1994; 3.88% inorganic carbon / 9.10% Total carbon = 43%, determined by combustion following Nelson and Sommers 1996). Alternatively, this enrichment may be due to the presence of benthic algae in the samples.

$\delta^{15}N$ Signatures- OSPM-affected and Reference Wetlands

The enrichment of ^{15}N at OSPM-affected wetlands may be due to a number of factors. Enrichment in the samples collected from Test Pond 7 was not as pronounced as the enrichment of samples from Natural Wetland. Test Pond 7 is turbid and contains high levels of bitumen in the fine tailings that make up the substrate (see Chapter 5). Perhaps turbidity limits photosynthetic production. This could lead to an increased relative importance of microbial production resulting from the degradation of bitumen in the mature fine tails substrate of Test Pond 7. Bacterial production, if prominent enough, could add extra trophic levels to the food web via the microbial loop at the base of the benthic and pelagic food webs (Sherr et al. 1986, Sherr and Sherr 1988, Riemann and Christoffersen 1993). It is also possible that amines added to the oil sands ore during the extraction of bitumen (M. MacKinnon 2002, Syncrude Canada Ltd., pers. comm.) are responsible for the elevated $\delta^{15}N$ signatures. Nitrogen is not detectable in any forms in the water of Test Pond 7 (Chapter 2) in part because it is subject to rapid bacterial nitrification processes (Fine Tails Fundamentals Consortium 1995b). As a result of this nitrification, nitrogen from the amines are incorporated into the wetland food web and can potentially alter (increase) the $\delta^{15}N$ signatures of food web components.

The enrichment of ^{15}N at Natural Wetland is harder to explain as the signatures are very enriched in ^{15}N and are even higher than what is typical of top vertebrate predators in lakes and streams (Fry 1991). The water of Natural Wetland is quite clear so the relative importance of bacterial production compared to photosynthetic production should be less than at Test Pond 7. Therefore, the microbial loop is less likely to contribute extra trophic

positions to the food web at Natural Wetland. Since consolidated/composite tailings water was continuously pumped through Natural Wetland in the summer of 2001, it is likely that amines were also continuously entering the wetland. If this is the case, the relatively constant influx of amines could have led to $\delta^{15}N$ signatures in food web components even higher than observed at Test Pond 7. As with Test Pond 7, nitrogen was not detectable in any forms in the water of Natural Wetland (Chapter 2) suggesting that it is rapidly removed by nitrification processes and thus rapidly incorporated into the food web.

2) Trophic Positions, Bioaccumulation Potential, and Food Web Structure

The lack of pattern between OSPM-affected and reference wetlands with respect to the trophic position estimates of the 3 size fractions of plankton collected may be due to variation in the relative composition of the phyto- and zooplankton in these samples. It may also be because using detrital $\delta^{15}N$ signatures as the baseline is not appropriate for samples consisting mainly of primary producers. Plankton samples from all sites also contained come suspended detritus, possibly obscuring the true isotopic signatures of the plankton.

The apparently higher trophic positions of taxa at OSPM-affected wetlands compared to reference wetlands suggest that there is a greater potential to biomagnify PAHs among the macroinvertebrate taxa living in OSPM-affected wetlands. This is true for Tanypodinae at OSPM-affected wetlands compared to Tanypodinae at reference wetlands as well as for *Chaoborus* at OSPM-affected wetlands compared to *Chaoborus* at reference wetlands. The fact that PAHs are at higher levels in the water and sediment at OSPM-affected wetlands

(Chapter 5) compounds differences in trophic position in determining the bioaccumulation potential.

The trophic position of *Chaoborus* was higher than the trophic position of Tanypodinae at both OSPM-affected and reference wetlands. Consequently, *Chaoborus* has a greater potential to bioaccumulate PAHs than Tanypodinae at both OSPM-affected and reference wetlands. However, other factors can affect bioaccumulation potential, such as the diet of organisms and where they spend most of their time in the aquatic environment.

If the food of Tanypodinae is enriched in PAHs compared to that of *Chaoborus*, then the higher trophic position of *Chaoborus* will not necessarily result in higher relative PAH body burdens. Tanypodinae eat benthic invertebrates (some of which ingest sediment) as well as sediment (Berg 1995). On the other hand, *Chaoborus* eat mainly planktonic material (Fedorenko 1975), but have also been observed to consume benthic organisms such as chironomids and oligochaetes (Swüste et al. 1973). These observations are consistent with the $\delta^{13}C$ signatures of *Chaoborus* at the study wetlands (Fig. 4.1). By virtue of their respective diets and considering that most PAHs in aquatic systems are in the sediment (Neff 1979, Chapter 5), Tanypodinae may have a greater potential to bioaccumulate PAHs than *Chaoborus* even though Tanypodinae occupy a lower trophic position. Furthermore, if naphthenic acids are the cause of the bitumen-like $\delta^{13}C$ signatures in *Chaoborus*, then it is doubtful that *Chaoborus* will accumulate higher levels of PAHs since this implies that bitumen is not an important source of energy (and hence PAHs) at the base of the pelagic food web.

Habitat differences may also confer greater PAH bioaccumulation potential in Tanypodinae than in *Chaoborus* independent of trophic position estimates. Tanypodinae live in direct contact with the sediment where the majority of PAHs are whereas *Chaoborus* spend most of their time in the water column where there are relatively few PAHs. *Chaoborus* larvae collected from Test Pond 7 were reared in the laboratory in the summer of 2001 and the first and possibly second instars were observed to built cases much like those that chironomids build. This behaviour suggests that they may be benthic for a part of their larval life. They may be as susceptible to bioaccumulation of PAHs as Tanypodinae if their feeding habits are similar at this time. Regardless, Tanypodinae still spend more time in direct contact with the sediment than *Chaoborus*. Thus, direct PAH uptake from the environment may confer greater bioaccumulation potential in Tanypodinae although this route of exposure is less important in the process of bioaccumulation than the dietary route (Gobas and Morrison 2000).

The higher trophic positions observed at OSPM-affected wetlands do not necessarily reflect longer food chains in these water bodies as might first appear if one compared only average baseline-corrected trophic positions at OSPM-affected vs. reference sites. Xu et al. (1999) reviewed the literature pertaining to the structural changes of freshwater ecosystems exposed to organic chemical contamination. This and other studies (Giddings et al. 1984, Cushman and Goyert, 1984, Whelly 1999, McCormick, 2000, Leung et al. 2001) document that freshwater ecosystems respond in characteristic ways to organic chemical contamination (Table 4.5). Evidently, such stress tends to reduce species diversity in both benthic and pelagic food webs. Benthic macroinvertebrate richness is lower at OSPM-affected wetlands

Table 4.5. Responses of freshwater ecosystem attributes to chemical contamination.

Type of Contamination	Component of Freshwater Ecosystem	Response Following Contamination
organic chemicals in general (Xu et al. 1999)	Phytoplankton Community	- abundance greatly reduced or - abundance greatly increased
	Zooplankton Community	- biomass greatly reduced - species richness greatly reduced
	Macrozooplankton	- abundance greatly reduced (especially *Daphnia*) - large cladocerans and copepods more sensitive than small
	Microzooplankton	- abundance increased (especially rotifers)
	ratio of zooplankton to phytoplankton	- declined
Oil (Giddings et al. 1984)	Phytoplankton Community	-total algal biomass increased, algal blooms at higher oil levels -species diversity reduced - diatoms eliminated - euglenophytes and chlorophytes became dominant
	Zooplankton Community	- total biomass reduced - species diversity reduced
	Macrozooplankton	- abundance of large cladocerans and copepods greatly reduced at high oil levels
	Microzooplankton	- abundance increased
	ratio of zooplankton to phytoplankton biomass	- declined

Table 4.5 cont. Responses of freshwater ecosystem attributes to chemical contamination.

Type of Contamination	Component of Freshwater Ecosystem	Response Following Contamination
Synthetic Crude Oil (Cushman and Goyert 1984)	Benthic Macroinvertebrates	- total diversity decreased with increasing dose - abundance generally higher in control ponds immediately after exposure, however some chironomids were more abundant in the medium-dose ponds - 2 months after dosing, all taxa except Caenidae were more abundant in high- or medium-dose ponds than in controls - within 1 month, acute effects on benthos observed at doses representing a 5-fold dilution of laboratory measured 48-h LC_{50} in *C. tentans*.
Oil Sands Process Materials	Benthic Macroinvertebrates (Whelly 1999)	- richness decreased at OSPM sites (low conductivity ref > high conductivity ref > OSPM) - abundance: highest at high conductivity reference sites; intermediate at OSPM, lowest at low conductivity reference - relative abundance of chironomids higher at OSPM wetlands
	Zooplankton (McCormick 2000)	- total abundance and biomass reduced
	Phytoplankton (Leung et al. 2001)	- composition of community changes but size structure not affected

than at reference wetlands (Whelly 1999), and chironomid genus richness is generally higher at reference wetlands as is total benthic macroinvertebrate family richness (C. Leonhardt University of Windsor, pers. comm., unpublished data). Similarly, fewer taxa of plankton were observed in the 3 different size fractions collected from OSPM-affected wetlands compared to reference wetlands (Appendix 4.1A-C)

Food web organization theory says that the expected number of trophic links increases linearly (slope ~ 2) as the number of trophic species (a set of organisms with identical prey species and identical predators) present in the food web increases over the range of 5 to 50 (Cohen and Newman 1988). This implies that reduced diversity in benthic and pelagic food webs due to environmental stress at OSPM-affected wetlands would lead to fewer trophic links, or to a decrease in the incidence of omnivory in these two food webs.

Studies of food web structure in lakes have shown that an increased incidence of omnivory leads to a depression in the baseline corrected $\delta^{15}N$ signatures (i.e., trophic position) of organisms (Cabana and Rasmussen 1994). Thus, the reduced species diversity and resulting reduction in omnivory at OSPM-affected wetlands should lead to increased estimates of trophic position of taxa inhabiting these waterbodies relative to reference wetlands, which is what was observed in this study.

Predatory chironomids (Tanypodinae) are generalists, feeding on whatever prey are available (Berg 1995). Dietary information for *Chaoborus americanus* and *C. trivittatus* suggests that *Chaoborus* is also a generalist predator (Fedorenko 1975). Fedorenko (1975) showed that the diet of the first three larval instars of *Chaoborus* was determined primarily by prey size, and the diet of the larger 4th instars was determined primarily by the spatial

availability of the prey. If, as other work suggests, there are indeed fewer types of prey available to Tanypodinae and *Chaoborus* in OSPM-affected wetlands compared to reference wetlands, then trophic positions at OSPM-affected wetlands are greater due to a reduction in the number of pathways by which biomass (energy) can possibly flow in the food web. Although organisms may occupy the same trophic position in OSPM-affected and reference wetlands in that they are both omnivorous secondary consumers (e.g., Tanypodinae and *Chaoborus*), differences in the structure of the underlying food webs appear to affect the functional parameters of the food webs (biomass and PAH flow and export). This conjecture is consistent with findings of studies on cause-effect relationships in energy flow and trophic structure (Hairston and Hairston 1993).

Hairston and Hairston (1993) found that trophic structure controls the fraction of energy consumed at each trophic level, rather than energetics controlling trophic structure. This observation was based on measurements of the efficiency of energy transfer between trophic levels and was true for a wide variety of systems - freshwater pelagic systems, forest ecosystems, and prairie ecosystems - suggesting that it is a wide-spread phenomenon. The presence of mine process materials possibly eliminates intolerant taxa resulting in lower diversity, which in turn has the potential to alter the competitive and predator-prey relationships (i.e., trophic structure) among the remaining taxa. The result is a change in trophic structure and food web function at OSPM-affected wetlands relative to reference wetlands, leading to higher trophic in the OSPM-affected wetlands. This may ultimately lead to greater potential for PAH bioaccumulation in predators at OSPM-affected wetlands.

The results of this chapter suggest that additional studies should be done related to stable isotopes and the structure and function of macroinvertebrate food webs in stressed ecosystems, OSPM-affected wetlands in particular. First, to confirm that trophic positions are indeed higher at OSPM-affected wetlands, a suite of macroinvertebrates from a number of other OSPM-affected and reference wetlands should be sampled for stable isotope analysis. In conjunction with these samples, the richness of benthic and pelagic communities should be determined. Benthic macroinvertebrate food web structure is also affected by salinity (Lovvorn et al. 1999) as is the structure of phytoplankton communities (Leung et al. 2001). Thus, measurements of salinity or conductivity should also be taken into account at these wetlands. Measurements of factors present at OSPM-affected wetlands that could potentially cause stress to organisms such as the PAH concentration in the sediment and water, and naphthenic acid concentration in the water should also be measured. Ideally, a suite of wetlands varying in the degree of salinity and concentration of potentially toxic constituents should be chosen to permit one to determine the relative importance of salinity, PAHs, and naphthenic acids in altering food web structure. The stable isotope data, consisting of trophic position and food source data, in conjunction with data on macroinvertebrate richness may provide insight into the mechanisms by which food web structure is altered by the above mentioned stresses.

In summary, bitumen and/or naphthenic acids appear to be a major source of carbon at the base of both the benthic and pelagic food webs at OSPM-affected wetlands, and slightly less so at the reference High Sulphate wetland where the substrate is partly composed of lean oil sands. At the reference wetland Shallow Wetland South Ditch, autochthonously

produced carbon is likely the major carbon source for benthic and pelagic food webs. The trophic position of benthic and pelagic taxa at OSPM-affected wetlands was significantly higher than that of taxa at reference wetlands. With respect to benthic and pelagic predatory dipterans, Tanypodinae and *Chaoborus* inhabiting OSPM-affected wetlands had higher trophic positions ($\delta^{15}N_{\text{baseline corrected}}$) than their counterparts at reference wetlands resulting in a greater potential for these taxa to bioaccumulate PAHs at OSPM-affected wetlands. Within all wetlands sampled *Chaoborus* had higher trophic positions ($\delta^{15}N_{\text{baseline corrected}}$) than Tanypodinae suggesting that *Chaoborus* has a greater potential to bioaccumulate PAHs. However, habitat and dietary differences between these two taxa likely results in Tanypodinae having a greater potential to bioaccumulate PAHs.

CHAPTER 5

Accumulation and Export of Polycyclic Aromatic Hydrocarbons by Benthic and Pelagic Predatory Dipterans Inhabiting Tailings-affected and Reference Wetlands in the Athabasca Oil Sands

INTRODUCTION

Insects with aquatic larval stages and terrestrial adult stages that inhabit water bodies containing bioaccumulative compounds pose a potential threat to the terrestrial organisms that feed on the adult insects because they are capable of transferring these substances as well as their biomass to their predators (Menzie 1980, Larsson 1984, Reinhold et al. 1999). Wetlands are being considered for use as a tool in the storage and detoxification of OSPM, and will certainly be incorporated into the reclaimed landscape when oil sands mine leases are closed (Oil Sands Wetlands Working Group 2000). This brings aquatic organisms that inhabit these water bodies into contact with PAHs, which can be incorporated into their tissues. The extent of PAH accumulation and export via predatory benthic (Tanypodinae) and pelagic (*Chaoborus*) dipterans inhabiting OSPM-affected and reference water bodies will be addressed in this chapter.

The importance of predatory larval insects in accumulating and exporting hydrophobic organic compounds such as PAHs may depend on the specific habitat that they occupy within wetlands. Regardless of the specific habitat requirements of larval insects, there are two major routes by which aquatic biota take up hydrophobic organic chemicals such as PAHs. Uptake can occur from water across respiratory surfaces (bioconcentration; (Gobas and Morrison 2000). Uptake via food (biomagnification) is the predominant means by which organisms acquire hydrophobic compounds (Gobas and Morrison 2000). This

suggests that benthic organisms, which live in direct contact with the sediment where the majority of PAHs are located in water bodies (Neff 1979), and feed either directly on sediment material or organisms that do so themselves, are capable of accumulating larger amounts of PAHs than pelagic organisms. Consequently, predatory benthic insects may export more PAHs to terrestrial insectivores than their predatory pelagic counterparts, regardless of the amount of biomass exported by these taxa.

The processes of bioconcentration and biomagnification can combine to result in food-chain bioaccumulation, or simply bioaccumulation (Gobas and Morrison 2000). In this process, a progressive increase is observed in the lipid-normalized concentration (i.e., fugacity) of particular compounds such as PAHs with an increase in trophic level. The occurrence of this can be tested for by calculating biota-sediment accumulation factors (BSAFs), which are a measure of the concentration in an organism normalized to its capacity to store the chemical divided by the concentration in the sediment, which has been normalized to the capacity of the sediment to hold the chemical (Gobas and Morrison 2000).

Typical values of BSAFs for benthic macroinvertebrates vary widely. In crayfish, BSAFs range from 0.01 to 0.40 for a wide variety of PAH congeners with the highest BSAFs observed for alkylated PAH congeners (Thomann and Komlos 1999). In mayflies in Lake Erie, BSAFs of individual PAH congeners were reported to vary from 0.001 to approximately 8 (Gewurtz et al. 2000). Oligochaetes were reported to have BSAFs for PAH congeners ranging from 1.0 to 2.6 (Brunson et al. 1998).

The magnitude of a BSAF relative to 1 is a reflection of the degree to which a chemical is subject to metabolism and elimination. At chemical equilibrium, a

bioaccumulation factor of 1.0 is expected. However, equilibrium is rarely achieved for high molecular weight, very hydrophobic compounds (Morrison et al. 1996). Bioaccumulation factors depend greatly on chemical properties and behaviour of the chemical within an organism, resulting in estimates of less than 1 (indicating metabolism or growth dilution of the chemical) or greater than 1 (indicating bioaccumulation). Thus, bioaccumulation factors provide information on key processes affecting the transfer of hydrophobic organic chemicals in food webs.

Aquatic invertebrates can incorporate PAHs into their tissues (Thomann et al. 1992, Clements et al. 1994, Harkey et al. 1994, Bell 1995, Harkey et al. 1995, Baumard et al. 1998, Gewurtz et al. 2000) and potentially transfer them to their vertebrate predators (Smits et al. 2000). Numerous studies have also been conducted on the uptake dynamics of PAHs and/or PCBs in benthic macroinvertebrates (Kirso and Irha 1998, Gewurtz et al. 2000) and phytoplankton (Swackhamer and Skoglund 1993, Kilham 1998). Most of the PAHs in the Athabasca oil sands are alkylated derivatives of parent compounds (Madill et al. 1999, Smits et al. 2000). Relatively little information is available on either the bioaccumulation potential or toxicity potential of alkylated versus parent PAHs. Recent work (Brown et al. 2001, Hodson et al. 2001) suggests that metabolites of alkylated PAHs can be more toxic than parent compounds, with toxicity increasing with the number, size, and location of the alkyl substitutions (Hodson et al. 2001). However, PAHs extracted from pore water of oil sands tailings showed a low potential to induce the production of potentially toxic metabolites (Madill et al. 2001).

Metabolism of PAHs by invertebrates is limited (Neff 1979, McElroy et al. 1989) so it is particularly important to study the accumulation of PAHs in these animals. If they bioaccumulate PAHs then they may act as vectors transporting these compounds to insectivorous vertebrates. Vertebrates have a well-developed mixed-function oxygenase (MFO) system and thus can rapidly metabolize PAHs, transforming them into potentially toxic free radical metabolites (Varanasi et al. 1989). Indirect measures are used to assess exposure in vertebrates, such as altered liver MFO activity (e.g., ethoxyresorufin-*0*-deethylase or EROD activity (van den Heuvel et al. 1999, Smits et al. 2000, Madill et al. 2001) or the presence of PAH metabolites in the bile as biliary excretion is the major route of PAH metabolite excretion (van den Heuvel et al. 1999).

In adult yellow perch (*Perca flavescens*), which feed mainly on benthic invertebrates and larger zooplankton, both altered MFO activity and bile PAH equivalents occurred in populations developing in OSPM-affected waterbodies. However, these perch appeared to exhibit no adverse ecological effects (van den Heuvel et al. 1999).

EROD activity in nestling tree swallows (*Tachycineta bicolor*) adjacent to OSPM-affected wetlands confirmed the presence of xenobiotics (most likely PAHs) in the diets of the swallows (Smits et al. 2000), which forage mainly (84%) on terrestrial adult insects with aquatic larval stages (Smits et al. 2000). However, this study found no relationship between the presence of nearby tailings or tailings pond water and ecological endpoints such as reproductive success, nestling growth rate, and immune response.

The objective of this chapter is to compare the concentration of PAH congeners in benthic and pelagic predatory larval midges inhabiting OSPM-affected and reference

wetlands and to assess whether or not bioaccumulation of PAHs occurs in these organisms. The concentration of PAHs in the larvae will also be contrasted with the concentration observed in adult insects with aquatic larval stages. Finally, using the PAH burdens of benthic and pelagic larval predatory midges in conjunction with annual production estimates of these taxa (Chapter 3), the potential annual export via benthic and pelagic predatory midges at OSPM-affected and reference wetlands will be assessed. This export will be related to the export of toxic substances in other water bodies and to an ecological study of swallows nesting adjacent to constructed wetlands in the Athabasca oil sands.

METHODS

1) Study Sites

Samples for PAH analysis were collected from two OSPM-affected wetlands (Natural Wetland and Syncrude Test Pond 7), and two reference wetlands (High Sulphate and Shallow Wetland South Ditch). Detailed environmental characteristics of each wetland were reported in Chapter 2.

2) Field Collection Methods

Samples Collected for PAH Analysis

See Figure 1.2 for a diagrammatic summary of the food webs that were sampled at each wetland and an illustration of the potential sources of PAHs to biota in a wetland. A summary of samples collected for PAH analysis (Summer, 2001) at all wetlands is presented

in Table 5.1. At High Sulphate, predatory Tanypodinae were not abundant enough to be collected for PAH analysis. Chironomini were collected in its place.

Water

To quantify concentrations of freely dissolved PAH congeners in the water column, semi-permeable membrane devices (SPMDs - Huckins et al. 1993, Sabaliunas and Södergren 1997, Metcalfe et al. 2000, Baussant et al. 2001) were deployed at each wetland (see Figs. 2.2, 2.4, 2.6, and 2.8 for locations). Each SPMD was constructed in a Class 100 clean room at Trent University by pipetting 1-mL of high purity triolein (99%) into a 40-cm length of low density polyethylene layflat tubing.

The SPMDs were assembled following the protocols of Metcalfe et al. (2000). Each sampling container consisted of a metal stove pipe shroud within which 3 individual SPMDs were mounted. Each shroud had 5 rows of 2-cm diameter holes cut in it to facilitate water exchange between the inside and outside of the shroud.

At Natural Wetland, one shroud was placed at the inflow of the wetland and one at the outflow (Fig. 2.2). The remaining sites lacked an inflow and outflow so shrouds were placed at opposite ends of the wetland (Fig. 2.4, 2.6, and 2.8), oriented perpendicular to the prevailing wind direction. All SPMDs were deployed on July 7, 2001 and collected 28 d later on August 6, 2001. On the collection day, SPMDs were removed from their housing, placed in glass jars, and stored frozen at -20°C for subsequent PAH analysis.

When SPMDs are deployed and collected they are exposed to the air and can potentially accumulate PAHs from the air. To correct for this possibility, a single SPMD (a

Table 5.1. Samples collected for PAH analysis at OSPM-affected and reference wetlands.

Site	Sample			
	Water	Sediment	Predatory benthic dipteran	Predatory pelagic dipteran
Natural Wetland (OSPM)	SPMDs	180 - 500-µm detritus	Tanypodinae	*Chaoborus*
Test Pond 7 (OSPM)	SPMDs	180 - 500-µm detritus	Tanypodinae	*Chaoborus*
High Sulphate (Reference)	SPMDs	180 - 500-µm detritus	n/a, Chironomini	*Chaoborus*
Shallow Wetland South Ditch (Reference)	SPMDs	180 - 500-µm detritus	Tanypodinae	*Chaoborus*

"trip length") was hung from a nearby tree with a twist tie through the loop on one of the ends for the duration of SPMD deployment and collection. A separate trip length SPMD was used at each wetland and the same trip length was used for deployment and collection. Only the trip length from Shallow Wetland South Ditch was analysed for PAH content.

Sediment

To quantify concentrations of PAHs in the sediments of the study sites, 180 - 500-µm detritus was collected. These samples were collected at the same time as those for stable isotope analysis and the collection methods can be found in 'Benthic Collections - Detritus' section of the Field Collection Methods in Chapter 4.

Tanypodinae

Tanypodinae were collected for PAH analysis from each wetland at the same time as collections were made to determine their stable carbon and nitrogen isotope signatures. Collection methods were identical to those outlined in Chapter 4 ('Benthic Collections - Chironomidae, Gastropoda, and Odonata (Zygoptera and Anisoptera)' section of the Field Collection Methods). Tanypodinae larvae were also collected for PAH analysis from Test Pond 7 in late June / early July, 2002.

Chaoborus

Chaoborus was collected for PAH analysis from each wetland at the same time as collections were made to determine their stable carbon and nitrogen isotope signatures.

Collection methods were identical to those outlined in the 'Collections of Taxa in the Pelagic Food Web - *Chaoborus*' section of the Field Collection Methods of Chapter 4. *Chaoborus* larvae were also collected for PAH analysis from Test Pond 7 in late June / early July, 2002.

Adult Aquatic Insects

Adult aquatic insects were collected using 12-V DC modified Pennsylvania-style ultraviolet (UV) lights (Kovats and Ciborowski 1989) to estimate the concentration of PAH congeners in adults insects that had emerged from a given wetland. Light trap collections were made in 1998 in the Natural Wetland /Hummock Wetland area (OSPM) and adjacent to Shallow Wetland (reference).

A white sheet was placed over the hood of the field truck in order to reflect light from the traps and attract more insects. At each sample site, 3 separate light traps were set up at dusk and powered from the truck's battery for two h. Thereafter, the contents of the collecting reservoirs were emptied into a glass jar, which was labelled, wrapped in tin foil, and stored frozen at -20°C. The sheet was gently folded and placed in the freezer as well. The following day insects that had remained on the sheet were combined with the rest of the sample.

3) Laboratory Methods

Sample Storage, Mass Estimation, and Allocation

Upon return to Windsor, all samples were stored frozen at -80°C for subsequent PAH analysis. Samples for stable isotope and PAH analyses were collected at the same time and in the same vials. Most samples were composites collected over a number of days, and hence

a single sample usually consisted of more than one vial. Since the sample mass required for stable isotope analysis (a few mg) is substantially less than that required for PAH analysis (~ 3- 5-g w.w.), a portion of the samples was removed for stable isotope analysis (see Chapter 4 Laboratory Methods - Sample Storage, Mass Estimation, and Allocation). The remainder of the sample was left for PAH analysis.

Shipping of Samples

Samples collected in 2001 for PAH analysis (Tanypodinae, Chironomini, *Chaoborus*, SPMDs, 180 - 500-μm detritus) were shipped on dry ice to Enviro-Test Laboratories in Edmonton, AB on April, 6, 2002. To minimize the risk of sample contamination due to handling, components of composite samples collected over a number of days were shipped in their individual collection vials rather than being combined prior to shipment.

Tanypodinae and *Chaoborus* samples collected from Test Pond 7 in 2002 were shipped on ice from Fort McMurray to Enviro-Test on July 18, 2002.

Adult insect samples collected in 1998 were shipped on dry ice to AXYS Analytical Services Ltd., Sidney, British Columbia.

Sample Processing

Among the invertebrate samples collected in 2001 (Tanypodinae, *Chaoborus*, and Chironomini), there was concern that bitumen stuck to the outside of the organisms would result in estimated PAH concentrations that were not representative of PAHs incorporated into tissues. Consequently, a methanol wash was performed at Enviro-Test Laboratories

(Edmonton, AB) on the invertebrate samples collected in 2001 to remove external, residual bitumen.

The methanol wash was not performed on the Tanypodinae and *Chaoborus* samples collected in 2002. Instead, total extractable hydrocarbon content was determined to permit calculation of a correction factor that would account for any PAHs on the outside of the organisms.

All sample extracts (Tanypodinae, Chironomini, *Chaoborus*, SPMDs, and 180 - 500-μm detritus) were analysed using low-resolution gas chromatography and mass spectroscopy (EPA 3540/8270-GC/MS). The lipid content of the invertebrate extracts was also determined (AOAC 24.005). Total organic carbon (loss on ignition, LOI) content of detritus samples was measured by combustion at Enviro-Test Laboratories (Edmonton, AB), following the methods of Nelson and Sommers (1996). Table 5.2 summarizes the target PAH congeners for all samples collected for PAH analysis, their molecular weights, and abbreviations used in this study.

The adult insect samples were analysed by high-resolution gas chromatography and mass spectroscopy (Method PH-T-01/Ver.2 AXYS Analytical Services Ltd. Sidney, BC). The lipid content of these samples was not determined.

For the most part, the same congeners assayed for samples processed at Enviro-Test Laboratories were also assayed in the adult insect samples analysed by AXYS Analytical Services Ltd. However, some congeners were only evaluated in the adult insect samples and some were only evaluated in the samples collected in 2001 and 2002 (Appendix 5.1).

Table 5.2. List of target PAH congeners in all samples collected for PAH analysis, their molecular weights, and abbreviations used in this study.

PAH Congener	Molecular Wt.	Abbrev.
Naphthalene*	128	N
Methyl Naphthalene	142	N1
C2 Naphthalene	156	N2
C3 Naphthalene	170	N3
C4 Naphthalene	184	N4
Acenaphthylene*	152	AC
Acenaphthene*	153	AE
Methyl Acenaphthene	166	AE1
Fluorene*	166	F
Methyl Fluorene	180	F1
C2 Fluorene	194	F2
Biphenyl	154	BPh
Methyl Biphenyl	168	BPh1
C2 Biphenyl	182	BPh2
Phenanthrene*	178	P
Anthracene*	178	A
Methyl Phenanthrene / Anthracene	192	PA1
C2 Phenanthrene / Anthracene	206	PA2
C3 Phenanthrene / Anthracene	220	PA3
C4 Phenanthrene / Anthracene	234	PA4
Dibenzothiophene*	184	D
Methyl Dibenzothiophene	198	D1
C2 Dibenzothiophene	212	D2
C3 Dibenzothiophene	226	D3
C4 Dibenzothiophene	240	D4
Fluoranthene*	202	FL
Pyrene*	202	PY
Methyl Fluoranthene / Pyrene	216	FL1/PY
Benzo(a)anthracene* / Chrysene	228	BA/C
Methyl Benzo(a)anthracene / Chrysene	242	BA1/C
C2 Benzo(a)anthracene / Chrysene	256	BA2/C
Benzo(b&k)fluoranthene*	252	BbkF
Benzo(a)pyrene*	252	BAP
Methyl Benzo(b&k)fluoranthene / Benzo(a)pyrene	266	BbK1/BAP
C2 Benzo(b&k)fluoranthene / Benzo(a)pyrene	280	BbK2/BAP
Indeno(c,d-123)pyrene*	276	IP
Dibenzo(a,h)anthracene*	278	DA
Benzo(ghi)perylene*	276	BP

*=Priority PAHs US EPA Protocol- Method 625

4) Determination of Bioaccumulation of PAHs

Total PAHs

Bioaccumulation was assessed only for Tanypodinae and *Chaoborus* samples collected from Test Pond 7 in 2002. This was done using the ratio [ΣPAH] : [bitumen], where [ΣPAH] was the sum of the concentrations of individual PAH congeners, and [bitumen] was the bitumen content observed in either the invertebrate samples or in the mature fine tails that compose the substrate of Test Pond 7. The total extractable hydrocarbon (TEH) content was the measure used to represent bitumen content of samples. These measures were not directly available at Test Pond 7 so the ratio of the concentration of individual PAH congeners to bitumen content at depths of 1-m, 6-m, 10-m, 20-m, and 30-m in the fine tails zone of Mildred Lake Settling Basin in 2000 and 2001 was used as a surrogate for the ratio of total PAHs to bitumen in Test Pond 7 (data courtesy of M. MacKinnon, Syncrude Canada Ltd., Appendix 5.2A-B). This was the source of the mature fine tails used in the construction of Test Pond 7.

The sum of the concentrations of all individual PAH congeners in the fine tails was plotted against TEH for various depths from 2000 and 2001. A linear regression analyses was performed to predict the total PAH content expected in a sample based on its TEH concentration. The regression equation was used to estimate the expected total PAH concentration of Tanypodinae and *Chaoborus* collected at Test Pond 7 in 2002 as a function of their TEH concentration. This assumes that all of the TEH present in the invertebrate samples was derived from bitumen. As a result, estimates of PAH concentrations in these organisms can be considered high as lipids will also contribute to TEH content. Following

this, a bioaccumulation factor was calculated for both invertebrate samples by dividing the observed total PAH concentration of each invertebrate sample by the total PAH concentration predicted from its TEH concentration. These bioaccumulation factors were compared to a value of 1.0 in order to assess whether or not bioaccumulation of PAHs had occurred in Tanypodinae and *Chaoborus*.

Individual PAH congeners

Bioaccumulation factors of individual PAH congeners were also examined. Each congener that was detected in Tanypodinae and *Chaoborus* samples from Test Pond 7 in 2002 was expressed on a $mg_{congener}$ / kg $bitumen_{Tanypodinae\ or\ Chaoborus}$ basis and divided by the average bitumen-based concentration of that congener observed from all depths in the 2000 and 2001 Mildred Lake Settling Basin samples (mg PAH / kg $bitumen_{average\ MLSB\ fine\ tails\ 2000\ \&\ 2001}$).

5) Estimation of PAH Export

Annual production estimates of Tanypodinae and *Chaoborus* larvae (Chapter 3) were multiplied by the total PAH concentrations in these organisms to provide an estimate of maximum potential annual PAH export per unit area via benthic and pelagic predatory dipterans at OSPM-affected and reference wetlands.

Concentrations of PAH congeners in all Tanypodinae (except at Test Pond 7 where bitumen was visible on the outside of the organisms) and *Chaoborus* samples collected in 2001 were below the detection limit (this is believed to be an analytical error - see Results

and Discussion below [1]). Therefore, a theoretical maximum potential PAH export via these organisms at all sites was estimated by assuming that each congener was at its detection limit. Some congeners were at quantifiable levels in the Tanypodinae sample from Test Pond 7 in 2001. However, it was assumed all other congeners were at detection limits when total PAH export was calculated. This was done as it seemed likely that the PAHs detected in this sample were adsorbed to the outside of the organisms in the form of bitumen and hence these PAHs would not be exported to the terrestrial environment when the adult insect emerged.

Tanypodinae were present at High Sulphate but could not be collected in sufficient quantities for PAH analysis. Instead chemical analyses were performed on the more abundant Chironomini. It was assumed that if Tanypodinae had been collected from High Sulphate their body burdens of PAHs would have been below the detection limits (which were identical for all invertebrate samples) since this was the general pattern with samples collected in 2001 regardless of the amount of PAHs present in the wetlands (Figs. 5.1 and 5.2). This assumption permitted estimation of total PAH export via Tanypodinae at this wetland and hence PAH export via Chironomini was not calculated.

Potential maximum total PAH export via Tanypodinae and *Chaoborus* was also estimated at Test Pond 7 using the PAH data from these organisms collected in 2002. In this case, the congeners that were not detected were assumed to be at the detection limit in order to obtain an estimate of total PAH export that would be more comparable with the samples collected in 2001.

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[1]Many of the congeners in these samples were observed to be present in the chromatograms (data not presented), but did not meet quantification criteria.

RESULTS AND DISCUSSION

1) PAH Fingerprint of Samples

Water

The total PAH concentration freely dissolved in the water column was highest at Test Pond 7 (3.9 μg / mL Triolein) followed by Natural Wetland (0.53 μg / mL Triolein), Shallow Wetland South Ditch (0.33 μg / mL Triolein), and High Sulphate (0.30 μg / mL Triolein) (see Appendix 5.3 for raw data; see Figs. 2.2, 2.4, 2.6, and 2.8 for deployment locations of SPMDs used in analysis). The trip length SPMD used at Shallow Wetland South Ditch did not have detectable levels of any of the congeners (Appendix 5.3).

With respect to individual congeners, C3 phenanthrene/anthracene and C4 phenanthrene/anthracene were detectable in the SPMDs at all four study sites (Fig. 5.1), with the highest concentrations (C3 congener: 0.77 μg/L, C4 congener: 1.20 μg/L) occurring at Test Pond 7. This is expected as the water in Test Pond 7 is largely mature fine tailings release water. Concentrations of the C3 congener were similar at the other three wetlands (0.07 - 0.10 μg/L). Levels of the C4 congener were higher at the two reference wetlands (0.17 and 0.23 μg/L at High Sulphate and Shallow Wetland South Ditch, respectively) than at the remaining OSPM-affected wetland, Natural Wetland (0.07 μg/L). Alkylated PAH congeners are more abundant in petroleum sources than parent PAHs. They are more resistant to environmental degradation than the parent compounds (Headley et al. 2001), and therefore are still present at detectable levels in the water column, even at reference sites.

The widest variety of congeners was present in the water at Test Pond 7 followed by Natural Wetland, High Sulphate, and Shallow Wetland South Ditch (Fig. 5.1). This and the

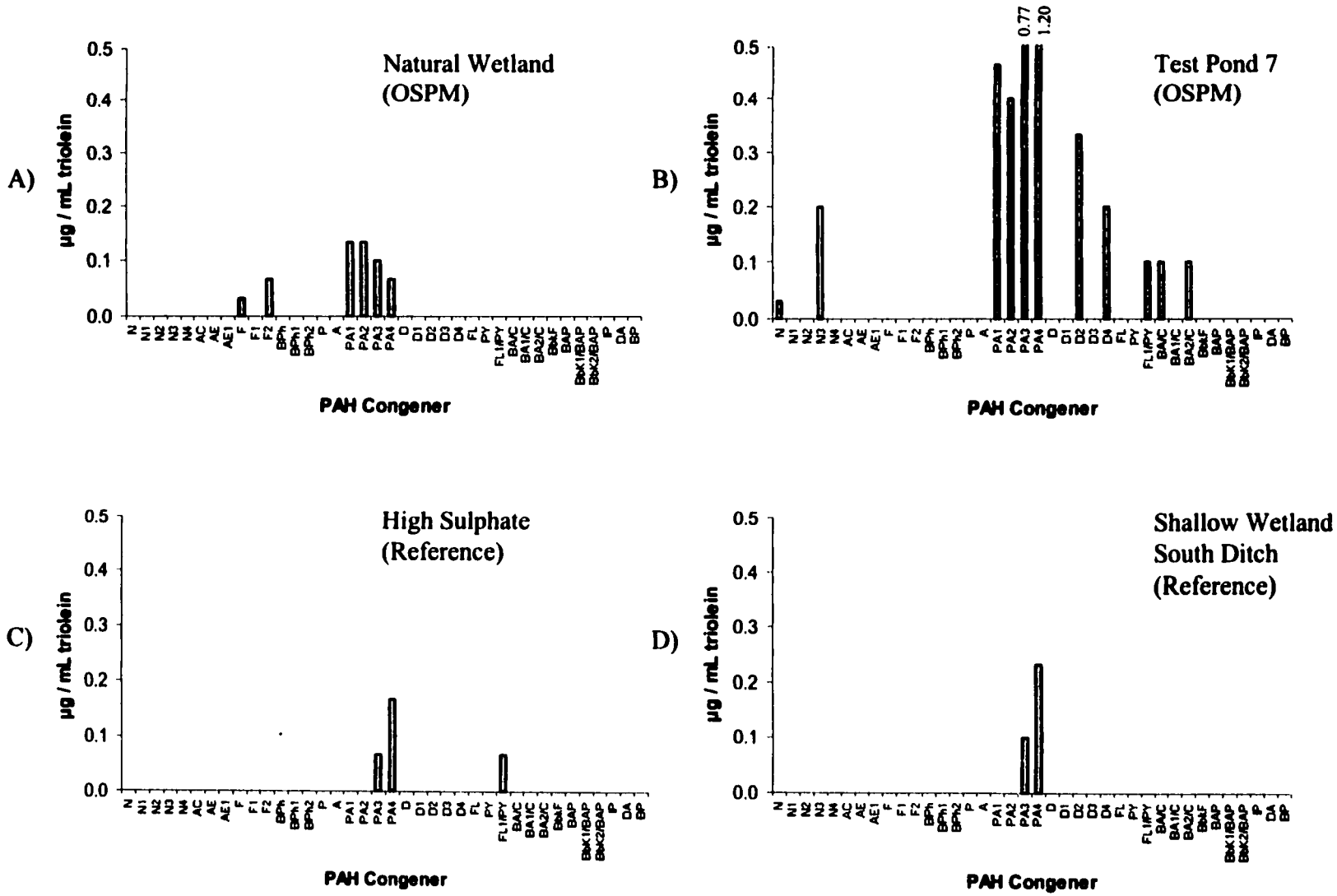

Fig. 5.1. Concentrations of PAH congeners in semi-permeable membrane devices at 4 constructed wetlands.

fact that higher concentrations of PAHs were observed in the water at the two OSPM-affected sites suggests that PAH levels in the sediment are higher at OSPM-affected wetlands than reference wetlands which is consistent with results of previous research (Smits et al. 2000).

Sediment

At all wetlands, the sediment data reported are for the 180 - 500-μm detritus that was collected in 2001 except at Test Pond 7 (Fig. 5.2). The sediment data from Test Pond 7 is based on the average PAH profile at different depths of the fine tails zone of Mildred Lake Settling basin in 2000 and 2001, which was the source of the mature fine tailings to fill Test Pond 7 during its creation. Data from the sediment of Test Pond 7 itself is not presented in Fig. 5.2 (although it is presented in Appendix 5.4 and Fig. 5.3) because bioaccumulation of PAHs in invertebrate samples from Test Pond 7 in 2002 were based on the PAH profile of Mildred Lake Settling Basin. As was the case with the water, the total PAH concentration in the sediment was highest at Test Pond 7, followed by Natural Wetland, Shallow Wetland South Ditch, and High Sulphate (Appendix 5.4). The widest variety of congeners was present in the sediment at Test Pond 7 followed by Natural Wetland, High Sulphate, and Shallow Wetland South Ditch (Fig. 5.2). This is the same pattern that was observed with the SPMDs (Fig. 5.1). As with the SPMDs, most congeners detected in the sediment were alkylated forms characteristic of petroleum sources.

Generally, the congeners that were detected freely dissolved in the water column by the SPMDs were at higher concentrations in the sediments than the congeners that were undetected in the SPMDs. However, exceptions occurred, particularly at Shallow Wetland

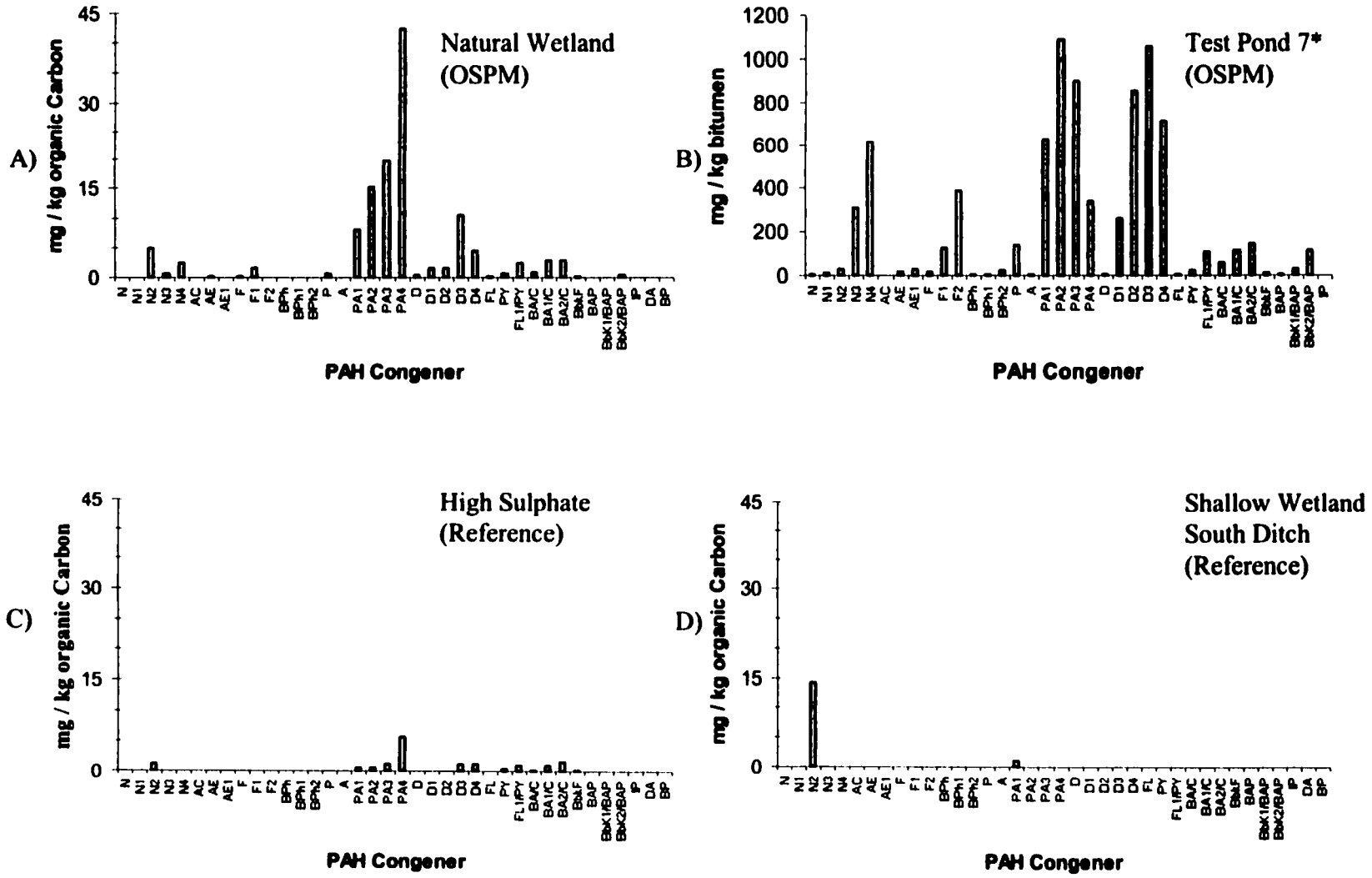

Fig. 5.2. Organic carbon normalized* PAH concentrations in 4 constructed wetlands, summer 2001.

* Data for Test Pond 7 is the average mg PAH / kg bitumen from depths of 6, 10, 20, and 30 m from July 2000 and July 2001 in the fine tails zone of the Mildred Lake Settling Basin (Appendix 5.2ab), see Appendix 5.4 for sediment PAH data at Test Pond 7.

South Ditch (c.f. Figs. 5.1D and 5.2D). At all sites, several congeners that were detected in the sediment were not detected in the water. Perhaps these congeners are more susceptible to photodegradation in the water column (Dutta and Harayama 2001). Dutta and Harayama found that *n*-alkylbenzenes were resistant to photooxidation whereas other alkylated PAHs (e.g., *n*-alkylbenzothiophenes) were almost completely photooxidized. It is also possible that some congeners are not present at high enough levels in the sediment to confer levels in the water column that are detectable by SPMDs.

Predatory Benthic and Pelagic Dipterans - Tanypodinae and Chaoborus

The PAH data from invertebrate samples collected in 2001 are presented in Appendix 5.5 and will not be presented in the Results and Discussion section of this chapter. All 2001 samples submitted for analysis were reported as being below detection limits for all congeners with the exception of Tanypodinae collected from Test Pond 7.

It is thought that the methanol wash that was performed on these samples to remove external bitumen (and PAHs) resulted in loss of accumulated (internal) PAHs and possibly lipids as well. PAHs and lipids are both somewhat soluble in methanol (Chen and Delfino 1997). Of all the samples collected in 2001, Tanypodinae from Test Pond 7 was the only sample to have visible particles of bitumen on their cuticle. This was also the only invertebrate sample in which any PAH congeners were detected, suggesting that the methanol wash was ineffective in completely removing adsorbed bitumen (PAHs) but capable of removing internal, or accumulated PAHs.

The methanol wash was not performed on the organisms collected from Test Pond 7 in 2002. In this case, PAHs were detected in the *Chaoborus*, which were visibly free of external bitumen, whereas no PAHs had been detected when the methanol wash was performed on the 2001 Test Pond 7 *Chaoborus* sample. Consequently, only the PAH data from Tanypodinae and *Chaoborus* collected at Test Pond 7 in 2002 are presented (Fig. 5.3, raw data in Appendix 5.6). The data in Fig. 5.3 are expressed on a mg PAH/kg bitumen basis, where bitumen represents the concentration of total extractable hydrocarbons present in the organisms.

As with the PAHs present in the water and sediment of Test Pond 7, the PAHs present in Tanypodinae and *Chaoborus* collected from this wetland were dominated by the alkylated congeners, particularly the phenanthrene/anthracenes (0.23 - 0.85 mg/kg dry $weight_{Tanypodinae}$ and 0.04 - 0.12 mg/kg dry $weight_{Chaoborus}$) and dibenzothiophenes (0.03 - 0.42 mg/kg dry $weight_{Tanypodinae}$ and 0.04 mg/kg dry $weight_{Chaoborus}$). These concentrations encompass the low end of the range observed for fluoranthene (0.15 - 100 mg/kg dry weight) and benzo[a]pyrene (0.01 - 6 mg/kg dry weight) in *Chironomus riparius* (Clements et al. 1994). Compared to other PAH congeners, the observed concentrations also suggest that alkylated derivatives, in addition to being more resistant to environmental degradation (Headley et al. 2001), are more resistant to metabolism than their parent compounds. These congeners may also be more resistant to metabolism (Dutta and Harayama 2001) or elimination than other alkylated congeners which were not observed in the organisms such as derivatives of naphthalene, fluorene, and benzo(a)anthracene/chrysene. These congeners were present at

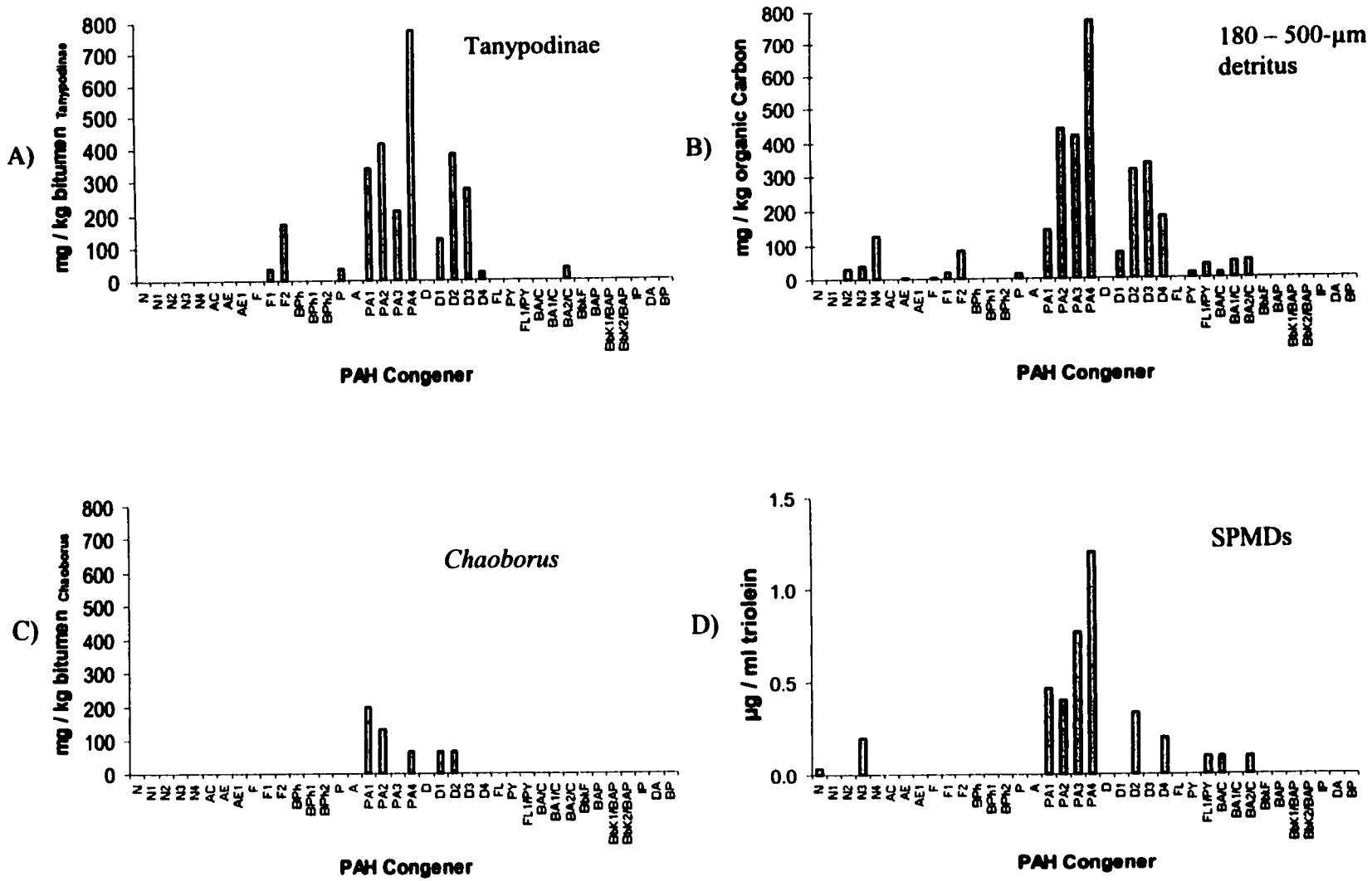

Fig. 5.3. PAH concentrations observed in A)Tanypodinae,* B) 180-500-µm detritus**, C) *Chaoborus**, and D) SPMDs*** collected from Test Pond 7 on July 2002*, June 15, 2001**, and July 7 – Aug. 6, 2001*** (see Table 5.2 for a list of congeners and their molecular weights).

concentrations in the sediment that were comparable with levels of phenanthrene/anthracene and dibenzothiophene alkylated derivatives, yet were not detected in the invertebrate samples.

The congeners that were present in both organisms occurred at higher levels in Tanypodinae than *Chaoborus*, suggesting either greater uptake or reduced elimination for Tanypodinae relative to *Chaoborus*. Exposure is likely the more important determinant of body burdens in this case, as Tanypodinae live in the sediment whereas *Chaoborus* live mainly in the water column in fishless water bodies (Dawidowicz et al. 1990). This results in a higher level of contact between PAHs and Tanypodinae (see water and sediment PAH data for Test Pond 7, Fig. 5.3; note: the sediment data in this figure is from Test Pond 7 itself and not Mildred Lake Settling Basin). It is also possible that this observation was due to external bitumen particles observed on Tanypodinae but not on *Chaoborus*.

Diet may also play a role in determining the observed differences in PAH body burdens. Tanypodinae ingest sediment material and benthic organisms (Berg 1995) whereas *Chaoborus* typically feed on planktonic organisms such as rotifers, nauplii, copepods, and cladocerans (Fedorenko 1975), but also consume benthic organisms such as chironomids and oligochaetes (Swüste et al. 1973). Thus, in addition to habitat differences, dietary differences also appear to confer higher PAH exposure for Tanypodinae.

The trophic position estimate of *Chaoborus* at Test Pond 7 (2.31; Table 4.3) was higher than that of Tanypodinae (*Derotanypus* = 1.67, other Tanypodinae = 1.77; Table 4.3) at Test Pond 7, suggesting a greater potential for *Chaoborus* to bioaccumulate PAHs in the absence of PAH metabolism by their food. This is consistent with other work done on trophic position estimates with stable nitrogen isotopes and the bioaccumulation of organic

compounds (Rasmussen et al. 1990, Kiriluk et al. 1994, Vander Zanden and Rasmussen 1996, Kidd et al. 2001) and inorganic compounds (e.g., mercury - Cabana and Rasmussen 1994, Vander Zanden and Rasmussen 1996, Atwell et al. 1998). However, reduced exposure through habitat and dietary differences combined with elimination in both organisms appears to obscure any effect of higher trophic positions resulting in higher levels of PAHs in the pelagic predator than the benthic predator.

By virtue of the similarity in the stable carbon isotope signatures of Tanypodinae ($\delta^{13}C$ = -29.59‰) and *Chaoborus* ($\delta^{13}C$ = -28.88‰) at Test Pond 7 with that of bitumen ($\delta^{13}C$ = -30.3‰), it appears that bitumen, and hence PAHs, may be an important source of carbon at the base of both benthic and pelagic food webs. However, higher concentrations of PAHs were observed in Tanypodinae than in *Chaoborus* despite the fact that *Chaoborus* has a greater potential to accumulate PAHs than Tanypodinae. This suggests that the bitumen-like $\delta^{13}C$ signatures of *Chaoborus* may be due to the presence of bitumen-derived naphthenic acids in the water of Test Pond 7 (see Chapter 4) which would confer a lower exposure rate to PAHs for *Chaoborus* than for Tanypodinae.

2) Bioaccumulation of PAHs in Predatory Benthic and Pelagic Dipterans

Total PAHs

Plotting total PAH concentration (mg total PAHs / kg sample) vs. total extractable hydrocarbon content or TEH (i.e., bitumen content - mg bitumen / kg sample) (Fig. 5.4) yielded a linear relationship between total PAH content and total bitumen content. The

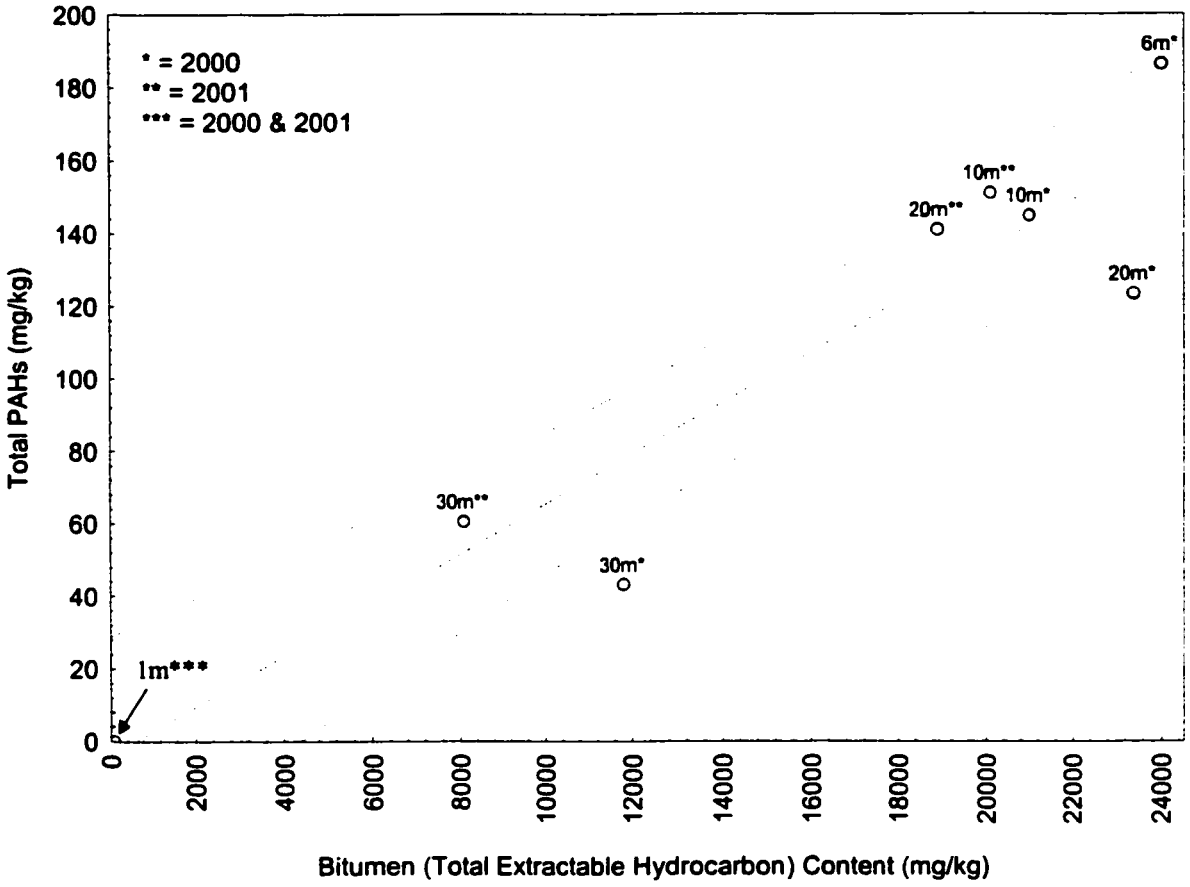

Fig. 5.4. The relationship between total PAH content (mg / kg dry weight) and bitumen (total extractable hydrocarbon) content (mg / kg dry weight) from various depths of Mildred Lake Settling Basin in August 2000 and 2001 (Data courtesy of M. MacKinnon, Syncrude Canada Ltd.) with 95% confidence bands. The regression equation takes the form: Total PAHs (mg/kg) = -4.38 + 0.007*TEH, $R^2 = 0.91$, $p < 0.00007$.

regression equation describing this relationship is:

$$\text{Total PAHs (mg / kg sample)} = -4.38 + (0.007 \times \text{TEH}) \quad (R^2 = 0.91, p < 0.0007)$$

The intercept (-4.38) has a standard error of 7.50 which is not significantly different than 0 (t-test, $p > 0.50$) thereby permitting the calculation of BSAFs for Tanypodinae and *Chaoborus* using expected PAH concentrations derived from the above equation. The observed TEH content of the Tanypodinae sample was 1100 mg/kg and for *Chaoborus* it was 610 mg/kg resulting in expected total PAH concentrations of 3.32 $mg/kg_{Tanypodinae}$ and -0.11 $mg/kg_{Chaoborus}$.

Biota-sediment accumulation factors were estimated by dividing the observed total PAH concentration by the expected total PAH concentration. This resulted in a calculated BSAF of 0.97 for Tanypodinae (Obs/Exp = 3.11 mg/kg / 3.32 mg/kg) and of -2.91 for *Chaoborus* (Obs./Exp. = 0.32 mg/kg / -0.11 mg/kg). A negative BSAF is not logical and is due to the small number of observations used to determine the relationship between total PAH concentration and TEH content of mature fine tailings. It is also due to most of the observations occurring at TEH levels much higher than observed in *Chaoborus*. These two factors result in low confidence in the estimate of total PAH concentration in *Chaoborus* based on *Chaoborus* TEH content (this point applies to Tanypodinae as well). Standard errors for the BSAFs were calculated but are not reported because they were very large due to the small number of data points used to determine the relationship between total PAH concentration and TEH content of the mature fine tailings. In order to increase confidence

in the estimates of BSAFs in the future, more data points for the PAH content of fine tailings needs to be obtained, particularly near the range of TEH contents observed in the organisms (610 - 1100 mg/kg). Taking into account the large standard errors in the BSAF estimates it can be said that the observed total PAH concentrations in both organisms were not significantly different than expected (assuming chemical equilibrium) based on the above regression equation (t-test, $p > 0.50$). In other words, the BSAFs for Tanypodinae and *Chaoborus* were not significantly different than 1.

As both of these bioaccumulation factors are not significantly different than 1.0, it suggests that these organisms are at chemical equilibrium with their environment with respect to PAHs (Gewurtz et al. 2000, Gobas and Morrison 2000). However, there is large variability in the BSAF estimates and despite this, the estimated BSAFs are still relatively close to and less than 1. The BSAF estimate for Tanypodinae is very close to 1 suggesting that they may be at chemical equilibrium with their environment with respect to PAH concentrations. Alternatively, this may suggest that a significant fraction of the PAHs detected in this sample was adsorbed to the outside of the organisms. If this was the case, then the PAH:TEH of Tanypodinae should be equivalent to the PAH:TEH of bitumen, resulting in a BSAF close to 1 in the Tanypodinae sample. The BSAF estimate for *Chaoborus* is not informative (because it is negative), but the lower concentrations of total PAHs observed in *Chaoborus* compared to Tanypodinae suggests that processes such as metabolism, direct elimination, and growth dilution (Gobas and Morrison 2000) may be important factors in determining the PAH body burden of *Chaoborus*. The observation that these processes seem to be more important for *Chaoborus* than Tanypodinae may be related to their relative exposures to

PAHs by virtue of their preferred habitats (benthic Tanypodinae likely have a higher exposure to PAHs than pelagic *Chaoborus*, see water and sediment PAH data in Figs. 5.1 and 5.2). If both organisms are capable of eliminating the PAHs at similar rates, then a reduced PAH exposure for *Chaoborus* would result in lower total PAH concentrations. This suggests that exposure to PAHs may be an important determinant of observed concentrations in these organisms. It also suggests that adsorption of bitumen (PAHs) to *Chaoborus* may not be as extensive as adsorption to Tanypodinae. This is supported by the observation of bitumen particles on the outside of Tanypodinae collected from Test Pond 7 and the absence of these particles on the outside of *Chaoborus* collected from this wetland.

The observed concentrations of total PAHs in Tanypodinae and *Chaoborus* at Test Pond 7 suggest that the benthic predatory dipterans have a greater capacity to export PAHs to the terrestrial environment than the pelagic predatory dipterans. This is assuming that adsorption of PAHs is not the only factor contributing to the difference in total PAH concentrations. Likewise, the benthic predatory dipterans studied also have a greater potential to transfer PAHs to terrestrial insectivores such as swallows.

Individual PAH Congeners

Of the 12 individual PAH congeners detected in Tanypodinae collected from Test Pond 7 in 2002, 9 exhibited bioaccumulation factors less of than 0.5 (Fig. 5.5.A, raw data for calculation in Appendix 5.7A-C). The remaining 3 congeners had bioaccumulation factors of 0.55, 0.66, and 2.79 (C4 substituted phenanthrene/anthracene). The 5 individual congeners of PAHs detected in the *Chaoborus* sample collected in 2002 from Test Pond 7 all exhibited

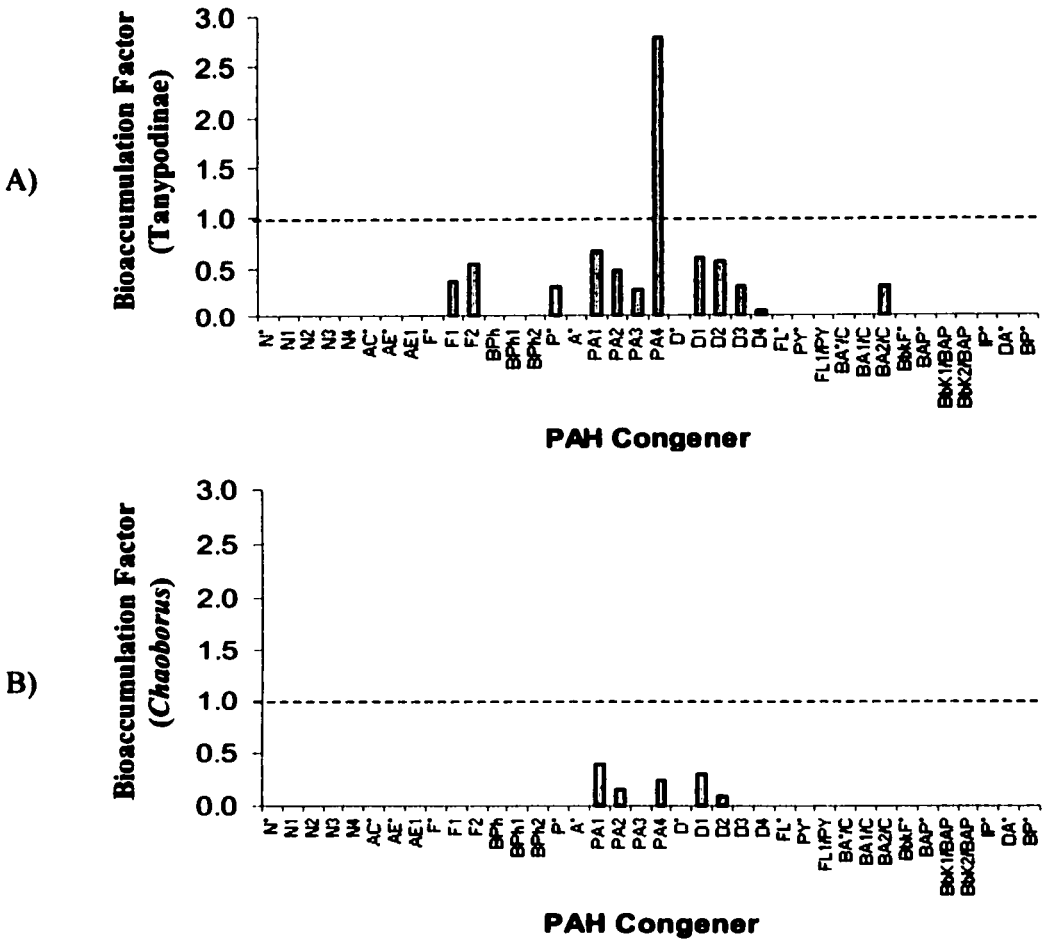

Fig. 5.5. Bioaccumulation factors of individual PAH congeners in A) Tanypodinae and B) *Chaoborus* collected from Test Pond 7 in 2002 where the bioaccumulation factor is:
(mg PAH / kg bitumen$_{organism}$) / (mg PAH / kg bitumen$_{average\ MLSB\ fine\ tails\ 2000\ \&\ 2001}$).
The line represents predicted equilibrium values.
See appendix 5.7A-C for calculations and Table 5.2 for a list of all congeners corresponding to the abbreviations used in this figure.

bioaccumulation factors of less than 0.5 (Fig. 5.5B, see Appendix 5.7 a-c for raw data and calculation of bioaccumulation factors). These results are consistent with those of Reinhold et al. (1999) who estimated BSAFs of 0.004 - 0.1 for total PAHs (US Environmental Protection Agency priority PAHs) in the entire benthic chironomid community. They are also consistent with results of Gewurtz et al. (2000) who observed BSAFs of less than 1 (most of which were less than 0.1) for the majority of individual US EPA priority PAH congeners in mayflies, mussels, amphipods, and crayfish. However, the results also contrast those of Bell (1995) who estimated BSAFs of 2.6 - 3.6 for fluoranthene in larvae of *Chironomus tentans*.

These results also suggest that Tanypodinae have a greater potential to accumulate individual PAH congeners than *Chaoborus*. This is the case even though a greater proportion of PAHs detected in the Tanypodinae sample were adsorbed to the outside of organisms, because this adsorption confers a higher PAH exposure for Tanypodinae. However, in both organisms the apparent rate of elimination (direct elimination of unmetabolized PAHs, metabolism, and growth dilution - Gobas and Morrison 2000) of individual PAH congeners exceeded that of uptake in that bioaccumulation factors were typically less than 1.0. Since concentrations of PAHs are highest at Test Pond 7 (Fig. 5.1 and Fig. 5.2), individual PAH congeners seem not to be susceptible to bioaccumulation in these organisms inhabiting either OSPM-affected or reference wetlands in the Athabasca oil sands. The only exception to this was C4 substituted phenanthrene/anthracene at Test Pond 7 in 2002, which was bioaccumulated (bioaccumulation factor > 1.0, but likely not significantly different from 1) in Tanypodinae. Several other PAH congeners were present at similar or higher levels in the

sediment of Test Pond 7 (Fig. 5.2), suggesting that this congener is more resistant to metabolism by Tanypodinae than other congeners.

3) Annual PAH Export (per m^2) via Predatory Benthic and Pelagic Dipterans

2001 samples

The estimated maximum potential annual PAH export via Tanypodinae and *Chaoborus* at all study sites is presented in Table 5.3. These results indicate that the benthic predatory dipteran Tanypodinae has a much greater potential to export PAHs to the terrestrial environment and to insectivorous predators than the pelagic predatory dipteran *Chaoborus*. This is true within each wetland sampled as well as among all wetlands.

The results from 2001 are independent of observed PAH concentrations in the organisms as these estimates were generated by assuming that all PAH congeners were at detection limits (which were the same for all samples). Thus, all differences reported among 2001 data are due solely to differences in annual production among taxa and among wetlands. Considering that the relative amounts of PAHs in the sediments and water are higher at OSPM-affected wetlands compared to reference wetlands (Figs. 5.1 and 5.2), relative differences in the estimates of PAH export at reference wetlands compared to OSPM-affected wetlands are conservative. Thus, differences in potential PAH export are expected to be even greater than observed among Tanypodinae or among *Chaoborus* inhabiting OSPM-affected versus reference wetlands. All estimates of PAH export can be considered high estimates as typically only 25% of larval chironomid biomass produced leaves a water body in terms of adult emergence (Menzie 1980).

Table 5.3. Annual total PAH* export via benthic (Tanypodinae) and pelagic (*Chaoborus*) predatory dipterans at OSPM-affected and reference wetlands.

Site		Annual Total PAH* Export via *Chaoborus* ($ng/m^2/y$)	Annual Total PAH* Export via Tanypodinae ($ng/m^2/y$)
Natural Wetland (OSPM)	**2001**	1	34 519
Test Pond 7 (OSPM)	**2001** **2002**	447365	12 274 37 130
High Sulphate (Reference)	**2001**	224	4 601
Shallow Wetland South Ditch (Reference)	**2001**	40	1 863

* see Table 5.2 for a complete list of individual PAH congeners

The maximum potential annual export of PAHs via *Chaoborus* collected in 2001 was greatest at Test Pond 7 followed by High Sulphate, Shallow Wetland South Ditch, and Natural Wetland (Table 5.3). This result highlights the fact that exposure to PAHs is not the only factor influencing potential PAH export. Secondary production also plays a large role in determining the magnitude of the export value. *Chaoborus* larvae accumulated smaller body burdens of PAHs at High Sulphate and Shallow Wetland South Ditch than at Natural Wetland (Fig. 5.1, 5.2), yet the greater values of *Chaoborus* annual production at these two reference sites conferred greater potential PAH export than what occurred at the OSPM-affected Natural Wetland. However, given the uncertainty in the actual PAH content of the *Chaoborus* samples at this site, it cannot be concluded with certainty that PAH export is indeed higher at these reference wetlands compared to Natural Wetland.

The maximum potential annual export of PAHs via Tanypodinae collected in 2001 was greatest at Natural Wetland followed by Test Pond 7, High Sulphate, and Shallow Wetland South Ditch (Table 5.3). As with the estimates of total PAH export via *Chaoborus*, these estimates largely reflect differences in Tanypodinae annual production (and associated uncertainties such as estimates of density and the number of cohorts per growing season) among these 4 wetlands. As with potential PAH export via *Chaoborus*, annual PAH export via Tanypodinae is not definitively greater at OSPM-affected wetlands than at reference wetlands. However, there were large differences in potential annual PAH export between OSPM-affected and reference wetlands (Table 5.3), and PAHs are at much higher concentrations in the sediment of OSPM-affected wetlands (Fig. 5.2). This suggests that

annual PAH export via Tanypodinae is likely greater at OSPM-affected wetlands than at reference wetlands.

2002 samples

The maximum potential estimates of annual PAH export via *Chaoborus* and Tanypodinae from 2002 at Test Pond 7 were not compared with 2001 estimates at Test Pond 7 or any of the other wetlands because detection limits were different for some of the PAH congeners (2001 data and detection limits in Appendix 5.5, 2002 raw data and detection limits in Appendix 5.6). As with the 2001 samples, the potential annual PAH export was greater (by an order of magnitude) via the predatory benthic midge, Tanypodinae which exceeded the predatory pelagic midge, *Chaoborus* in terms of both PAH accumulation and annual production. Since the magnitude of the difference in the annual production estimates for these two taxa is greater than the magnitude of the difference in the total PAH content of the samples, the difference in maximum potential annual PAH export is due mainly to differences in annual production.

Estimated Time for PAH Removal

It is possible to estimate the time that it would take to remove the PAHs from Test Pond 7 if Tanypodinae emergence was assumed to be the only mechanism of removal (removal via *Chaoborus* is assumed to be insignificant relative to Tanypodinae due to higher production and PAH accumulation in Tanypodinae). Using the regression equation describing the relationship between total PAH concentration and TEH content of mature fine tailings

(the substrate of Test Pond 7), the average TEH content (assumed to be 2% or 20 000 mg/kg) results in a total PAH concentration of 135.62 mg/kg. The density of the substrate at Test Pond 7 was assumed to be 1250 kg/m^3 (Fine Tails Fundamentals Consortium 1995b). This density was multiplied by the total PAH concentration in the sediment and the volume of fine tailings in the biologically active zone (top 10-cm, = surface area (797 m^2) x depth (0.1 m) = 79.7 m^3; M. MacKinnon 2002, Syncrude Canada Ltd., pers. comm.) to obtain the amount of total PAHs present in Test Pond 7 (135.62 mg PAHs/kg sediment x 1250 kg sediment/m^3 sediment x 79.7 m^3 sediment x 1 kg/10^6 mg = 13.51 kg PAHs in the top 10-cm of mature fine tails in Test Pond 7). Total annual PAH export via Tanypodinae at Test Pond 7 in 2002 (Table 5.3) was converted to from ng/m^2/yr to kg/m^2/yr and multiplied by 0.25 since typically only 25% of produced chironomid biomass emerges from water bodies (Menzie 1980). This value was then multiplied by the surface area of Test Pond 7 (Table 2.3) to obtain the potential amount of PAHs exported via Tanypodinae (= (37 130 ng/m^2/yr) x (1 kg/10^{12} ng) / (0.25) x (797 m^2) = 7.39 x 10^{-6} kg PAHs exported via Tanypodinae/y). The estimated time for PAH removal from Test Pond 7 via Tanypodinae was then calculated by dividing the total amount of PAHs in the top 10-cm of the MFT in Test Pond 7 (13.51 kg PAHs) by the rate of export via Tanypodinae (7.39 x 10^{-6} kg/y) with a result of 1.8 x10^6 y.

This lengthy time for PAH removal from a water body containing mature fine tailings can be considered a very high overestimate. PAHs will also be removed from these systems via metabolism and export via other chironomids and components of the benthic and pelagic communities with aquatic larval stages and terrestrial adult stages. Processes such as photodegradation (Hatch and Burton 1999, Duxbury et al. 1994, Bell 1995, Dutta and

Harayama 2001), volatization (Sage and Sage 2000), and bacterial degradation (Dutta and Harayama 2001) of PAHs are undoubtedly important processes in the removal of PAHs from these systems and should be taken into account to obtain a realistic estimate of PAH removal from a water body.

4) PAH Export - Wetlands of the Athabasca Oil Sands vs. Polluted Urban Areas

To give some context to the potential export of PAHs from wetlands in the Athabasca oil sands to the terrestrial environment, export was expressed in $ng/m^2/d$ (Table 5.4) and compared to the aerial deposition of certain PAH congeners (in $ng/m^2/d$) in Lake Erie (Table 5.5, International Atmospheric Deposition Network 1998) whose surrounding area is heavily urbanized. Daily atmospheric deposition into Lake Erie of the single compound phenanthrene (Table 5.5) was higher than the potential estimated maximum daily export of total PAHs via Tanypodinae and *Chaoborus* combined at each of the study wetlands (Table 5.4, see Table 5.2 for a list of all congeners). The remaining 4 congeners for which atmospheric deposition into Lake Erie was measured had higher rates of deposition than what was observed for potential total PAH export via *Chaoborus* at all study sites and via Tanypodinae at Shallow Wetland South Ditch.

These results suggest that if the atmospheric deposition rate of the same number of PAH congeners were measured in Lake Erie as in the Tanypodinae and *Chaoborus* samples, then the rate of atmospheric deposition in Lake Erie would greatly exceed the rate of PAH export via Tanypodinae and *Chaoborus* at either reference or OSPM-affected wetlands. In order to obtain a better comparison between these two measures, one should measure all

Table 5.4. Daily total PAH* export via benthic (Tanypodinae) and pelagic (*Chaoborus*) predatory dipterans at OSPM-affected and reference wetlands.

Site		Daily Total PAH* Export via *Chaoborus* ($ng/m^2/d$)	Daily Total PAH* Export via Tanypodinae ($ng/m^2/d$)
Natural Wetland (OSPM)	**2001**	0.1	345.2
Test Pond 7 (OSPM)	**2001**	4.5	122.7
	2002	3.7	371.3
High Sulphate (Reference)	**2001**	2.2	46
Shallow Wetland South Ditch (Reference)	**2001**	0.4	18.6

* see Table 5.2 for a complete list of individual PAH congeners

Table 5.5. Daily atmospheric deposition of individual PAH congeners in 1998 as measured by the International Atmospheric Deposition Network.

PAH congener	Aerial Deposition into Lake Erie ($ng/m^2/d$)
Phenanthrene	440
Pyrene	81
Benzo(b&k)fluoranthene	77
Benzo(a)pyrene	25
Indeno(c,d-123)pyrene	36
Sum	659

possible sources of PAH export from these wetlands, including volatization and export via other insects possessing larval or nymphal aquatic stages and terrestrial adult stages.

The higher rate of aerial deposition of PAHs in Lake Erie likely results in the biota inhabiting the region surrounding Lake Erie having greater exposure to PAHs than biota exposed to PAHs in emergent insects from OSPM-affected wetlands via their diets. For example, swallows living in the area immediately surrounding Lake Erie are exposed to PAHs in the air constantly when they breathe and these PAHs are also deposited on their insect prey and in water bodies when larvae of their insect prey live. In contrast, swallows living adjacent to constructed OSPM-affected wetlands are likely exposed to PAHs only when they consume their insect prey. Thus, in terms of exposing terrestrial insectivores to PAHs, benthic and pelagic predatory dipterans inhabiting wetlands of the Athabasca oil sands may pose less of a threat than exposure of atmospheric PAHs to similar animals living in heavily urbanized areas.

5) PAH Body Burdens in Adult and Larval Aquatic Insects - Implications for PAH Export

The body burdens of total PAHs (see Table 5.2 for a list of congeners) exported by Tanypodinae and *Chaoborus* at Test Pond 7 were estimated to be 3.11 mg/kg dry mass and 0.32 mg/kg dry mass respectively (see Appendix 5.6 for concentrations of individual congeners). The concentration of total PAHs (see Appendix 5.1 for a list of congeners) observed in adult mosquitos, chironomids, and trichopterans collected near Shallow Wetland in 1998 was 1.8 mg / kg dry mass (Appendix 5.1). The concentration of total PAHs (same congeners as for Shallow Wetland sample) observed in adult dipterans collected from the

Natural Wetland / Hummock Wetland area was 1.0 mg / kg dry mass (Appendix 5.1) Although there were differences in the target PAH congeners between the larval and adult samples (see Appendix 5.1), the total number of congeners was similar (38 for larvae, 36 for adults) thereby justifying comparisons.

The adult insects collected near Shallow Wetland likely originated in both reference and OSPM-affected wetlands as Shallow Wetland is a reference water body adjacent to a water body with consolidated tailings as a substrate. Furthermore, there are numerous small Test Ponds (similar to and including Test Pond 7) approximately 100 m from Shallow Wetland. Adults collected in the Natural Wetland / Hummock wetland area likely came solely from OSPM-affected water bodies as most of the surface water in this area is from OSPM seepage from the adjacent tailings pond.

The higher concentrations of total PAHs observed in larval Tanypodinae at Test Pond 7 than observed in either of the two adult insect samples suggests that some of the PAHs detected in the larval samples were adsorbed to the outside of the organisms, and as such, will not be exported to the terrestrial environment. Alternatively, metabolism of PAHs may occur between larval and adult stages. Bell (1995) studied bioaccumulation of fluoranthene at different life-stages of the chironomid *Chironomus tentans*. Bell (1995) found that approximately 85% of fluoranthene present in the larvae of *C. tentans* was metabolized during pupation and/or eliminated with the pupal exuviae when the adult insect emerged. Consequently, bioaccumulation factors of 3.6 in the 4th instar larvae were reduced to 0.2 in the adults. Reinhold et al. (1999) have also demonstrated significant losses of a variety of PAH congeners from larval to adult stages for chironomids. This high degree of PAH

elimination reaffirms that the previous estimates of PAH export via Tanypodinae and *Chaoborus* are likely maximum potential estimates because a significant portion of the PAHs present in the larvae were likely metabolized and/or eliminated during pupation and eclosion. It also suggests that the majority of the PAHs found in the adult insects came from those individuals with benthic larvae as opposed to pelagic larvae, because the total PAH concentration observed in pelagic *Chaoborus* from Test Pond 7 was much lower than in either of the two adult insect samples.

6) Swallow Dietary PAH Intake and Ecological Endpoints

In a study of tree swallows (*Tachycineta bicolor*) being reared in nest boxes adjacent to OSPM-affected and reference wetlands (including Natural Wetland and the Shallow Wetland South Ditch area), Smits et al. (2000) found no among-wetland difference with respect to ecologically relevant endpoints (reproductive success, nestling growth rate, and immune response) that could be attributed to the presence of OSPM (Smits et al. 2000). This is consistent with the relatively low levels of estimated maximum potential PAH export via Tanypodinae and *Chaoborus* from either reference or OSPM-affected wetlands. Further reductions in potential export could occur via PAH loss during pupation (Larsson 1984, Bell 1995, Reinhold et al. 1999). The PAH data from the adult and larval insects may explain the presence of xenobiotics in the diets of swallows nesting adjacent to OSPM-affected sites as represented by increased hepatic EROD activity at productive (for larval insects) OSPM-affected wetlands.

SUMMARY

In summary, the concentrations of total PAHs were higher in the water and sediment of OSPM-affected wetlands than reference wetlands, and in all cases the dominant congeners detected were alkylated derivatives of parent compounds. Similarly, PAH congeners detected in Tanypodinae and *Chaoborus* samples from Test Pond 7 (2002) were dominated by alkylated congeners. The concentrations of PAHs in these Test Pond 7 samples were higher in Tanypodinae (benthic) than in *Chaoborus* (pelagic) even though the trophic position of *Chaoborus* confers a higher potential to accumulate PAHs. This suggests that habitat and dietary PAH exposure play a key role in determining the PAH body burdens in these organisms. Bioaccumulation of total PAHs was not observed in these samples. When the bioaccumulation of individual congeners was addressed, only C4 substituted phenanthrene/anthracene was observed to bioaccumulate and only in Tanypodinae. Elimination of PAHs via metabolism and direct elimination of unmetabolized compounds may be important and effective PAH elimination mechanism in these organisms. Growth dilution also possibly contributes to the lack of bioaccumulation of PAHs.

Tanypodinae likely export more PAHs to the terrestrial environment (and to tree swallows) when these larval insects emerge as adults by virtue of their higher annual production and higher PAH body burdens relative to *Chaoborus*. Tanypodinae at OSPM-affected wetlands export more PAHs than Tanypodinae at reference wetlands due to greater annual production alone. The PAH body burdens in Tanypodinae of OSPM-affected wetlands is also probably greater than those in Tanypodinae of reference wetlands and this will also contribute to greater export via Tanypodinae at OSPM-affected wetlands. This export on its

own is insignificant in terms of removal of PAHs from water bodies with mature fine tailings substrates. The amount of PAH export via Tanypodinae and *Chaoborus* at both wetland types is likely much less than the rate of aerial PAH deposition in heavily urbanized areas. The detection of PAHs in terrestrial adult insects with aquatic larval stages in concentrations consistent with the maxima postulated in this study, confirms that Tanypodinae and *Chaoborus* do potentially contribute PAHs to the diet of tree swallows, although apparently not enough to induce detectable ecological effects in the swallows.

CHAPTER 6.

Thesis Conclusions

The results of this study indicate that the benthic chironomid communities of the OSPM-affected wetlands examined are not consistently less productive than reference wetlands. This may in part be due to the relative maturity of the OSPM-affected wetlands sampled. However, the productivity of some chironomid subfamilies and tribes was greater at OSPM-affected wetlands (Tanypodinae) and less at OSPM-affected wetlands (Chironomini). These differences may reflect different feeding habits of different chironomid taxa, combined with intolerance of certain taxa to OSPM. There were no clear effects of wetland type (OSPM-affected vs. reference) on the secondary production of the pelagic predatory midge *Chaoborus*. The annual production of the predatory benthic Tanypodinae was consistently higher than the annual production of the predatory pelagic *Chaoborus* in the same wetland.

Carbon and nitrogen stable isotope signatures of organisms collected in the benthic and pelagic food webs of OSPM-affected and reference wetlands suggest that the structure and function of these food webs in OSPM-affected wetlands is different than that in reference wetlands. Trophic positions of macroinvertebrates at OSPM-affected wetlands were found to be significantly higher than those at reference wetlands. This is possibly caused by a reduction in biodiversity resulting from the elimination of taxa that are intolerant to OSPM. Supporting evidence for this conjecture could be obtained by conducting a detailed assessment of biodiversity in the benthic and pelagic communities of OSPM-affected and reference wetlands and relating it to trophic position estimates. Stable nitrogen isotope

signatures indicate that trophic positions, and hence bioaccumulation potentials, are higher for *Chaoborus* than for Tanypodinae. Stable carbon isotope signatures suggest that the benthic and pelagic food webs of OSPM-affected wetlands depend on PAH-rich bitumen as an energy source at the base of the food webs. This implies that PAH concentrations should be higher in *Chaoborus* than Tanypodinae based on trophic position estimates. However naphthenic acids, which are a component of bitumen, are water soluble and likely have a stable carbon isotope signature identical to bitumen. This may confer bitumen-like stable carbon isotope signatures in pelagic organisms through the presence of naphthenic acids in the water inside of the organisms. The bitumen-like stable carbon isotope signatures may also be the result of microbial degradation of naphthenic acids at the base of the pelagic food web. In either case, it is implied that fewer PAHs flow through pelagic food webs than benthic food webs. This is consistent with the higher PAH concentrations observed in Tanypodinae than in *Chaoborus* (see below).

Bioaccumulation of PAHs (total PAHs and most individual congeners) in predatory benthic and pelagic dipterans inhabiting OSPM-affected and reference wetlands does not seem to occur. However, Tanypodinae do accumulate greater body burdens of PAHs than *Chaoborus*. This is likely due to differences in habitat and feeding habits (i.e., differences in PAH exposure) and may be related to greater adsorption of bitumen to Tanypodinae. The greater amount of PAHs accumulated by Tanypodinae combined with higher annual production confers greater potential for PAH export from these wetlands to the terrestrial environment via Tanypodinae than via *Chaoborus*. If Tanypodinae was the only agent of PAH removal from the wetlands it would take hundreds of thousands of years to remove all

of the PAHs. However, other taxa are also capable of exporting PAHs in the same manner. Furthermore, PAHs are broken down or removed from water bodies by other mechanisms as well. This suggests that the actual time for PAH removal is substantially less than hundreds of thousands of years.

The maximum potential export via these organisms from wetlands when they emerge as adults is likely less than aerial deposition of PAHs in heavily urbanized areas. The relatively low amounts of PAHs exported by predatory benthic and pelagic dipterans are consistent with the findings of a previous study (Smits et al. 2000) which found evidence of PAHs in the diet of tree swallows feeding on these and other terrestrial adult insects with aquatic larval stages but no ecologically relevant endpoints that could be attributed to wetland type (OSPM-affected vs. reference).

In conclusion, food web structures are altered at OSPM-affected wetlands compared to reference wetlands, and despite this, Tanypodinae and *Chaoborus* attain significant levels of annual production in both wetland types. These organisms also appear to export relatively small amounts of PAHs to the terrestrial environment in general, and to terrestrial insectivores in particular.

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Appendix 2.1A: Physical and chemical measurements collected at Natural Wetland, summer 2001.

Date	Start Time	Finish Time	Air Temp (°C)	Water Temp (°C)	Salinity (‰)	Conductivity (µS/cm)	D.O. surface (mg/L)	D.O. bottom (mg/L)	Water pH	Sediment pH	Water ORP	Sediment ORP	Water Depth (cm)	Depth Change (cm)
05/11/01	13:40	16:15	16.0	15.2	0.9	730			9.2	8.6	101	-355		
05/14/01	08:45	09:15	10.0	11.5	0.4	710	10.8		9.3	8.4	149	-130		
05/15/01	13:50	16:40	11.5	13.5	0.3	720	11.0		9.4	8.8	203	11		
05/16/01	13:45	16:20	18.0	15.5	0.2	800			9.4	8.6	190	-260		
05/17/01	09:25	16:30	11.0	13.0	0.2	760	10.4		9.5	8.4	147	-179		
05/18/01	13:45	16:00	13.0	14.0	0.4	800	10.8		9.6	8.8	170	-240		
05/21/01	13:30	18:40	15.0	12.8	0.8	1090	10.9		9.4	8.8	190	-131	57.0	
05/22/01	09:30	16:55	18.0	12.0	0.2	1020	10.3		9.3	8.7	205	-177	57.3	0.3
05/23/01	12:40	16:45	27.0	20.0	0.9	1350	9.2		9.0	8.5	205	-260	57.5	0.5
05/24/01	09:00	16:45	15.5	16.0	1.0	1300	8.4		9.0	8.5	219	-260	57.5	0.5
05/25/01	14:05	16:30	24.0	22.0	1.2	1650	11.0		8.9	8.6	207	-260	57.8	0.8
05/28/01	12:30	16:15	23.0	19.7	1.0	1630	8.9		9.0	8.7	212	-367	59.0	2.0
05/29/01	09:00	15:15	21.0	18.7	1.2	1620	7.4		9.0	8.6	232	-320	58.0	1.0
05/30/01	08:35	09:10	10.0	16.2	1.1	1520	7.7		9.1	8.8	220	-356	58.3	1.3
06/01/01	09:10	09:45	14.0	12.5	1.0	1920	9.4		8.7	8.5	241	-340	58.9	1.9
06/04/01	08:35	10:45	17.0	18.2	1.2	1680	7.3		8.9	8.9	45	-355	57.4	0.4
06/06/01	08:35	09:00	19.0	19.1	1.2	1680	7.3		8.9	8.2	138	-349	56.8	-0.2
06/08/01		16:10	20.0	24.7	1.4	2060	7.8		8.2	7.7	181	-274	56.5	-0.5
06/11/01	08:35	09:40	15.0	16.8	1.1	1710	4.0		8.7	8.4	201	-310	55.5	-1.5
06/15/01	17:45	18:35	18.0	18.5	1.5	1900	8.6		8.9	8.7	106	-325	57.6	0.6
06/18/01	14:40	16:45	17.0	24.7	1.1	1920	8.9		8.3	7.0	192	-267	52.5	-4.5
06/19/01	08:35	17:30	12.0	15.8	1.0	1680	7.1		9.2	8.7	236	-310	51.7	-5.3
06/22/01	08:50	09:50	16.8	19.5	1.2	1770	6.8		9.1	8.4	226	-340	53.8	-3.2
06/25/01	08:58		12.0	13.8	0.9	1498	7.9		8.8	8.0	190	-280	54.3	-2.7
06/29/01	09:18		16.0	16.0	1.1	1600	6.7		7.6	7.0	96	-161	53.4	-3.6
07/02/01	09:48		18.7	18.0	1.9	1700	9.3		11.6	10.4	200	-249	51.0	-6.0
07/02/06	09:10	09:50	21.0	19.8	1.2	1650	8.7*		12.4*	10.9	221	-240	48.7	-8.3
07/02/09	08:50	09:20	20.0	19.2	1.0	1400	8.2		11.3*	10.1*	130	-159	52.5	-4.5
07/13/01	08:50	09:15	18.0	20.0	1.1	1810	8.8		11.5*	10.2*	95	-187	56.3	-0.7
07/16/01	08:45	09:25	22.3	22.3	1.3	1970	6.7		6.4*	6*	227	-330	57.4	0.4
07/20/01	08:55	09:30	17.0	21.0	0.9	1890	6.5		8.7	8.2	30	-286	57.5	0.5
07/23/01	08:40	09:30	13.0	17.0					8.8		158	-390	56.5	-0.5
07/27/01	08:45	09:10	15.0	18.5	1.0	1800	6.4	5.5	11.1	10.4*	-40	-306	57.3	0.3
07/30/01	08:50	09:15	17.0	17.5	1.0	1740	5.0	4.6	8.1		82	-287	61.0	4.0
08/03/01	08:40	12:11	18.0	21.0	1.1	1850	5.2	5.0	8.3		104	-350	57.4	0.4
Mean (± S.D.)					**1.0 ± 0.4**	**1498 ± 417**	**8.2 ± 1.9**	**5.0 ± 0.5**	**9.1 ± 0.8**	**8.6 ± 0.8**	**163 ± 67**	**-268 ± 86**		

* values suspect, not included in means

Blanks represent missing values due to a broken meter. All data were collected at the start time.

Appendix 2.1B: Physical and chemical measurements collected at Test Pond 7, summer 2001.

Date	Start Time	Finish Time	Air Temp (°C)	Water Temp (°C)	Salinity (‰)	Conductivity (µS/cm)	D.O. surface (mg/L)	D.O. bottom (mg/L)	Water pH	Sediment pH	Water ORP	Sediment ORP	Water Depth (cm)	Depth Change (cm)
05/30/01	16:15	16:33	10.0	15.8	0.9	1320	8.3		8.5	7.4	219	-242		
06/01/01	13:45	17:10		18.5	0.9	1410	8.9		9.2	8.2	188	-215		
06/04/01	15:15	17:05	24.5	24.2	1.1	1650	6.8		9.0	7.9	162	-135	71.3	
06/05/01	09:30	15:35	20.0	18.8	1.0	1470	7.3		8.9	7.9	62	-130	71.0	-0.3
06/06/01	13:50	14:15	27.0	24.3	1.2	1690	7.1		8.9	8.0	168	-196	70.9	-0.4
06/07/01	09:30	14:55	26.0	24.5	1.2	1690	7.7		9.3	8.0	175	-102	70.0	-1.3
06/08/01	12:10	12:45	23.5	23.0	1.1	1720	7.8		9.2	8.0	192	-87	69.5	-1.8
06/11/01	15:05	16:50	21.5	20.9	1.1	1580	7.2		9.1	8.5	215	-146	69.6	-1.7
06/12/01	09:45	16:50	19.0	20.1	1.0	1550	7.1		9.3	8.1	151	-90	69.7	-1.6
06/15/01	11:15	15:00	18.5	18.5	1.1	1500	7.2		9.2	8.1	226	-129	68.9	-2.4
06/18/01	10:35	11:10	17.0	17.5	0.9	1490	8.2		8.0	7.2	224	-148	67.7	-3.6
06/22/01	14:25	15:05	24.2	23.3	1.1	1760	8.4		9.2	8.0	203	-209	65.5	-5.8
06/25/01	13:20		14.0	15.0	0.8	1400	8.4		9.4	8.1	170	-136	66.5	-4.8
06/29/01	12:49		16.0	16.2	1.0	1550	7.8		7.2*	6.6	125	-126	67.2	-4.1
07/02/01	15:45		22.0	22.2	1.3	1760			10.5*	9.7	185	-163	66.5	-4.8
07/06/01	14:00	14:30	25.5	21.3	1.3	1730			8.1	6.8	188	-157	64.5	-6.8
07/09/01	10:50	11:30		22.0	1.1	1720	8.5		11.2	9.8	158	-142	63.1	-8.2
07/13/01	10:35	11:10	22.0	20.8	1.2	1800	8.0		12.8	11.1	175	-215	61.7	-9.6
07/16/01	11:00	11:30	24.0	22.0	0.9	2020	8.0		10.8*	8.4	163	-211	60.8	-10.5
07/20/01	13:25	14:05	25.0	24.5	2.0	2270	7.8		8.8		136	-117	60.6	-10.7
07/23/01	12:30	13:15	22.2	23.8	1.0	1950	7.5		9.1		107	-217	59.3	-12.0
07/27/01	14:40	15:00	22.0	24.2	1.0	2080	8.6	7.6	9.0	9.8*	125	-185	58.3	-13.0
07/30/01	11:20	11:43	24.0	21.5	1.0	1800	6.6	7.9	8.8		83	-154	61.0	-10.3
08/03/01	17:55	19:35	26.0	24.0	1.1	2220	8.4	8.6	9.1		126	-195	60.4	-10.9
Mean (± S.D.)					**1.1 ± 0.2**	**1714 ± 251**	**7.8 ± 0.6**	**8.0 ± 0.5**	**9.2 ± 1.0**	**8.2 ± 1.1**	**164 ± 43**	**-160 ± 44**		

* values suspect, not included in means

Blanks represent missing values due to a broken meter. All data were collected at the start time.

Appendix 2.1C: Physical and chemical measurements collected at High Sulphate, summer 2001.

Date	Start Time	Finish Time	Air Temp (°C)	Water Temp (°C)	Salinity (‰)	Conductivity (µS/cm)	D.O. surface (mg/L)	D.O. bottom (mg/L)	Water pH	Sediment pH	Water ORP	Sediment ORP	Water Depth (cm)	Depth Change (cm)
05/14/01	14:20	17:00		15.2	0.7	1010	11.2		8.2	7.5	193	-83		
05/16/01	08:35	09:20	8.0	10.8	0.5	920			8.3		225	-140		
05/18/01	12:25	12:45	13.8	12.5	0.7	1040	9.3		8.4	7.7	141	-85		
05/21/01	12:25	12:40	13.0	12.2	0.8	1080	10.8		8.5	7.8	165	-176	60.0	
05/23/01	11:25	11:40	24.0	16.0	0.8	1020	9.0		8.5	7.7	205	-240		
05/25/01	13:20	13:45	23.0	21.0	1.0	1400	7.8		8.6	7.9	220	-146	58.5	-1.5
05/28/01	11:20	11:35	23.0	17.5	0.8	1400	8.4		8.6	8.0	207	-175	58.9	-1.1
05/30/01	17:00	17:25	9.0	15.8	1.0	1310	8.4		8.4	7.8	154	-190	60.0	0.0
06/01/01	10:05	10:20	13.0	13.5	0.8	1250	9.6		8.3	7.9	190	-188	60.3	0.3
06/04/01	10:50	11:50	21.5	20.0	1.1	1520	7.8		8.5	7.8	145	-175	58.5	-1.5
06/06/01	09:25	10:00	21.0	19.1	1.1	1540	7.4		8.3	7.8	126	-201	57.1	-2.9
06/08/01	17:20	17:40	20.0	23.7	1.2	1770	7.9		7.9	7.3	201	-142	55.5	-4.5
06/11/01	10:15	10:50	14.5	18.5	1.0	1570	6.8		7.7	7.9	175	-190	56.5	-3.5
06/15/01	16:45	17:30	19.5	20.3	1.1	1630	8.1		8.3	7.7	195	-190	54.8	-5.2
06/18/01	13:50	14:20		18.7	1.2	1690	8.4		7.5	7.1	196	-146	53.2	-6.8
06/22/01	10:20	11:00		20.6	0.4	810	8.5		8.4	7.8	145	-210	50.4	-9.6
06/25/01	10:40		13.0	14.2	1.0	1500	8.9		8.3	7.9	148	-172	51.7	-8.3
06/29/01	10:35		12.0	15.0	1.0	1580	9.2		6.5*	6.6*	120	-116		
07/02/01	10:38		21.0	19.0	1.0	1650	8.8		11.1*	9.9*	106	-147		
07/06/01	10:20	11:20	21.0	20.8	1.3	1770	8.3		12.2	10.3	147	-145	49.8	-10.2
07/09/01	14:30		26.5	27.0	1.2	2090	7.7		10.9	9.9	161	-232	47.3	-12.7
07/13/01	13:15	16:40	26.0	26.0	1.5	1800	7.8		7.6	3.1*	121	-112	46.5	-13.5
07/16/01	13:45	17:00	21.8	21.5	1.1	2280	9.0			6.6*	190	-325	45.7	-14.3
07/20/01	10:05	10:30	21.0	21.0	1.0	1850	7.8		8.2	9.7	40	-238	46.3	-13.7
07/23/01	09:55	10:55	17.2	18.0					8.0		123	-175	44.4	-15.6
07/27/01	09:50	10:10	17.0	19.0	1.0	1870	7.4	6.3	7.5	9.9*	23	-200	43.1	-16.9
07/30/01	09:40	10:00	20.5	19.0	1.0	1710	5.8	4.9	7.5		108	-238	46.8	-13.2
08/03/01	13:02	15:10	25.0	26.0	1.2	2260	8.8	6.2	7.8		103	-232	44.9	-15.1
Mean (± S.D.)					**1.0 ± .2**	**1530 ± 389**	**8.5 ± 1.1**	**5.8 ± 0.8**	**8.4 ± 1.0**	**8.1 ± 0.9**	**153 ± 50**	**-179 ± 52**		

* values suspect, not included in means

Blanks represent missing values due to a broken meter. All data were collected at the start time.

Appendix 2.1D: Physical and chemical measurements collected at Shallow Wetland South Ditch, summer 2001.

Date	Start Time	Finish Time	Air Temp (°C)	Water Temp (°C)	Salinity (‰)	Conductivity (µS/cm)	D.O. surface (mg/L)	D.O. bottom (mg/L)	Water pH	Sediment pH	Water ORP	Sediment ORP	Water Depth (cm)	Depth Change (cm)
05/10/01	09:30	12:15	11.0	11.0	0.2	460	11.5		8.5	7.7	29	204		
05/11/01	09:30	10:10	11.0	12.0	0.1	462	11.2		8.2	7.2	119	58		
05/14/01	10:50	11:15	16.0	13.0	0.1	500			8.2	7.3	141	93		
05/16/01	10:55	11:15	14.0	13.0	0.0	490	9.4		8.5	7.4	183	-79		
05/18/01	10:45	11:10	9.0	11.2	0.2	490	9.5		8.5	7.7	178	-89		
05/21/01	11:00	11:20	12.0	9.2	0.3	500	9.9		8.8	7.8	212	9	80.0	
05/23/01	09:45	10:15		15.2	0.2	530	9.4		8.7	7.7	186	-3	79.8	-0.2
05/25/01	11:20	11:40	21.0	18.0	0.2	500	8.8		8.7	7.9	229	-105	79.5	-0.5
05/28/01	10:00	10:25	21.0	17.5	0.2	590	11.8		8.7	7.8	183	-80	80.0	0.0
05/30/01	11:25	11:45	12.0	16.2	0.2	590	8.8		8.6	7.9	216	-254	79.8	-0.2
06/01/01	13:15	13:30	17.5	17.5	0.2	580	8.5		8.7	8.1	226	-146	81.0	1.0
06/04/01	14:50		24.0	23.2	0.4	700	8.8		8.7	7.9	167	-124	79.9	-0.1
06/06/01	13:05	13:40	26.0	24.2	0.4	690	8.4		8.5	7.5	200	-170	78.8	-1.2
06/08/01	11:45	12:00	22.0	22.3	0.0	690	8.2		8.6	7.9	205	-150	77.7	-2.3
06/11/01	14:00	15:00	17.5	20.3	0.2	620	8.7		7.6	6.8	204	-86	78.2	-1.8
06/15/01	10:00	11:05	19.0	19.8	0.2	630	9.4		8.7	7.8	131	-118	77.1	-2.9
06/18/01	09:40	10:30	18.0	18.0	0.1	580	9.8		9.0	7.8	228	-127	75.8	-4.2
06/22/01	13:40	14:15	24.0	22.0	0.1	640	9.4		9.0	7.9	195	-139	73.1	-6.9
06/25/01	12:30		14.0	15.1	0.0	530	9.6		9.1	8.1	175	-139		
06/29/01	11:50		12.0	15.0	0.0	600	8.8		6.3*	5.8	156	-106	75.3	-4.7
07/02/01	15:00	15:40	21.5	18.0	0.0	720			11.4	9.8	215	-148	74.1	-5.9
07/06/01	13:15	13:55		22.1	0.3	670			8.5	7.4	182	-197	72.1	-7.9
07/09/01	10:15	10:45	22.0	22.0	0.1	630	9*		11.1	9.6	141	-152	70.0	-10.0
07/13/01	10:00	10:30	21.0	21.0	0.1	640	8.8		10.8*	9.7*	3	-150	68.2	-11.8
07/16/01	10:20	10:50	24.0	23.0	0.8	780	9.8		8.9*	6.3	157	-71	66.5	-13.5
07/20/01	11:45	12:15	24.0	23.1	0.6	820	9.2		9.0	7.4	61	-163	65.9	-14.1
07/23/01	13:25	15:50	24.0	22.5	0.1	680	10.6		9.3		129	-160	65.5	-14.5
07/27/01	15:15	16:50	24.0	24.5	0.3	750	9.6		9.2	10.4*	85	-139	62.5	-17.5
07/30/01	11:50	16:50	21.0	21.0	0.2	700	6.4	6.0	8.6		79	-135	66.1	-13.9
08/03/01	15:50	17:45	24.0	24.0			9.0	8.5	9.1		95	-105	64.3	-15.7
Mean (± S.D.)					**0.2 ± 0.2**	**612 ± 98**	**9.4 ± 1.1**	**7.3 ± 1.8**	**8.9 ± 0.8**	**7.7 ± 0.8**	**157 ± 60**	**-100 ± 92**		

* values suspect, not included in means

Blanks represent missing values due to a broken meter. All data were collected at the start time.

Appendix 2.2: Water Quality at 4 constructed wetlands, 2000 and 2001
(Data courtesy of Mike MacKinnon (Syncrude Canada Ltd.)

Site	Year	Date	Temp (°C)	DO	pH	Cond (uS/cm)	NH_3	N (NH_4)	IC50 (Vol %)	IC20 (Vol %)	Naphthenic Acids (mg/L)	Na	K	Mg	Ca
HS	2000	Jul-15				2400		<0.01	100	100	15.3	295	14.80	90.0	262.00
HS	2001	Jun-14	20.8		7.6	1673			100	100	9.0	206	15.60	58.7	101.00
		Jul-17	13.9		7.9	1517			100	100	3.8	248	13.60	69.9	97.70
		Jul-25			7.6	2260	BDL	BDL	100	100	3.8	268	14.40	73.6	109.00
		Aug-20	18.8		7.6	1796			100	100	9.5	268	14.40	72.1	123.00
		Aug-28			7.8	2010	BDL	BDL	100	100	9.5	267	14.30	72.7	113.00
NW	2000	Jul-15				1880		1.4	100	21	65.3	441	15.60	18.2	25.30
NW	2001	Jun-14	21		8.3	1880			100	25	54.7	455	14.10	15.1	26.00
		Jul-18	19.5		8.3	1784			100	17	58.1	461	15.50	14.9	24.00
		Jul-25			8.6	2310	BDL	BDL	100	33	61.4	488	15.60	15.1	22.70
		Aug-20	20.8		8.5	1870			100	34	58.3	462	15.10	13.6	22.10
		Aug-28			8.6	1910	BDL	BDL	100	30	54.3	437	14.40	12.7	20.30
SWSD	2000	Jul-15				880						134	2.80	43.9	30.40
SWSD	2001	Jul-19			8.78	654	BDL	BDL	100	100	1.4	96.7	BDL	33.7	21.60
TP7	2000	May-09	10.2	9.1	8.87	997			100	100	14.1	335	5.79	9.4	13.50
		Jun-11	9.9	8.5	8.79	1433						397	7.11	10.9	13.80
		Jun-28	21.0	10	9.01	1704		<0.01	100	100	18.7	388	6.61	11.2	11.50
		Jul-20	22.6	9.1	9.03	1770						436	7.60	12.1	10.70
		Aug-09	19.6	10.6	9.12	1900			100	81	21.5	467	7.66	11.8	8.83
		Sep-28	10.4	9.6	8.93	1600			100	100	16.8	462	7.40	11.3	9.26
TP7	2001	Jun-21	22.0	7.9*	8.8	1822	BDL	BDL	100	100	19.3	880	14.40	22.9	24.50
		Jul-24	24.0	10	9.14	1980	BDL	BDL	100	100	23.6	494	7.66*	11.6	9.50
		Aug-20	16.2	5.1	8.91	1954	BDL	BDL				492	7.80*	11.1	8.44

concentrations in mg/L
BDL = Below Detection Limit
TRACE = Trace amount detected
blank = not measured
* measured in the laboratory at Syncrude Canada Ltd. Edmonton

Appendix 2.2 cont.: Water Quality at 4 constructed wetlands, 2000 and 2001.
(Data courtesy of Mike MacKinnon (Syncrude Canada Ltd.)

Site	Year	Date	F_e	Cl	SO_4	CO_3	HCO_3	NO_2	NO_3	PO_4	Al	B	Ba	Cd	Co	Cr	Cu
HS	2000	Jul-15	BDL	12	1600		160	BDL	BDL	BDL	BDL	1.060	BDL	BDL	BDL	BDL	BDL
HS	2001	Jun-14	BDL	0.2	700	0.0	254	BDL	BDL	BDL	BDL	0.464	0.028	BDL	BDL	BDL	BDL
		Jul-17	BDL	BDL	880	0.0	160	BDL	BDL	BDL	BDL	0.783	0.017	BDL	BDL	BDL	BDL
		Jul-25	BDL	TRACE	1100	0.0	185	BDL	BDL	BDL	BDL	0.657	BDL	BDL	BDL	BDL	BDL
		Aug-20	BDL	BDL	990	0.0	212	BDL	BDL	BDL	BDL	0.800	0.010	BDL	BDL	BDL	BDL
		Aug-28	BDL	BDL	1100	0.0	300	BDL	BDL	BDL	BDL	0.870	BDL	BDL	BDL	BDL	BDL
NW	2000	Jul-15	1.3	58	390		768	BDL	BDL	BDL	BDL	2.840	0.022	BDL	BDL	BDL	BDL
NW	2001	Jun-14	2	56	220	31.8	897				0.19	2.630	0.097	BDL	BDL	BDL	BDL
		Jul-18	2	68	210	27.0	917				BDL	2.930	0.085	BDL	BDL	BDL	BDL
		Jul-25	2.3	68.0	220	40.2	944	BDL	BDL	BDL	BDL	2.910	0.062	BDL	BDL	BDL	BDL
		Aug-20	2.1	79	220	25.2	900				0.50	2.870	0.082	BDL	BDL	BDL	BDL
		Aug-28	2.1	73.0	210	46.2	832	BDL	BDL	BDL	0.37	3.020	0.067	BDL	BDL	BDL	BDL
SWSD	2000	Jul-15	BDL	15	320		243	BDL	BDL	BDL	BDL	0.429	BDL	BDL	BDL	BDL	BDL
SWSD	2001	Jul-19	BDL	8.3	160	0.0	240	BDL	BDL	BDL	BDL	0.310	BDL	BDL	BDL	BDL	BDL
TP7	2000	May-09	0.98	88	68		749	BDL	BDL	BDL	2.97	1.020	BDL	BDL	BDL	BDL	BDL
		Jun-11	2	100	87		879	BDL	BDL	BDL	1.35	1.320	0.024	BDL	BDL	BDL	BDL
		Jun-28	1.4	110	96		820	BDL	BDL	BDL	0.79	1.410	0.018	BDL	BDL	BDL	BDL
		Jul-20	1.7	130	110		899	BDL	BDL	BDL	2.67	1.690	0.034	BDL	BDL	BDL	BDL
		Aug-09	4.4	120	110		1057	BDL	BDL	BDL	1.71	1.740	BDL	BDL	BDL	BDL	BDL
		Sep-28	5.6	120	94	88.0	764				2.87	1.620	BDL	BDL	BDL	0.03	BDL
TP7	2001	Jun-21	1.5	130	92	55.2	810	BDL	BDL	BDL	2.15	2.950	0.0*	BDL	BDL	BDL	BDL
		Jul-24	1.2	130	86	101.0	806	BDL	BDL	BDL	1.19	1.6*	BDL	BDL	BDL	BDL	BDL
		Aug-20	BDL	140	110	78.6*	833	BDL	BDL	BDL	1.25	1.0*	0.030	BDL	BDL	BDL	BDL

concentrations in mg/L
BDL = Below Detection Limit
TRACE = Trace amount detected
blank = not measured
* measured in the laboratory at Syncrude Canada Ltd. Edmonton

Appendix 2.2 cont.: Water Quality at 4 constructed wetlands, 2000 and 2001.
(Data courtesy of Mike MacKinnon (Syncrude Canada Ltd.)

Site	Year	Date	Fe	Li	Mn	Mo	Ni	Pb	S	Sb	Se	Si	Sr	Ti	V	Zn	Zr
HS	2000	Jul-15	BDL	BDL	BDL	BDL	BDL	BDL		BDL	BDL	0.56	2.37	BDL	BDL	BDL	BDL
HS	2001	Jun-14	0.135	BDL	0.054	BDL	BDL	BDL	282.0	BDL	BDL	BDL	1.31	0.031	BDL	BDL	BDL
		Jul-17	0.092	BDL	0.021	BDL	BDL	BDL	338.0	BDL	BDL	1.33	1.38	0.031	BDL	BDL	BDL
		Jul-25	0.105	BDL	0.013	BDL	BDL	BDL	356.0	BDL	BDL	1.85	1.420	BDL	BDL	BDL	BDL
		Aug-20	0.233	BDL	0.100	BDL	BDL	BDL	347.0	BDL	BDL	1.77	1.41	BDL	BDL	BDL	BDL
		Aug-28	0.129	BDL	0.032	BDL	BDL	BDL	352.0	BDL	BDL	BDL	1.400	0.018	BDL	BDL	BDL
NW	2000	Jul-15	0.237	BDL	0.019	0.26	BDL	BDL		BDL	BDL	8.18	0.584	BDL	BDL	BDL	BDL
NW	2001	Jun-14	0.350	BDL	0.047	0.26	BDL	BDL	86.0	BDL	BDL	3.8	0.534	0.037	BDL	BDL	BDL
		Jul-18	0.314	BDL	0.041	0.28	BDL	BDL	81.9	BDL	BDL	5.35	0.531	0.034	BDL	BDL	BDL
		Jul-25	0.364	BDL	0.015	0.29	BDL	BDL	85.7	BDL	BDL	5.63	0.529	0.015	BDL	BDL	BDL
		Aug-20	0.490	BDL	0.031	0.26	BDL	BDL	77.0	BDL	BDL	6.2	0.51	0.02	BDL	BDL	BDL
		Aug-28	0.364	BDL	0.016	0.29	BDL	BDL	76.8	BDL	BDL	4.61	0.465	0.027	BDL	BDL	BDL
SWSD	2000	Jul-15	BDL	BDL	0.018	BDL	BDL	BDL		BDL	BDL	0.126	0.346	BDL	BDL	BDL	BDL
SWSD	2001	Jul-19	BDL	BDL	BDL	BDL	BDL	BDL	60.5	BDL	BDL	BDL	0.235	BDL	BDL	BDL	BDL
TP7	2000	May-09	0.790	BDL	BDL	BDL	BDL	BDL		BDL	BDL	8.7	0.222	0.021	BDL	BDL	BDL
		Jun-11	0.318	BDL	BDL	BDL	BDL	BDL		BDL	BDL	5.68	0.231	0.036	BDL	BDL	BDL
		Jun-28	0.229	BDL	0.005	BDL	BDL	BDL		BDL	BDL	3.2	0.23	0.033	BDL	0.02	0.01
		Jul-20	0.690	BDL	0.007	BDL	BDL	BDL		BDL	BDL	11.7	0.241	0.084	BDL	BDL	0.03
		Aug-09	0.460	BDL	BDL	BDL	BDL	BDL		BDL	BDL	7.7	0.197	BDL	BDL	0.03	BDL
		Sep-28	0.660	BDL	BDL	BDL	BDL	BDL		BDL	BDL	10.1	0.203	BDL	BDL	BDL	BDL
TP7	2001	Jun-21	0.552	BDL	BDL	BDL	BDL	BDL	68.0*	ENA	BDL	13.4	0.408	0.0*	BDL	BDL	BDL
		Jul-24	0.24*	BDL	BDL	BDL	BDL	BDL	38.0*	BDL	BDL	0.0*	0.166	BDL	BDL	BDL	BDL
		Aug-20	0.340	BDL	0.012	BDL	BDL	BDL		BDL	BDL	5.3	0.189	0.02*	BDL	BDL	BDL

concentrations in mg/L
BDL = Below Detection Limit
TRACE = Trace amount detected
blank = not measured
* measured in the laboratory at Syncrude Canada Ltd. Edmonton

Appendix 2.3: Depth, area, and volume of study sites (See Chapter 2 for maps used to generate data).

Natural Wetland*

Area of Wetland (m^2)	Volume of Wetland (m^3)
12700	3300

* based on Bishay (1992)

Test Pond 7

Depth (m)	Area of Contour (m^2)	Average Depth (m)	Volume of Contour (m^3)
0.40	796.63	0.40	318.65
Total:	**796.63**		**318.65**

Shallow Wetland South Ditch

Depth (m)	Area of Contour (m^2)	Average Depth (m)	Volume of Contour (m^3)
0.20	282.37	0.35	98.83
0.50	298.79	0.60	179.27
0.70	61.78	0.85	52.51
1.00	79.17	1.10	87.09
Total:	**722.11**		**417.70**

Appendix 2.3 continued: Depth, area, and volume of study sites (See Chapter 2 for maps used to generate data).

High Sulphate

Depth (m)	Area of Contour (m^2)	Open Water Area (m^2)	Area in Cattails (m^2)	Average Depth (m)	Volume of Contour (m^3)	Volume of Open Water (m^3)	Volume of Water Inside Cattails (m^3)
0.00	93.41	0.00	93.41	0.025	2.34	0.00	2.34
0.05	244.85	0.00	244.85	0.075	18.36	0.00	18.36
0.10	223.80	0.00	223.80	0.125	27.98	0.00	27.98
0.15	203.75	14.69	189.06	0.175	35.66	2.57	33.09
0.20	212.46	51.90	160.56	0.225	47.80	11.68	36.13
0.25	188.01	185.39	2.62	0.275	51.70	50.98	0.72
0.30	141.18	94.74	46.44	0.325	45.88	30.79	15.09
0.35	139.11	117.92	21.19	0.375	52.17	44.22	7.95
0.40	187.45	183.72	3.73	0.425	79.67	78.08	1.59
0.45	176.94	176.94	0.00	0.475	84.05	84.05	n/a
0.50	135.93	135.93	0.00	0.525	71.36	71.36	n/a
0.55	102.90	102.90	0.00	0.575	59.17	59.17	n/a
0.60	83.07	83.07	0.00	0.625	51.92	51.92	n/a
0.65	59.26	59.26	0.00	0.675	40.00	40.00	n/a
0.70	1.08	1.08	0.00	0.725	0.78	0.78	n/a
Total:	**2193.20**	**1207.54**	**985.66**		**668.83**	**525.60**	**143.23**

Appendix 2.4: Oil and solids content of sediment pore water, bitumen content of sediment, and particle size distribution of the mineral solids of sediment collected from study wetlands. (Data courtesy of Mike MacKinnon, Syncrude Canada Ltd., Edmonton, AB)

Site	Oil in Sediment Pore Water (g/100g)	Solids in Sediment Pore Water (g/100g)	OWS* (Bitumen Content of Sediment) (%)	Particle Size Distribution (% less than)**							
				250 µm	125 µm	44 µm	22 µm	11 µm	5.5 µm	2.8 µm	1.0 µm
Natural	0.25	22.70	0.01	97	82.5	46.6	33.6	22.5	13.6	7.78	3.18
Wetland	0.27	38.08	0.01	75	42.4	24.3	18.4	13.5	9.1	5.72	2.35
(NW)	0.24	26.16	0.01	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
OSPM	0.36	34.19	0.01	95	71	40.7	30.2	21.1	13.4	8.00	3.33
	0.64	37.40	0.02	90.7	71.7	45.1	32.2	21.7	13.5	8.06	3.42
n	5	5	5	4	4	4	4	4	4	4	4
Mean ± S.D.	0.54±0.13	34.84±15.83	0.017±0.0045	85.2±16.0	69.1±16.6	45.1±10.8	32.9±7.4	23.3±4.9	15.5±3.1	9.48±1.71	3.60±0.64
Test	1.02	28.40	0.04	78	34.2	20.2	16.4	12.7	9.3	6.41	2.82
Pond 7	1.07	30.00	0.04	76	23.6	14.3	11.8	9.0	6.3	4.16	1.53
(TP7)	0.94	28.10	0.03	71	14.3	5.4	4.3	3.5	2.8	2.07	0.87
OSPM	0.97	28.60	0.03	95	53.5	29.5	24.2	19.3	14.0	9.11	3.51
	1.14	29.30	0.04	84	59	39.0	32.5	26.4	19.4	12.50	4.61
	1.04	29.80	0.03	100	83.1	58.4	50.1	41.6	30.9	19.80	7.30
n	6	6	6	6	6	6	6	6	6	6	6
Mean ± S.D.	1.03±.0.072	29.03±0.78	0.035±0.0019	84.1±11.4	44.7±25.5	27.8±19.0	23.2±16.4	18.7±13.7	13.8±10.2	9.01±6.44	3.44±2.32
High	0.40	21.44	0.02	88	70.3	44.4	31.8	22.0	14.4	8.67	3.15
Sulphate	0.41	19.43	0.02	93	80.5	53.2	38.3	26.6	17.3	10.30	3.69
(HS)	0.67	32.82	0.02	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Reference	0.60	57.52	0.01	62	45	30.1	22.9	16.9	11.7	7.54	3.09
	0.62	43.03	0.01	98.2	80.4	52.8	38.7	27.5	18.5	11.40	4.46
n	5	5	5	4	4	4	4	4	4	4	4
Mean ± S.D.	0.35±0.17	31.71±6.91	0.011±0.0038	89.4±10.0	66.9±17.2	39.2±10.2	28.6±6.9	19.7±4.2	12.4±2.2	7.39±1.12	3.07±0.49
Shallow	0.16	69.94	0.0023	100	100	99.3	89.7	80.2	68.6	53.7	22.5
Wetland	0.00	75.65	0.0000	100	99	83.4	75.2	66.9	56.7	43.9	18.4
South	0.03	80.88	0.0004	100	100	100	95.6	86.6	74.5	58.7	24.8
Ditch	0.10	70.50	0.0014	100	100	100	93.7	82.6	69.5	53.6	22.5
(SWSD)	0.04	79.69	0.0005	100	100	100	92.7	82	69.5	54.3	23.5
Reference	0.05	83.17	0.0006	100	100	100	95.2	86.2	74.4	59.1	26.4
	0.27	56.95	0.0047	100	98.1	81.1	69.9	58.7	46.1	32.5	12.1
	0.07	75.41	0.0009	100	100	99.1	88.1	76.8	63.6	47.6	18.4
	0.11	80.38	0.0014	100	100	99.3	89.8	79.9	67.7	52.8	22.6
	0.10	78.54	0.0013	100	100	94.5	86.4	78.4	67.2	52.5	22.7
	0.08	80.87	0.0010	100	100	94	89.3	83.7	74.1	58.6	23.6
n	11	11	11	11	11	11	11	11	11	11	11
Mean ± S.D.	0.092±0.074	75.63±7.53	0.0013±0.0013	100±0.0	99.7±0.62	95.5±6.9	87.8±6.2	78.4±6.5	66.5±6.5	51.57±7.83	21.59±3.94

* OWS = oil content / solids content of pore water, i.e., % bitumen of sediment

** Represents mineral solids; determined by laser diffraction after bitumen was removed

- All sediment samples were collected from the sediment-water interface
- NW and HS Samples: Collected in 2000 and 2001 by Margo Moore (Simon Fraser University)
- SWSD Samples: Collected in 2002 by Natalie Cooper (University of Alberta) from environmentally similar Shallow Wetland
- TP7 Samples: Oil and solids contents from 2001 and 2002 at TP7, samples collected by Mike MacKinnon (Syncrude Canada Ltd.) whereas particle size distribution is from 2000 and 2001 samples collected from Pond 5 (enviromentally similar to TP7, near Waste Area 11) by Margo Moore (SFU)
- All samples analysed by Syncrude Canada Ltd. (Edmonton Laboratory)

Appendix 3.1: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

Natural Wetland - Tanytarsini *see Appendix 4.1

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g/m^2)
0.5-1.1	1854.5	4.31E-06	7.99E-03				
1.1-1.7	6676.2	1.13E-05	7.56E-02	-4821.7	7.82E-06	-3.77E-02	-3.77E-01
1.7-2.3	3848.1	2.58E-05	9.94E-02	2828.1	1.86E-05	5.25E-02	5.25E-01
2.3-2.9	3059.9	4.54E-05	1.39E-01	788.2	3.56E-05	2.81E-02	2.81E-01
2.9-3.5	1785.0	7.43E-05	1.33E-01	1275.0	5.99E-05	7.63E-02	7.63E-01
3.5-4.1	857.7	1.11E-04	9.55E-02	927.3	9.28E-05	8.61E-02	8.61E-01
4.1-4.7	208.6	1.58E-04	3.30E-02	649.1	1.35E-04	8.74E-02	8.74E-01
4.7-5.3	115.9	2.05E-04	2.37E-02	92.7	1.81E-04	1.68E-02	1.68E-01
5.3-5.9	23.2	2.53E-04	5.85E-03	92.7	2.29E-04	2.12E-02	2.12E-01
5.9-6.5	23.2	3.89E-04	9.01E-03	0.0	3.21E-04	0.00E+00	0.00E+00
	18452.4			23.2	3.89E-04	9.01E-03	9.01E-02
		Cohort B (ΣB)	0.62			**Cohort P**	3.77
		Cohort P/B	6.07			***CPI Correction Factor**	10.87
		Annual P/B (yr^{-1})	65.99			**Annual P**	41.02

Natural Wetland - Chironomini *see Appendix 4.1

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g/m^2)
0.90-1.55	185.5	8.05E-06	1.49E-03				
1.55-2.20	115.9	2.12E-05	2.45E-03	69.5	1.46E-05	1.02E-03	1.02E-02
2.20-2.85	208.6	3.96E-05	8.25E-03	-92.7	3.04E-05	-2.82E-03	-2.82E-02
2.85-3.50	185.5	7.40E-05	1.37E-02	23.2	5.68E-05	1.32E-03	1.32E-02
3.50-4.15	231.8	1.11E-04	2.58E-02	-46.4	9.26E-05	-4.29E-03	-4.29E-02
4.15-4.80	231.8	1.69E-04	3.91E-02	0.0	1.40E-04	0.00E+00	0.00E+00
4.80-5.45	0.0	0.00E+00	0.00E+00	231.8	8.43E-05	1.95E-02	1.95E-01
5.45-6.10	0.0	0.00E+00	0.00E+00	0.0	0.00E+00	0.00E+00	0.00E+00
6.10-6.75	0.0	0.00E+00	0.00E+00	0.0	0.00E+00	0.00E+00	0.00E+00
6.75-7.40	69.5	4.85E-04	3.38E-02	-69.5	2.43E-04	-1.69E-02	-1.69E-01
	1228.6			69.5	4.85E-04	3.38E-02	3.38E-01
		Cohort B (ΣB)	0.12			**Cohort P**	0.56
		Cohort P/B	4.47			***CPI Correction Factor**	3.25
		Annual P/B (yr^{-1})	14.53			**Annual P**	1.81

Appendix 3.1 cont.: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

Natural Wetland - Tanypodinae *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.80-2.18	2480.4	1.20E-05	2.97E-02				
2.18-3.56	324.5	4.45E-05	1.45E-02	2155.9	2.82E-05	6.09E-02	6.09E-01
3.56-4.94	533.2	1.55E-04	8.27E-02	-208.6	9.98E-05	-2.08E-02	-2.08E-01
4.94-6.32	973.6	2.66E-04	2.59E-01	-440.4	2.11E-04	-9.27E-02	-9.27E-01
6.32-7.70	649.1	4.96E-04	3.22E-01	324.5	3.81E-04	1.24E-01	1.24E+00
7.70-9.08	255.0	6.54E-04	1.67E-01	394.1	5.75E-04	2.27E-01	2.27E+00
9.08-10.46	139.1	8.99E-04	1.25E-01	115.9	7.77E-04	9.00E-02	9.00E-01
10.46-11.84	92.7	1.48E-03	1.37E-01	46.4	1.19E-03	5.52E-02	5.52E-01
11.84-13.22	46.4	1.85E-03	8.57E-02	46.4	1.67E-03	7.72E-02	7.72E-01
13.22-14.6	46.4	2.39E-03	1.11E-01	0.0	2.12E-03	0.00E+00	0.00E+00
	5540.4			46.4	2.39E-03	1.11E-01	1.11E+00
		Cohort B (ΣB)	1.33			Cohort P	7.44
		Cohort P/B	5.58			*CPI Correction Factor	3.86
		Annual P/B (yr^{-1})	21.57			Annual P	28.77

Natural Wetland - Orthocladiinae *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.50-1.21	208.6	5.06E-06	1.06E-03				
1.21-1.92	486.8	1.45E-05	7.08E-03	-278.2	9.80E-06	-2.73E-03	-2.73E-02
1.92-2.63	255.0	3.41E-05	8.69E-03	231.8	2.43E-05	5.63E-03	5.63E-02
2.63-3.34	115.9	7.04E-05	8.16E-03	139.1	5.23E-05	7.27E-03	7.27E-02
3.34-4.05	46.4	9.66E-05	4.48E-03	69.5	8.35E-05	5.81E-03	5.81E-02
4.05-4.76	46.4	1.62E-04	7.49E-03	0.0	1.29E-04	0.00E+00	0.00E+00
4.76-5.47	46.4	2.22E-04	1.03E-02	0.0	1.92E-04	0.00E+00	0.00E+00
5.47-6.18	46.4	2.74E-04	1.27E-02	0.0	2.48E-04	0.00E+00	0.00E+00
6.18-6.89	69.5	4.19E-04	2.91E-02	-23.2	3.47E-04	-8.03E-03	-8.03E-02
6.89-7.60	46.4	5.12E-04	2.38E-02	23.2	4.66E-04	1.08E-02	1.08E-01
	1367.7			46.4	5.12E-04	2.38E-02	2.38E-01
		Cohort B (ΣB)	0.11			Cohort P	0.53
		Cohort P/B	4.72			*CPI Correction Factor	7.77
		Annual P/B (yr^{-1})	36.67			Annual P	4.14

Appendix 3.1 cont.: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

Test Pond 7 - Tanytarsini *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.70-1.23	370.9	6.27E-06	2.32E-03				
1.23-1.76	1506.8	1.38E-05	2.08E-02	-1135.9	1.00E-05	-1.14E-02	-1.14E-01
1.76-2.29	1692.2	2.68E-05	4.53E-02	-185.5	2.03E-05	-3.77E-03	-3.77E-02
2.29-2.82	2202.2	4.46E-05	9.83E-02	-510.0	3.57E-05	-1.82E-02	-1.82E-01
2.82-3.35	904.1	6.92E-05	6.26E-02	1298.2	5.69E-05	7.39E-02	7.39E-01
3.35-3.88	857.7	1.01E-04	8.62E-02	46.4	8.49E-05	3.94E-03	3.94E-02
3.88-4.41	1043.2	1.40E-04	1.46E-01	-185.5	1.20E-04	-2.23E-02	-2.23E-01
4.41-4.94	185.5	1.62E-04	3.01E-02	857.7	1.51E-04	1.29E-01	1.29E+00
4.94-5.47	139.1	2.32E-04	3.23E-02	46.4	1.97E-04	9.15E-03	9.15E-02
5.47-6.00	23.2	3.25E-04	7.54E-03	115.9	2.79E-04	3.23E-02	3.23E-01
	8924.8			23.2	3.25E-04	7.54E-03	7.54E-02
		Cohort B (ΣB)	0.53			Cohort P	2.56
		Cohort P/B	4.82			*CPI Correction Factor	10.87
		Annual P/B (yr^{-1})	52.43			Annual P	27.86

Test Pond 7 - Chironomini *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
1.90-3.18	92.7	3.21E-05	2.98E-03				
3.18-4.46	46.4	8.71E-05	4.04E-03	46.4	5.96E-05	2.76E-03	2.76E-02
4.46-5.74	0.0	0.00E+00	0.00E+00	46.4	4.35E-05	2.02E-03	2.02E-02
5.74-7.02	153.0	4.06E-04	6.22E-02	-153.0	2.03E-04	-3.11E-02	-3.11E-01
7.02-8.30	0.0	0.00E+00	0.00E+00	153.0	2.03E-04	3.11E-02	3.11E-01
8.30-9.58	23.2	9.28E-04	2.15E-02	-23.2	4.64E-04	-1.08E-02	-1.08E-01
9.58-10.86	0.0	0.00E+00	0.00E+00	23.2	4.64E-04	1.08E-02	1.08E-01
10.86-12.14	23.2	1.52E-03	3.52E-02	-23.2	7.60E-04	-1.76E-02	-1.76E-01
12.14-13.42	23.2	1.72E-03	4.00E-02	0.0	1.62E-03	0.00E+00	0.00E+00
13.42-14.70	23.2	2.58E-03	5.98E-02	0.0	2.15E-03	0.00E+00	0.00E+00
	384.9			23.2	2.58E-03	5.98E-02	5.98E-01
		Cohort B (ΣB)	0.23			Cohort P	1.06
		Cohort P/B	4.72			*CPI Correction Factor	3.25
		Annual P/B (yr^{-1})	15.34			Annual P	3.46

Appendix 3.1 cont.: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

Test Pond 7 - Tanypodinae *see Appendix 4.1

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g/m^2)
1.60-2.75	231.8	3.55E-05	8.23E-03				
2.75-3.90	301.4	8.84E-05	2.66E-02	-69.5	6.19E-05	-4.31E-03	-4.31E-02
3.90-5.05	162.3	1.70E-04	2.75E-02	139.1	1.29E-04	1.80E-02	1.80E-01
5.05-6.20	162.3	2.88E-04	4.67E-02	0.0	2.29E-04	0.00E+00	0.00E+00
6.20-7.35	92.7	4.20E-04	3.89E-02	69.5	3.54E-04	2.46E-02	2.46E-01
7.35-8.50	92.7	6.45E-04	5.98E-02	0.0	5.32E-04	0.00E+00	0.00E+00
8.50-9.65	46.4	8.08E-04	3.75E-02	46.4	7.26E-04	3.37E-02	3.37E-01
9.65-10.80	23.2	1.20E-03	2.77E-02	23.2	1.00E-03	2.32E-02	2.32E-01
10.80-11.95	69.5	1.38E-03	9.61E-02	-46.4	1.29E-03	-5.98E-02	-5.98E-01
11.95-13.10	92.7	1.78E-03	1.65E-01	-23.2	1.58E-03	-3.67E-02	-3.67E-01
	1275.0			92.7	1.78E-03	1.65E-01	1.65E+00
		Cohort B (ΣB)	0.53			**Cohort P**	2.65
		Cohort P/B	4.95			***CPI Correction Factor**	3.86
		Annual P/B (yr^{-1})	19.15			**Annual P**	10.23

Test Pond 7 - Orthocladiinae *see Appendix 4.1

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g/m^2)
2.30-2.97	46.4	3.73E-05	1.73E-03				
2.97-3.64	46.4	8.54E-05	3.96E-03	0.0	6.13E-05	0.00E+00	0.00E+00
3.64-4.31	0.0	0.00E+00	0.00E+00	46.4	4.27E-05	1.98E-03	1.98E-02
4.31-4.98	23.2	1.60E-04	3.71E-03	-23.2	8.00E-05	-1.85E-03	-1.85E-02
4.98-5.65	0.0	0.00E+00	0.00E+00	23.2	8.00E-05	1.85E-03	1.85E-02
5.65-6.32	0.0	0.00E+00	0.00E+00	0.0	0.00E+00	0.00E+00	0.00E+00
6.32-6.99	0.0	0.00E+00	0.00E+00	0.0	0.00E+00	0.00E+00	0.00E+00
6.99-7.66	23.2	4.68E-04	1.09E-02	-23.2	2.34E-04	-5.43E-03	-5.43E-02
7.66-8.33	23.2	6.42E-04	1.49E-02	0.0	5.55E-04	0.00E+00	0.00E+00
8.33-9.00	23.2	8.14E-04	1.89E-02	0.0	7.28E-04	0.00E+00	0.00E+00
	185.5			23.2	8.14E-04	1.89E-02	1.89E-01
		Cohort B (ΣB)	0.05			**Cohort P**	0.23
		Cohort P/B	4.20			***CPI Correction Factor**	7.77
		Annual P/B (yr^{-1})	32.67			**Annual P**	1.76

Appendix 3.1 cont.: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

High Sulphate - Tanytarsini *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.50-1.05	276.2	3.60E-06	9.95E-04				
1.05-1.60	1632.4	1.13E-05	1.85E-02	-1356.1	7.46E-06	-1.01E-02	-1.01E-01
1.60-2.15	1682.6	2.31E-05	3.88E-02	-50.2	1.72E-05	-8.64E-04	-8.64E-03
2.15-2.70	1707.7	3.99E-05	6.82E-02	-25.1	3.15E-05	-7.91E-04	-7.91E-03
2.70-3.25	652.9	6.62E-05	4.32E-02	1054.8	5.31E-05	5.60E-02	5.60E-01
3.25-3.80	301.4	9.45E-05	2.85E-02	351.6	8.04E-05	2.83E-02	2.83E-01
3.80-4.35	301.4	1.28E-04	3.86E-02	0.0	1.11E-04	0.00E+00	0.00E+00
4.35-4.90	25.1	1.83E-04	4.59E-03	276.2	1.56E-04	4.30E-02	4.30E-01
4.9-5.450	50.2	2.41E-04	1.21E-02	-25.1	2.12E-04	-5.32E-03	-5.32E-02
5.45-6.0	50.2	3.07E-04	1.54E-02	0.0	2.74E-04	0.00E+00	0.00E+00
	6680.1			50.2	3.07E-04	1.54E-02	1.54E-01
		Cohort B (ΣB)	0.27			**Cohort P**	1.43
		Cohort P/B	5.30			***CPI Correction Factor**	10.87
		Annual P/B (yr⁻¹)	57.64			**Annual P**	15.50

High Sulphate - Chironomini *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.90-2.58	4419.9	2.36E-05	1.04E-01				
2.58-4.26	3842.3	9.07E-05	3.49E-01	577.6	5.72E-05	3.30E-02	3.30E-01
4.26-5.94	4445.0	2.11E-04	9.37E-01	-602.7	1.51E-04	-9.09E-02	-9.09E-01
5.94-7.62	1054.8	4.30E-04	4.53E-01	3390.3	3.20E-04	1.09E+00	1.09E+01
7.62-9.30	979.4	6.93E-04	6.79E-01	75.3	5.61E-04	4.23E-02	4.23E-01
9.30-10.98	527.4	1.09E-03	5.76E-01	452.0	8.93E-04	4.04E-01	4.04E+00
10.98-12.66	376.7	1.61E-03	6.05E-01	150.7	1.35E-03	2.03E-01	2.03E+00
12.66-14.34	251.1	2.04E-03	5.12E-01	125.6	1.82E-03	2.29E-01	2.29E+00
14.34-16.02	75.3	2.50E-03	1.88E-01	175.8	2.27E-03	3.99E-01	3.99E+00
16.02-17.70	100.5	3.50E-03	3.52E-01	-25.1	3.00E-03	-7.54E-02	-7.54E-01
	16072.5			100.5	3.50E-03	3.52E-01	3.52E+00
		Cohort B (ΣB)	4.76			**Cohort P**	27.48
		Cohort P/B	5.78			***CPI Correction Factor**	3.25
		Annual P/B (yr⁻¹)	18.80			**Annual P**	89.39

Appendix 3.1 cont.: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

High Sulphate - Tanypodinae *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
1.20-1.87	125.6	1.34E-05	1.68E-03				
1.87-2.54	200.9	3.16E-05	6.34E-03	-75.3	2.25E-05	-1.69E-03	-1.69E-02
2.54-3.21	251.1	5.98E-05	1.50E-02	-50.2	4.57E-05	-2.30E-03	-2.30E-02
3.21-3.88	226.0	8.47E-05	1.91E-02	25.1	7.23E-05	1.81E-03	1.81E-02
3.88-4.55	150.7	1.52E-04	2.29E-02	75.3	1.18E-04	8.91E-03	8.91E-02
4.55-5.22	175.8	1.99E-04	3.50E-02	-25.1	1.75E-04	-4.41E-03	-4.41E-02
5.22-5.89	50.2	3.07E-04	1.54E-02	125.6	2.53E-04	3.17E-02	3.17E-01
5.89-6.56	100.5	3.53E-04	3.55E-02	-50.2	3.30E-04	-1.66E-02	-1.66E-01
6.56-7.23	25.1	4.01E-04	1.01E-02	75.3	3.77E-04	2.84E-02	2.84E-01
7.23-7.90	50.2	5.64E-04	2.83E-02	-25.1	4.83E-04	-1.21E-02	-1.21E-01
	1356.1			50.2	5.64E-04	2.83E-02	2.83E-01
		Cohort B (ΣB)	0.19			**Cohort P**	0.99
		Cohort P/B	5.24			***CPI Correction Factor**	3.86
		Annual P/B (yr^{-1})	20.25			**Annual P**	3.83

High Sulphate - Orthocladiinae *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.70-1.28	929.2	6.93E-06	6.44E-03				
1.28-1.86	1531.9	1.54E-05	2.35E-02	-602.7	1.11E-05	-6.72E-03	-6.72E-02
1.86-2.44	1331.0	2.83E-05	3.77E-02	200.9	2.19E-05	4.39E-03	4.39E-02
2.44-3.02	276.2	4.88E-05	1.35E-02	1054.8	3.86E-05	4.07E-02	4.07E-01
3.02-3.60	125.6	8.11E-05	1.02E-02	150.7	6.49E-05	9.78E-03	9.78E-02
3.60-4.18	50.2	1.20E-04	6.01E-03	75.3	1.00E-04	7.56E-03	7.56E-02
4.18-4.76	50.2	1.52E-04	7.64E-03	0.0	1.36E-04	0.00E+00	0.00E+00
4.76-5.34	25.1	1.91E-04	4.79E-03	25.1	1.71E-04	4.31E-03	4.31E-02
5.34-5.92	50.2	3.01E-04	1.51E-02	-25.1	2.46E-04	-6.17E-03	-6.17E-02
5.92-6.5	50.2	3.53E-04	1.77E-02	0.0	3.27E-04	0.00E+00	0.00E+00
	4419.9			50.2	3.53E-04	1.77E-02	1.77E-01
		Cohort B (ΣB)	0.14			**Cohort P**	0.84
		Cohort P/B	5.92			***CPI Correction Factor**	7.77
		Annual P/B (yr^{-1})	46.00			**Annual P**	6.56

Appendix 3.1 cont.: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

Shallow Wetland South Ditch - Tanytarsini *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.6-1.5	3546.8	8.81E-06	3.12E-02				
1.5-2.4	4705.8	2.38E-05	1.12E-01	-1159.1	1.63E-05	-1.89E-02	-1.89E-01
2.4-3.3	1738.6	5.44E-05	9.46E-02	2967.2	3.91E-05	1.16E-01	1.16E+00
3.3-4.2	718.6	1.04E-04	7.48E-02	1020.0	7.92E-05	8.08E-02	8.08E-01
4.2-5.1	69.5	1.71E-04	1.19E-02	649.1	1.38E-04	8.94E-02	8.94E-01
5.1-6.0	23.2	2.78E-04	6.44E-03	46.4	2.25E-04	1.04E-02	1.04E-01
6.0-6.9	0.0	0.00E+00	0.00E+00	23.2	1.39E-04	3.22E-03	3.22E-02
6.9-7.8	0.0	0.00E+00	0.00E+00	0.0	0.00E+00	0.00E+00	0.00E+00
7.8-8.7	0.0	0.00E+00	0.00E+00	0.0	0.00E+00	0.00E+00	0.00E+00
8.7-9.6	23.2	7.78E-04	1.80E-02	-23.2	3.89E-04	-9.02E-03	-9.02E-02
	10825.7			23.2	7.78E-04	1.80E-02	1.80E-01
		Cohort B (ΣB)	0.35			**Cohort P**	3.18
		Cohort P/B	9.11			***CPI Correction Factor**	10.87
		Annual P/B (yr^{-1})	99.04			**Annual P**	34.55

Shallow Wetland South Ditch - Chironomini *see Appendix 4.1

Size Group (mm)	N (# / m²)	W (g)	B (g / m²)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m²)	x 10 (g/m²)
0.7-1.54	950.4	8.20E-06	7.79E-03				
1.54-2.38	1182.3	2.33E-05	2.76E-02	-231.8	1.58E-02	-3.65E-03	-3.65E-02
2.38-3.22	718.6	5.18E-05	3.72E-02	463.6	1.02E+00	1.74E-02	1.74E-01
3.22-4.06	463.6	1.02E-04	4.71E-02	255.0	2.02E+00	1.95E-02	1.95E-01
4.06-4.9	255.0	1.63E-04	4.17E-02	208.6	3.02E+00	2.76E-02	2.76E-01
4.9-5.74	115.9	2.58E-04	2.99E-02	139.1	4.02E+00	2.93E-02	2.93E-01
5.74-6.58	92.7	3.19E-04	2.96E-02	23.2	5.02E+00	6.69E-03	6.69E-02
6.58-7.42	46.4	4.95E-04	2.30E-02	46.4	6.02E+00	1.89E-02	1.89E-01
7.42-8.26	0.0	0.00E+00	0.00E+00	46.4	7.02E+00	1.15E-02	1.15E-01
8.26-9.1	46.4	8.18E-04	3.79E-02	-46.4	8.02E+00	-1.90E-02	-1.90E-01
	3871.3			46.4	9.02E+00	3.79E-02	3.79E-01
		Cohort B (ΣB)	0.28			**Cohort P**	1.69
		Cohort P/B	5.99			***CPI Correction Factor**	3.25
		Annual P/B (yr^{-1})	19.50			**Annual P**	5.49

Appendix 3.1 cont.: Calculation of chironomid production by the Size-frequency Method (Benke, 1996)

Shallow Wetland South Ditch - Tanypodinae *see Appendix 4.1

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g/m^2)
0.9-1.77	417.3	8.14E-06	3.39E-03				
1.77-2.64	394.1	2.68E-05	1.05E-02	23.2	1.74E-05	4.04E-04	4.04E-03
2.64-3.51	394.1	5.10E-05	2.01E-02	0.0	3.89E-05	0.00E+00	0.00E+00
3.51-4.38	162.3	9.98E-05	1.62E-02	231.8	7.54E-05	1.75E-02	1.75E-01
4.38-5.25	92.7	1.57E-04	1.46E-02	69.5	1.28E-04	8.93E-03	8.93E-02
5.25-6.12	69.5	2.48E-04	1.72E-02	23.2	2.03E-04	4.69E-03	4.69E-02
6.12-6.99	0.0	0.00E+00	0.00E+00	69.5	1.24E-04	8.62E-03	8.62E-02
6.99-7.86	0.0	0.00E+00	0.00E+00	0.0	0.00E+00	0.00E+00	0.00E+00
7.86-8.73	23.2	5.98E-04	1.39E-02	-23.2	2.99E-04	-6.93E-03	-6.93E-02
8.73-9.6	23.2	2.30E-06	5.34E-05	0.0	3.00E-04	0.00E+00	0.00E+00
	1576.3			23.2	2.30E-06	5.34E-05	5.34E-04
		Cohort B (ΣB)	0.10			**Cohort P**	0.40
		Cohort P/B	4.19			***CPI Correction Factor**	3.86
		Annual P/B (yr^{-1})	16.18			**Annual P**	1.55

Shallow Wetland South Ditch - Orthocladiinae *see Appendix 4.1

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g/m^2)
0.6-1.21	278.2	5.15E-06	1.43E-03				
1.21-1.82	278.2	1.36E-05	3.78E-03	0.0	9.38E-06	0.00E+00	0.00E+00
1.82-2.43	231.8	2.73E-05	6.33E-03	46.4	2.05E-05	9.48E-04	9.48E-03
2.43-3.04	208.6	5.31E-05	1.11E-02	23.2	4.02E-05	9.32E-04	9.32E-03
3.04-3.65	69.5	7.41E-05	5.16E-03	139.1	6.36E-05	8.85E-03	8.85E-02
3.65-4.26	23.2	1.14E-04	2.65E-03	46.4	9.43E-05	4.37E-03	4.37E-02
4.26-4.87	23.2	1.98E-04	4.60E-03	0.0	1.56E-04	0.00E+00	0.00E+00
4.87-5.48	23.2	2.02E-04	4.67E-03	0.0	2.00E-04	0.00E+00	0.00E+00
5.48-6.09	46.4	3.09E-04	1.43E-02	-23.2	2.55E-04	-5.92E-03	-5.92E-02
6.09-6.7	23.2	4.14E-04	9.60E-03	23.2	3.62E-04	8.39E-03	8.39E-02
	1205.4			23.2	4.14E-04	9.60E-03	9.60E-02
		Cohort B (ΣB)	0.06			**Cohort P**	0.33
		Cohort P/B	5.20			***CPI Correction Factor**	7.77
		Annual P/B (yr^{-1})	40.39			**Annual P (g/m^4/yr)**	2.57

Appendix 3.2: Calculation of *Chaoborus* production by the Size-frequency Method (Benke, 1996)

Natural Wetland - *Chaoborus*

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g / m^2)
2.04-3.41	6.3E-02	2.90E-06	1.82E-07				
3.41-4.79	2.0E-01	8.05E-06	1.62E-06	-1.4E-01	5.48E-06	-7.59E-07	-7.59E-06
4.79-6.16	2.4E-01	2.03E-05	4.79E-06	-3.5E-02	1.42E-05	-5.00E-07	-5.00E-06
6.16-7.53	1.8E-01	4.08E-05	7.35E-06	5.6E-02	3.05E-05	1.71E-06	1.71E-05
7.53-8.91	1.8E-01	6.27E-05	1.16E-05	-4.3E-03	5.18E-05	-2.21E-07	-2.21E-06
8.91-10.28	8.5E-02	9.97E-05	8.47E-06	9.9E-02	8.12E-05	8.08E-06	8.08E-05
10.28-11.65	1.8E-02	2.54E-04	4.48E-06	6.7E-02	1.77E-04	1.19E-05	1.19E-04
11.65-13.02	2.1E-02	3.66E-04	7.84E-06	-3.7E-03	3.10E-04	-1.16E-06	-1.16E-05
13.02-14.40	1.7E-02	5.20E-04	8.90E-06	4.3E-03	4.43E-04	1.90E-06	1.90E-05
14.40-15.77	8.6E-03	6.94E-04	5.93E-06	8.6E-03	6.07E-04	5.19E-06	5.19E-05
	1.0E+00			8.6E-03	6.94E-04	5.93E-06	5.93E-05
		Cohort B (ΣB)	6.11E-05			**Cohort P**	3.47E-04
		Cohort P/B	5.68			***CPI Correction Factor**	2.65
		Annual P/B (yr^{-1})	15.02			**Annual P ($g/m^2/yr$)**	0.00092

Test Pond 7 - *Chaoborus*

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g / m^2)
2.08-3.13	30.2	3.58E-06	1.08E-04				
3.13-4.18	34.9	1.03E-05	3.58E-04	-4.7	6.93E-06	-3.26E-05	-3.26E-04
4.18-5.22	53.3	2.25E-05	1.20E-03	-18.4	1.64E-05	-3.02E-04	-3.02E-03
5.22-6.27	108.8	4.19E-05	4.56E-03	-55.5	3.22E-05	-1.79E-03	-1.79E-02
6.27-7.32	141.1	6.56E-05	9.26E-03	-32.3	5.38E-05	-1.74E-03	-1.74E-02
7.32-8.37	63.5	8.93E-05	5.67E-03	77.6	7.75E-05	6.01E-03	6.01E-02
8.37-9.42	14.8	1.32E-04	1.95E-03	48.7	1.10E-04	5.38E-03	5.38E-02
9.42-10.46	5.6	1.72E-04	9.61E-04	9.3	1.52E-04	1.40E-03	1.40E-02
10.46-11.51	0.7	2.55E-04	1.86E-04	4.9	2.13E-04	1.04E-03	1.04E-02
11.51-12.56	8.E-03	4.04E-04	3.07E-06	0.7	3.29E-04	2.38E-04	2.38E-03
	453.0			8.E-03	3.07E-06	2.33E-08	2.33E-07
		Cohort B (ΣB)	4.35E-02			**Cohort P**	1.41E-01
		Cohort P/B	3.23			***CPI Correction Factor**	2.65
		Annual P/B (yr^{-1})	8.55			**Annual P ($g/m^2/yr$)**	0.37

Appendix 3.2 cont.: Calculation of *Chaoborus* production by the Size-frequency Method (Benke, 1996)

High Sulphate - *Chaoborus*

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g / m^2)
0.75-1.90	45.1	5.48E-07	2.47E-05				
1.90-3.04	266.6	2.12E-06	5.65E-04	-221.4	1.33E-06	-2.95E-04	-2.95E-03
3.04-4.19	181.2	7.15E-06	1.30E-03	85.3	4.64E-06	3.96E-04	3.96E-03
4.19-5.34	123.3	1.73E-05	2.14E-03	57.9	1.22E-05	7.09E-04	7.09E-03
5.34-6.49	51.9	3.56E-05	1.85E-03	71.4	2.65E-05	1.89E-03	1.89E-02
6.49-7.632	38.8	6.15E-05	2.39E-03	13.1	4.86E-05	6.36E-04	6.36E-03
7.632-8.78	18.3	9.22E-05	1.69E-03	20.5	7.69E-05	1.58E-03	1.58E-02
8.78-9.926	12.9	1.62E-04	2.09E-03	5.4	1.27E-04	6.87E-04	6.87E-03
9.926-11.07	8.6	2.31E-04	1.98E-03	4.4	1.96E-04	8.57E-04	8.57E-03
11.07-12.22	89.2	7.00E-06	6.24E-04	-80.6	1.19E-04	-9.59E-03	-9.59E-02
	836.1			89.2	3.50E-06	3.12E-04	3.12E-03
		Cohort B (ΣB)	1.76E-02			**Cohort P**	7.06E-02
		Cohort P/B	4.01			***CPI Correction Factor**	2.65
		Annual P/B (yr^{-1})	10.60			**Annual P (g/m^2/yr)**	0.19

Shallow Wetland South Ditch - *Chaoborus*

Size Group (mm)	N (# / m^2)	W (g)	B (g / m^2)	delta N	Weight at Loss W (g)	Weight Loss W*deltaN (g / m^2)	x 10 (g / m^2)
1.85-3.15	0.6	3.18E-06	1.96E-06				
3.15-4.45	7.8	9.80E-06	7.66E-05	-7.2	6.49E-06	-4.67E-05	-4.67E-04
4.45-5.75	11.9	2.16E-05	2.57E-04	-4.1	1.57E-05	-6.40E-05	-6.40E-04
5.75-7.05	10.6	4.56E-05	4.82E-04	1.3	3.36E-05	4.39E-05	4.39E-04
7.05-8.36	4.3	7.92E-05	3.41E-04	6.3	6.24E-05	3.92E-04	3.92E-03
8.36-9.66	2.3	1.34E-04	3.03E-04	2.1	1.07E-04	2.20E-04	2.20E-03
9.66-10.96	1.5	2.10E-04	3.10E-04	0.8	1.72E-04	1.35E-04	1.35E-03
10.96-12.26	0.8	3.11E-04	2.40E-04	0.7	2.61E-04	1.83E-04	1.83E-03
12.26-13.56	0.3	4.30E-04	1.14E-04	0.5	3.71E-04	1.88E-04	1.88E-03
13.56-14.86	4.E-02	5.29E-04	2.33E-05	0.2	4.80E-04	1.06E-04	1.06E-03
	40.0			4.E-02	2.33E-05	1.02E-06	1.02E-05
		Cohort B (ΣB)	2.47E-03			**Cohort P**	1.27E-02
		Cohort P/B	5.12			***CPI Correction Factor**	2.65
		Annual P/B (yr^{-1})	13.55			**Annual P (g/m^2/yr)**	0.03

Appendix 3.3: Development times (days) of chironomid and *Chaoborus* larvae at 15 °C.

Taxa	Larval Development Time at 15 °C (days)
Tanytarsini	
Cladotanytarsus atridorsum (K.)	10
Rheotanytarsus photophilus (G.)	8.4
Average	**9.20**
Chironomini	
Chironomus sp.	20.5
Chironomus annularius	43.3
Chironomus dorsalis	34.9
Chironomus heterodentatus	42.9
Chironomus plumosus	60.4
Glyptotendipes pallens (K.)	22.9
Limnochironomus pulsus	8.4
Limnochironomus nervosus	48
Cryptochironomus pararostratus gp.	37.5
Parachironomus biannulatus	7.4
Microtendipes chloris	29.4
Polypedilum convictum	13.4
Polypedilum nubeculosum (K.)	35
Phaenopsectra flavipes	35.8
Apedilum elachistus	20
Chironomus decorus	32
Average	**30.74**
Tanypodinae	
Ablabesmyia monilis (L.)	16.95
Procladius choreus	34.8
Average	**25.88**
Orthocladiinae	
Synorthocladius semivirens (K.)	8.7
Cricotopus bicinctus (Meig.)	14.7
Cricotopus sylvestris (Fabr.)	17.5
Cricotopus algarum (K.)	24.9
Microcricotopus bicolor (Zett.)	5.4
Metriocnemus hirticollis (Staeg.)	13.9
Corynoneura coronata (Edw.)	5
Average	**12.87**
Chaoborus	
Chaoborus flavicans	37.8

data for *Apedilum elachistus* and *Chironomus decorus* from Balci & Kennedy (2002)
data for the rest of the chironomids from Mackey (1977a)
data for *Chaoborus* from Hanazato and Yasuno (1989)

Appendix 4.1A: Composition of 0.5 - 20-µm plankton samples from study wetlands.*

Taxa	Natural Wetland	Test Pond 7	High Sulphate	Shallow Wetland South Ditch
Anabaena	0	n/a**	0	3
Anacystis	4	n/a**	9	3
Fragilaria	0	n/a**	6	2
Gloecystis	47	n/a**	48	80
Pareuglypha	2	n/a**	8	5
Spondylosium	0	n/a**	0	5
Chlorophyta	212	n/a**	143	90

* numbers refer to single organisms identified in 1 mL of the 100 mL sample
absolute abundances among wetlands not comparable, only relative abundances.

** sample was lost and hence composition could not be evaluated

Appendix 4.1B: Composition of 20 - 180-µm plankton samples from study wetlands.*

Taxa	Natural Wetland	Test Pond 7	High Sulphate	Shallow Wetland South Ditch
Phytoplankton				
Spondylosium	0	n/a**	10	0
Gloeocystis	22	n/a**	42	162
Anabaena	0	n/a**	2	2
Ceratium	2	n/a**	0	41
Fragilaria	5	n/a**	0	0
Chlorophyta	0	n/a**	133	26
Blue-Green cells	0	n/a**	0	10
Animals				
Cyclopoida	0	n/a**	11	0
Diaphanosoma	0	n/a**	22	0
Daphnia	1	n/a**	1	6
Keratella quadrata	2	n/a**	10	1

* numbers refer to single organisms identified in 1 mL of the 100 mL sample
absolute abundances among wetlands not comparable, only relative abundances.

** sample was lost and hence composition could not be evaluated

Appendix 4.1C: Composition of 180 - 500-µm plankton samples from study wetlands.*

Taxa	Natural Wetland	Test Pond 7	High Sulphate	Shallow Wetland South Ditch
Phytoplankton				
Anabaena	0	69	0	9
Fragilaria	1	0	0	0
Pareuglypha	0	1	0	0
Spirochaeta	0	1	0	0
Spondylosium	0	0	103	0
Chlorophyta	108	13	327	84
Cyanophyta	1	0	0	0
Animals				
Odonata	0	0	0	1
Plecoptera	0	1	0	0
Chaoborus	6	2	7	4
Cyclopoida	12	0	6	1
Ephemeroptera	0	0	1	0
Amphipoda	0	0	0	11
Orthocladiinae	5	0	0	0
Ostracoda	0	0	4	0
Daphnia	13	4	9	11
Diaphanosoma	26	0	7	1
Other Crustacea	0	0	0	2
Keratella cochlearis	0	0	5	0
Keratella quadrata	2	28	83	1

Appendix 4.2A: Raw data from stable isotope analysis and trophic position calculations based on detrital $\delta^{15}N$ signatures at Natural Wetland.

Sample	Abbrev.	Collection Date (2001)	%C	%N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Trophic Position: $1+(^{15}N_{sample} - {}^{15}N_{base})/3.4$		
							(avg baseline**)	(min baseline**)	(max baseline**)
Benthic									
0.5-20 µm detritus	ud	May 21	7.68	0.54	-28.31	11.56	1.71	1.00	2.26
20-180 µm detritus	fd	May 21	20.15	1.23	-27.92	8.57	0.83	0.12	1.38
180-500 µm detritus	d	May 21	31.27	1.58	-28.05	7.28	0.45	-0.26	1.00
Derotanypus	D	May 21-29	45.00	8.19	-30.19	13.64	2.32	1.61	2.87
Chironomus	CH	Aug. 5-9	39.24	8.66	-30.36	16.15	3.06	2.35	3.61
Tanypodinae	T	May 22-29	44.47	9.46	-28.94	14.82	2.67	1.96	3.22
Anisoptera	A	May 24-29	44.36	10.31	-30.10	17.99	3.60	2.89	4.15
Zygoptera	Z	May 24-25	46.50	10.11	-29.56	18.11	3.64	2.93	4.19
Misc. chironomids	M	May 15-22	42.12	8.72	-28.87	15.60	2.90	2.19	3.45
Pelagic									
0.5-20 µm plankton	up	June 20	4.52	0.49	-28.57*	10.64	1.44	0.73	1.99
20-180 µm plankton	fp	June 20	26.72	2.22	-26.17	9.42	1.08	0.37	1.63
180-500 µm plankton	p	June 19-21	29.62	3.91	-32.02	22.46	4.92	4.21	5.46
Chaoborus	C	June 18-21	46.91	9.92	-30.83	19.38	4.01	3.30	4.56
Dytiscidae	DY	May 25-29	49.13	10.94	-30.57	6.41	0.20	-0.51	0.74
Daphnia	DA	June 19	37.34	6.84	-31.04	16.74	3.24	2.52	3.78

* Below Detection Limit

Carbon range is $5 * 10^{-6}$ to $28 * 10^{-6}$

Nitrogen range is $2 * 10^{-6}$ to $23 * 10^{-6}$

**Baseline Correction

Avg Baseline $\delta^{15}N$ ((ufd + fd + d)/3):	9.14
Max Baseline $\delta^{15}N$ (d)	7.28
Min Baseline $\delta^{15}N$ (ud)	11.56

Appendix 4.2B: Raw data from stable isotope analysis and trophic position calculations based on detrital $\delta^{15}N$ signatures at Test Pond 7.

Sample	Abbrev.	Collection Date (2001)	%C	%N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Trophic Position: $1+(^{15}N_{sample} - {}^{15}N_{base})/3.4$		
							(avg baseline**)	(min baseline**)	(max baseline**)
Benthic									
0.5-180 µm detritus	ufd	June 16	9.92	0.39	-25.70	4.15	0.95	0.89	1.00
180-500 µm detritus	d	June 15	25.58	1.01	-25.95	4.51	1.05	1.00	1.11
Derotanypus	D	May 30-June 5	44.53	8.14	-29.85	6.62	1.67	1.62	1.73
Tanypodinae	T	May 30-June 5	33.29	6.81	-29.33	6.94	1.77	1.71	1.82
Anisoptera	A	May 30-June 5	44.24	8.75	-28.75	7.86	2.04	1.99	2.09
Zygoptera	Z	May 30-June 4	47.45	10.30	-28.93	8.58	2.25	2.20	2.30
Misc. chironomids	M	June 1-5	45.47	8.62	-29.67	7.31	1.88	1.82	1.93
Gastropoda	G	June 5	42.27	10.34	-26.24	2.37	0.42	0.37	0.48
Pelagic									
0.5-20 µm plankton	up	June 7	4.31	0.25	-29.10*	4.59*	n/a	n/a	n/a
20-180 µm plankton	fp	June 7	20.91	3.36	-27.81	6.62	1.67	1.62	1.73
180-500 µm plankton	p	June 13-14	35.80	5.03	-28.40	8.03	2.09	2.04	2.14
Notonectidae	N	June 5	50.01	10.57	-28.47	5.20	1.26	1.20	1.31
Chaoborus	C	June 15-16	42.63	10.52	-28.88	8.77	2.31	2.25	2.36
Daphnia	DA	June 15	35.88	6.71	-29.40	7.18	1.84	1.79	1.89
Dytiscidae	DY	June 1	48.73	10.99	-25.41	4.98	1.19	1.14	1.25

* Below Detection Limit

Carbon range is $5 * 10^{-8}$ to $28 * 10^{-8}$

Nitrogen range is $2 * 10^{-8}$ to $23 * 10^{-8}$

**Baseline Correction

Avg Baseline δ15N ((ufd + fd)/2):	4.33
Max Baseline δ15N (ufd)	4.15
Min Baseline δ15N (d)	4.51

Appendix 4.2C: Raw data from stable isotope analysis and trophic position calculations based on detrital $\delta^{15}N$ signatures at High Sulphate.

Sample	Abbrev.	Collection Date (2001)	%C	%N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Trophic Position: $1+(^{15}N_{sample} - {}^{15}N_{base})/3.4$		
							(avg baseline**)	(min baseline**)	(max baseline**)
Benthic									
0.5-180 µm detritus	ufd	July 4	11.45	0.45	-27.44	3.10	1.16	1.00	1.49
20-180 µm detritus	fd	July 4	17.20	0.71	-27.24	3.09	1.16	1.00	1.49
180-500 µm detritus	d	July 4	28.59	1.48	-27.30	1.44	0.68	0.51	1.00
Chironomus	CH	July 3-5	45.84	10.65	-31.83	4.21	1.49	1.33	1.81
Misc. chironomids	M	July 3-5	43.30	9.23	-31.12	1.80	0.78	0.62	1.11
Anisoptera	A	July 3-5	42.86	10.46	-30.41	3.71	1.34	1.18	1.67
Zygoptera	Z	July 3-5	45.51	11.01	-31.01	4.14	1.47	1.31	1.79
Gastropoda	G	July 16	41.90	9.70	-29.95	2.56	1.00	0.84	1.33
Pelagic									
0.5-20 µm plankton	up	July 12	3.54	0.34	-28.52*	1.51*	n/a	n/a	n/a
0.5-180 µm plankton	ufp	July 12	2.41	0.24	-28.23*	1.39*	n/a	n/a	n/a
20-180 µm plankton	fp	July 12	26.75	2.61	-26.79	4.05	1.44	1.28	1.77
180-500 µm plankton	p	July 11-13	21.68	1.58	-27.74	7.82	2.55	2.39	2.88
Chaoborus	C	July 11-17	29.66	7.32	-30.45	4.98	1.72	1.55	2.04
Notonectidae	N	July 17	45.71	11.64	-28.91	3.81	1.37	1.21	1.70
Daphnia	DA	July 17	24.35	4.09	-25.89	2.64	1.03	0.86	1.35

* Below Detection Limit Carbon range is $5 * 10^{-6}$ to $28 * 10^{-6}$
Nitrogen range is $2 * 10^{-6}$ to $23 * 10^{-6}$

**Baseline Correction

Avg Baseline delta15N ((ufd + fd + d)/3):	2.54
Max Baseline δ15N (d)	1.44
Min Baseline δ15N (ufd)	3.10

Appendix 4.2D: Raw data from stable isotope analysis and trophic position calculations based on detrital $\delta^{15}N$ signatures at Shallow Wetland South Ditch.

Sample	Abbrev.	Collection Date (2001)	%C	%N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Trophic Position: $1+(^{15}N_{sample} - {}^{15}N_{base})/3.4$ (avg baseline**)	(min baseline**)	(max baseline**)
Benthic									
0.5-20 µm detritus	ud	July 23	3.15	0.21	-24.13*	-3.65*	n/a	n/a	n/a
0.5-180 µm detritus	ufd	July 23	5.40	0.33	-21.78*	-0.08	0.94	0.70	1.11
20-180 µm detritus	fd	July 23	5.10	0.32	-22.38*	-0.46	0.82	0.59	1.00
180-500 µm detritus	d	July 23	10.21	0.57	-17.25	0.95	1.24	1.00	1.41
Chironomus	CH	July 20-26	37.39	9.19	-28.21	2.23	1.62	1.38	1.79
Tanypodinae	T	July 20-30	44.52	10.46	-29.11	2.12	1.58	1.34	1.76
Anisoptera	A	July 24-26	41.94	10.67	-27.99	2.86	1.80	1.56	1.98
Zygoptera	Z	July 24	47.17	11.52	-27.90	3.51	1.99	1.75	2.17
Gastropoda	G	July 31	42.92	9.25	-27.68	1.31	1.35	1.11	1.52
Pelagic									
0.5-20 µm plankton	up	Aug. 1	2.72	0.27	-26.03*	2.03*	n/a	n/a	n/a
20-180 µm plankton	fp	Aug. 1	29.02	2.54	-22.34	3.98	2.13	1.89	2.31
180-500 µm plankton	p	Aug. 2	33.84	4.13	-25.64	11.60	4.37	4.13	4.55
Daphnia	DA	July 31	4.05	0.72	-25.9*	2.74	1.77	1.53	1.94
Notonectidae	N	July 31	46.32	11.72	-26.00	3.28	1.92	1.69	2.10
Chaoborus	C	July 28-31	45.27	11.28	-25.40	4.48	2.28	2.04	2.45

* Below Detection Limit

Carbon range is $5 * 10^{-8}$ to $28 * 10^{-8}$

Nitrogen range is $2 * 10^{-8}$ to $23 * 10^{-8}$

**Baseline Correction

Avg Baseline delta15N ((ufd + fd + d)/3):	0.14
Max Baseline δ15N (fd)	-0.46
Min Baseline δ15N (ud)	0.95

Appendix 5.1: Fresh weight concentrations of PAHs in adult insects collected near Shallow Wetland and in the Natural Wetland / Hummock Wetland area in 1998 (note: Total concentrations are converted to a dry weight basis). Source: J. Ciborowski, M. Whelly, C. Leonhardt, and D. Laing, University of Windsor, unpublished data.

Congener**	Shallow Wetland mosquitos, chironomids, and trichopterans (ng/g wet weight)	Natural Wetland / Hummock area dipterans (ng/g wet weight)
Napthalene*	180	180
Methyl naphthalene	54	100
C2 Naphthalene	32	81
C3 Naphthalene	32	62
C4 Naphthalene	58	33
Acenaphthylene*	3.2	5
Acenaphthene*	< 1.1	11
Fluroene*	< 0.42	13
Methyl fluorene	< 0.85	< 1.4
C2 Fluorene	< 1.4	< 1.6
Phenanthrene*	18	16
Anthracene*	< 0.43	< 0.76
Methyl phenanthrene/anthracene	59	23
C2 Phenanthrene/anthracene	300	82
C3 Phenanthrene/anthracene	79	18
C4 Phenanthrene/anthracene	100	27
Methyl dibenzothiophene	35	8.6
C2 Dibenzothiophene	130	39
C3 Dibenzothiophene	180	40
Fluoranthene*	4.9	3.2
Pyrene*	9.4	3.5
Methyl fluoranthene/pyrene	14	3.2
Benzo(a)anthracene*/Chrysene	8.8	2.7
Benzo(a)pyrene*	< 1.5	< 0.53
Indeno(c,d-123)pyrene*	< 0.21	< 0.12
Dibenzo(a,h)anthracene*	< 1.5	< 0.43
Benzo(ghi)perylene*	< 0.26	< 0.07
Benzo(e)pyrene	< 1.2	< 0.72
Benzofluoranthenes	< 1.8	< 0.61
C3 Fluorene	< 2.3	< 2.7
C4 Fluorene	66	2.7
benzo(b)naphtho(1,2-d)thiophene	25	5.9
C2 Fluoranthene/pyrenes	24	6
C3 Fluoranthene/pyrenes	25	4.9
C4 Fluoranthene/pyrenes	14	2.2
Perylene	1.2	< 0.75
% Moisture	**19**	**18**
Total of quanitfiable PAHs (ng/g dry weight)	**1816.7**	**964.5**
Total of quantifiable PAHs (mg/kg dry weight)	**1.8**	**1.0**

* Priority PAHs US EPA Protocol- Method 625

** bold faced congeners were not measured in larval samples from 2001 and 2002;Methyl acenaphthene, Biphenyl, Methyl biphenyl, C2 Biphenyl, Dibenzothiophene*, C4 Dibenzothiophene, Methyl benzo(a)anthracene/Chrysene, C2 Benzo(a)anthracene/Chrysene, Benzo(b&k)fluoranthene*, Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene, and C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene were measured in 2001 and 2002 larval samples but not in the above adult samples

Appendix 5.2A: Concentration of PAH congeners at various depths of the fine tails zone of Mildred Lake Settling Basin in 2000 (data courtesy of M. MacKinnon, Syncrude Canada Ltd.).

Congener	Molecular Weight	Concentration (mg/kg tailings) at Depth (m) 1	6	10	20	30
Napthalene*	128	0.0001	0.16	0.03	0.09	< 0.01
Methyl naphthalene	142	< 0.0001	0.49	0.1	0.26	0.02
C2 Naphthalene	156	< 0.0001	0.93	0.29	0.58	0.09
C3 Naphthalene	170	< 0.0001	6.8	3.4	5.4	1.2
C4 Naphthalene	184	< 0.0001	14	5.5	9.9	2.1
Acenaphthylene*	152	< 0.0001	< 0.01	< 0.01	< 0.01	< 0.01
Acenaphthene*	153	< 0.0001	0.5	0.27	0.35	0.08
Methyl acenaphthene	166	< 0.0001	0.21	0.1	0.15	0.05
Fluroene*	166	< 0.0001	0.51	0.37	0.37	0.11
Methyl fluorene	180	< 0.0001	3	2.3	2.5	0.77
C2 Fluorene	194	< 0.0001	12	7.4	8.7	2.4
Biphenyl	154	< 0.0001	0.1	0.04	0.07	0.03
Methyl biphenyl	168	< 0.0001	0.15	0.102	0.13	0.04
C2 Biphenyl	182	< 0.0001	< 0.02	< 0.02	< 0.02	< 0.02
Phenanthrene*	178	< 0.0001	3.5	2.7	2.5	0.78
Anthracene*	178	< 0.0001	0.21	0.16	0.12	0.05
Methyl phenanthrene/anthracene	192	0.0002	9.1	7.8	6.1	2
C2 Phenanthrene/anthracene	206	0.0012	29	20	16	5.1
C3 Phenanthrene/anthracene	220	0.007	19	18	16	6
C4 Phenanthrene/anthracene	234	0.0038	7.3	6.9	4.9	2
Dibenzothiophene*	184	< 0.0001	0.24	0.15	0.18	0.07
Methyl dibenzothiophene	198	0.0006	5.7	4.6	4.2	1.6
C2 Dibenzothiophene	212	0.0025	21	17	14	4.9
C3 Dibenzothiophene	226	0.0055	27	25	17	6.4
C4 Dibenzothiophene	240	0.0048	15	11	4.9	3.6
Fluoranthene*	202	< 0.0001	0.3	0.24	0.17	0.07
Pyrene*	202	0.0003	1.1	0.87	0.73	0.28
Methyl fluoranthene/pyrene	216	0.0011	3.3	3.2	2.1	0.9
Benzo(a)anthracene*/Chrysene	228	< 0.0001	1.4	1.2	1	0.41
Methyl benzo(a)anthracene/Chrysene	242	0.0011	2.2	2	1.6	0.7
C2 Benzo(a)anthracene/Chrysene	256	0.0015	0.4	2.8	2.1	0.93
Benzo(b&k)fluoranthene*	252	< 0.0001	0.27	0.24	0.22	0.09
Benzo(a)pyrene*	252	< 0.0001	0.12	0.07	0.09	0.03
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	266	0.0004	0.82	0.73	0.52	0.21
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	280	0.0003	0.4	0.27	0.24	0.1
Indeno(c,d-123)pyrene*	276	< 0.0001	0.04	0.04	< 0.01	0.01
Dibenzo(a,h)anthracene*	278	< 0.0001	0.03	0.02	0.18	0
Benzo(ghi)perylene*	276	< 0.0001	0.07	0.05	0.04	0.02
Total PAHs		0.0304	186.4	144.94	123.39	43.14
Total Extractable Hydrocarbon Content (i.e., bitumen content)		<100	24000	21000	23400	11800
Proportion of bitumen in sample		< 0.0001	0.024	0.021	0.0234	0.0118

* Priority PAHs US EPA Protocol- Method 625

Appendix 5.2B: Concentration of PAH congeners at various depths of the fine tails zone of Mildred Lake Settling Basin in 2001 (data courtesy of M. MacKinnon, Syncrude Canada Ltd.).

Congener	Molecular Weight	Concentration (mg/kg tailings) at Depth (m)				
		1	6	10	20	30
Napthalene*	128	0.00004	0.21	0.08	0.05	0.02
Methyl naphthalene	142	0.00004	0.53	0.22	0.12	0.04
C2 Naphthalene	156	0.00006	1.4	0.49	0.34	0.15
C3 Naphthalene	170	0.00005	15	5	6.2	2.8
C4 Naphthalene	184	< 0.00004	32	11	12	5.1
Acenaphthylene*	152	< 0.00002	0.03	0.01	0.01	< 0.01
Acenaphthene*	153	< 0.00002	0.88	0.32	0.37	0.17
Methyl acenaphthene	166	< 0.00004	1.6	0.63	0.68	0.37
Fluroene*	166	< 0.00002	0.72	0.25	0.3	0.15
Methyl fluorene	180	0.00007	5.5	2	1.9	0.83
C2 Fluorene	194	0.00036	15	6.5	5.3	2.3
Biphenyl	154	0.00015	0.27	0.17	0.08	0.05
Methyl biphenyl	168	< 0.00004	0.37	0.11	0.14	0.07
C2 Biphenyl	182	0.00012	1.2	0.99	0.57	0.26
Phenanthrene*	178	< 0.00002	6.4	2.4	2.4	0.84
Anthracene*	178	< 0.00002	0.07	0.11	0.04	< 0.01
Methyl phenanthrene/anthracene	192	0.00007	34	14	13	5.6
C2 Phenanthrene/anthracene	206	0.00023	50	17	18	8.2
C3 Phenanthrene/anthracene	220	0.00054	35	18	16	6.2
C4 Phenanthrene/anthracene	234	0.00014	14	5.3	6.3	3.1
Dibenzothiophene*	184	< 0.00002	0.19	0.06	0.12	0.08
Methyl dibenzothiophene	198	0.0006	12	4.4	4.3	2
C2 Dibenzothiophene	212	< 0.00004	37	14	13	6.3
C3 Dibenzothiophene	226	0.00067	39	22	16	7.2
C4 Dibenzothiophene	240	< 0.00004	40	13	14	4.4
Fluoranthene*	202	< 0.00002	0.22	0.16	0.09	0.04
Pyrene*	202	0.00003	0.5	0.33	0.25	0.15
Methyl fluoranthene/pyrene	216	0.00005	4.8	0.91	0.98	0.66
Benzo(a)anthracene*/Chrysene	228	0.00027	2.6	1	0.76	0.38
Methyl benzo(a)anthracene/Chrysene	242	0.00048	5.8	2.5	1.9	0.76
C2 Benzo(a)anthracene/Chrysene	256	< 0.00004	7.8	2.9	2.7	1
Benzo(b&k)fluoranthene*	252	0.00005	0.47	0.26	0.15	0.09
Benzo(a)pyrene*	252	< 0.00002	0.2	0.09	0.07	0.03
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	266	< 0.00004	0.81	0.65	0.43	0.19
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	280	0.00066	7.9	4.1	2.4	1
Indeno(c,d-123)pyrene*	276	< 0.00002	0.08	0.03	0.02	< 0.01
Dibenzo(a,h)anthracene*	278	< 0.00002	0.08	0.03	0.02	< 0.01
Benzo(ghi)perylene*	276	0.00004	0.2	0.08	0.05	0.02
Total PAHs		0.00472	373.8	151.1	141.04	60.55
Total Extractable Hydrocarbon Content (i.e., bitumen content)		<100	18900	20100	18900	8100
Proportion of bitumen in sample		< 0.0001	0.019	0.02	0.0189	0.008

* Priority PAHs US EPA Protocol- Method 625

Appendix 5.3: Concentrations of PAH congeners in semi-permeable membrane devices (SPMDs) at OSPM-affected and reference wetlands in 2001.

Congener	Mol. Weight	Concentration (µg / mL triolein)					
		Site					
		NW (OSPM)	TP7 (OSPM)	HS (Ref)	SWSD (Ref)	SWSD Trip Length	DL (µg)
Napthalene*	128	BDL	0.03	BDL	BDL	BDL	0.1
Methyl naphthalene	142	BDL	BDL	BDL	BDL	BDL	0.2
C2 Naphthalene	156	BDL	BDL	BDL	BDL	BDL	0.2
C3 Naphthalene	170	BDL	0.20	BDL	BDL	BDL	0.2
C4 Naphthalene	184	BDL	BDL	BDL	BDL	BDL	0.2
Acenaphthylene*	152	BDL	BDL	BDL	BDL	BDL	0.1
Acenaphthene*	153	BDL	BDL	BDL	BDL	BDL	0.1
Methyl acenaphthene	166	BDL	BDL	BDL	BDL	BDL	0.2
Fluroene*	166	0.03	BDL	BDL	BDL	BDL	0.1
Methyl fluorene	180	BDL	BDL	BDL	BDL	BDL	0.2
C2 Fluorene	194	0.07	BDL	BDL	BDL	BDL	0.2
Biphenyl	154	BDL	BDL	BDL	BDL	BDL	0.2
Methyl biphenyl	168	BDL	BDL	BDL	BDL	BDL	0.2
C2 Biphenyl	182	BDL	BDL	BDL	BDL	BDL	0.2
Phenanthrene*	178	BDL	BDL	BDL	BDL	BDL	0.1
Anthracene*	178	BDL	BDL	BDL	BDL	BDL	0.1
Methyl phenanthrene/anthracene	192	0.13	0.47	BDL	BDL	BDL	0.2
C2 Phenanthrene/anthracene	206	0.13	0.40	BDL	BDL	BDL	1.2
C3 Phenanthrene/anthracene	220	0.10	0.77	0.07	0.10	BDL	0.2
C4 Phenanthrene/anthracene	234	0.07	1.20	0.17	0.23	BDL	0.2
Dibenzothiophene*	184	BDL	BDL	BDL	BDL	BDL	0.1
Methyl dibenzothiophene	198	BDL	BDL	BDL	BDL	BDL	0.2
C2 Dibenzothiophene	212	BDL	0.33	BDL	BDL	BDL	0.2
C3 Dibenzothiophene	226	BDL	BDL	BDL	BDL	BDL	0.2
C4 Dibenzothiophene	240	BDL	0.20	BDL	BDL	BDL	0.2
Fluoranthene*	202	BDL	BDL	BDL	BDL	BDL	0.1
Pyrene*	202	BDL	BDL	BDL	BDL	BDL	0.1
Methyl fluoranthene/pyrene	216	BDL	0.10	0.07	BDL	BDL	0.2
Benzo(a)anthracene*/Chrysene	228	BDL	0.10	BDL	BDL	BDL	0.1
Methyl benzo(a)anthracene/Chrysene	242	BDL	BDL	BDL	BDL	BDL	0.2
C2 Benzo(a)anthracene/Chrysene	256	BDL	0.10	BDL	BDL	BDL	0.2
Benzo(b&k)fluoranthene*	252	BDL	BDL	BDL	BDL	BDL	0.1
Benzo(a)pyrene*	252	BDL	BDL	BDL	BDL	BDL	0.1
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	266	BDL	BDL	BDL	BDL	BDL	0.2
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	280	BDL	BDL	BDL	BDL	BDL	0.2
Indeno(c,d-123)pyrene*	276	BDL	BDL	BDL	BDL	BDL	0.1
Dibenzo(a,h)anthracene*	278	BDL	BDL	BDL	BDL	BDL	0.1
Benzo(ghi)perylene*	276	BDL	BDL	BDL	BDL	BDL	0.1
Total PAHs:		**0.53**	**3.90**	**0.30**	**0.33**	**0.00**	

* Priority PAHs US EPA Protocol- Method 625

Appendix 5.4: Total organic carbon normalized PAH concentrations** in the 180 - 500-µm detritus collected from OSPM-affected and reference wetlands in 2001.

Congener	Mol. Wt.	Concentration in 180 - 500-µm detritus (mg/kg organic carbon)** Site NW (OSPM)	TP7** (OSPM)	HS (Ref)	SWSD (Ref)	Det Lim (mg/kg)	TP7 Det Lim (mg/kg) ***
Napthalene*	128	BDL	BDL	BDL	BDL	0.04	0.2
Methyl naphthalene	142	BDL	BDL	BDL	BDL	0.08	0.4
C2 Naphthalene	156	5.13	31.25	1.33	25.00	0.08	0.4
C3 Naphthalene	170	0.73	41.67	BDL	BDL	0.08	0.4
C4 Naphthalene	184	2.53	127.08	BDL	BDL	0.08	0.4
Acenaphthylene*	152	BDL	BDL	BDL	BDL	0.04	0.2
Acenaphthene*	153	0.22	4.17	BDL	BDL	0.04	0.2
Methyl acenaphthene	166	BDL	BDL	BDL	BDL	0.08	0.4
Fluroene*	166	0.22	4.17	BDL	BDL	0.04	0.2
Methyl fluorene	180	1.61	20.83	BDL	BDL	0.08	0.4
C2 Fluorene	194	BDL	85.42	BDL	BDL	0.08	0.4
Biphenyl	154	BDL	BDL	BDL	BDL	0.08	0.4
Methyl biphenyl	168	BDL	BDL	BDL	BDL	0.08	0.4
C2 Biphenyl	182	BDL	BDL	BDL	BDL	0.08	0.4
Phenanthrene*	178	0.59	14.58	BDL	BDL	0.04	0.2
Anthracene*	178	BDL	BDL	BDL	BDL	0.04	0.2
Methyl phenanthrene/anthracene	192	8.42	141.67	0.74	1.92	0.08	0.4
C2 Phenanthrene/anthracene	206	15.75	437.5	0.66	BDL	0.08	0.4
C3 Phenanthrene/anthracene	220	20.51	416.67	1.37	BDL	0.08	0.4
C4 Phenanthrene/anthracene	234	43.96	770.83	5.86	BDL	0.08	0.4
Dibenzothiophene*	184	0.37	BDL	BDL	BDL	0.04	0.2
Methyl dibenzothiophene	198	1.65	77.08	BDL	BDL	0.08	0.4
C2 Dibenzothiophene	212	1.54	312.5	BDL	BDL	0.08	0.4
C3 Dibenzothiophene	226	10.99	333.33	1.41	BDL	0.08	0.4
C4 Dibenzothiophene	240	4.76	179.17	1.25	BDL	0.08	0.4
Fluoranthene*	202	0.18	BDL	BDL	BDL	0.04	0.2
Pyrene*	202	0.62	14.58	0.35	BDL	0.04	0.2
Methyl fluoranthene/pyrene	216	2.60	39.58	1.09	BDL	0.08	0.4
Benzo(a)anthracene*/Chrysene	228	1.03	14.58	0.23	BDL	0.04	0.2
Methyl benzo(a)anthracene/Chrysene	242	3.00	47.92	1.05	BDL	0.08	0.4
C2 Benzo(a)anthracene/Chrysene	256	3.00	52.08	1.72	BDL	0.08	0.4
Benzo(b&k)fluoranthene*	252	0.18	BDL	0.23	BDL	0.04	0.2
Benzo(a)pyrene*	252	BDL	BDL	BDL	BDL	0.04	0.2
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	266	BDL	BDL	BDL	BDL	0.08	0.4
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	280	0.37	BDL	BDL	BDL	0.08	0.4
Indeno(c,d-123)pyrene*	276	BDL	BDL	BDL	BDL	0.04	0.2
Dibenzo(a,h)anthracene*	278	BDL	BDL	BDL	BDL	0.04	0.2
Benzo(ghi)perylene*	276	BDL	BDL	BDL	BDL	0.04	0.2
Total PAHs		**129.96**	**3166.66**	**17.30**	**26.92**		
% Organic Carbon**:**		**27.3**	**4.8****	**25.6**	**5.2**		

* Priority PAHs US EPA Protocol- Method 625

** organic carbon content of the 180 - 500µm detritus was not determined at Test Pond 7 so the average of Test Ponds 1 (4.7%), 2 (5.7%), and 5 (4.1%) was used

*** detection limits raised due to low sample mass

**** see Nelson and Sommers 1996 for methods used to determine organic carbon contents

Appendix 5.5: Dry weight concentrations of PAHs in invertebrate samples collected from OSPM-affected and reference wetlands in 2001.

Congener	Concentration (mg / kg sample)								DL (mg/kg)
	Chaoborus				Tanypodinae (Chironomini at HS)				
	NW	TP7	HS	SWSD	NW	TP7	HS	SWSD	
Napthalene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Methyl naphthalene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
C2 Naphthalene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.04
C3 Naphthalene	BDL	BDL	BDL	BDL	BDL	0.22	BDL	BDL	0.04
C4 Naphthalene	BDL	BDL	BDL	BDL	BDL	0.38	BDL	BDL	0.04
Acenaphthylene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Acenaphthene*	BDL	BDL	BDL	BDL	BDL	0.02	BDL	BDL	0.02
Methyl acenaphthene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.04
Fluroene*	BDL	BDL	BDL	BDL	BDL	0.02	BDL	BDL	0.02
Methyl fluorene	BDL	BDL	BDL	BDL	BDL	0.12	BDL	BDL	0.04
C2 Fluorene	BDL	BDL	BDL	BDL	BDL	0.31	BDL	BDL	0.04
Biphenyl	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.04
Methyl biphenyl	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.04
C2 Biphenyl	BDL	BDL	BDL	BDL	BDL	0.06	BDL	BDL	0.04
Phenanthrene*	BDL	BDL	BDL	BDL	BDL	0.06	BDL	BDL	0.02
Anthracene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Methyl phenanthrene/anthracene	BDL	BDL	BDL	BDL	BDL	0.55	BDL	BDL	0.04
C2 Phenanthrene/anthracene	BDL	BDL	BDL	BDL	BDL	0.71	BDL	BDL	0.04
C3 Phenanthrene/anthracene	BDL	BDL	BDL	BDL	BDL	0.57	BDL	BDL	0.04
C4 Phenanthrene/anthracene	BDL	BDL	BDL	BDL	BDL	0.81	BDL	BDL	0.04
Dibenzothiophene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Methyl dibenzothiophene	BDL	BDL	BDL	BDL	BDL	0.24	BDL	BDL	0.04
C2 Dibenzothiophene	BDL	BDL	BDL	BDL	BDL	0.65	BDL	BDL	0.04
C3 Dibenzothiophene	BDL	BDL	BDL	BDL	BDL	0.82	BDL	BDL	0.04
C4 Dibenzothiophene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.04
Fluoranthene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Pyrene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Methyl fluoranthene/pyrene	BDL	BDL	BDL	BDL	BDL	0.11	BDL	BDL	0.04
Benzo(a)anthracene*/Chrysene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Methyl benzo(a)anthracene/Chrysene	BDL	BDL	BDL	BDL	BDL	0.07	BDL	BDL	0.04
C2 Benzo(a)anthracene/Chrysene	BDL	BDL	BDL	BDL	BDL	0.09	BDL	BDL	0.04
Benzo(b&k)fluoranthene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Benzo(a)pyrene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.04
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.04
Indeno(c,d-123)pyrene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Dibenzo(a,h)anthracene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Benzo(ghi)perylene*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
Lipid Content (%)	1.2	0.4	0.5	0.4	1.5	1.1	0.4	0.5	

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Appendix 5.6: Dry weight and bitumen corrected concentration of PAHs in Tanypodinae and *Chaoborus* collected from Test Pond 7, 2002.

Congener	Mol. Wt.	Tanypodinae		*Chaoborus*		dry weight Det Lim (mg/kg)
		(mg/kg dry weight)	(mg/kg bitumen)	(mg/kg dry weight)	(mg/kg bitumen)	
Napthalene*	128	BDL	BDL	BDL	BDL	0.02
Methyl naphthalene	142	BDL	BDL	BDL	BDL	0.02
C2 Naphthalene	156	BDL	BDL	BDL	BDL	0.02
C3 Naphthalene	170	BDL	BDL	BDL	BDL	0.02
C4 Naphthalene	184	BDL	BDL	BDL	BDL	0.02
Acenaphthylene*	152	BDL	BDL	BDL	BDL	0.02
Acenaphthene*	153	BDL	BDL	BDL	BDL	0.02
Methyl acenaphthene	166	BDL	BDL	BDL	BDL	0.02
Fluroene*	166	BDL	BDL	BDL	BDL	0.02
Methyl fluorene	180	0.04	36.36	BDL	BDL	0.02
C2 Fluorene	194	0.19	172.73	BDL	BDL	0.02
Biphenyl	154	BDL	BDL	BDL	BDL	0.02
Methyl biphenyl	168	BDL	BDL	BDL	BDL	0.02
C2 Biphenyl	182	BDL	BDL	BDL	BDL	0.02
Phenanthrene*	178	0.04	36.36	BDL	BDL	0.02
Anthracene*	178	BDL	BDL	BDL	BDL	0.02
Methyl phenanthrene/anthracene	192	0.37	336.36	0.12	196.72	0.02
C2 Phenanthrene/anthracene	206	0.46	418.18	0.08	131.15	0.02
C3 Phenanthrene/anthracene	220	0.23	209.09	BDL	BDL	0.02
C4 Phenanthrene/anthracene	234	0.85	772.73	0.04	65.57	0.02
Dibenzothiophene*	184	BDL	BDL	BDL	BDL	0.02
Methyl dibenzothiophene	198	0.14	127.27	0.04	65.57	0.02
C2 Dibenzothiophene	212	0.42	381.82	0.04	65.57	0.02
C3 Dibenzothiophene	226	0.30	272.73	BDL	BDL	0.02
C4 Dibenzothiophene	240	0.03	27.27	BDL	BDL	0.02
Fluoranthene*	202	BDL	BDL	BDL	BDL	0.02
Pyrene*	202	BDL	BDL	BDL	BDL	0.02
Methyl fluoranthene/pyrene	216	BDL	BDL	BDL	BDL	0.02
Benzo(a)anthracene*/Chrysene	228	BDL	BDL	BDL	BDL	0.02
Methyl benzo(a)anthracene/Chrysene	242	BDL	BDL	BDL	BDL	0.02
C2 Benzo(a)anthracene/Chrysene	256	0.04	36.36	BDL	BDL	0.02
Benzo(b&k)fluoranthene*	252	BDL	BDL	BDL	BDL	0.02
Benzo(a)pyrene*	252	BDL	BDL	BDL	BDL	0.02
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	266	BDL	BDL	BDL	BDL	0.02
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	280	BDL	BDL	BDL	BDL	0.02
Indeno(c,d-123)pyrene*	276	BDL	BDL	BDL	BDL	0.02
Dibenzo(a,h)anthracene*	278	BDL	BDL	BDL	BDL	0.02
Benzo(ghi)perylene*	276	BDL	BDL	BDL	BDL	0.02
Total PAHs		**3.11**	**2827.27**	**0.32**	**524.59**	
Total Extractable Hydrocarbon Content (i.e. bitumen content - mg / kg dry weight)		**1100**		**610**		**5**
proportion of bitumen by dry weight		**0.0011**		**0.0006**		

* Priority PAHs US EPA Protocol- Method 625

Appendix 5.7A: Ratio of individual PAHs to bitumen content at various depths of Mildred Lake Settling Basin, 2000.
Source: Mike MacKinnon, Syncrude Canada Ltd., Edmonton, AB

Congener	Mildred Lake Settling Basin (MLSB) PAH Concentrations (mg/kg bitumen)**, 2000				
	Depth (m)				
	1	6	10	20	30
Napthalene*	1.00	6.67	1.43	3.85	0.00
Methyl naphthalene	0.00	20.42	4.76	11.11	1.69
C2 Naphthalene	0.00	38.75	13.81	24.79	7.63
C3 Naphthalene	0.00	283.33	161.90	230.77	101.69
C4 Naphthalene	0.00	583.33	261.90	423.08	177.97
Acenaphthylene*	0.00	0.00	0.00	0.00	0.00
Acenaphthene*	0.00	20.83	12.86	14.96	6.78
Methyl acenaphthene	0.00	8.75	4.76	6.41	4.24
Fluroene*	0.00	21.25	17.62	15.81	9.32
Methyl fluorene	0.00	125.00	109.52	106.84	65.25
C2 Fluorene	0.00	500.00	352.38	371.79	203.39
Biphenyl	0.00	4.17	1.90	2.99	2.54
Methyl biphenyl	0.00	6.25	4.86	5.56	3.39
C2 Biphenyl	0.00	0.00	0.00	0.00	0.00
Phenanthrene*	0.00	145.83	128.57	106.84	66.10
Anthracene*	0.00	8.75	7.62	5.13	4.24
Methyl phenanthrene/anthracene	2.00	379.17	371.43	260.68	169.49
C2 Phenanthrene/anthracene	12.00	1208.33	952.38	683.76	432.20
C3 Phenanthrene/anthracene	70.00	791.67	857.14	683.76	508.47
C4 Phenanthrene/anthracene	38.00	304.17	328.57	209.40	169.49
Dibenzothiophene*	0.00	10.00	7.14	7.69	5.93
Methyl dibenzothiophene	6.00	237.50	219.05	179.49	135.59
C2 Dibenzothiophene	25.00	875.00	809.52	598.29	415.25
C3 Dibenzothiophene	55.00	1125.00	1190.48	726.50	542.37
C4 Dibenzothiophene	48.00	625.00	523.81	209.40	305.08
Fluoranthene*	0.00	12.50	11.43	7.26	5.93
Pyrene*	3.00	45.83	41.43	31.20	23.73
Methyl fluoranthene/pyrene	11.00	137.50	152.38	89.74	76.27
Benzo(a)anthracene*/Chrysene	0.00	58.33	57.14	42.74	34.75
Methyl benzo(a)anthracene/Chrysene	11.00	91.67	95.24	68.38	59.32
C2 Benzo(a)anthracene/Chrysene	15.00	16.67	133.33	89.74	78.81
Benzo(b&k)fluoranthene*	0.00	11.25	11.43	9.40	7.63
Benzo(a)pyrene*	0.00	5.00	3.33	3.85	2.54
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	4.00	34.17	34.76	22.22	17.80
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	3.00	16.67	12.86	10.26	8.47
Indeno(c,d-123)pyrene*	0.00	1.67	1.90	0.00	0.85
Dibenzo(a,h)anthracene*	0.00	1.25	0.95	7.69	0.00
Benzo(ghi)perylene*	0.00	2.92	2.38	1.71	1.69

* Priority PAHs US EPA Protocol- Method 625

** for 1m depth, proportion of bitumen in sample assumed to be 0.0001, congeners below detection limits assumed to be at a concentration of 0, see Appendix 5.2A for bitumen contents at other depths

Appendix 5.7B: Ratio of individual PAHs to bitumen content at various depths of Mildred Lake Settling Basin, 2001.
Source: Mike MacKinnon, Syncrude Canada Ltd., Edmonton, AB

Congener	Mildred Lake Settling Basin (MLSB) PAH Concentrations (mg/kg bitumen)**, 2001				
	Depth (m)				
	1	6	10	20	30
Napthalene*	0.40	11.11	3.98	2.65	2.47
Methyl naphthalene	0.40	28.04	10.95	6.35	4.94
C2 Naphthalene	0.60	74.07	24.38	17.99	18.52
C3 Naphthalene	0.50	793.65	248.76	328.04	345.68
C4 Naphthalene	0.00	1693.12	547.26	634.92	629.63
Acenaphthylene*	0.00	1.59	0.50	0.53	0.00
Acenaphthene*	0.00	46.56	15.92	19.58	20.99
Methyl acenaphthene	0.00	84.66	31.34	35.98	45.68
Fluroene*	0.00	38.10	12.44	15.87	18.52
Methyl fluorene	0.70	291.01	99.50	100.53	102.47
C2 Fluorene	3.60	793.65	323.38	280.42	283.95
Biphenyl	1.50	14.29	8.46	4.23	6.17
Methyl biphenyl	0.00	19.58	5.47	7.41	8.64
C2 Biphenyl	1.20	63.49	49.25	30.16	32.10
Phenanthrene*	0.00	338.62	119.40	126.98	103.70
Anthracene*	0.00	3.70	5.47	2.12	0.00
Methyl phenanthrene/anthracene	0.70	1798.94	696.52	687.83	691.36
C2 Phenanthrene/anthracene	2.30	2645.50	845.77	952.38	1012.35
C3 Phenanthrene/anthracene	5.40	1851.85	895.52	846.56	765.43
C4 Phenanthrene/anthracene	1.40	740.74	263.68	333.33	382.72
Dibenzothiophene*	0.00	10.05	2.99	6.35	9.88
Methyl dibenzothiophene	6.00	634.92	218.91	227.51	246.91
C2 Dibenzothiophene	0.00	1957.67	696.52	687.83	777.78
C3 Dibenzothiophene	6.70	2063.49	1094.53	846.56	888.89
C4 Dibenzothiophene	0.00	2116.40	646.77	740.74	543.21
Fluoranthene*	0.00	11.64	7.96	4.76	4.94
Pyrene*	0.30	26.46	16.42	13.23	18.52
Methyl fluoranthene/pyrene	0.50	253.97	45.27	51.85	81.48
Benzo(a)anthracene*/Chrysene	2.70	137.57	49.75	40.21	46.91
Methyl benzo(a)anthracene/Chrysene	4.80	306.88	124.38	100.53	93.83
C2 Benzo(a)anthracene/Chrysene	0.00	412.70	144.28	142.86	123.46
Benzo(b&k)fluoranthene*	0.50	24.87	12.94	7.94	11.11
Benzo(a)pyrene*	0.00	10.58	4.48	3.70	3.70
Methyl benzo(b&k)fluoranthene/Benzo(a)pyrene	0.00	42.86	32.34	22.75	23.46
C2 Benzo(b&k)fluoranthene/Benzo(a)pyrene	6.60	417.99	203.98	126.98	123.46
Indeno(c,d-123)pyrene*	0.00	4.23	1.49	1.06	0.00
Dibenzo(a,h)anthracene*	0.00	4.23	1.49	1.06	0.00
Benzo(ghi)perylene*	0.40	10.58	3.98	2.65	2.47

* Priority PAHs US EPA Protocol- Method 625

** for 1m depth, proportion of bitumen in sample assumed to be 0.0001, congeners below detection limits assumed to be at a concentration of 0, see Appendix 5.2B for bitumen contents at other depths

Appendix 5.7C: Calculation of bioaccumulation factors for congeners detected in Tanypodinae and *Chaoborus* collected from Test Pond 7 in 2002 based on the the ratio of PAHs to bitumen in the samples divided by the average ratio of PAHs to bitumen at depths of 1, 6, 10, 20, and 30 m in the fine tails zone of Mildred Lake Settling Basin, 2000 and 2001.
Source: Mike MacKinnon, Syncrude Canada Ltd., Edmonton, AB

Congener	MLSB fine tails Avg (2000 & 2001 depths 1 - 30 m) (mg/kg bitumen)	Std. Dev	Tanypodinae (mg/kg bitumen)	*Chaoborus* (mg/kg bitumen)	Bioaccumulation Factors (mg/kg bitumen $_{organism}$ / mg/kg bitumen $_{avg\ MLSB\ fine\ tails}$)	
					Tanypodinae	*Chaoborus*
N*	3.35	3.37	BDL	BDL	BDL	BDL
N1	8.87	9.13	BDL	BDL	BDL	BDL
N2	22.05	21.73	BDL	BDL	BDL	BDL
N3	249.43	227.74	BDL	BDL	BDL	BDL
N4	495.12	486.92	BDL	BDL	BDL	BDL
AC*	0.26	0.51	BDL	BDL	BDL	BDL
AE*	15.85	13.34	BDL	BDL	BDL	BDL
AE1	22.18	27.42	BDL	BDL	BDL	BDL
F*	14.89	10.96	BDL	BDL	BDL	BDL
F1	100.08	80.50	36.36	BDL	0.36	BDL
F2	311.26	230.63	172.73	BDL	0.55	BDL
BPh	4.63	4.17	BDL	BDL	BDL	BDL
BPh1	6.12	5.52	BDL	BDL	BDL	BDL
BPh2	17.62	24.25	BDL	BDL	BDL	BDL
P*	113.61	94.47	36.36	BDL	0.32	BDL
A*	3.70	3.16	BDL	BDL	BDL	BDL
PA1	505.81	526.24	336.36	196.72	0.66	0.39
PA2	874.70	746.58	418.18	131.15	0.48	0.15
PA3	727.58	509.09	209.09	BDL	0.29	BDL
PA4	277.15	206.07	772.73	65.57	2.79	0.24
D*	6.00	3.84	BDL	BDL	BDL	BDL
D1	211.19	174.11	127.27	65.57	0.60	0.31
D2	684.29	544.18	381.82	65.57	0.56	0.10
D3	853.95	594.26	272.73	BDL	0.32	BDL
D4	575.84	599.03	27.27	BDL	0.05	BDL
FL*	6.64	4.47	BDL	BDL	BDL	BDL
PY*	22.01	14.93	BDL	BDL	BDL	BDL
FL1/PY	90.00	75.28	BDL	BDL	BDL	BDL
BA*/C	47.01	37.75	BDL	BDL	BDL	BDL
BA1/C	95.60	83.65	BDL	BDL	BDL	BDL
BA2/C	115.68	118.09	36.36	BDL	0.31	BDL
BbkF*	9.71	6.95	BDL	BDL	BDL	BDL
BAP*	3.72	2.95	BDL	BDL	BDL	BDL
BbK1/BAP	23.44	13.58	BDL	BDL	BDL	BDL
BbK2/BAP	93.03	134.09	BDL	BDL	BDL	BDL
IP*	1.12	1.33	BDL	BDL	BDL	BDL
DA*	1.67	2.47	BDL	BDL	BDL	BDL
BP*	2.88	2.95	BDL	BDL	BDL	BDL

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