Determining food web impacts on experimental aquatic systems from the disposal of oil sands process-affected waste materials

by

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Abstract

Current mining operators in the Athabasca oil sands deposit of Alberta, Canada have made commitments to zero discharge of oil sands process-affected waste materials (OSPM) from the mine site and rehabilitation of mined lands to a pre-mining state. As part of aquatic reclamation efforts, experimental test sites that contain a range of OSPM (solid and liquid components) were constructed to monitor the evolution and viability of aquatic habitats used as disposal sinks for OSPM produced by mining activities. In the present study, stable isotopes of carbon, nitrogen and sulphur were used to gauge some of the potential effects of OSPM site construction methods on aquatic food webs. Carbon and nitrogen isotopic signatures of sediment, dissolved inorganic carbon, dissolved organic carbon, particulate organic matter, periphytic material, plants, plankton, aquatic invertebrates and fish were used to assess differences related to the naphthenic acid (NA) concentration in OSPM and reference sites. For statistical analyses, sites were grouped into low (0 to 4 mg/L), medium (4 to 15 mg/L) and high (> 15 mg/L) NA concentrations. There were no significant differences in food web area or food web length among the low, medium and high NA concentration sites. In most cases, sample carbon isotope analyses of low, medium and high NA concentration sites were not significantly different, suggesting food web carbon sources did not include significant contributions from OSPM materials at OSPM sites. Significant differences, however, were found in the sample nitrogen isotope signatures between low, medium and high NA concentration sites. Ammonia from OSPM is suggested to be the main contributor to $\delta^{15}N$ enrichment.

To determine the potential effects of site construction and OSPM within experimental test sites, carbon and sulphur stable isotopes of water, plankton, aquatic invertebrates and fish were analyzed. With the exception of *Chaoborus* and *Haliplus,* all carbon isotope signatures were not

significantly different in constructed and reference sites. Also with the exception of *Haliplus*, sulphur isotope values for aquatic organisms from constructed and reference sites were significantly different. Aquatic organisms and water samples from constructed sites built in, or close, to the boundary of Kcw clays typically had $\delta^{34}S < 0$ ‰. Coinciding with depleted $\delta^{34}S$ signatures found in these aquatic systems were elevated sulphate concentrations. The waters at experimental test sites are in direct contact with the soil materials that facilitate the accumulation of sulphates as a result of the oxidation of substrate sulphide minerals. In general the results of the study suggest that aquatic food web structure and function do not change with the introduction of OSPM. Shifts in isotopic signatures suggestive of changes in food web structure, however, do occur when site construction exposes Kcw clays in the substrate.

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Chapter 1. General Introduction

1.1 Overview

Canada possesses three quarters of the world's oil sands deposits. One of these, the Athabasca Deposit in northeastern Alberta, is located close to the surface and is easily exploited with surface mining. Syncrude Canada Ltd. (SCL), is the largest of the three mining companies currently operating in the area, with over 500 kt of ore being processed daily. Since its inception in 1978, the mining activities of SCL have disturbed an area of over 18 000 Ha. During the extraction and refining of bitumen from oil sands, large volumes of process-affected water (15 m^3 per m^3 of synthetic crude oil) and tailings are produced (MacKinnon, 1989). The waste, or oil sands process-affected waste materials (OSPM), must be disposed of in an environmentally safe manner. As production from the oil sands grows, the problem of disposal will become more acute, and the need to improve our understanding of the consequences of various disposal options will become ever more important.

Oil sands deposits are typically located 30-90 m beneath the earth's surface. Access to deposits requires the removal of overburden material (vegetation, topsoil and clays) (Fine Tailings Fundamentals Consortium, 1995). The exposed oil-bearing sands are then removed and transported to the extraction plant, where oil is extracted from the sand using the Clark Hot Water Extraction method. Hot water, caustic (sodium hydroxide) and steam are mixed with the oil sands in a large industrial tumbler to separate the bitumen from the sand. The bitumen floats to the top and is removed and refined into synthetic crude oil. The remaining mixture, an aqueous slurry of water, silt, sand, clay and unrecovered bitumen referred to as tailings (Fine Tailings Fundamentals Consortium, 1995) is pumped into large settling basins. In the settling basin, the tailings segregate. Coarse sand and clays form the edges, while the

finer clays and sands fill the basin. After a period of time, the tailings densify to form mature fine tails (MFT), a mixture of water, fines and clays, and residual bitumen (Boerger et al., 1992). At the present rates of oil sands extraction, over 1 billion m^3 of OSPM will be created during the life of the existing leases and require disposal in an environmentally responsible manner (Nelson et al., 1995).

SCL has considered several different reclamation methods to prevent negative impacts associated with the disposal of process wastes on the mining site and in the local receiving environment. One of the proposed reclamation methods, the "wet landscape" approach, involves capping MFT with a layer of OSPM or non-OSPM water to form a constructed lake. The water layer must be of sufficient depth (>3 m) to ensure that mixing and re-suspension of MFT materials does not occur. If mixing does not occur, a lake ecosystem can develop naturally in the capping water. Since the "wet landscape" approach will take several years to implement, several aquatic experimental test sites were developed to monitor and study the evolution of aquatic habitats differentially affected by process-affected waste materials. It is expected that studies from smaller scale experimental test sites related to aquatic food web structure and function will aid in the design and development of larger scale constructed lakes and wetlands for OSPM disposal.

In general, stable isotope techniques provide a powerful tool for the study of food webs (Peterson et al., 1985) and may help in understanding the impacts of OSPM on local food webs found on oil sands deposits. In recent years the use of stable isotopes to evaluate aquatic food web structure and function has become increasingly popular in ecological studies because the predictable differences in the carbon (C), nitrogen (N) and sulphur (S) isotope ratios between consumers and their diet provide information on energy flows,

nutrient sources and trophic relationships (Goering et al., 1990; Peterson and Fry, 1987; Yoshioka and Wada, 1994). The C and S isotopic composition of animals are usually similar to their diets and can indicate the dietary source(s) important for consumers (Peterson and Fry, 1987). Consumption-related changes in carbon appear to be modest, with increases ranging from 0.0 to 1.0 ‰ per trophic transfer (DeNiro and Epstein, 1978; Fry and Sherr, 1984). Sulphur isotopes also have limited fractionation (< 1 ‰ per trophic transfer) and can be used as a reliable indicator of which plant or bacterial food sources are most important for consumers (Mekhtiyeva et al., 1976; Peterson et al., 1986; Peterson and Fry, 1987). Nitrogen isotopic signatures, however, change more dramatically with each trophic transfer, increasing an average of 3 to 5 ‰ with each trophic link (Peterson and Fry, 1987). Since C, S and N isotopes behave differently from one another when passed between trophic levels, it is possible to cross reference observed isotopic changes between predator and prey to infer trophic structure and food web connectivity, thereby creating a more accurate picture of food web relationships (Fry, 1983; Peterson et al., 1985) and providing a possible means by which the cascade impacts of contaminants through aquatic food webs may be studied (Power et al., 2002).

The potential effects of OSPM on biota have been studied using a variety of organisms and techniques with mixed results. Whelly (1998) found that the relative abundance of chironomids was always higher at OSPM sites than at comparable reference sites. However, the composition of wetland benthic invertebrate communities in general, and chironomid communities in particular, were different in OSPM sites than in reference wetlands (Whelly, 1998). Bendell-Young et al. (2000) noted that while invertebrate abundance was equal in OSPM sites and reference sites, invertebrate richness differed. Work

by Leung et al. (2001) determined there were no significant effects on phytoplankton communities in OSPM sites greater than 5 years old. Leonhardt (2003) also noted that within five years of construction, zoobenthic abundance in OSPM sites was similar to reference sites, and that within 5-7 years of construction the richness in the zoobenthic community of OSPM sites was also similar to reference wetlands. Overall, previous research has tended to support the argument that age is a significant factor in reducing OSPM toxicity to aquatic organisms. Although each of these studies has contributed to an improved understanding of the possible ecological risks associated with OSPM reclamation, all of the studies focused on a specific species or trophic level. To date, no attempt has been made to examine the wider food web related impacts of OSPM introduction, or to trace the possible bioaccumulative effects of OSPM in aquatic environments

To better understand the "wet landscape" approach as a disposal method for OSPM and its implications for the establishment of naturalized ecosystems, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ signatures of representative inorganic and biotic samples were used to determine some of the potential effects of OSPM and site construction on elements of aquatic food webs in the experimental test sites. Specifically, this thesis examines selected components of reference and OSPM aquatic food webs to determine if OSPM sites differ from reference sites. In particular, the thesis has two objectives. The first objective was to compare and contrast key elements of the food webs found in OSPM sites with those found in reference wetland areas located on, or near, oil sands lease properties (Chapter 2). The second objective was to determine if there were any detectable effects of wetland construction on the aquatic food webs and to determine the implications of construction for the establishment of naturalized aquatic ecosystems (Chapter 3).

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Chapter 2. The use of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to determine food web impacts on experimental aquatic systems from the disposal of oil sands process-affected waste materials.*

Abstract

Current mining operations in the Athabasca oil sands deposit of Alberta, Canada follow a zero discharge policy for oil sands process-affected waste materials (OSPM) from their sites. In this study, stable isotopes of carbon and nitrogen have been used to gauge some of the potential effects of OSPM on aquatic food webs. This has involved the measurement of $\delta^{13}C$ and $\delta^{15}N$ in sediment, DIC, DOC, POM, periphytic material, plants, plankton, aquatic invertebrates and fish from sites with and without OSPM. For statistical analyses sites were grouped into low (0 to 4 mg/L), medium (4 to 15 mg/L) and high (> 15 mg/L) naphthenic acid (NA) concentrations, as an indicator of the degree of similarity to OSPM impacted sites. Results of consumer food web area and food web length comparisons from low, medium and high NA concentration sites showed no significant differences. In most cases carbon isotope analyses of samples from low, medium and high NA concentration sites were not significantly different, suggesting food web carbon sources did not include significant contributions from OSPM materials at OSPM sites. Significant differences, however, were shown for nitrogen isotope signatures between low, medium and high NA concentration sites. Analysis suggests ammonia originating from OSPM was the main contributor to $\delta^{15}N$ enrichment.

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2.1 Introduction

Canada possesses three quarters of the world's oil sands deposits. One of these, the Athabasca deposit, Alberta, Canada, is located close to the surface and can be accessed with surface mining technology. Syncrude Canada Ltd. (SCL) is the largest mining operator on the Athabasca deposit and the largest single source producer of synthetic crude oil in Canada. When mining operations began in 1978, SCL committed to zero discharge of oil sands process-affected waste materials (OSPM) from the mine site and undertook to rehabilitate mined lands to a pre-mining state. Integral to this commitment has been the development of OSPM disposal strategies involving water capping of mining-related liquid and solid wastes through the construction of naturalized lakes and wetlands (Fine Tailings Fundamentals Consortium, 1995). To date, a number of studies have been completed to characterize and quantify the potential aquatic impacts of OSPM water capping including: water chemistry characterization (van den Heuvel et al., 1999a; van den Heuvel et al., 1999b), plankton dynamics (Leung, 2001), benthic invertebrate communities (Gould, 2000) and fish population responses (van den Heuvel et al., 1999a; van den Heuvel et al., 1999b; Murchie and Power, 2004). Although each of these studies has contributed to an improved understanding of the possible ecological risks associated with water capping, all of the studies focused on specific species or trophic levels. To date, no attempt has been made to examine wider food web related impacts of water capping or to trace the possible bioaccumulative effects of OSPM in aquatic environments.

One of the more studied water capping methods, the “wet landscape” approach, involves covering a mixture of water, fine clays (<22 μm) and residual bitumen (Boerger et al., 1992) referred to as mature fine tails (MFT), with a layer of OSPM or non-OSPM water

to form a constructed lake. The water layer must be of sufficient depth (>3 m) to ensure that mixing and resuspension of MFT materials does not occur, thus allowing a viable lake ecosystem to develop in the capping water. The scale of oil sands mining and waste disposal implies that any reclamation solution will need to be implemented on a large scale. Therefore, to test the feasibility of the proposed "wet landscape" option for reclamation and to monitor the evolution of water capped systems containing OSPM under natural conditions, several aquatic experimental test sites were constructed at SCL's Mildred Lake site. The experimental sites vary in size, water cap composition (OSPM water or non-OSPM water) and MFT content.

Naphthenic acids (NAs), naturally occurring surfactants associated with the bitumen in the oil sands, are found in OSPM water. NAs are the principal acute toxicants produced in OSPM water resulting from oil sands processing (Alberta Environmental Protection, 1996; Schramm et al., 2000). Studies on aquatic organisms have demonstrated that different species and life stages show differential sensitivity to NAs (Patrick et al., 1968; Dokholyan and Magomedoz, 1984; Verbek et al., 1994). Verbeek et al. (1994) examined the effects of NAs from OSPM (Mildred Lake Settling Basin) on rainbow trout (*Onchorynchus mykiss*) and found that they were approximately three times more sensitive than bacterial endpoints (Microtox ® bacterial assay) and approximately seven times more sensitive than *Daphnia magna*. The complex nature of NAs, as well as the simultaneous presence of other OSPM constituents such as alkylated polycyclic aromatic hydrocarbons, however, make it difficult to assess the overall ecotoxicological significance of the OSPM mixture. Furthermore, the differential sensitivities of test organisms suggest that holistic ecosystem approaches will be required to assess the overall ecotoxicological significance of exposure to OSPM.

In recent years, the use of stable isotopes to evaluate aquatic food web structure and dynamics has increased in ecological studies because predictable differences in the carbon (C) and nitrogen (N) isotope ratios between consumers and their diet provide information on energy flows, nutrient sources and trophic relationships (Goering et al., 1990; Peterson and Fry, 1987; Yoshioka and Wada, 1994). The C isotopic composition of animals is similar to their diets, increasing between 0.0 to 1.0 ‰ per trophic level (DeNiro and Epstein, 1978; Fry and Sherr, 1984). Nitrogen isotopes, however, change more dramatically with each trophic transfer, increasing an average of 3 to 5 ‰ with each trophic link (Peterson and Fry, 1987). Since C and N isotopes behave differently from one another when passed between trophic levels, it is possible to cross-reference observed isotopic changes between predator and prey to infer trophic structure and food web connectivity, thereby creating a more accurate picture of food web relationships (Fry, 1983; Peterson et al., 1985) and the possible means by which contaminant impacts cascade through aquatic food webs (Power et al., 2002).

In view of the deficiency of food web related impact information on the water capping of OSPM and the wide availability of stable isotope analytical technologies, this study was undertaken to improve understanding of the trophic impacts of water capping. Specifically, the objective of the study was to compare and contrast the isotopic signatures of OSPM resident taxa from multiple trophic levels with similar taxa found in reference wetlands located on and near oil sands lease properties. As a working hypothesis, the study postulated that differences among common taxa from the study sites would be related to the differences in the concentration of NA compounds found in each site.

2.2 Materials and Methods

The study was conducted in 2003 at sites located on and/or near the SCL oil sands lease, northeast of Fort McMurray, Alberta (56°39'N, 111°13'W) (Fig. 1). Eight study sites were chosen in total (Fig. 1), four reference sites and four oil sands process-affected sites (Table 1). Sites were chosen based on age, accessibility, and the presence/absence of fish, as well as to include a gradient of OSPM. All sites were > 10 years old when sampled. Beaver Creek Reservoir (BCR) and Demonstration Pond (DP) are the only sites that contain fish. Of the eight sites, four contain neither OSPM water nor MFT (E1, BCR, Barge Marsh (BM), Shallow Wetland (SWL)), two contain no OSPM water and MFT (E3, DP), one contains OSPM water and MFT (E5) and one contains only MFT with water consolidated from MFT (E7) (Table 1). E1, E3, E5 and E7 were originally excavated to a maximum depth of 5 metres in 1989 and all but E1 were filled with MFT (Table 1). E1 received 1000 m^3 of soil substrate to bring it to a common depth with the other test sites, and was filled with 1000 m^3 of non-OSPM water. E3 and E5 were both capped with 1000 m^3 of water, however E3 received non-OSPM water and E5 received OSPM water. DP was excavated to a depth of >12 metres in 1993, filled with MFT and then capped with 2.5 metres or 70,000 m^3 of non-OSPM water. E1, E3 and DP were filled with local muskeg drainage water, considered here as non-OSPM water. Details of site water chemistry and NA concentrations are given in Table 1.

Reference sites selected for study included E1, BM, BCR and SWL. None of the selected reference sites contain OSPM water and/or MFT (Table 1). E1 is physically similar to the other selected experimental tests sites and was constructed at the same time and in the same manner (Table 1). BM is a shallow pit, formed in 1977 and located approximately 6.5

km north of the SCL lease site (Fig. 1, Table 1). BCR is located on the mine lease and was formed by the impoundment of the Upper Beaver Creek in 1975. SWL is also located on the SCL lease site and was created in 1993 when a berm was built during the construction of the DP.

The experimental test sites (E3, E5, E7 and DP) were built to allow for monitoring of aquatic ecosystems influenced by OSPM and will be referred to hereafter as OSPM sites. Surface run-off and rain water have been the only known sources of water entering both OSPM and reference sites since construction.

Study sites were grouped by NA concentrations (Table 1), and included low (0 to 4 mg/L), medium (4 to 15 mg/L) and high (> 15 mg/L) NA concentration categories. Low NA concentration sites included all reference sites (E1, BCR, BM, SWL). Medium NA concentration sites included sites that contained no OSPM water, but did contain MFT (E3, DP). High NA concentration sites included sites that contained MFT and either OSPM water or water consolidated from MFT (E5, E7).

All sites were sampled twice using identical methods, once in the month of July and once in late August or early September, 2003. Plankton were sampled using 63 µm and 153 µm mesh plankton nets (30 cm diameter). To obtain standardized sample volumes for assessment, 3 vertical hauls from substrate to surface were conducted in randomly chosen locations at each study site. Immediately following collection, plankton samples were brought to the lab and sorted by size into three categories, 64 - 153 µm, 154 - 500 µm and >500 µm (using 63, 153 and 500 µm mesh screens). Once sorted, plankton were examined under a dissecting microscope to ensure sand, leaf litter, detritus and other biota were removed. The samples were then placed in glass petri dishes and dried at 40^{o}C for 24 hrs.

Qualitative samples of aquatic invertebrates were taken from the littoral zone (<2m) of all study sites at five randomly selected locations using a D-frame kick-net. Following collection, samples were rinsed through a series of stacked sorting sieves (1000, 500, 355, 250 and 125 μm mesh sizes). The contents of the sieves were rinsed directly into shallow light-coloured pans and taxa were picked out by hand. Any invertebrates found were identified using a dissecting microscope (up to 25X power) and placed in site specific 2.2 μm filtered water for 4 hrs to allow for gut clearance. Gastropod flesh was removed from the shell and cleaned of biogenic carbonates and grit before drying. All invertebrates were dried in glass vials at 40^{o}C for 48 hrs. Six invertebrate taxa were chosen for analysis based on relative abundance at the study sites and feeding guild, including scrapers (Gastropoda), shredders (*Hyalella azteca*, *Haliplus*), predators (*Enallagma*, *Chaoborus*) and collectors (Chironomidae).

In DP and BCR, fish were collected using minnow traps (6.35 mm mesh) and seining (6.35 mm mesh). All fish taxa were frozen for later identification. Fathead minnow (*Pimephales promelas*) was the only species present at both sites and were used for comparison purposes. In the lab dorsal muscle tissue was removed posterior to the dorsal fin and above the lateral line and dried at 40^{o}C for 24 hrs following standardized protocols (e.g. Power et al., 2002). Similar sampling effort was expended at all other sites to confirm fish absences.

Macrophytes were sampled in the littoral zone of all study sites. Mature, emergent, submergent and floating macrophytes were collected and stored in polyethylene bags for later identification. Plant samples were collected by hand to include leaves and stems. In the laboratory, plant samples were sorted to genus and washed with deionized water to obtain

clean samples for stable isotope analysis. *Typha latifolia* was the only species found at all test sites and was used for the comparative analysis of carbon and nitrogen isotopes. All analyzed samples were cut into smaller sections, placed in glass Petri dishes and dried for 24 hrs at 40^{o}C.

Periphytic samples were scraped from the surface of submerged rocks and filtered onto pre-combusted (500 ^{o}C for 6 hours) Whatman QMA quartz fibre filters (47 mm diameter and 2.2 μm pore size). Periphytic material collected on filters were dried on glass petri dishes for 24 hrs at 40^{o}C.

Water samples for particulate organic matter (POM) were passed through a 63 μm mesh to eliminate large particles and collected on pre-combusted Whatman QMA quartz fibre filters. POM was therefore defined as material > 2.2 μm and < 63 μm. POM filters were allowed to dry on glass pedri dishes for 24 hrs at 40^{o}C.

Three replicate water samples were collected subsurface in 1-L polyethylene bottles for standard water chemistry, as well as stable isotope analyses of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC). Water chemistry (Table 1) was completed at Syncrude's Research Facility in Edmonton following standardized protocols (SCL, 1995). DIC water samples were refrigerated and analyzed within two weeks of sampling (Drimmie et al., 1990). DOC water samples were frozen prior to analysis (Drimmie et al., 2004).

Surface sediment samples were collected in the littoral zone of all study sites at a water depth of approximately 0.5 metres using 500 mL glass jars. Glass jars were submerged in water until all air pockets were removed and dragged through the first 1 mm of surface sediment until approximately half of the jar contained sediment. Bank side overburden material was sampled 2 metres from the waters edge of each study site with a small shovel.

Overburden samples were dug to a depth of 15cm, where only the 5 to 15 cm portion was removed and placed into 500mL glass jars.

All sediment samples were dried for 48 hrs at 40^{o}C prior to analysis. Approximately 500 mg of dried and ground surface and overburden sediment were treated with a 10% hydrochloric acid (HCl) solution in 500 mL glass beakers to remove the inorganic fraction of the sample (Baker and Burns, 1985). Small amounts of the HCl solution were added until the reaction was complete, as evidenced by termination of carbon dioxide escape. The quantity of the HCl solution added, therefore, depended on the carbonate content of the sample. After acidification the remaining HCl solution was removed with three distilled water rinses. Sediment samples were then re-dried for 48 hrs at 40^{o}C. Both acidified and unacidified sediment samples were analyzed, with δ^{13}C values being obtained from the acidified sample and corresponding δ^{15}N values being obtained from the unacidified sample to avoid any potential effects of acidification on nitrogen stable isotope ratios.

All samples used for stable isotope analysis were ground to a fine powder using a Retsch MM 2000 ball mill grinder (F. Kurt Retsch, GMBH and Co., Haan, Germany). For simultaneous analysis of carbon and nitrogen isotopes, approximately 1 mg of the homogenate was used for plankton, invertebrates and fish, 2 - 7 mg for macrophytes, 2.5 mg for periphytic material (without fibre filter), 5 – 85 mg for surface sediment and 15 - 60 mg for overburden sediment material. Depending upon the amount of POM or periphytic material on the quartz fibre filter, quarter, half or whole filters were used for isotope analyses.

Carbon and nitrogen isotope compositions were determined using a Micromass VG Isochrom continuous-flow isotope-ratio mass spectrometer connected to a Carlo Erba

elemental analyzer (CHNS-O EA1108), with an analytical precision of ± 0.2‰. All isotope analyses were completed at the Environmental Isotope Laboratory, University of Waterloo (Waterloo, Ontario, Canada). Duplicate sample analysis (1 in 8) was completed for purposes of determining machine analytical variability. Measurement precision was established by repeat analysis of commercially available laboratory standards (International Atomic Energy Agency (IAEA) standard CH6: $\delta^{13}C = -10.4 \pm 0.1‰$ and IAEA-N1: $\delta^{15}N = 0.4 \pm 0.2‰$).

Stable isotope ratios are expressed as delta values (δ) and are measures of the parts per thousand difference (‰) between the isotope ratio of a sample and that of an international standard determined as follows:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{Sample}-R_{Standard})/R_{Standard}] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, ratio of the sample or the standard. International reference standards used here included carbonate rock from the Peedee Belemnite formation for $\delta^{13}C$ (Craig, 1957) and atmospheric nitrogen for $\delta^{15}N$ (Mariotti, 1983). By convention, all international standards are set at a value of 0‰.

To examine the potential impacts of OSPM on the wider food web, consumer food web area ($‰^2$) and food web length (‰) measures were compared between low, medium and high NA concentration sites. Consumer food web areas were represented as the minimum convex polygonal area obtained by bounding the extreme mean ± standard error $\delta^{13}C$ and $\delta^{15}N$ measures of all sampled consumer taxa (excluding fish). Polygon areas were computed using a standard polygon area formula (O'Rourke, 1998). Food web length was defined as the $\delta^{15}N$ difference from the base of the food web (surface sediment or periphytic material) to the most enriched invertebrate taxa (*Chaoborus*) in each of the low, medium and high NA

concentration sites and averaging the length estimates obtained within NA concentration categories.

All statistical analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago). Tukey's *post-hoc* HSD test was used for multiple comparison of means to determine significant differences in the $\delta^{13}C$ or $\delta^{15}N$ values for common inorganic and biotic samples from sites in each of the low, medium and high NA groupings. Where comparisons were made between two sites only, two sample t-tests selected for equal or unequal variances were used. Significance in all statistical testing was set at $\alpha = 0.05$.

2.3 Results

NA concentrations in a priori groupings of low (0 to 4 mg/L), medium (4 to 15 mg/L) and high (> 15 mg/L) concentration sites were significantly different from one another (Tukey's $P < 0.05$). Consumer food web area and food web lengths did not differ significantly among low, medium and high NA concentration sites (Tukey's $P > 0.05$). Consumer food web area and lengths averaged respectively, 14.17‰^{2} and 5.75 ‰ for low, 20.06‰^{2} and 6.42‰ for medium, and 23.53‰^{2} and 6.88‰ for high NA concentration sites (Fig. 2 and 3). No significant differences were found in $\delta^{13}C$ (Tukey's $P > 0.05$) when low, medium and high NA concentration sites were compared for DOC, DIC, surface sediment, POM, *Typha latifolia*, plankton (64 to 153 µm, 154 to 500 µm and > 500 µm size fractions), Gastropoda, *Haliplus*, *Chaoborus* and Chironomidae. Significant differences were found between low and medium NA concentration sites of overburden sediment (Tukey's $P < 0.05$) (Table 2) (Fig. 4). Significant differences were found between low and high NA concentration sites of periphytic material, *Hyalella azteca* and Enallagma (Tukey's $P < 0.05$) (Table 2) (Fig. 4). Significant differences were found between medium and high NA concentration sites of *Hyalella azteca* and Enallagma (Tukey's $P < 0.05$) (Table 2) (Fig. 4). Tukey's HSD *post-hoc* test results for $\delta^{15}N$ between low, medium and high NA concentration sites are shown in Table 2 and Fig. 5 and 6. Independent two sample t-testing revealed that *Pimephales promelas* from BCR and DP differed significantly in $\delta^{13}C$ and $\delta^{15}N$ (t-test $P < 0.05$).

2.4 Discussion

Consumer food web area and food web length comparisons showed no significant differences between low, medium and high concentrations of NA compounds. Carbon isotopes of representative taxa collected from low, medium and high NA sites also showed no significant differences for DOC, DIC, surface sediment, POM, *Typha latifolia*, plankton (64 to 153 μm, 154 to 500 μm and > 500 μm size fractions), as well as Gastropoda, *Haliplus*, *Chaoborus* and Chironomidae. Nitrogen stable isotope signatures within surface sediment and all biota examined, however, showed significant differences among NA sites. Results from food web area and length suggest that large scale shifts in food web structure and function do not occur, although changes to the relative positions of organisms are evident in the stable nitrogen isotope signatures.

Previous studies (Leung et al., 2001; Leonhardt, 2003) have shown that OSPM impacts on aquatic food web structure and function are related to the time since OSPM introduction to a system. Using microcosm experiments Leung et al. (2001) determined that there were no significant impacts on phytoplankton community composition in OSPM sites greater than 5 years in age. Leonhardt (2003) also found that within five years of OSPM introduction the zoobenthic abundance in OSPM sites were similar to reference sites and that within 5-7 years of construction the richness in the zoobenthic community of OSPM sites were similar to reference wetlands. In this study, all sites were older than 10 years of age when sampled, supporting the claim that OSPM sites greater than 7 years of age support aquatic ecosystems similar to reference sites, based on broad scale descriptors of food web structure (consumer food web area and length).

The apparent lack of OSPM impact on overall food web structure and function may be a result of the degradation of NAs, as well as other OSPM constituents, over time. NAs, the principal acutely toxic component in OSPM water (Alberta Environmental Protection, 1996; Schramm et al., 2000), are known to decrease rapidly in toxicity at OSPM sites (Fine Tailings Consortium, 1995). Indigenous microbial communities degrade bitumen and its constituent components (i.e. NAs) in both OSPM water (Herman et al., 1994; Holowenko et al., 2002) and at OSPM sites (Moore et al., 2002). All OSPM sites had well-developed macrophyte communities (pers. ob.), which could contribute to the reduction of OSPM toxicity in two ways. First, macrophyte-derived organic materials provide a good substrate for the formation of biofilms, and aiding in microbial degradation (Herman et al., 1994; Fine Tailings Fundamentals Consortium, 1995; Gould, 2000; Holowenko et al., 2002). Naphthenate-degrading bacteria are located in the sediment, at the sediment-water interface, and in biofilms attached to surfaces such as plant stalks and detritus (Nix et al., 1994). Macrophytes such as *Typha latifolia* can provide increased surface area for biofilm development thereby increasing NA degradation rates. Second, organic materials accumulating at the sediment-water interface, if thick enough, may isolate the underlying material (OSPM) from surface water leading to a decrease in the rate of transfer of soluble materials to the water column NA concentration. In this study no detectable changes in food web area and length existed between OSPM and reference sites which may indicate that degradation processes of NAs occur over time.

Carbon isotope signatures of low, medium and high NA site groupings were not significantly different, suggesting that OSPM is not likely the main carbon source for these systems. The carbon isotope signatures most likely reflect atmospheric CO_2. The OSPM

were transferred to the experimental sites > 10 years before this study was conducted and have had the opportunity to degrade. Since the time of construction an equilibrium with the atmosphere has most likely occurred, thereby influencing the carbon signatures of these environments. The ^{13}C content of components of the carbon cycle of freshwaters vary widely depending on the source of the dissolved CO_2 (i.e. from the atmosphere or respired organic matter) (Peterson and Fry, 1987). CO_2 can also enter the food web from bacterial degradation of bitumen within the OSPM. To provide evidence for bacterial degradation the carbon isotopic values of DIC from OSPM and reference sites can be compared. The $\delta^{13}C$ of oil sands constituents (bitumen) from the mine site is -30.3‰ (Dixon and Farwell, 2002) and can be a significant carbon source for bacteria. If OSPM carbon was entering the food web, as respired CO_2 from bacterial degradation, organisms would be expected to have more depleted $\delta^{13}C$ signatures. The similarity of DIC $\delta^{13}C$ signatures among sites, however, suggests CO_2 is incorporated via diffusion from the atmosphere. The similarity of carbon isotopic values from surface sediments of low, medium and high NA sites also suggests that the degradation of carbon sources from the underlying MFT (sediments) is not sufficient to create environments that are affected differently. *Pimephales promelas* did show an enrichment in the carbon isotopic signature between the OSPM (DP) and reference (BCR) sites, however this may be a result of low replication of sites containing fish or fish specific sensitivities. Patrick et al. (1968) demonstrated that bluegill sunfish (*Lepomis macrochirus*) were more sensitive to commercial NAs compared to diatoms (*Nitzschia linearis*) or snails (*Physa heterostropha*) in freshwater environments using survival as an endpoint. *Pimephales promelas* could also have similar NA sensitivities enriching the carbon isotopic signatures at OSPM sites. Since most of the samples did not show significant differences in the carbon

isotopic signatures of OSPM and reference sites we can suggest that sources of carbon for the study sites are most likely dominated by atmospheric CO_2 and not carbon from OSPM.

In contrast to the C situation, OSPM does appear to be a significant N source for biota at OSPM influenced sites. The nitrogen isotope signatures of most samples were significantly different when low, medium and high NA sites were compared. During the oil sands upgrading process ammonia is released as a by-product and can be found in the OSPM water and MFT at OSPM sites. The ammonia is either consumed by biota directly or indirectly through nitrification. Since all study sites discussed here are oxic, nitrification processes produces nitrates and nitrites from the ammonia (Axler et al., 1981; Lean and Knowles, 1987; Rudd et al., 1988), which is readily available for nutrient uptake by biota. Leggett et al. (2000) studied biota at the base of the pelagic food chain in Lake Ontario and found that the available ammonia in lakes is derived largely from the sediments and through regeneration from algal and zooplankton excretion. Leggett et al. (2000), suggested that NH_4^+ produced from the sediment is able to diffuse through the entire water column in a mixed system and, furthermore, that NH_4^+ produced through sediment diagenesis is likely to have a $\delta^{15}N$ characteristic of the surface sediment from which it is generated. Vander Zanden and Rasmussen (1999) also demonstrated that nitrogen isotope signatures will reflect differences in known nitrogen substrates. Since all sites in this study are > 10 years old, it is likely that the ammonium at OSPM sites has been continuously recycled since OSPM introduction, as indicated by the enriched nitrogen isotopic signatures of samples throughout the food web (Fig. 5 and 6). If ammonia was introduced through OSPM, it would be found throughout the food web including substrate materials and continuously returned through the sediments.

A study by M.D. MacKinnon (Syncrude Canada Ltd., pers. comm., Appendix 1), followed ammonia concentrations in OSPM water after a single introduction into E5 to achieve 2 mg/L. The initially high NH_3 concentration decreased quickly, while there was an elevation in nitrate and nitrite. This increase in nitrate and nitrite remained for a short period of time; however, once ammonia was removed the nitrite and nitrate levels dropped below detection levels (approximately < 0.01mg/L). The rapid uptake of all nitrogen species suggests that biota are quickly consuming the nitrite and nitrate that is readily available through ammonia nitrification. The present study as well as previously analyzed water samples from E7 (Ganshorn, 2002), showed no detectable forms of nitrogen. Rapid bacterial nitrification processes at OSPM sites may be responsible for the water nitrogen results (Fine Tails Fundamentals Consortium, 1995). If bacterial production is prominent enough in OSPM sites, it may be causing an enrichment in the nitrogen signatures via the microbial loop, thereby changing the relative position of organisms (Sherr et al., 1986; Sherr and Sherr, 1988; Riemann and Christoffersen, 1993). Ganshorn (2002) and Murchie and Power (2004) studied nitrogen stable isotope signatures of organisms collected in the benthic and pelagic food webs of OSPM and reference sites in the Athabasca oil sands region. Murchie and Power (2004) attributed the nitrogen enrichment between OSPM and reference sites to oil sands process-wastes such as ammonia and NAs. Ganshorn (2002) also found that nitrogen enrichment occurred in macroinvertebrates at OSPM sites compared to reference sites. Ganshorn (2002) attributed the nitrogen enrichment of food web components to bacterial nitrification processes which incorporate nitrogen from amines. Our results also indicate that the enrichment of nitrogen at OSPM sites is reflecting the ammonia found in OSPM. Once

the ammonia from OSPM is incorporated into the food web, the relative position of organisms demonstrated in the nitrogen signatures of biota at OSPM sites reflect a change.

The present study has provided evidence of subtle food web related changes in aquatic communities exposed to OSPM. Although OSPM does not appear to be directly re-worked or used as an energy source, compounds within OSPM shift the relative position of organisms within food webs. The enriched $\delta^{15}N$ signatures of key taxa, therefore, may be viewed as diagnostic of an effect of OSPM water-capping, but not as evidence of substantive changes in food web function or aged (> 10 years) OSPM sites. Nevertheless, more experimental manipulations of OSPM affected sites is required before general conclusions concerning the overall food web related impacts of OSPM can be drawn. Of particular importance would be studies of possible bacterial degradation of OSPM materials and associated basal food web effects.

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Table 2.1. The physical and chemical characteristics of study sites on, or near, the Syncrude Canada Ltd. Lease, Fort McMurray, Alberta. Water sampling was completed coincident with aquatic taxa sampling. All measurements are in mg/L unless otherwise indicated. Sites are grouped into low (0 to 4 mg/L), medium (4 to 15 mg/L) and high (> 15 mg/L) NA concentrations. NA* denotes NAs. Non-OSPM water comes from local muskeg drainage water and/or precipitation. MFT release water represents water generated through mature fine tails densification and de-watering and OSPM water is oil sands process material(s) water from Mildred Lake settling basin.

Site group	Study Site	Surface area (Ha)	Average water depth (m)	Mature fine tails (MFT) (m^3)	Water cap	pH	Cond (uS/cm)	Salinity (ppt)	DO	DOC (ppm)	NA*	Na	Ca+Mg	Cl	SO_4	HCO_3 +CO_3
Low	E1	0.05	1	0	Non-OSPM water	8.27	760	0.4	11.9	17.17	2.3	74.5	77.2	5.8	276.0	185.0
	Shallow Wetland	0.8	0.5	0	Non-OSPM water	9.45	473	0.2	6.4	26.86	3.1	76.5	36.0	27.0	9.1	306.6
	Barge Marsh	0.6	1	0	Non-OSPM water	7.42	350	0.0	9.4	15.16	1.1	24.2	44.7	37.0	7.8	159.0
	Beaver Creek Resevoir	220	2	0	Non-OSPM water	7.41	270	0.1	8.0	23.69	1.5	29.3	35.2	1.1	13.3	187.3
Medium	E3	0.05	1	1000	Non-OSPM water + MFT release water	8.81	767	0.4	10.1	38.5	4.7	149.0	28.0	31.0	61.5	365.4
	Demonstration Pond	4.0	2	70000	Non-OSPM water + MFT release water	8.76	1360	0.7	8.9	44.62	10.0	334.0	33.0	92.0	162.0	556.9
High	E5	0.05	1	1000	OSPM water + MFT release water	9.21	2660	1.4	12.1	58.32	20.3	620.0	44.0	140.0	716.0	451.3
	E7	0.05	0.4	2000	MFT release water + precipitation	8.55	1960	1.0	7.8	79.75	21.7	509.0	26.0	130.0	104.0	955.1

Table 2.2. Mean $\delta^{13}C$ and $\delta^{15}N$ values of sample types collected from low, medium and high NA concentration sites. Superscripts used to denote common means as determined by Tukey's *post-hoc* HSD tests ($P > 0.05$).

			Common Means	
Sample Type	Treatments	n	$\delta^{13}C$	$\delta^{15}N$
Dissolved Organic Carbon	Low	4	-27.34 ^{A}	N/A
	Medium	2	-27.00 ^{A}	N/A
	High	2	-26.97 ^{A}	N/A
Dissolved Inorganic Carbon	Low	4	-7.50 ^{A}	N/A
	Medium	2	-5.02 ^{A}	N/A
	High	2	-4.18 ^{A}	N/A
Overburden Sediment	Low	8	-28.72 ^{A}	0.39 ^{A}
	Medium	4	-25.60 ^{B}	2.08 ^{A}
	High	4	-28.28 ^{AB}	2.25 ^{A}
Surface Sediment	Low	24	-28.08 ^{A}	1.04 ^{A}
	Medium	8	-27.80 ^{A}	2.34 ^{B}
	High	8	-27.95 ^{A}	2.85 ^{B}
Periphytic Material	Low	14	-18.65 ^{A}	0.05 ^{A}
	Medium	7	-19.51 ^{A}	1.69 ^{AB}
	High	7	-25.49 ^{B}	3.28 ^{A}
Particulate Organic Matter	Low	8	-28.28 ^{A}	1.20 ^{A}
	Medium	4	-27.79 ^{A}	4.69 ^{B}
	High	4	-28.19 ^{A}	4.52 ^{B}
Typha latifolia	Low	24	-28.50 ^{A}	3.05 ^{A}
	Medium	8	-28.74 ^{A}	4.64 ^{AB}
	High	8	-28.76 ^{A}	7.04 ^{B}
Plankton Size Fractions				
64 to 153 µm	Low	16	-28.47 ^{A}	1.86 ^{A}
	Medium	6	-26.99 ^{A}	3.77 ^{B}
	High	7	-28.36 ^{A}	4.85 ^{B}
154 to 500 µm	Low	16	-29.71 ^{A}	2.74 ^{A}
	Medium	8	-28.37 ^{A}	5.64 ^{B}
	High	7	-28.00 ^{A}	5.52 ^{B}
> 500 µm	Low	16	-30.42 ^{A}	3.95 ^{A}
	Medium	6	-28.37 ^{A}	6.88 ^{B}
	High	8	-28.30 ^{A}	8.60 ^{B}
Invertebrates				
Gastropoda	Low	92	-27.29 ^{A}	2.30 ^{A}
	Medium	23	-27.24 ^{A}	4.47 ^{B}
	High	4	-26.44 ^{A}	3.22 ^{AB}
Hyalella azteca	Low	27	-25.60 ^{A}	2.68 ^{A}
	Medium	11	-25.46 ^{AB}	5.50 ^{B}
	High	7	-23.67 ^{B}	3.68 ^{AB}
Haliplus	Low	13	-26.77 ^{A}	2.92 ^{A}
	Medium	6	-25.57 ^{A}	5.76 ^{B}
	High	6	-26.34 ^{A}	5.51 ^{B}
Enallagma	Low	22	-27.92 ^{A}	4.34 ^{A}
	Medium	8	-27.55 ^{AB}	5.79 ^{AB}
	High	11	-25.75 ^{B}	6.46 ^{B}
Chaoborus	Low	17	-27.92 ^{A}	5.80 ^{A}
	Medium	4	-28.00 ^{A}	5.08 ^{A}
	High	9	-28.38 ^{A}	8.76 ^{B}
Chironomidae	Low	24	-28.02 ^{A}	2.80 ^{A}
	Medium	8	-27.46 ^{A}	5.69 ^{B}
	High	8	-27.64 ^{A}	5.80 ^{B}

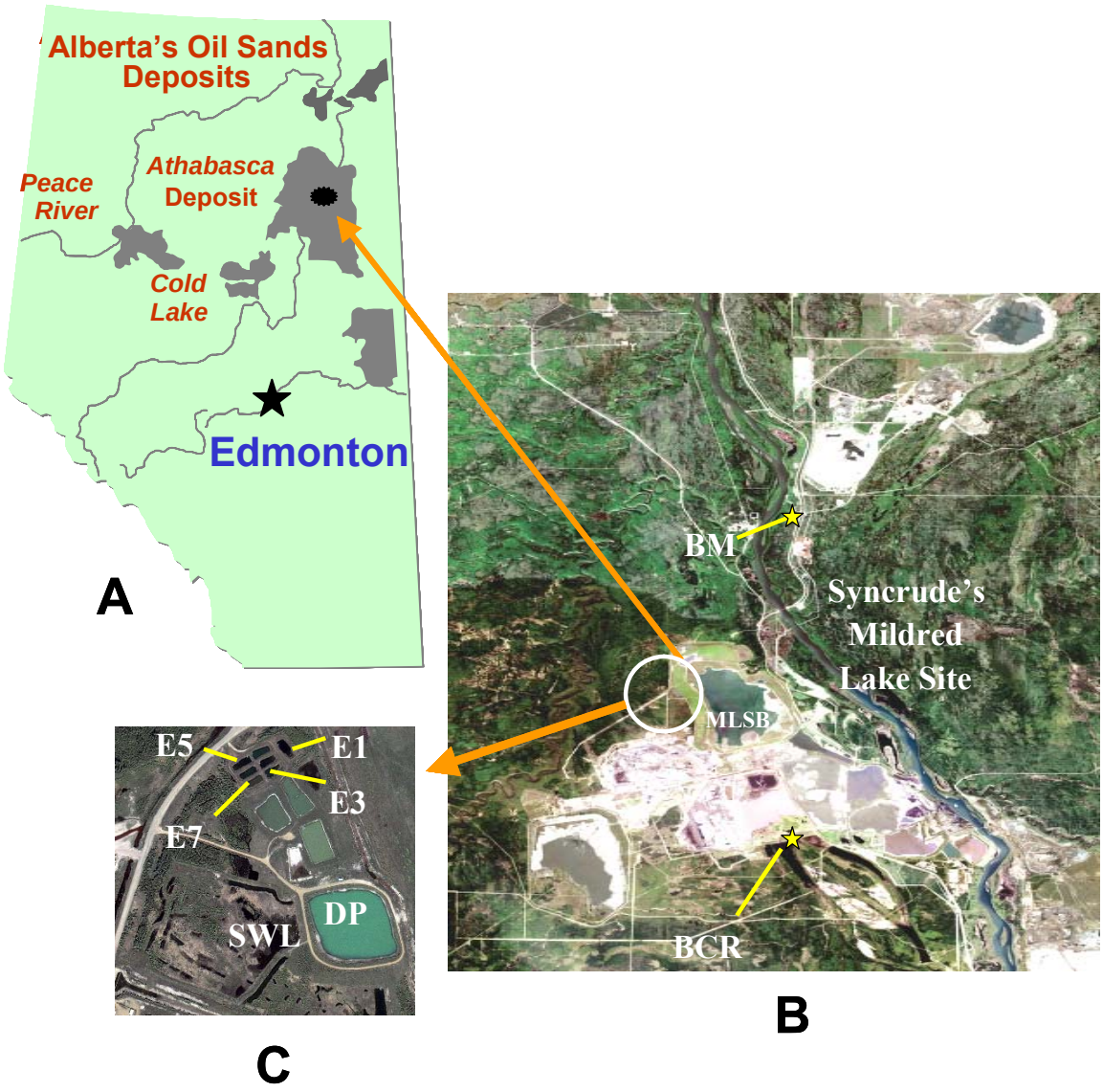

Fig. 2.1. Relative geographic location of study sites within the Province of Alberta, Canada (panel A) and the general location of study sites on the Syncrude Canada Ltd. lease (panel B and C). DP, SWL, BCR, BM and MLSB represent Demonstration Pond, Shallow Wetland, Beaver Creek Reservoir, Barge Marsh and Mildred Lake Settling Basin.

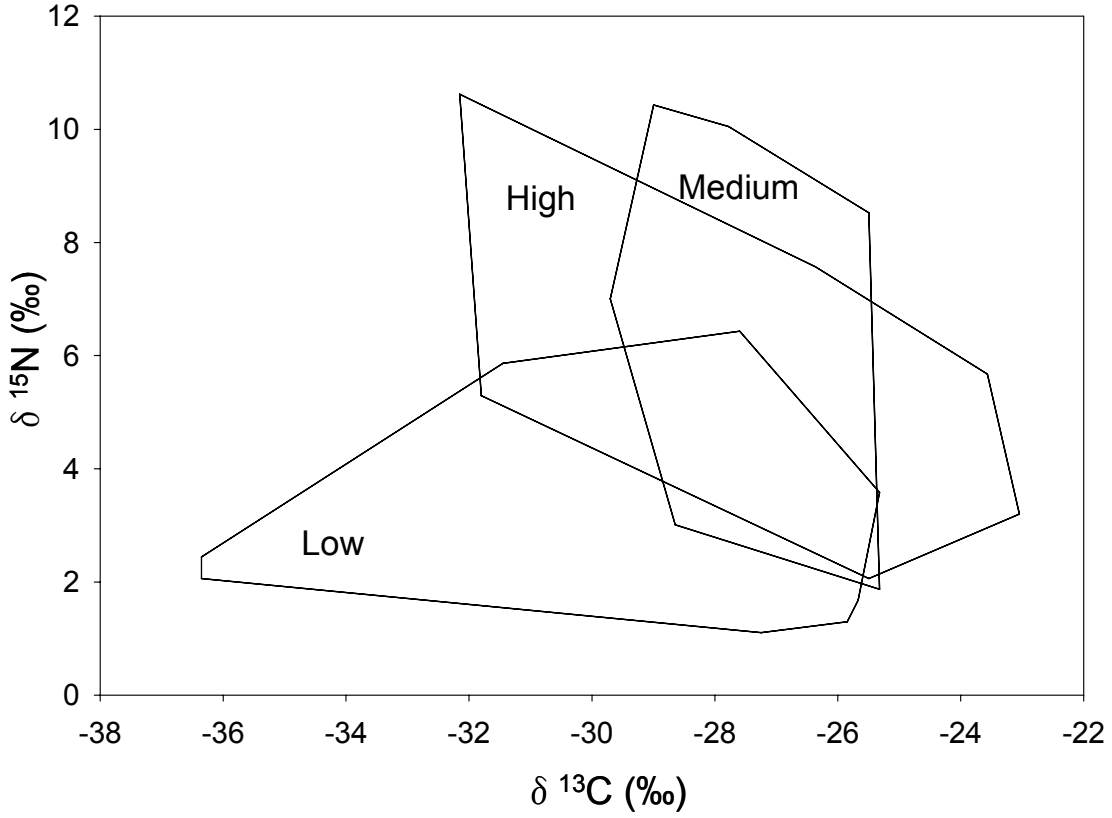

Fig. 2.2. Averaged consumer food web areas obtained by bounding the mean ± standard error $\delta^{13}C$ and $\delta^{15}N$ measures of sample consumer taxa (excluding fish) in each of the low, medium and high NA concentration sites with a minimum convex polygon and averaging the area estimates obtained within NA concentration categories. Low NA concentration sites averaged 14.17 $‰^2$ (n = 4), medium NA concentration sites averaged 20.06 $‰^2$ (n = 2) and high NA concentration sites averaged 23.53 $‰^2$ (n = 2).

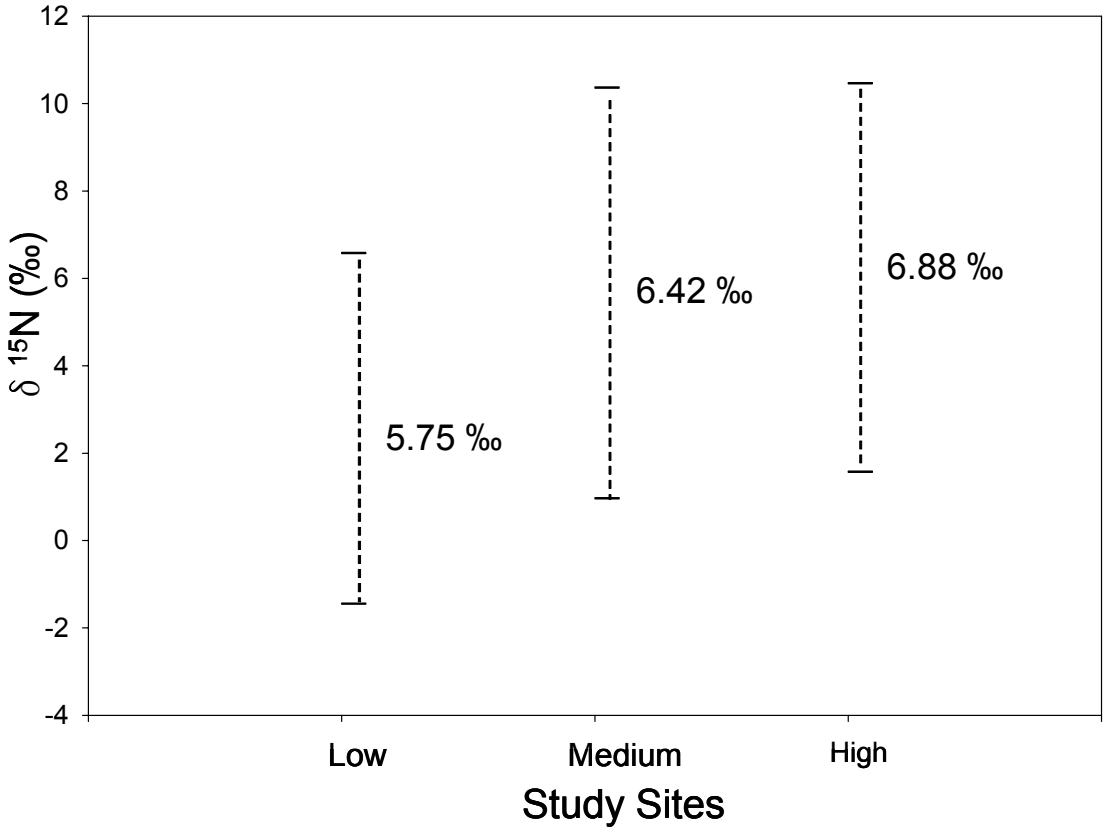

Fig. 2.3. Estimates of average food web length in the low, medium and high NA concentration groupings. Length defined as the $\delta^{15}N$ isotopic separation between the base of the food web (surface sediment or periphytic material) and the most enriched invertebrate taxa.

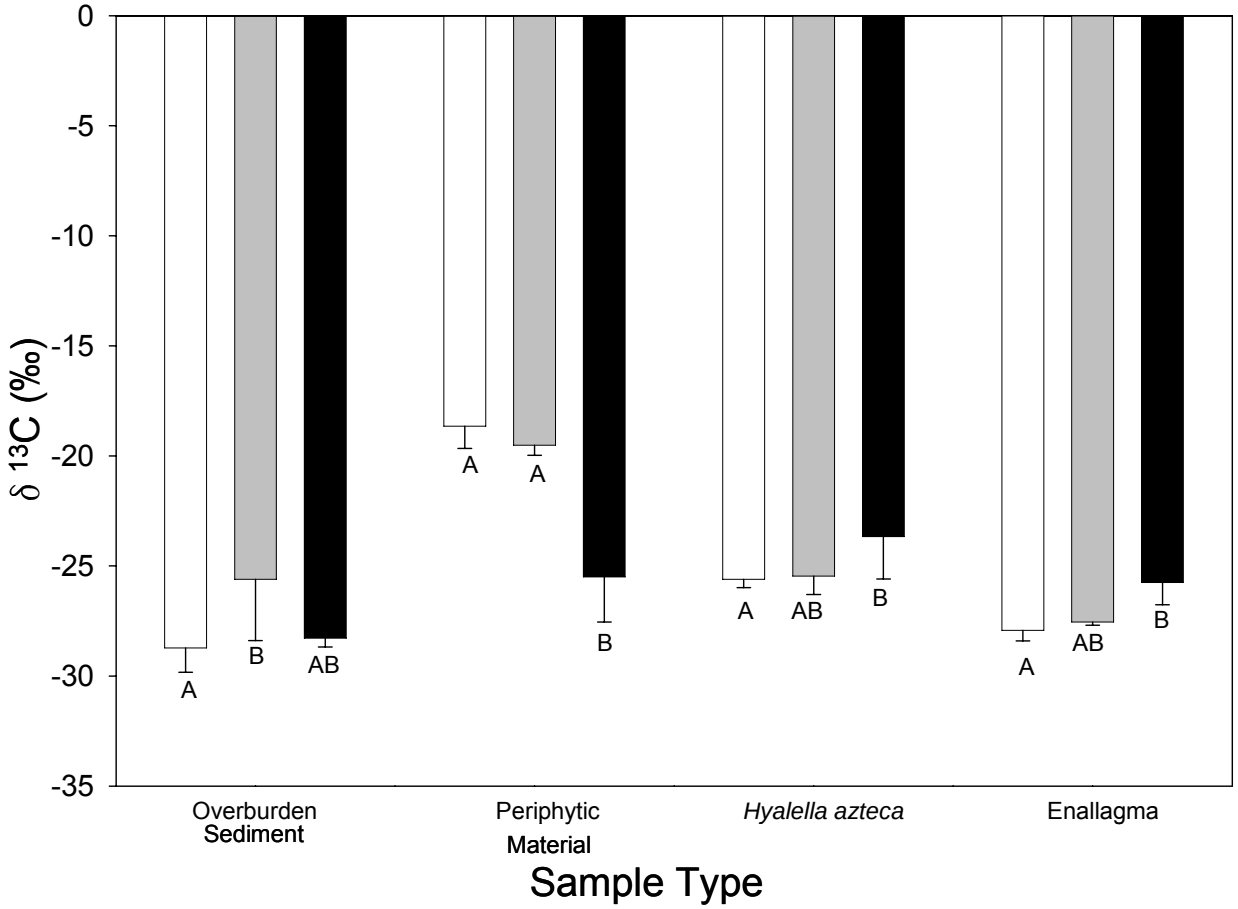

Fig. 2.4. Mean $\delta^{13}C$ measures by sample type in each of the low (white bars), medium (grey bars) and high (black bars) NA concentration sites. Letters A and B used to denote common means as determined using Tukey's *post-hoc* HSD test ($P > 0.05$). Error bars represent ± one standard error.

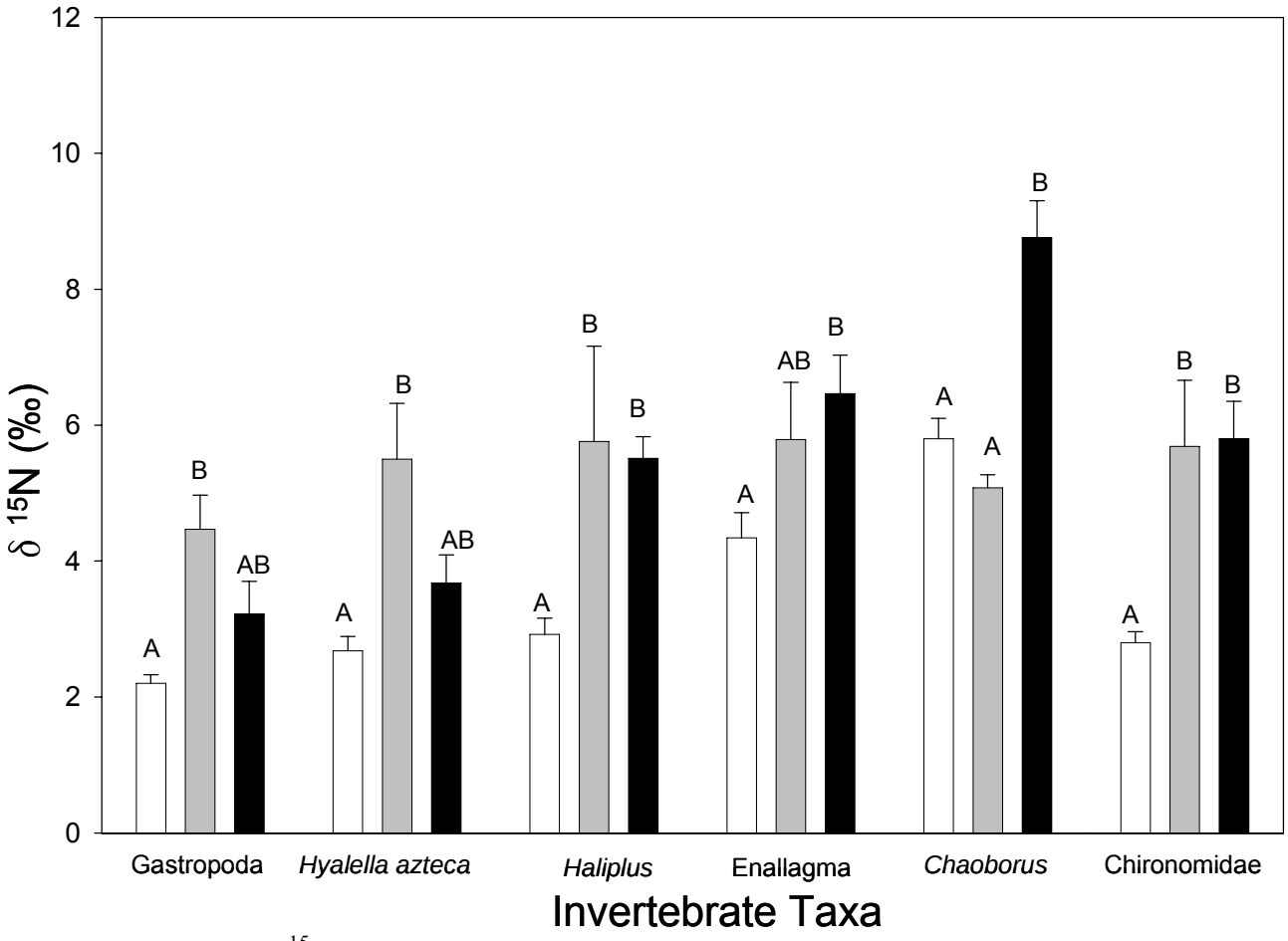

Fig. 2.5. Mean $\delta^{15}N$ measures by invertebrate in each of the low (white bars), medium (grey bars) and high (black bars) NA concentration sites. Letters A and B used to denote common means as determined using Tukey's *post-hoc* HSD test ($P > 0.05$). Error bars represent ± one standard error.

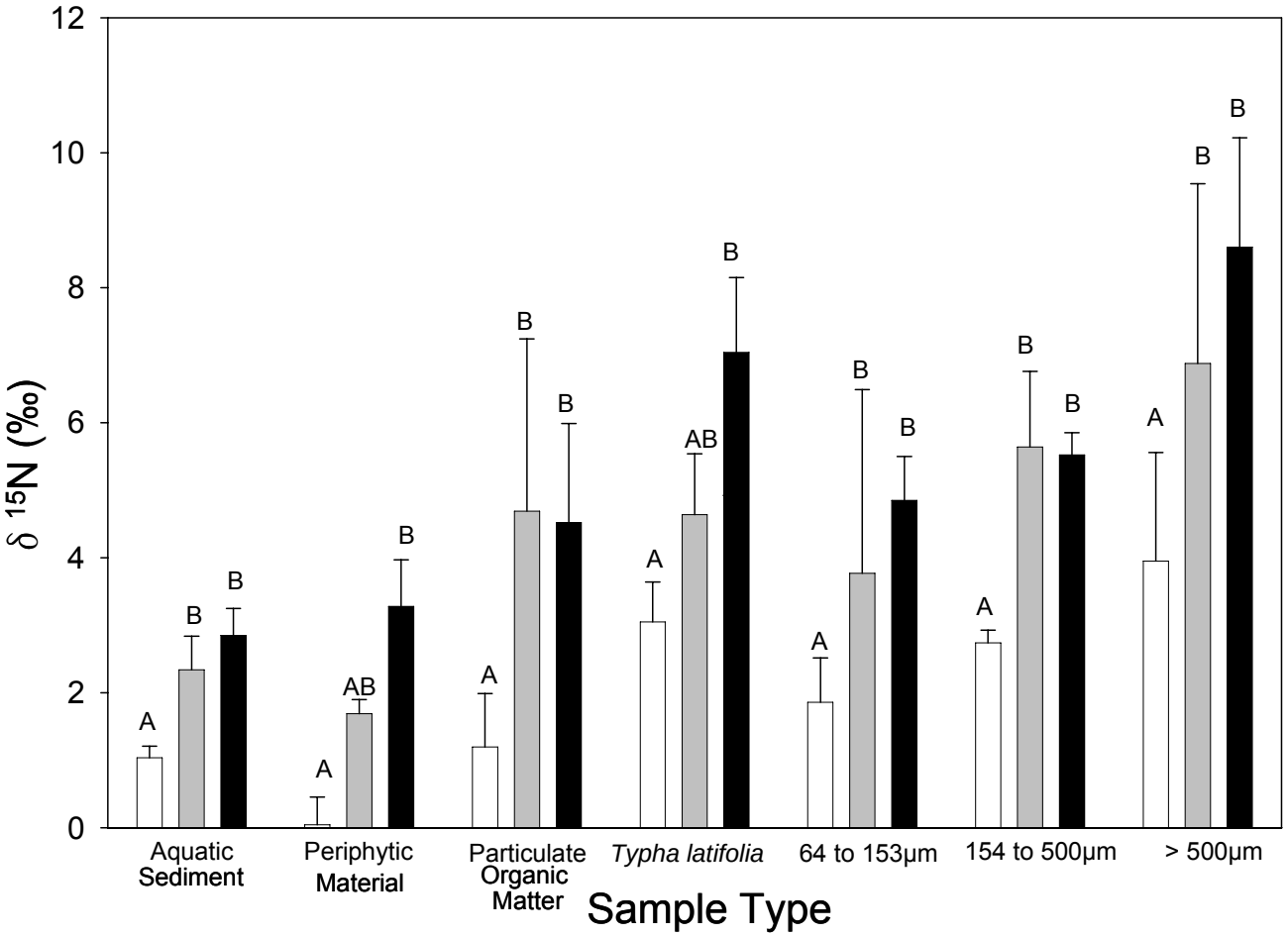

Fig. 2.6. Mean $\delta^{15}N$ measures by sample type in each of the low (white bars), medium (grey bars) and high (black bars) NA concentration sites. The three size fractions of plankton are represented by 64 to 153µm, 154 to 500µm and >500µm. Letters A and B used to denote common means as determined using Tukey's *post-hoc* HSD test ($P > 0.05$). Error bars represent ± one standard error.

Chapter 3. Tracing aquatic food web impacts of oil sands developments using carbon ($\delta^{13}C$) and sulphur ($\delta^{34}S$) stable isotopes.*

Abstract

Current mining operators in the Athabasca oil sands deposit of Alberta, Canada have made a commitment to zero discharge of oil sands process-affected waste materials (OSPM) from the mine site and to rehabilitate mined lands to a pre-mining state. As part of aquatic reclamation, experimental test sites that contain a range of OSPM have been constructed to monitor the evolution of aquatic habitats and their viability as stable aquatic ecosystems (lakes and wetlands). To determine the potential effects of construction and OSPM within these test sites, carbon and sulphur stable isotopes of water, plankton, aquatic invertebrates and fish were analyzed. In all cases, except with *Chaoborus* and *Haliplus,* carbon isotope signatures were not significantly different in constructed and reference sites. With the exception of *Haliplus*, sulphur isotope values for aquatic organisms from constructed and reference sites were significantly different. Aquatic organisms and water samples from constructed sites built in or close to the boundary of Cretaceous Clearwater (Kcw) clays typically had $\delta^{34}S < 0$ ‰, often associated with weathering of marine cretaceous rocks. Coinciding with depleted $\delta^{34}S$ signatures found in these aquatic systems were elevated sulphate concentrations.

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3.1 Introduction

Canada possesses three quarters of the world's oil sands deposits. One of these, the northern Alberta Athabasca deposit, is located close to the surface and can be exploited with surface mining. Syncrude Canada Ltd. (SCL) is the largest mining operator on the Athabasca deposit and has committed to zero discharge of oil sands process-affected waste materials (OSPM) from its mining and refining operations. To implement the policy, SCL has developed a number of remediation and waste material disposal strategies aimed at incorporating mining-related liquid and solid wastes with the construction of naturalized lakes and wetlands (Fine Tailings Fundamentals Consortium, 1995). SCL has also actively supported scientific assessment of its reclamation strategies (e.g., Hesslein and Ramlal, 1993; Whelly et al., 1998; Power and van den Heuvel, 1999; van den Heuvel et al., 1999; Evanson and Van Der Kraak, 2001; Dixon and Farwell, 2002; Tetreault et al., 2003; Murchie and Power, 2004) with the aim of characterizing and quantifying potential aquatic impacts. From these studies only a select few have concentrated on the implications of OSPM reclamation strategies for food web relationships (e.g., studies of yellow perch, *Perca flavescens*, populations, Murchie and Power 2004, and plankton dynamics, Leung et al. 2001).

One of the proposed disposal waste material methods, the “wet landscape” approach, consists of covering mature fine tails (MFT), a process-waste consisting of 65% water, fine clay particles (<22 μm) and residual bitumen (Boerger et al., 1992), with a layer of water to form a constructed lake. This water cap layer could be either OSPM water or non-OSPM water, but the intent is that a lake ecosystem will develop over time. The water layer must be of sufficient depth to ensure that mixing with the OSPM in the MFT zone does not occur. Over time, detrital material will collect and act as the bottom sediment to provide a platform

for microbial degradation. Natural detoxification processes should occur during microbial degradation, allowing a lake ecosystem to develop naturally (Fine Tailings Fundamentals Consortium, 1995). Time is an important variable in the "wet landscape" approach, but it is expected that within 1-2 years colonization by biota will succeed in moving the lake ecosystem towards natural conditions (Leonhardt, 2003). To date, studies in constructed test sites ranging from less than 0.1Ha to >4Ha have indicated that evolution of aquatic habitats will proceed differentially depending on the source, chemical characteristics and age of the incorporated OSPM materials.

Stable isotope studies are one means by which the evolution of experimental test sites can be monitored. Stable isotope tracers are present in natural systems and their distribution reflects the history of physical and metabolic processes within an ecosystem (Peterson and Fry, 1987). Accordingly, the use of stable isotopes to evaluate aquatic food web dynamics has become increasingly popular in ecological studies because differences in carbon (C) and sulphur (S) isotope ratios between consumers and their diet can provide information on energy flows and nutrient sources (Goering et al., 1990; Peterson and Fry, 1987; Yoshioka and Wada, 1994). For example, the C and S isotopic composition of animals is usually similar to their diets and can indicate the dietary source(s) important for consumers (Peterson and Fry, 1987). Consumption-related changes in carbon appear to be modest, with increases ranging from 0.0 to 1.0 ‰ per trophic transfer (DeNiro and Epstein, 1978; Fry and Sherr, 1984). Sulphur isotopes also have limited fractionation (< 1 ‰ per trophic transfer) and can be used as a reliable indicator of which plant or bacterial food sources are most important for consumers (Mekhtiyeva et al., 1976; Peterson et al., 1986; Peterson and Fry, 1987).

Accordingly, to better understand the effectiveness of the "wet landscape" approach as a disposal method for OSPM and its implications for the establishment of naturalized ecosystems, $\delta^{13}C$ and $\delta^{34}S$ signatures of representative taxa from constructed and naturally occurring wetland sites on the oil sands were compared. As a working hypothesis, the study postulated that the $\delta^{13}C$ and $\delta^{34}S$ positions of organisms from constructed sites used for reclamation purposes would differ significantly from similar organisms resident in reference sites.

3.2 Materials and Methods

The study was conducted at sites located on and/or near the Syncrude Canada Ltd. oil sands lease, northeast of Fort McMurray, Alberta (56°39'N, 111°13'W) (Fig. 1). Eight study sites were chosen in total, four reference sites and four oil sands process-affected sites (Table 1). Sites were chosen based on age, accessibility, and the presence/absence of fish, as well as to include a gradient of OSPM. All sites were > 10 years old when sampled. Beaver Creek Reservoir (BCR) and Demonstration Pond (DP) are the only sites that contain fish. Of the eight sites, four contain neither OSPM water nor MFT (E1, BCR, Barge Marsh (BM), Shallow Wetland (SWL)), two contain no OSPM water and MFT (E3, DP), one contains OSPM water and MFT (E5) and one contains only MFT with water consolidated from MFT (E7) (Table 1). E1, E3, E5 and E7 were originally excavated to a maximum depth of 5 metres in 1989 and all but E1 were filled with MFT (Table 1). E1 received 1000 m^3 of soil substrate to bring it to a common depth with the other test sites, and was filled with 1000 m^3 of non-OSPM water. E3 and E5 were both capped with 1000 m^3 of water, however E3 received non-OSPM water and E5 received OSPM water. DP was excavated to a depth of >12 metres in 1993, filled with MFT and then capped with 2.5 metres or 70,000 m^3 of non-OSPM water. E1, E3 and DP were filled with local muskeg drainage water, considered here as non-OSPM water. Table 1 reports the gradient of process-affected waste materials in greater detail and gives associated sulphate levels.

Reference sites selected for study included E1, BM, BCR, and SWL. None of these designated study sites contained OSPM, but they had differing histories for their clay liner substrates (Table 2). E1 is physically similar to E3, E5 and E7 and was constructed at the same time and in the same manner (Table 2). BM is a shallow pit and was discovered in 1977

during a highway construction. It is located approximately 6.5 km north of the Syncrude lease site and is recharged from surface waters draining the surrounding muskeg landscape (Fig. 1, Table 1). BCR is located on the mine lease and was formed by the impoundment of the Upper Beaver Creek in 1975, with recharge waters coming from the Beaver Creek watershed. SWL is located on the SCL lease on the same experimental test area where the test sites were constructed. It was formed when a berm was built during the construction of the DP in 1993. Its waters will be a mix of muskeg drainage waters and precipitation.

The experimental test sites (E1, E3, E5, E7 and DP) were built to provide test systems analogous to possible conditions expected in lease-closure pit lakes and reclamation habitats and will be referred to here after as constructed sites. All other sites (BM, BCR and SWL) will be termed reference sites. The materials used to create the constructed sites covered a range of OSPM properties. The constructed sites have allowed the study of these environmentally stressed areas to monitor biota colonization and successes (Harris, 2001). OSPM waters and MFT, as well as overburden clays that can be expected in the "wet landscape" reclamation options, are included. All sites were built at or near the boundary of a zone of Pleistocene lacustrine (Pl) and Cretaceous Clearwater (Kcw) clays that form an impervious liner so that release or re-charge to local groundwater aquifers is negligible (Table 2). The water chemistry in the various test sites reflect the release of OSPM waters from MFT, the leaching of ions from the clay liners, and the re-charge from surface run-off and precipitation waters. None of the other sites (BM, BCR and SWL) were excavated for experimental purposes, and most contain sediments dominated by Pl and basal tills (Table 2). Barge Marsh differs slightly from the other reference sites, as it sits in Pleistocene Glaciofluvial (Pf) sand (Table 2).

Water, plankton, aquatic invertebrate and fish were sampled at all sites July 2003 using identical methods. Plankton were sampled using 63 µm and 153 µm mesh plankton nets (30 cm diameter). To obtain standardized sample volumes for assessment, 3 vertical hauls from substrate to surface were conducted in randomly chosen locations at each study site. Immediately following collection, plankton samples were brought to the lab and sorted by size into three categories, 64 - 153 µm, 154 - 500 µm and >500 µm (using 63, 153 and 500 µm mesh screens). Once sorted, plankton were examined under a dissecting microscope to ensure sand, leaf litter, detritus and other biota were removed. The samples were then placed in glass petri dishes and dried at 40^{o}C for 24 hrs.

Qualitative samples of aquatic invertebrates were taken from the littoral zone (< 2 m) of all study sites at five randomly selected locations using a D-frame kick-net. Following collection, samples were rinsed through a series of stacked sorting sieves (1000, 500, 355, 250 and 125 µm mesh sizes). The contents of the sieves were rinsed directly into shallow light-coloured pans and taxa were picked out by hand. Any invertebrates found were identified using a dissecting microscope (up to 25X power) and placed in site specific 2.2 µm filtered water for 4 hrs to allow for gut clearance. Gastropod flesh was removed from the shell and cleaned of biogenic carbonates and grit before drying. All invertebrates were dried in glass vials at 40^{o}C for 48 hrs. Six invertebrate taxa were chosen for analysis based on relative abundance and feeding guild, including scrapers (Gastropoda), shredders (*Hyalella azteca*, *Haliplus*), predators (*Enallagma*, *Chaoborus*) and collectors (Chironomidae).

In the DP and BCR, fish were collected using minnow traps (6.35 mm mesh) and seining (6.35 mm mesh). All fish taxa were frozen for later identification. Fathead minnow (*Pimephales promelas*) was the only species present at both sites and were used for

comparison purposes. In the lab dorsal muscle tissue was removed posterior to the dorsal fin and above the lateral line and dried at 40^{o}C for 24 hrs following standardized protocols (e.g. Power et al., 2002). Similar sampling effort was expended at all other sites to confirm fish absences.

Three replicate water samples were collected subsurface in 1-L polyethylene bottles for standard water chemistry, as well as stable isotope analyses of dissolved inorganic carbon (DIC) and barium sulphate. Water chemistry (Table 1) was completed at Syncrude's Research Facility in Edmonton following standardized protocols (SCL, 1995). DIC water samples were refrigerated and analyzed within two weeks of sampling (Drimmie, 1990). Water samples were converted to barium sulphate in the lab via $BaSO_4$ precipitation (Heemskerk, 1994) to yield sample material for $\delta^{34}S$ analysis, with the exception of the water sample from SWL. SWL had a low SO_4 concentration, therefore the ion exchange technique was used to prepare the water sample for sulphur analysis (Heemskerk, 1994). All isotope analysis was completed at the Environmental Isotope Laboratory, University of Waterloo.

All samples used for stable isotope analysis were ground to a fine powder using a Retsch MM 2000 ball mill grinder (F. Kurt Retsch, GMBH and Co., Haan, Germany). Approximately 1 mg of the homogenate was used for carbon isotope analysis and approximately 3 - 5 mg of material was used for sulphur isotope analysis. Carbon isotope compositions were determined using a Micromass VG Isochrom continuous-flow isotope-ratio mass spectrometer connected to a Carlo Erba elemental analyzer (CHNS-O EA1108), with an analytical precision of ± 0.2‰. Sulphur isotope analyses were completed on a Europa TracerMass/Roboprep system, with an analytical precision of ± 0.3‰. All isotope analyses were completed at the Environmental Isotope Laboratory, University of Waterloo

(Waterloo, Ontario, Canada). Duplicate sample analysis (1 in 8) was completed for purposes of determining machine analytical variability. Measurement precision was established by repeat analysis of commercially available laboratory standards (International Atomic Energy Agency standard CH6: $\delta^{13}C = -10.4 \pm 0.1‰$ and NBS-127: $\delta^{34}S = 20.30 \pm 0.3‰$).

Stable isotope ratios are expressed as delta values (δ) and are measures of the parts per thousand difference (‰) between the isotope ratio of a sample and that of an international standard.

$$\delta^{13}C \text{ or } \delta^{34}S = [(R_{Sample}-R_{Standard})/R_{Standard}] \times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{34}S/{}^{32}S$ ratio of the sample or the standard. International reference standards used here included: carbonate rock from the Peedee Belemnite formation (Craig, 1957) for $\delta^{13}C$ and primordial sulphur from the Canyon Diablo meteorite (Rees et al., 1978) for $\delta^{34}S$. By convention, all international standards are set at a value of 0‰.

Statistical analyses of the data were preformed using SPSS version 14.0 (SPSS Inc., Chicago) and included use of independent two sample t-tests to compare carbon and sulphur isotopic values for samples from constructed and reference study sites. Linear regression analyses were used on water samples to compare barium sulphate $\delta^{34}S$ values, as well as salinity values to sulphate levels in constructed and reference study sites. Significance in all statistical testing was set at $\alpha = 0.05$.

3.3 Results

In Fig. 2, the mean results of stable carbon ($\delta^{13}C$) and sulphur ($\delta^{34}S$) isotopes for plankton, aquatic invertebrates and fish samples are plotted against each other, and a grouping by construction is indicated. With the exception of *Haliplus* all aquatic organisms in unconstructed sites ranged in $\delta^{34}S$ from -0.74 to 4.64 ‰ and in constructed sites from -11.99 to -5.93 ‰ (Fig. 2). Comparison of isotope signatures from reference and constructed sites showed no significant differences (t-test $P > 0.05$) in $\delta^{13}C$ for all taxa, except *Haliplus* and *Chaoborus* (t-test $P < 0.05$) (Table 3). Significant differences in $\delta^{34}S$ (t-test $P < 0.05$) were found for all taxa, except *Haliplus* (t-test $P > 0.05$) (Table 3). The range of $\delta^{34}S$ for the various physical and species groupings is plotted in Fig. 3. Linear regression analysis revealed that barium sulphate, $\delta^{34}S$, was significantly and negatively related to sulphate concentration in the waters from the various locations (Regression F-statistic $P = 0.05$, $r^2 = 0.49$) (Fig. 4). When analysed using linear regression, no significant relationship between salinity (conductivity) and sulphate concentration was found (Regression F-statistic $P > 0.05$, $r^2 = 0.43$).

3.4 Discussion

In most cases, carbon isotopes of representative taxa collected from constructed and reference sites were not significantly different. With the exception of *Haliplus*, all sulphur isotopic signatures of plankton, aquatic invertebrates and fish obtained from constructed wetlands differed significantly from the signatures of the same taxa obtained from reference sites. Results suggest the hypothesis that the $\delta^{34}S$ signatures of organisms from constructed sites would differ significantly from the signatures of like organisms from reference sites. No support; however, was found for the hypothesis concerning significant differences in organism of $\delta^{13}C$ signatures.

With respect to the single non-significant $\delta^{34}S$ result, it should be noted that adult Haliplidae are capable of sustained flight (Jäch, 1997), and *Haliplus* may be dispersing among OSPM and reference sites to find better food sources or new habitats. The migration in and out of study sites, therefore, is probably responsible for the fact that stable isotopes in *Haliplus* were not significantly different between constructed and reference sites.

Stable sulphur isotopic ratios of aquatic organisms will reflect the geological setting of their environment, with increasing negative $\delta^{34}S$ values typically being associated with weathering of marine Cretaceous rocks (Hitchon and Krouse, 1972). Positive values are generally found in systems where S inputs come predominantly via atmospheric deposition (Nriagu and Coker, 1978a,b) or from other mineral sources, including evaporates and some igneous rocks (Hitchon and Krouse, 1972). Most native soils in Alberta range in $\delta^{34}S$ values from -30 to +5‰ (Lowe et al., 1971, Krouse and Case, 1981). All constructed sites were built directly into Pl clay near the boundary of the Kcw clay strata, which is generally associated with depleted ^{34}S isotope signatures. The waters (pelagic and substrate

porewaters) in constructed sites are in direct contact with the soil materials, such that accumulation of sulphate will occur more readily as a result of oxidation of the sulphide minerals in the water body substrates. Coinciding with depleted $\delta^{34}S$ signatures, it was expected that there would be elevated sulphate concentrations resulting from the oxidation processes. Increase in sulphate concentrations are consistent with the results found in this study. Wells around the Peace River region of Alberta with high sulphate concentrations have also been associated with depleted $\delta^{34}S$ values (Krouse and Case, 1981). Hesslein et al. (1988) also found high sulphate concentrations and depleted $\delta^{34}S$ signatures in lakes of northwestern Ontario, but related the observed effects to fractionation of sulfur isotopes, where retention of $\delta^{32}S$ in the lakes is favored by either sulfate reduction or sorption.

All current study sites were oxic (Table 1), with no evidence of reducing conditions (negative Eh) being reported within their pelagic zones. Bacterial reduction of sulphate is unlikely to occur within the water column or in the epibenthic zones, while anaerobic conditions within the sediments and soft tailings are common and support an active anaerobic microbial community (Sobolewski, 1999; Holowenko, 2000). Sulphates enter the aquatic food web through algae, bacteria and rooted higher aquatic plants (Cook and Schindler, 1983). Since there is no S isotope fractionation associated with plant metabolism of sulphate (Thode, 1980), and no evidence for isotope fractionation in the decay of organic matter, the $\delta^{34}S$ pyrite in the sediments (clays, MFT, OSPM) in the various study sites, will likely account for the sulphates in the waters as a result of oxidation processes. Release at the water to substrate interfaces, as well as through surface runoff from precipitation will add to the sulphate increases seen.

Reference site waters are not in direct contact with fresh clays, except in E1 where the site was excavated prior to filling with water. Even though E1 is a reference site, it has seen an increase in sulphate content in the water column through release from the exposed Pl and Kcw clays and resulting oxidation of pyretic sulphides.

At SWL, the non-OSPM water was added to the site after weathering of the clays and the establishment of vegetative cover, including grasses and plants. Little available pyretic sulphur for oxidation and release into post water-filling was likely, both as a result of its weathering and the shielding effect between the water and the soil surface provided by the vegetation; this is reflected in the low SO_4 levels measured. Most of the water entering SWL, as well as the other reference sites (BM, BCR), will be from rainfall or surface runoff, and the SO_4 levels are low (Table 1) with no evidence of its addition from the sediment or riparian contacts being evident. Krouse et al. (1991) suggest that lakes with very low sulphate concentrations (0.5-4.0 mg of SO_4/L) (Fig. 4) in remote areas of continental shield terrain, have no evidence of sulphur isotopic fractionation due to sulphate reducers. Therefore, the $\delta^{34}S$ values of the sulphate in precipitation-dominated systems will reflect that of the rain water in the absence of other natural sources. The S signature of precipitation will be that which will be found in the S signature of the barium sulphate (water fraction) and aquatic life sampled. Nriagu and Harvey (1978) reported $\delta^{34}S$ values in precipitation of 3-8 ‰ in the Sudbury, Ontario area. Nriagu and Soon (1985) reported mean delta values for precipitation around 3.5 ‰ from Turkey Lakes, which are near Sault Ste. Marie, Ontario. Peterson and Fry (1987) reported average $\delta^{34}S$ continental precipitation between +1 to +7 ‰. Since the values associated with reference sites fall into these ranges, it would appear that the $\delta^{34}S$ values measured in this study represent that of the precipitation recharge waters.

The $\delta^{34}S$ value differences between the study sites provide us with an understanding of the pathways and interactions of the waters and biota within constructed wetlands. Study results are important since constructed wetlands will be a part of reclamation projects in the oil sands region of Alberta. Sulphur results allow researchers to follow and predict the source, flux and sinks for mobilized S associated with OSPM and the construction of wetlands. If an OSPM building material for these "wet landscape" reclamation components has a high sulphate concentration, or has the potential to release solubilized species of S during post-depositional processes, it is likely that the resulting high sulphate concentrations will be passed through various trophic levels, and pose potential effects on the development and sustainability of healthy aquatic ecosystems with stable food webs. Different species have different tolerances for sulphate levels (Singleton, 2000; Davies et al., 2003), and these differences should be considered during the design and implementation of wetlands and lake environments in the reclaimed landscapes so that the success of possible sensitive species can be anticipated and performance of the resulting aquatic systems are not compromised.

The values of S signatures in both the water and substrate materials can help researchers determine the approximate concentration levels of a study site and determine if it is appropriate to utilize. If an environment high in sulphates must be used for wetland construction, consideration to reduce the sulphate levels of introduced substances should be made to allow for the success of sulphate sensitive species. The present study has demonstrated that soils in direct contact with the water column can lead to significantly elevated sulphate concentrations and change $\delta^{34}S$ values. Study results determined that S signatures of constructed vs. reference sites displayed a shift change in the aquatic food web

structure. More detailed future studies could focus on soil and plant $\delta^{34}S$ values and a broader range of sites to determine isotopic fingerprints for future studies.

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Table 3.1. The physical and chemical characteristics of study sites on, or near, the Syncrude Canada Ltd. Lease, Fort McMurray, Alberta. Water sampling was completed coincident with aquatic taxa sampling. All measurements are in mg/L unless otherwise indicated. Sites are grouped into low (0 to 4 mg/L), medium (4 to 15 mg/L) and high (> 15 mg/L) naphthenic acid (NA) concentrations. NA* denotes NAs. Non-OSPM water comes from local muskeg drainage water and/or precipitation. MFT release water represents water generated through mature fine tails densification and de-watering and OSPM water is oil sands process material(s) water from Mildred Lake settling basin.

Study Site	Surface area (Ha)	Average water depth (m)	Mature fine tails (MFT) (m^3)	Water cap (m^3)	pH	Cond (uS/cm)	Salinity (ppt)	DO	DOC (ppm)	NA*	Na	Ca+Mg	Cl	SO_4	HCO_3 +CO_3
E1	0.05	1	0	Non-OSPM water	8.27	760	0.4	11.9	17.17	2.3	74.5	77.2	5.8	276.0	185.0
E3	0.05	1	1000	Non-OSPM water + MFT release water	8.81	767	0.4	10.1	26.86	4.7	149.0	28.0	31.0	61.5	365.4
E5	0.05	1	1000	OSPM water + MFT release water	9.21	2660	1.4	12.1	15.16	20.3	620.0	44.0	140.0	716.0	451.3
E7	0.05	0.4	2000	MFT release water + precipitation	8.55	1960	1.0	7.8	23.69	21.7	509.0	26.0	130.0	104.0	955.1
Demonstration Pond	4.0	2	70000	Non-OSPM water + MFT release water	8.76	1360	0.7	8.9	38.5	10.0	334.0	33.0	92.0	162.0	556.9
Shallow Wetland	0.8	0.5	0	Non-OSPM water	9.45	473	0.2	6.4	44.62	3.1	76.5	36.0	27.0	9.1	306.6
Barge Marsh	0.6	1	0	Non-OSPM water	7.42	350	0.0	9.4	58.32	1.1	24.2	44.7	37.0	7.8	159.0
Beaver Creek Resevoir	220	2	0	Non-OSPM water	7.41	270	0.1	8.0	79.75	1.5	29.3	35.2	1.1	13.3	187.3

Table 3.2. Geology of study sites, including approximate depths and description of underlying soils.

Site	Geology	Depths (m)
E1, E3, E5, E7 and Demonstration Pond	Pleistocene Lacustrine (Pl) + some Cretaceous Clearwater (Kcw) with reclamation soil (peat/soil mix) on riparian areas	0.0 - 2.0
	Pl + Kcw clays	2.0 - 6.0
	Kcw clays	6.0 +
Shallow Wetland	Holocene muskeg	0.0 - 0.5
	Pl + Kcw clays	2.0 - 6.0
	Kcw clays	6.0 +
Barge Marsh	Holocene muskeg	0.0 - 0.5
	Pleistocene Glaciofluvial (Pf) sand	0.5 - 3.0
	Cretaceous McMurray formation (Km) (oil sands)	3.0 +
Beaver Creek Resevoir	Holocene muskeg	0.0 - 0.5
	Pl clays	0.5 - 10
	Km (oil sands)	10 +

Table 3.3. P-value from independent two sample t-tests comparing $\delta^{13}C$ and sulphur $\delta^{34}S$ in plankton, invertebrates and fish collected from constructed and reference sites.

	P-value	
Taxa	**$\delta^{13}C$**	**$\delta^{34}S$**
Plankton Size Fractions		
64 to 153 µm	0.650	<0.001
154 to 500 µm	0.665	<0.001
> 500 µm	0.424	<0.001
Invertebrates		
Gastropoda	0.239	<0.001
Hyalella azteca	0.784	<0.001
Haliplus	0.026	0.281
Enallagma	0.127	<0.001
Chaoborus	0.024	<0.001
Chironomidae	0.183	0.002
Fish		
Pimephales promelas	0.424	<0.001

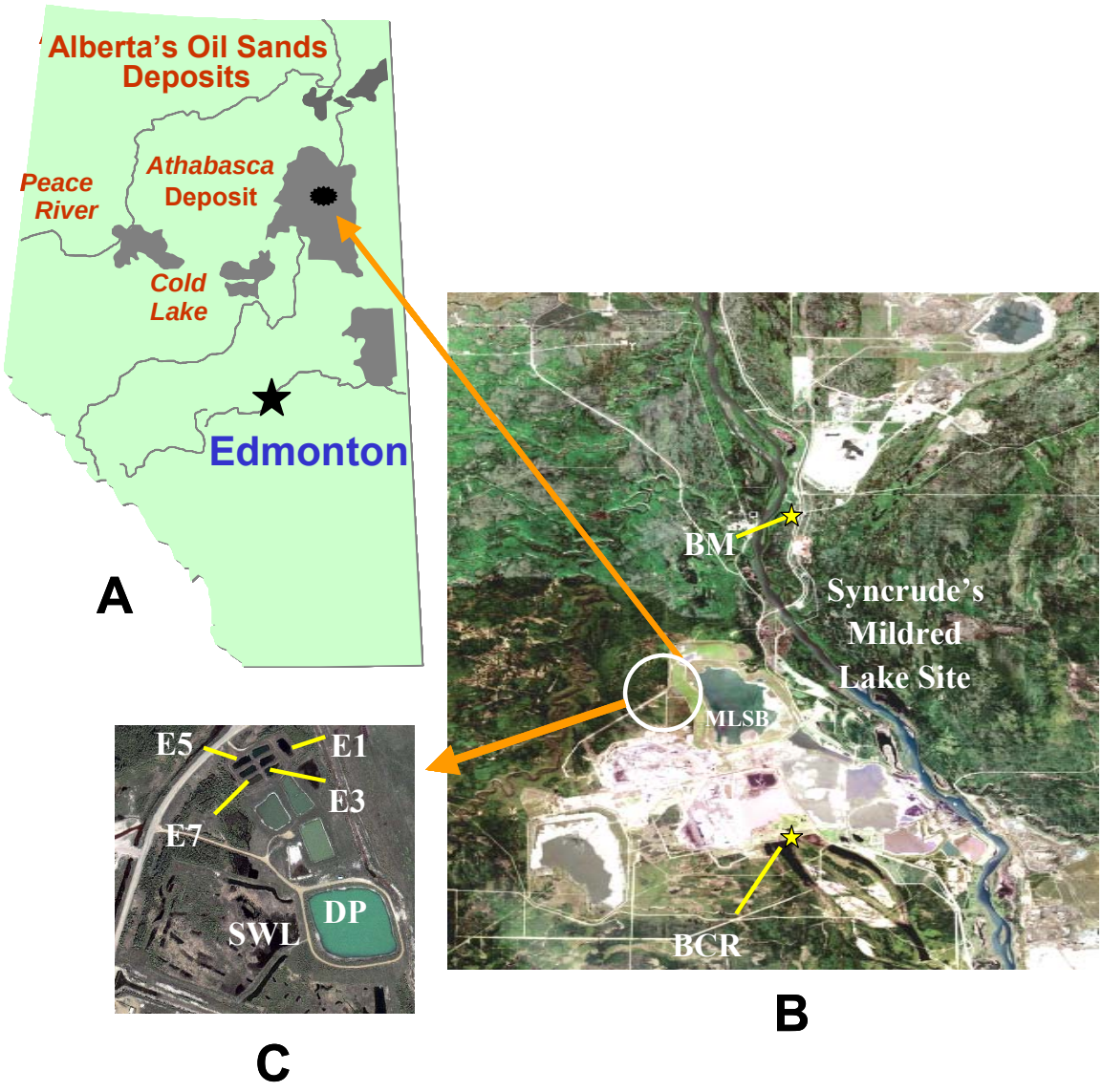

Fig. 3.1. Relative geographic location of study sites within the Province of Alberta, Canada (panel A) and the general location of study sites on the Syncrude Canada Ltd. lease (panel B and C). DP, SWL, BCR, BM and MLSB represent Demonstration Pond, Shallow Wetland, Beaver Creek Reservoir, Barge Marsh and Mildred Lake Settling Basin.

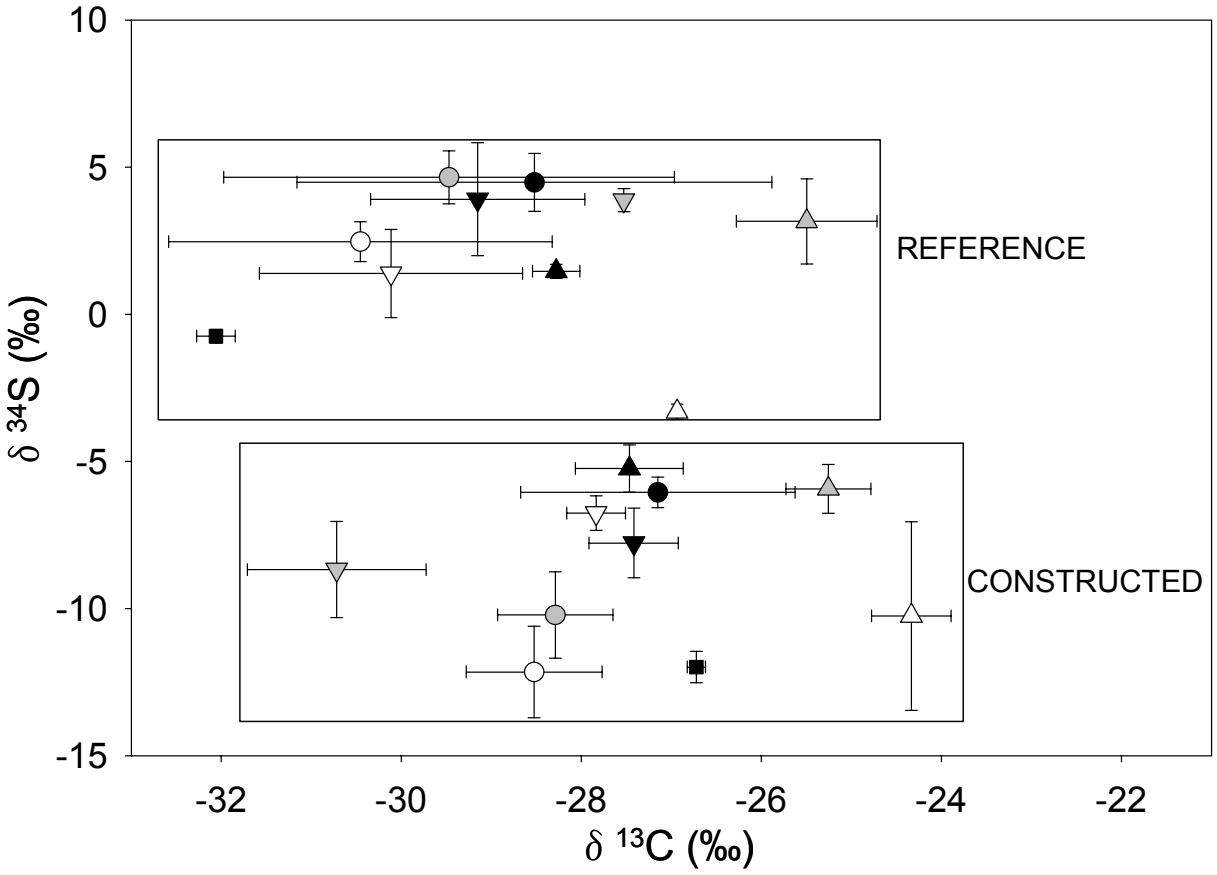

Fig. 3.2. Mean isotopic values for three size fractions of plankton (circle) (64 to 153 µm – black, 154 to 500 µm – grey, > 500 µm - white), invertebrates (up and down triangle) (Gastropoda – black up, *Hyalella azteca* – grey up, *Haliplus* – white up, *Enallagma*- black down, *Chaoborus* – grey down, Chironomidae – white down) and *Pimephales promelas* (square) of reference and constructed study sites. Error bars represent ± one standard error.

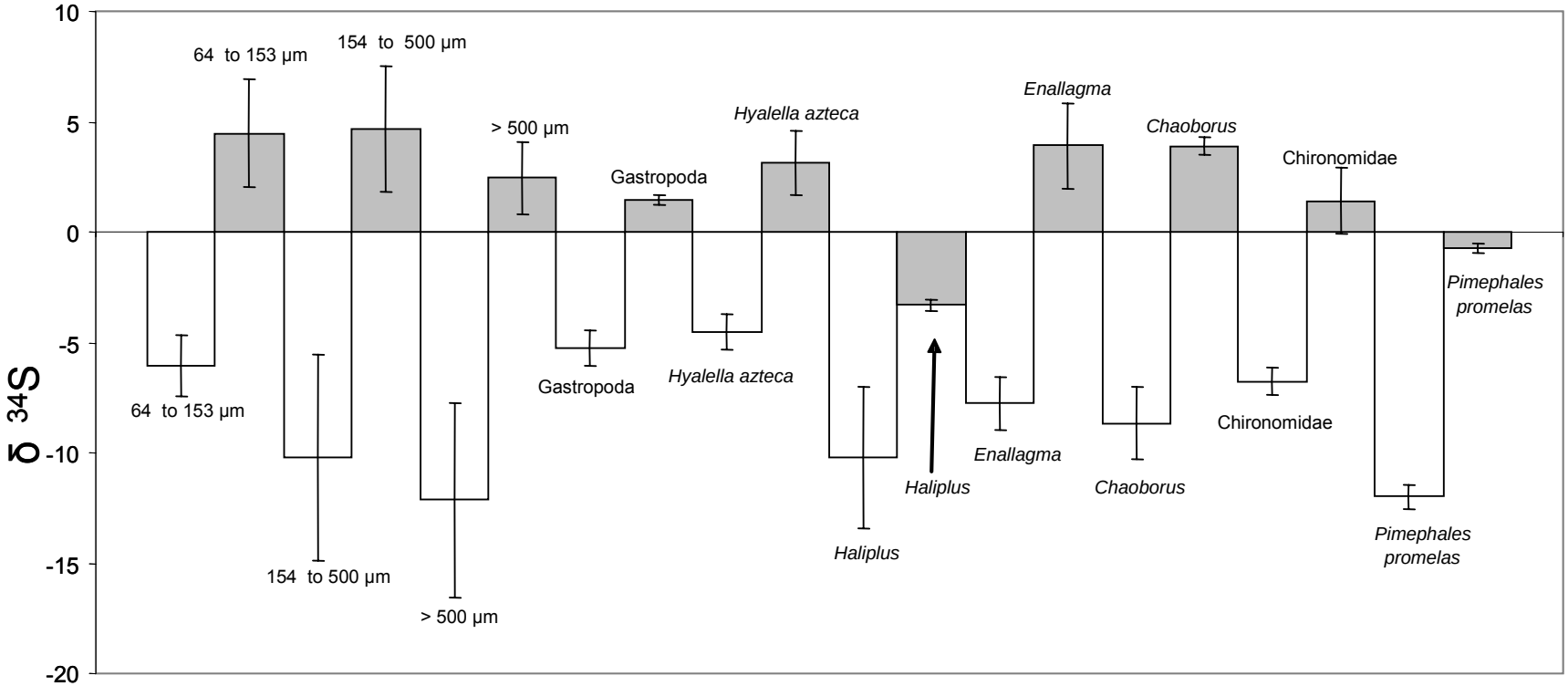

Fig. 3.3. Mean sulphur isotopic values for plankton, invertebrates and fish. White bars represent the grouping of all constructed sites and gray bars represent the grouping of all reference sites. The three size fractions of plankton are represented by 64 to 153µm, 154 to 500µm and >500µm. Error bars represent ± one standard error.

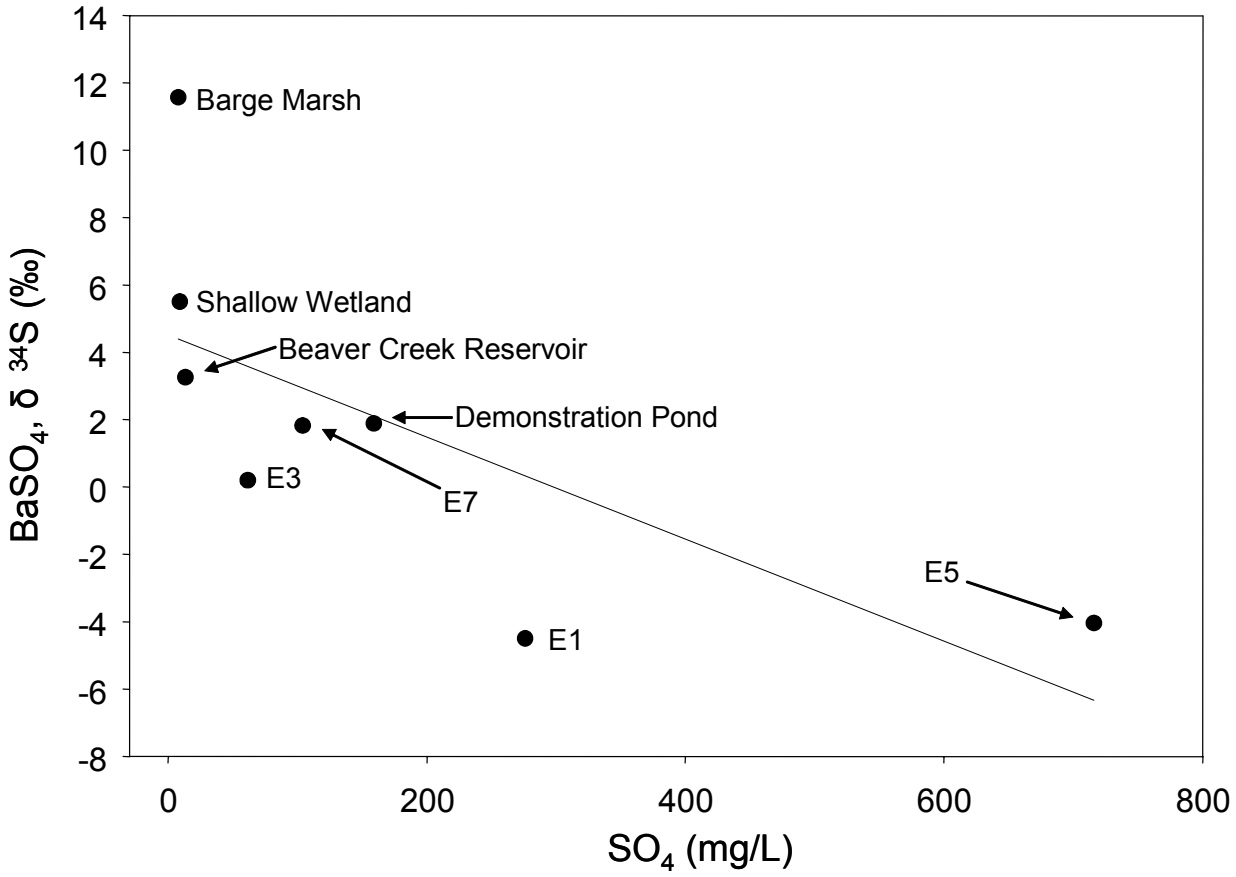

Fig. 3.4. General results for sulphur isotopic values of barium sulphate, $\delta^{34}S$ (‰) vs. sulphate concentrations (mg/L) of all study sites. Linear regression analyses is plotted as a solid line ($r^2 = 0.492$

Chapter 4. General Conclusions

In this thesis, representative components of aquatic food web structure and function were investigated in OSPM sites and reference sites using stable isotope ratios of carbon, sulphur and nitrogen. Carbon stable isotope analysis showed that 10 years after OSPM introduction, differences between OSPM sites and reference sites (Chapter 2) were not detectable. However, a significant isotopic enrichment in nitrogen was apparent for individual taxa, indicating that the relative position of taxa in OSPM sites differ from reference sites (Chapter 2). Sulphur isotope analysis revealed that construction, not OSPM introduction, can change food web position (Chapter 3).

To assess the effects of OSPM on the aquatic food web, Chapter 2 examined changes in stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios of sediment, DIC, DOC, POM, periphytic material, plants, plankton, aquatic invertebrates and fish. Comparisons between low, medium and high NA concentration sites showed no significant differences in consumer food web area and food web length. In most cases carbon isotope signatures of individual taxa were not significantly different from each other when low, medium and high NA concentration sites were compared. The results demonstrate that OSPM is not the main contributor of carbon to the aquatic food web, the most likely source being atmospheric CO_2. The nitrogen isotope signatures of most individual samples were significantly different when low, medium and high NA concentration sites were compared, suggesting that compounds from OSPM, such as ammonia, are likely enriching nitrogen signatures at OSPM sites. Since OSPM sites are oxic, nitrification processes produce nitrates and nitrites from the ammonia that are readily available for nutrient uptake by biota. Once biota at the base of the food web (e.g., periphytic material, POM) incorporate nitrogen isotopes that are enriched, the nitrogen

signature will travel from the base of the food web to the top predators of a system. Food web structure and function (determined by food web area and length) of OSPM sites were similar to reference sites > 10 years of age. However, nitrogen isotope results demonstrate that subtle changes in the relative positions of organisms occur in OSPM sites.

In Chapter 3, carbon and sulphur stable isotope ratios were used to determine the potential effects of site construction and OSPM on water, plankton, aquatic invertebrates and fish. In most cases, as in Chapter 2, carbon isotopes of representative taxa collected from constructed and reference sites were not significantly different. However, most sulphur isotopic signatures of plankton, aquatic invertebrates and fish obtained from constructed wetlands differed significantly from the signatures of the same taxa obtained from reference sites. Stable sulphur isotopic ratios of aquatic organisms will reflect the geological setting of their environment, with increasing negative $\delta^{34}S$ values typically being associated with weathering of marine Cretaceous rocks. Coinciding with depleted $\delta^{34}S$ signatures found in these aquatic systems were elevated sulphate concentrations. Accordingly, the stable isotopic results support the hypothesis that reclamation activities, specifically site construction, can shift food webs, resulting in identifiably different ecosystems.

Overall, this work demonstrates that carbon isotopes do not change significantly with OSPM content or site construction. Nitrogen isotopes do not differ when food web structure and position are analyzed, but do change with regard to the relative position of organisms between OSPM and reference sites. Sulphur isotopes change between constructed and reference sites. Although OSPM did not have a significant effect on studied food webs, OSPM did alter the $\delta^{15}N$ positions of organisms. Construction of sites into cretaceous substrates altered food web $\delta^{34}S$ signatures. The natural substrate materials of potential

aquatic reclamation sites should be determined in advance of construction. Substrate materials in contact with water may release toxic/unsuitable materials to aquatic organisms. If an OSPM building material for these "wet landscape" reclamation components has a high sulphate concentration, or has the potential to release solubilized sulphur species during post-depositional processes, it is believed that the resulting high sulphate concentrations will be passed through various trophic levels, and pose potential effects such as sulphate toxicity. If an environment high in sulphates must be used for wetland construction, consideration to reduce the sulphate levels of introduced substances should be made.

Future studies could look at a wider range of species including those with long life cycles. Invertebrates with longer life cycles (weeks to months) such as dragonflies, fingernail clams and snails experience longer exposure to wetland conditions and may be better indicators of bioaccumulation of OSPM constituents. A variety of soil types in the surrounding area should also be investigated to help determine which substrates are most suitable for OSPM reclamation. Sites of various ages and sizes should also be included in future study designs to help determine if these factors influence food web structure, function and relative positions of organisms.

Appendix A.1. Change in nitrogen species (NH3, NO2 and NO3) in OSPM (oil sands process-affected waste material) water when aged under aerobic conditions. Examples of OSPM water in E5 from July 1989 to August 1992 where OSPM water was isolated under aerobic conditions. (MacKinnon, pers. comm.)