

**IMPACTS OF THE CN RAIL OIL SPILL ON SOFTSTEM BULRUSH-DOMINATED  
LACUSTRINE MARSHES IN WABAMUN LAKE**

A report submitted

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to

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## EXECUTIVE SUMMARY

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A train derailment occurred on Canadian National Railway Co.'s primary route through northern Alberta on August 03, 2005, spilling Bunker 'C' oil and small quantities of Imperial Pole Treating oil (PTO) into the water on the north shore of Wabamun Lake. Subsequently, oil spread along the northern, eastern, and southern shores of Wabamun Lake, negatively affecting crucial habitat for numerous aquatic organisms, waterbirds, and waterfowl. The impacts of the oil spill and subsequent treatment regimes on the vegetation forming the lacustrine marshes have not been assessed as of August 2007. We were hired to assess the ecosystem health of previously treated, contaminated, and unaffected lacustrine marshes of Wabamun Lake and to provide a long-term prognosis for the viability of these marshes. Therefore, we measured (1) aboveground biomass, height, density, degree of oiling, and fecundity of the dominant emergent macrophyte species, *Schoenoplectus tabernaemontani* (K.C. Gmel.) Palla (the new name of the softstem bulrush *Scirpus validus* Vahl.); (2) the species richness, density, and percent water volume inhabited (PVI) by submerged aquatic vegetation (SAV); (3) concentrations of polycyclic aromatic hydrocarbon (PAH) and naphthenic acids (NA; cytotoxic, carcinogenic, mutagenic, and teratogenic components of Bunker 'C' oil and PTO) in lacustrine marsh sediments and *S. tabernaemontani* rhizomes; and (4) a suite of standard limnological variables of the surface water in lacustrine marshes in Wabamun Lake. The study design tried to sample a suite of sites representing a gradient of oil spill impact, e.g., oiling on plants and altered water and sediment chemistry (control – unaffected; Rizzie Beach – moderately affected; Ascot Beach and Grebe Reedbed – heavily affected). Rizzie and Ascot Beaches and the Grebe Reedbeds had areas that were classified as unaffected; however, we found that all sites previously classified as “unaffected” were indeed affected by some measure; only the control site west of the oil spill location was completely unaffected by the spill.

We show that aboveground biomass of softstem bulrush (*S. tabernaemontani*) falls within the range previously reported for this and similar freshwater marsh bulrush species in North America. Hence, the oil spill itself (the residual PAHs, NAs, etc.) did not have any

residual effects on the bulrush plants; however, the activities (treatments) used to remove and clean up the oil has had negative effects in some areas that were likely exacerbated by prevailing area characteristics. For example, treatment effects varied among areas, with significantly lower biomass at Ascot Beach, but significant increases in biomass at Rizzie Beach. This different response may be the result of physical characteristics prevalent at these two sites, including water depth, wind and wave exposure, and treatment intensity. Areas of shallower water had higher biomass, regardless of oil or treatment effects. Overall, the oil spill seemed to have had no negative effects on *S. tabernaemontani* biomass, cover, height, and seedhead production. This was particularly evident at the Grebe Reedbed, which was heavily affected by the oil spill and these variables were similar between the affected and untreated site and the “unaffected” site. Despite the lack of effect on the plants, several PAHs were still present in detectable quantities in lake sediments; however, concentrations were below Canadian environmental guidelines. NAs were generally below detection limits in sediments and *S. tabernaemontani* rhizomes. There were no noticeable negative effects on SAV species richness, cover, or PVI in any of the sites. Overall, treatments removed oil from both the plants and from the sediments; however, the question is at what cost to the reedbed health, since the plants were not negatively affected by the oiling alone.

To remove oil from the reedbed, emergent macrophytes were cut just above the sediment surface or just below the water line, and the sediments in the reedbeds were flushed and/or vacuumed several times in various locations. Treatment regimes were successful in reducing oiling on plants, but had a severe impact on *S. tabernaemontani* rhizome density, and consequently rhizome biomass. For example, treatment intensity was more aggressive at Ascot Beach than at Rizzie Beach, consequently, recovery success from those treatment regimes differed between these two areas. There appeared to be a relationship between disturbance from treatments, water depth, and recovery success, with the shallower sites having greater recovery success from the treatment than deeper sites, likely in response to increased rates of seed germination, seedling growth, and rhizome survival and re-growth. Therefore, future treatment regimes should take into consideration water depth to ensure successful macrophyte re-growth from either seeds or

rhizomes. Water levels have risen on average about 40 cm from 2005-2007, with the effect that treatment impacts were more severe in deeper water. The long-term prognosis of the treatment areas (where the rhizome and plant cover is still low) remains uncertain due to high water levels, which reduce seed germination, and generally slow lateral growth rates of *Schoenoplectus* rhizomes. Thus, while the oil spill itself did not place the reedbeds in jeopardy, the treatment impacts in the deeper reedbeds reduced both plant and rhizome density and may have long term consequences. These areas may remain largely open or sparsely vegetated for several years, thereby providing lower quality habitat for fish, waterbirds, and waterfowl, which depend on these marshes for spawning, rearing young, and feeding habitat.

We recommend some continued monitoring of the reedbeds in selected areas. In addition, we recommend that in any future spills, treatments, such as high pressure sediment flushing or sediment vacuuming, or any regime that significantly disturbs lacustrine marsh sediments be avoided, particularly in marshes with deep water.

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## 1.0 INTRODUCTION

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Prairie and southern boreal forest marshes are dynamic ecosystems characterized by alternating flooding and drying cycles. Their dynamic nature results from environmental factors interacting with the dominant marsh plant species. In western Canada, water level fluctuations and water chemistry are prominent among these environmental factors (Walker and Coupland 1968). These variables interact with ecological life history features of the dominant plant species, such as their life-span, reproductive mode (vegetative, sexual, or both), and tolerance to flood, drought, and salinity (Harris and Marshall 1963, van der Valk 1981, Lieffers and Shay 1982). The combined effects of hydrology, local bedrock geology, and wetland morphology (hydrogeomorphic variables) tend to be the primary regional determinants of emergent and submerged plant community structure in wetlands and littoral systems (Minc 1997, Keough et al. 1999).

Wetlands of prairie and (southern) boreal shallow lakes and ponds are dominated by species of the emergent macrophytes *Carex* (sedges), *Eleocharis* (spike rush), *Schoenoplectus* (softstem bulrush), and *Typha* (cattails) and the submerged aquatic vegetation (SAV) species *Myriophyllum* (water milfoil), *Potamogeton*, (pondweed), *Ranunculus* (buttercup), and *Utricularia* (bladderwort; Walker and Coupland 1968, Millar 1973). These plant communities provide seasonal or permanent habitat and food sources for a myriad of invertebrates and vertebrates. For example, waterfowl and waterbirds, such as ducks, geese, grebes, terns, loons, herons, and rails, commonly breed in or along the edges of lacustrine marshes, taking advantage of nesting materials, cover, and food sources, including (in)vertebrates, algae, SAV, and macrophytes (Scheffer 2004). Also, many fish species, such as *Esox lucius* L. (northern pike) and *Culaea inconstans* Kirtland (stickleback) use marshes as spawning, rearing young, and feeding habitat.

Wetland plants have been used previously as indicators of geology, groundwater, soil type, bedrock composition, and minerals (Chikishev 1965), and to delineate wetland boundaries; however, until recently, few attempts have been made to use wetland plants

as indicators of biological condition (Mack et al. 2000). As an indicator assemblage, plants offer several advantages: (1) they are a ubiquitous feature of wetlands and, in most cases, can be identified to species-level with minimal training (U.S. E.P.A. 2002), (2) they are immobile and therefore susceptible to physical, chemical, and biological changes in the surrounding environment, (3) plant communities possess a number of attributes easily measured and quantified, and (4) many plant community attributes, as well as individual species, are sensitive to anthropogenic and natural disturbances.

Anthropogenic and natural disturbances, including, but not limited to, eutrophication, climate change, shoreline alterations, and commercial and residential developments, affect the water quality and quantity and sediment quality of lacustrine wetlands (Day et al. 1988, Barko et al. 1991). For example, light availability is a primary factor determining photosynthetic potential of emergent and submerged aquatic vegetation and can be reduced by non-algal and algal turbidity, including periphyton growth (Phillips et al. 1978), which subsequently affects plant and animal communities, hydrology, thermal regimes, and nutrient dynamics. Anthropogenic habitat losses over the past half century have significantly affected northern pike (*E. lucius*) production in North America. Northern pike are large keystone piscivores important in “top-down” predatory regulation of fish communities. They tolerate a wide range of environmental conditions, but they are primarily mesothermal (cool-water) fish adapted to shallow, productive, mesotrophic to eutrophic environments. They are a common and abundant species, found in 45% of the total freshwater area of North America (Carlander et al. 1978). Casselman and Lewis (1996) reiterated the importance of macrophyte cover as an important feature of nursery habitat for pike that not only reduces susceptibility to predation but also provides cover for important prey species and for young pike to lie in wait for prey. They concluded that the optimal vegetative density for nursery habitat ranged from 40-90% cover.

A train derailment occurred on Canadian National Railway Co.’s (from hereon CN) primary route through northern Alberta running east-west, parallel to Wabamun Lake, AB, on August 03, 2005. The derailment was caused by at least 13 defects in a 12-m section of rail track (TSB 2007), resulting in the release of about 712,500 L of Bunker

'C' oil and about 88,000 L of Imperial Pole Treating oil (PTO) onto the ground in the vicinity of the derailment and into the water of Wabamun Lake. Bunker 'C' oil is a complex mixture of aliphatic, olefinic, naphthenic (NA), and polycyclic aromatic hydrocarbons (PAH). PTO is a mineral oil consisting of a mixture of aliphatic and aromatic hydrocarbons. The PAHs with the five highest concentrations in the Bunker 'C' oil and the PTO that spilled into Wabamun Lake were naphthalene ( $380 \mu\text{g g}^{-1}$ ), acenaphthene ( $300\text{-}330 \mu\text{g g}^{-1}$ ), fluorine ( $470\text{-}1,400 \mu\text{g g}^{-1}$ ), acridine ( $490\text{-}1,800 \mu\text{g g}^{-1}$ ), and phenanthrene ( $1,700\text{-}2,700 \mu\text{g g}^{-1}$ ) (data from samples collected August 09, 2005; CN Railway Co. 2006).

PAHs are a diverse class of organic compounds that contain two or more fused aromatic (benzene) rings (Fetzer 2000, CCME 2007) that can be toxic to aquatic biota at elevated concentrations (CCME 2007). Differences in the structure and size of individual PAHs result in substantial variability in the physical and chemical properties of these substances (CCME 2007). The primary sources of emissions of reportable size are petroleum refineries, fossil fuel power plants (coal, oil), coal-tar production plants, coking plants, bitumen and asphalt production plants, paper mills, wood products manufacturers, aluminum production plants, and industrial machinery manufacturers. Other emitters of PAHs are asphalt roads, road and road tar, coal, coal tar, fires of all types (bush, forest, agricultural, home heating, cooking, etc), and the manufacture and use of preserved wood (creosote). Natural sources include fire, e.g., bush or forest fires, crude oil, shale oil, coal tars, and active volcanoes. NAs are oily liquids, which are classified as monobasic carboxylic acids of the general formula R-COOH, where R represents the naphthene moiety consisting of cyclopentane and cyclohexane derivatives. NAs are composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids, with smaller amounts of acyclic aliphatic acids. The cycloaliphatic acids include single and fused multiple cyclopentane and cyclohexane rings. The carboxyl group is usually attached to a side chain rather than directly to the ring. Aromatic, olefinic, hydroxyl, and dibasic acids are present as minor components (see review in Headley and McMartin 1992). NAs recovered from refinery streams occur naturally in crude oil and are not formed during the refining process. Heavy crude oils have the highest acid content, and paraffinic crude

oils usually have low acid content. NAs are obtained by caustic extraction of petroleum distillates, primarily kerosene and diesel fractions. Several PAHs and NAs are known or suspected cytotoxins and endocrine disruptors, carcinogens, mutagens, and/or teratogens (causing birth defects) and have been linked to health problems in humans, other mammals, and aquatic organisms, including fish and invertebrates (Rogers et al. 2002, Luch 2005).

The derailment site was located in the community of Whitewood Sands, about 7.5 km west of the village of Wabamun. The area directly affected by the derailment covered a distance of about 1 km, an estimated 300 m of which were directly affected by petroleum hydrocarbons resulting from the rupture of the tank cars. The affected area included CN's right-of-way and intermittent parcels of adjacent land to the south of the rail line towards the north shore of Wabamun Lake. Over subsequent days and weeks, the oil dispersed along the northern, eastern, and southern shores of Wabamun Lake. Since August 03, 2005, several studies have examined the impacts of the oil spill on sediment microbial, algal, limited macrophyte, fish, invertebrate, and zooplankton communities (e.g., Foght 2006, CN Railway Co. 2007a, b); however, the impacts on the vegetation of the lacustrine wetlands of Wabamun Lake have not been adequately assessed to date.

Several of the affected lacustrine marshes have previously been treated with a combination of vegetation cutting, vacuuming, and/or low-pressure flushing. Treatment guidance was specific to three categories of bulrush/reed beds: (1) accessible from shore, patchy distribution,  $\leq 0.4 \text{ m}^2$  in area, harvesting is done manually; (2) accessible from shore,  $> 0.4 \text{ m}^2$  in area, and not designated as "very sensitive areas", harvesting is done manually; and (3) inaccessible from shore,  $> 0.4 \text{ m}^2$ , and designated as "very sensitive areas", harvesting was done primarily using a reed cutter with manual supplements as required. Harvesting of each lacustrine marsh was done according to site-specific deployment instructions. Treatments were designed to (a) avoid trampling new macrophyte shoots, (b) avoid trampling sunken oil, (c) minimize disturbance of fish habitat, (d) minimize disturbance and re-suspension of soft substrata (e.g., silt/clay), (e) minimize disturbance of migratory waterfowl nesting/brooding habitat, and (f) avoid

trampling of back-shore areas of macrophyte beds. Low-pressure water flushing was used in conjunction with vegetation removal to remove oil from sediments, shores with vegetation, and lacustrine marshes in Wabamun Lake in an effort to minimize damage to plants and animals. Subsequent vacuuming removed most of the remaining tar balls from the sediment. Treatment regimes varied among the lacustrine marshes due to environmental characteristics (e.g., composition of sediments, water depths) and accessibility (C. Brock, C. Emmerton, Alberta Environment, personal communication). The goal of these treatments was to reduce oil on the vegetation, surface of the lake, and in the lake sediment; however, short- and long-term impacts on the lacustrine wetlands in response to various treatment approaches remain unknown from the perspectives of plant biodiversity, plant productivity, and suitability as future habitat for waterbird, waterfowl, and fish populations.

## 2.0 OBJECTIVES

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This study was designed to assess the ecosystem health of previously treated, contaminated, and unaffected lacustrine marshes of Wabamun Lake and to provide a long-term prognosis for the viability of these marshes. Our objectives were to:

1. measure aboveground biomass, height, density, degree of oiling, and fecundity of the dominant macrophyte species, *S. tabernaemontani*;
2. assess the species richness, percent volume inhabited (PVI), and cover by submersed aquatic vegetation (SAV);
3. measure chl. *a* concentrations as a proxy for algal productivity;
4. determine concentrations of PAHs and NAs in Wabamun Lake sediments and *S. tabernaemontani* rhizomes;
5. collect standard limnological data at various locations along the north-shore of Wabamun Lake (surface water N and P concentrations, pH, water depth, Secchi disk depth); and
6. provide a prognosis for the recovery of lacustrine marshes in Wabamun Lake.

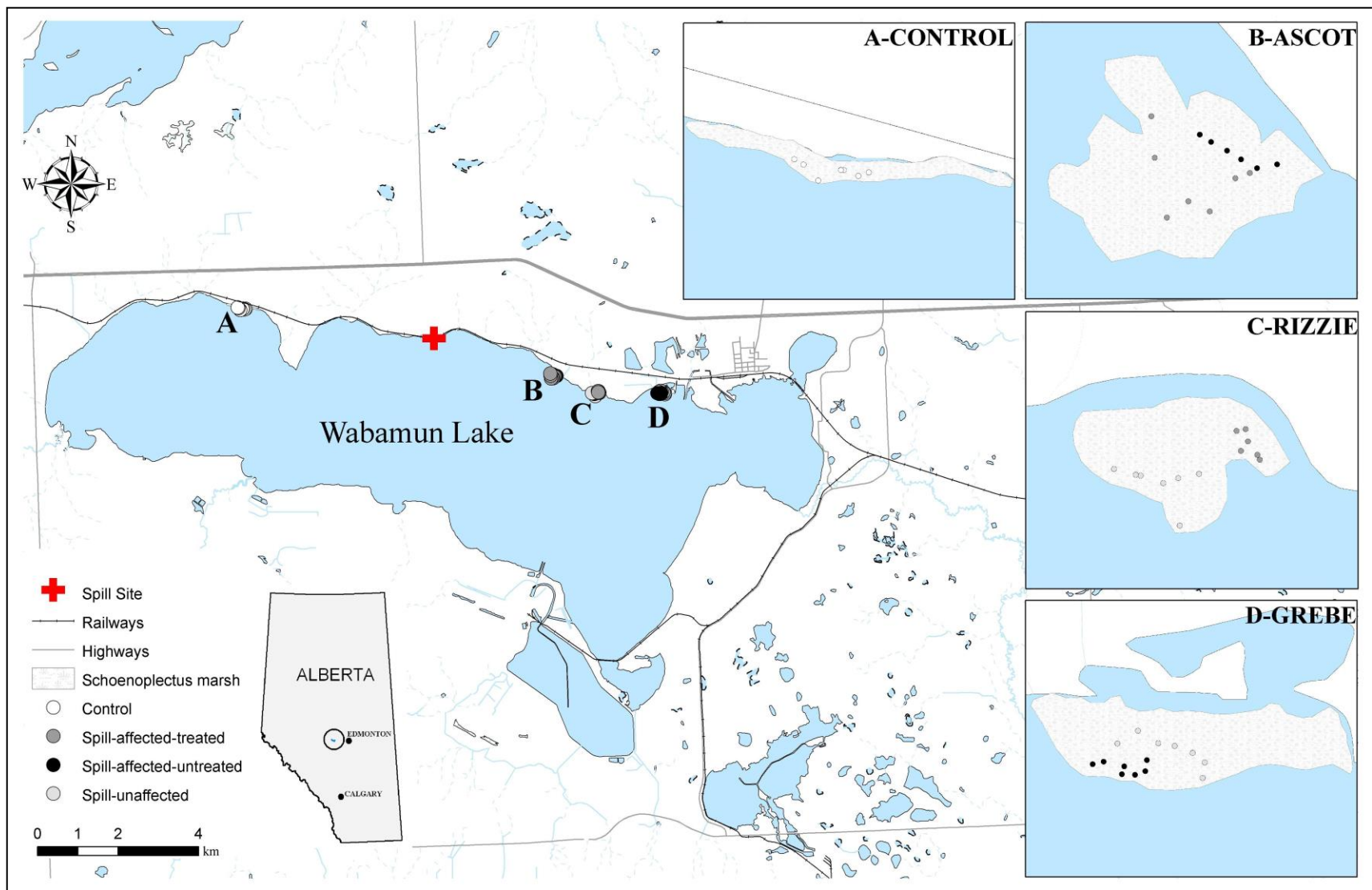
### 3.0 METHODS

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#### 3.1. SITE SELECTION

Four study areas were selected based on the degree of treatment following the oil spill. The affected areas were Ascot Beach, Rizzie Beach, and the “Grebe Reedbed” (a misnomer, because the marsh is dominated by bulrushes, not reeds). These areas are located on the northern shore of Wabamun Lake about 3-5 km west of the village of Wabamun and about 3-5.5 km east of the CN oil spill location at Whitewood Sands (Figure 1). The areas varied in their treatment intensities, ranging from the aggressively-treated Ascot Beach to the more moderately-treated Rizzie Beach and Grebe Reedbed. The control area, unaffected by the oil spill, was located about 4 km west of the oil spill location on the northern shore of Wabamun Lake (Figure 1).

Sampling locations were established in the control area along a transect parallel to the shoreline. In the affected areas, two transects were established, each with seven sampling locations generally parallel to the shoreline. At Ascot Beach, transect 1 was located in the oil spill affected and treated *Schoenoplectus* marsh, while transect 2 ran along a northern-most pre-existing transect on the eastern expanse of the *Schoenoplectus* marsh. This part of Ascot Beach was previously classified as unaffected by the oil spill, and hence never treated; however, direct observations showed the presence of oil on *S. tabernaemontani* plants and oil sheens and tar balls on/in the water. This part of the study area was re-classified as affected but untreated (Figure 1). At Rizzie Beach, transect 1 was located in the unaffected eastern expanse of the *Schoenoplectus* marsh, and transect 2 was located in the previously treated south-western expanse of the *Schoenoplectus* marsh (Figure 1). At the Grebe Reedbed, transect 1 was located in the heavily affected, untreated, boomed area, while transect 2 was located between the shore and the boomed reedbed in the unaffected *Schoenoplectus* marsh (Figure 1). Thus, there were seven study sites in total. All transects and sampling locations were established randomly in each of the sites (control – unaffected; Ascot Beach – affected and treated, affected and untreated; Rizzie Beach – unaffected, affected and treated; Grebe Reedbed – unaffected, affected and untreated). Coordinates were obtained with a Garmin™ GPS.



**Figure 1.** Locations of the four study areas in Wabamun Lake, AB.



### **3.2. *SCHOENOPLECTUS TABERNAEMONTANI* BIOMASS, PLANT AND RHIZOME DENSITY, HEIGHT, SEEDHEAD PRODUCTION, AND DEGREE OF OILING**

Aboveground biomass was destructively sampled in randomly-placed 0.5 x 0.5 m plots (0.25 m<sup>2</sup>) at each of the seven sites (n = 7 plots/site). Long-handled clippers were used to cut stems just above the sediment. Plants were then cut into 30-cm segments, placed into paper bags, dried to constant mass at 50 °C, and weighed to the nearest gram. Final biomass values were expressed as g m<sup>-2</sup>.

We also measured plant and rhizome density (% cover m<sup>-2</sup>), height with a meter stick (cm; n = 25 plants/plot, n = 7 plots/site), and the degree of oiling of 25 individual *S. tabernaemontani* plants adjacent to each of the seven harvested plots per site. The degree of oiling was visually assessed by applying a rating from 0-10 to each of the plants, whereby a rating of “0” indicated the absence of oil on an individual plant and a rating of “10” indicated a plant entirely covered in oil. This rating provided an indication of the relative abundance of oil on *S. tabernaemontani* plants in each of the seven sites. The proportion of *S. tabernaemontani* plants with/without seedheads was determined from the harvested vegetation (% of plants with/without seedheads m<sup>-2</sup>).

### **3.3. LACUSTRINE MARSH SEDIMENT, *SCHOENOPLECTUS TABERNAEMONTANI* RHIZOME, AND WATER CHEMISTRY AND DEPTH**

#### **3.3.1. LACUSTRINE MARSH SEDIMENT AND *SCHOENOPLECTUS TABERNAEMONTANI* RHIZOME CHEMISTRY**

Sediment samples were collected manually by a diver from the top 5 cm of the lake sediment and placed in 250 ml and 125 ml screw-top, glass jars (n = 4/site). Samples were stored on ice in a cooler during transport and in a refrigerator until analyses within 14 days. Rhizomes of *S. tabernaemontani* (1-3 healthy-appearing segments, each 5-15 cm long) were cut with clippers from rhizome conglomerates embedded in the lake sediment (n = 3 plots/site). These rhizomes are massive structures, with tussocks emerging from the sediments. Samples were stored in Ziploc™ bags on ice in a cooler and subsequently in a refrigerator. PAH and NA analyses were done at ALS Laboratories in Edmonton and Fort McMurray, AB.

PAHs were extracted from sediment and rhizome samples using either a Soxhlet extractor or with a shake/vortex/sonication procedure (EPA method 3540, modified for use of a rotary evaporator and ETL glassware) according to CCME (Canadian Council of Ministers for the Environment) guidelines. The extract was then concentrated and analyzed by gas chromatography-mass spectrometry (GC/MS) in the selective ion monitoring (SIM) mode (EPA method 8270, modified for SIM analysis). All samples were analyzed for benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene, and quinoline. These compounds represent a limited suite of PAHs and are listed by the CCME as potentially harmful to aquatic organisms. Thus, they should be monitored under the Protection of Aquatic Life (PAL) guidelines.

Naphthenic acids were extracted from sediment and rhizome samples using dichloromethane and then quantified using a combination of the Dean and Stark and Soxhlet methods according to Clescerl et al. (method 5520 A, B, C, F; 1998).

### **3.3.2. WATER CHEMISTRY AND DEPTH**

One surface water sample (vol. = 2 L) was collected at each of the seven sites. This was sufficient, since the lake is well mixed throughout the ice-free season. Samples were stored on ice in a cooler during transport and in a refrigerator at 4 °C until analyses. All samples were analyzed for total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), nitrite/nitrate ( $\text{NO}_2^-/\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), total nitrogen, (TN), total dissolved nitrogen (TDN), pH, and chlorophyll *a* (chl. *a*). After filtration, all perishable analyses were done within four weeks at the Biogeochemical Analytical Laboratory in the Department of Biological Sciences, University of Alberta, Edmonton, AB.

$\text{NO}_2^-/\text{NO}_3^-$  and  $\text{NH}_4^+$  samples were filtered, fixed with conc.  $\text{H}_2\text{SO}_4$ , and analyzed within four weeks. TDN samples were filtered and analyzed within 4 weeks.  $\text{NO}_2^-/\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and TDN concentrations were measured on a Technicon Auto Analyser II. TDP and TP were analyzed following Bierhuizen and Prepas (1985) and SRP was analyzed following Menzel and Corwin (1965). N and P analyses were done using a Lachat Quick Chem

Flow Injection Analyzer (Lachat Instruments, Milwaukee, WI, USA). Chl. *a* samples were filtered within 24 hrs., and chl. *a* was extracted with 95% ethanol and analyzed fluorometrically with a Shimadzu RF-1501 Fluorescence Spectrophotometer (Shimadzu North America, Columbia, MD, USA) according to Bergmann and Peters (1980). Acidity/alkalinity was measured using an Accumet Model 50 pH meter (Fisher Scientific Canada, Ottawa, ON, Canada).

Water depth and Secchi disk depth (an indicator of turbidity) were measured to the nearest cm with a meter stick at each site ( $n = 7/\text{site}$ ).

#### **3.4. SAV SPECIES RICHNESS, PVI, AND COVER**

The species richness of submerged aquatic vegetation (SAV) was determined by raking the sediment surface in four 1-m<sup>2</sup> plots at each sampling location (4 m<sup>2</sup>/sampling location,  $n = 7$  sampling locations/site, therefore 28 m<sup>2</sup>/site). Plant samples were collected and identified to species. A PVI (percent volume inhabited) value for SAV was obtained by visually estimating the percent occupation by the SAV community in the water column (water volume = depth x 1 m<sup>2</sup>; e.g., Canfield et al. 1984). The cover of SAV was visually estimated prior to SAV species collections (% cover m<sup>-2</sup>). Plant taxonomy followed the USDA (2007) plants database.

#### **3.5. STATISTICAL ANALYSES**

Analyses of variance (ANOVA) with *post-hoc* Tukey LSD tests were used to analyze all data among the seven sites and four areas (independent variables: sites, areas; dependent variables: *S. tabernaemontani* biomass, *S. tabernaemontani* height, *S. tabernaemontani* density, SAV density, SAV PVI, *S. tabernaemontani* oiling rating, water depth, Secchi disc depth). ANOVAs are an appropriate analysis since they are resilient to some deviations from data normality and homogeneity of variances (Zar 1998).

Simple linear regressions were done between *S. tabernaemontani* biomass and *S. tabernaemontani* and SAV density, proportion of *S. tabernaemontani* plants with seedheads, *S. tabernaemontani* plant height, and water depth. All statistical analyses were done using Systat, v. 11 (Systat Software, Inc., San Jose, CA, USA).

### 3.6. RATIONALE FOR EXPERIMENTAL DESIGN

Our experimental design was chosen to reduce environmental variability among sampling plots, provide a better indication of plant community characteristics within sites, and allow us to detect meaningful differences among sites with high statistical power. Our approach was based on widely accepted methods to assess ecosystem characteristics. For example, our sampling transects were oriented roughly parallel to the open water of Wabamun Lake within each study site. This is the preferred transect orientation, because distinct plant community zones form in many wetlands due to different environmental conditions with increasing distances from the open water, e.g., differences in water depth, exposure, organic matter content, and nutrient dynamics (e.g., Mitsch and Gosselink 2000, Whitehouse and Bayley 2005). Also, our sample size ( $n = 7$  plots/site) falls within the range used most frequently in studies of this nature (generally  $n = 5-10$  plots/site; e.g., Hopkins et al. 1978, Thormann and Bayley 1997, Chimner and Cooper 2003), and our quadrat (0.5 x 0.5 m) was of appropriate size for sampling the biomass of *S. tabernaemontani* (Hopkins et al. 1978).

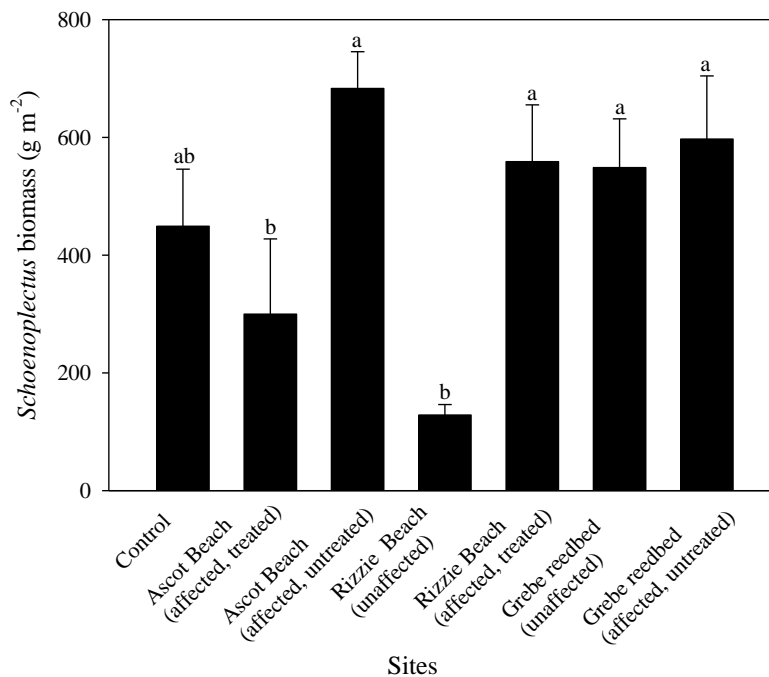
All other variables, e.g., water and Secchi disk depths, *S. tabernaemontani* height, density, fecundity, and oiling, SAV community characteristics, and sediment, *S. tabernaemontani* rhizome, and surface water chemistry samples, were measured or collected within 1 m distance from the biomass sampling plots, since these variables are not independent of each other. Similar to the biomass sampling protocol, our experimental design conformed to widely accepted methods and maintained sample integrity and quality, e.g., for sediment, rhizome, and water chemistry samples. Lastly, sample sizes for these variables were appropriate to detect differences among sites.

Substantial deviations from these refined and widely accepted experimental designs, e.g., different orientations of sampling transects, smaller sample sizes, and/or the use of smaller quadrats, are unacceptable, because they generally lack the power to detect among-site differences, which render them useless for ecological studies of this nature.

## 4.0 RESULTS

### 4.1. *SCHOENOPLECTUS TABERNAEMONTANI* BIOMASS, PLANT AND RHIZOME DENSITY, HEIGHT, SEEDHEAD PRODUCTION, AND DEGREE OF OILING

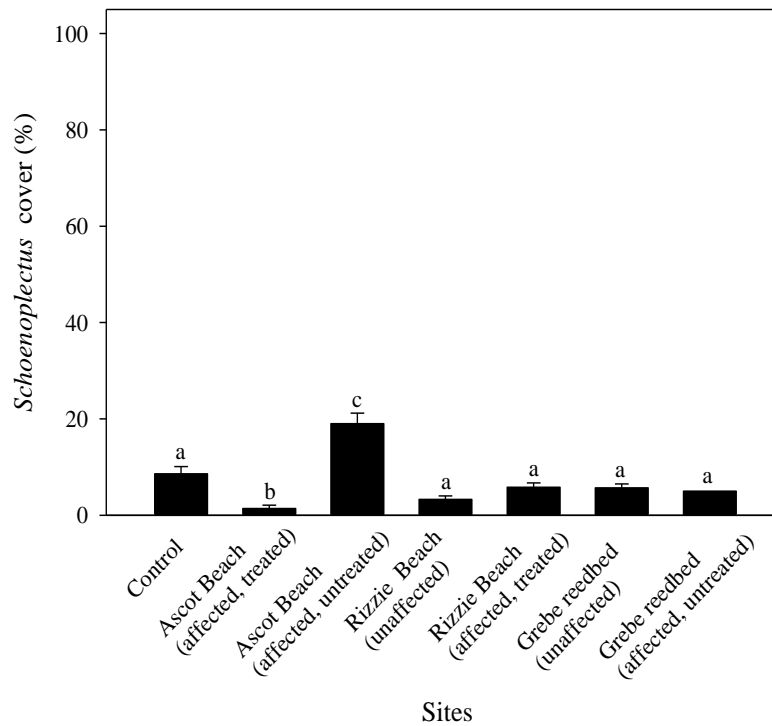
Biomass differed significantly among sites ( $128\text{--}683\text{ g m}^{-2}$ ;  $F = 4.773$ , d.f. = 6,  $p = 0.001$ ; Figure 2). Most notably, Rizzie Beach (unaffected) had the lowest *S. tabernaemontani* biomass ( $128\text{ g m}^{-2}$ ), while the remaining sites generally had similar biomass values ( $449\text{--}683\text{ g m}^{-2}$ ; Figure 2). Post oil spill treatment significantly reduced *S. tabernaemontani* biomass at Ascot Beach ( $683\text{ g m}^{-2}$  to  $300\text{ g m}^{-2}$ ) but not at Rizzie Beach (Figure 2).



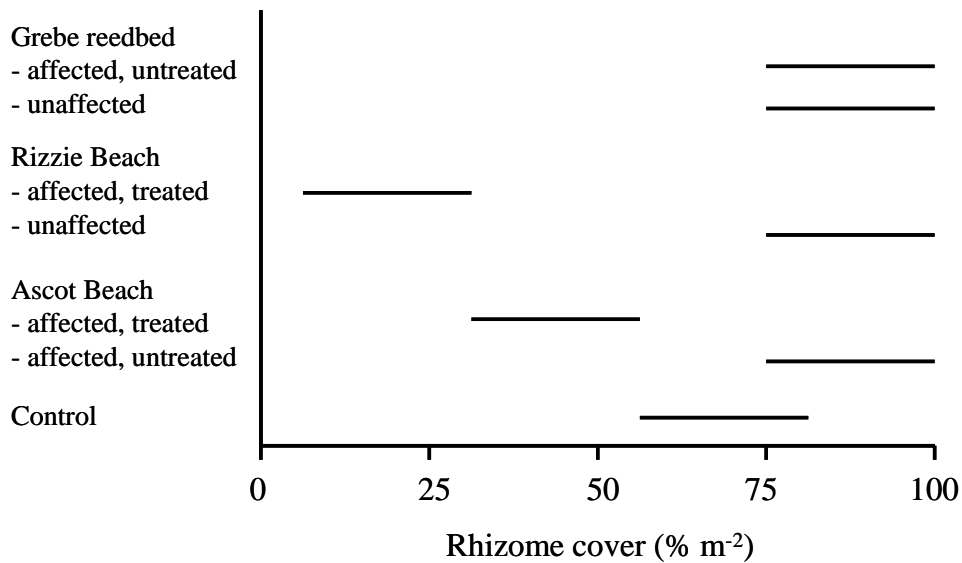
**Figure 2.** Aboveground biomass (means  $\pm$  SE) of *Schoenoplectus tabernaemontani* in lacustrine marshes in Wabamun Lake, AB. Different letters indicate significant differences among sites ( $p < 0.05$ ).

*Schoenoplectus tabernaemontani* plant cover differed significantly among sites ( $F = 27.460$ , d.f. = 6,  $p < 0.0001$ ; Figure 3). It was generally low (3-9%), except at Ascot Beach (affected and untreated), where it was 19% (Figure 3). Similarly, *S. tabernaemontani* rhizome cover differed among sites, with four of the seven sites having a rhizome cover  $> 75\% \text{ m}^{-2}$  (Figure 4). Post oil spill treatment significantly reduced *S. tabernaemontani* plant cover at Ascot Beach (from 19% to 1.4%) and rhizome cover at

Rizzie Beach and Ascot Beach (from 75-100% to 0-25% and from 75-100% to 36-56%, respectively; Figure 4).

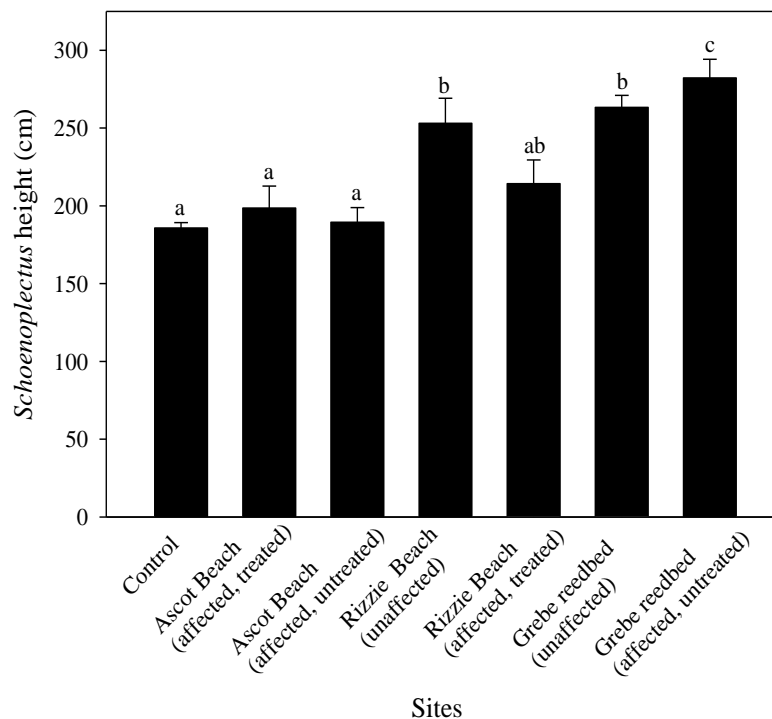


**Figure 3.** Cover (means  $\pm$  SE) of *Schoenoplectus tabernaemontani* in lacustrine marshes in Wabamun Lake, AB. Different letters indicate significant differences among sites ( $p < 0.05$ ).



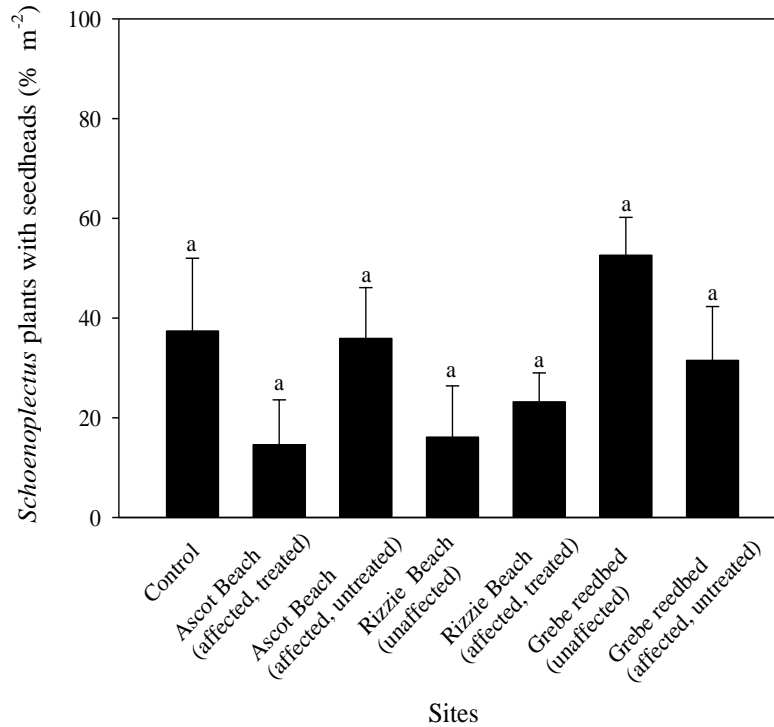
**Figure 4.** Range of cover of *Schoenoplectus tabernaemontani* rhizomes on the sediment in lacustrine marshes in Wabamun Lake, AB.

The height of *S. tabernaemontani* plants differed significantly among sites ( $F = 295.192$ ,  $d.f. = 6$ ,  $p < 0.0001$ ; Figure 5), with those at Grebe Reedbed and at Rizzie Beach having the tallest plants (214-282 cm). Plants at Ascot Beach and at the control site were the smallest ones (196-199 cm; Figure 5). In addition, there was significant variation in *S. tabernaemontani* plant height among plots within several sites (Ascot Beach, affected and treated –  $F = 1029.286$ ,  $d.f. = 6$ ,  $p < 0.0001$ ; Rizzie Beach, affected and treated –  $F = 53.142$ ,  $d.f. = 6$ ,  $p < 0.0001$ ; Rizzie Beach, unaffected –  $F = 39.065$ ,  $d.f. = 6$ ,  $p < 0.0001$ ; Grebe Reedbed, unaffected –  $F = 14.760$ ,  $d.f. = 6$ ,  $p < 0.0001$ ; data not shown).



**Figure 5.** Height of *Schoenoplectus tabernaemontani* plants (means  $\pm$  SE) in lacustrine marshes in Wabamun Lake, AB. Different letters indicate significant differences among sites ( $p < 0.05$ ).

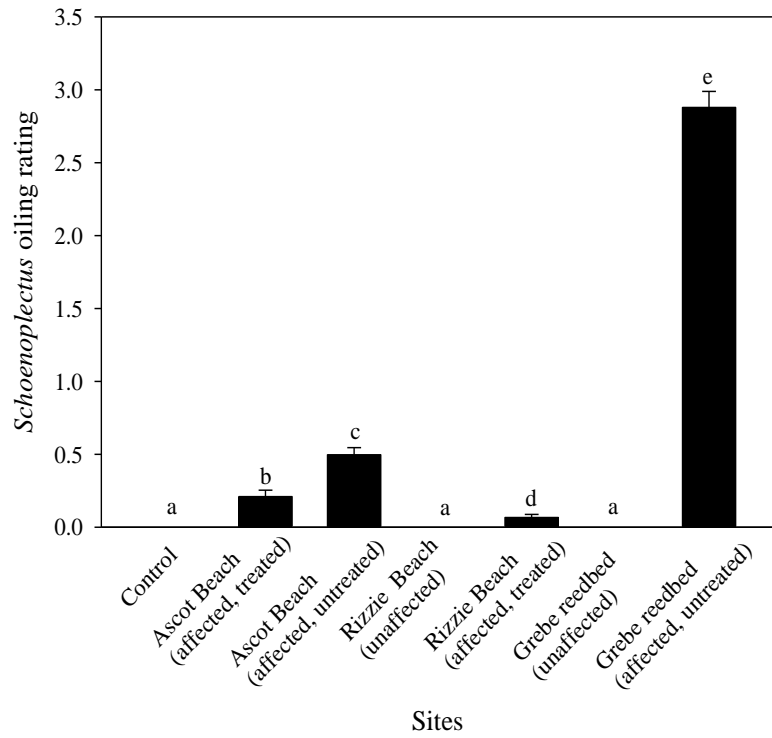
The number of *S. tabernaemontani* plants that produced seedheads did not differ significantly among sites ( $F = 1.072$ ,  $d.f. = 6$ ,  $p < 0.397$ ), ranging from a low of 15%  $m^{-2}$  (Ascot Beach, affected and treated) to a high of 53%  $m^{-2}$  (Grebe Reedbed, unaffected; Figure 6). On average, 30%  $m^{-2}$  of *S. tabernaemontani* plants produced seedheads at Wabamun Lake.



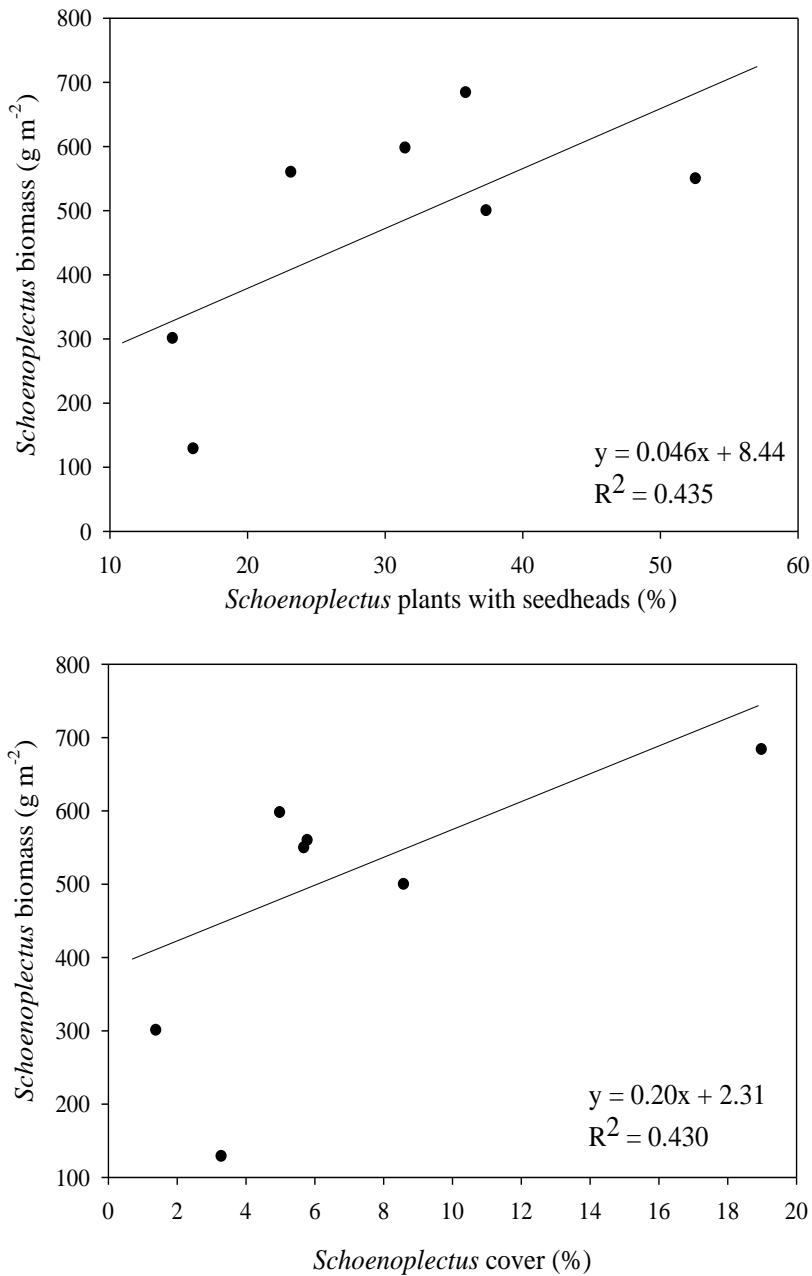
**Figure 6.** Proportion of *Schoenoplectus tabernaemontani* plants (means  $\pm$  SE) with seedheads in lacustrine marshes in Wabamun Lake, AB.

Post oil spill treatment of the affected areas of the Wabamun Lake lacustrine marshes significantly reduced oiling on *S. tabernaemontani* plants, as was clearly demonstrated at Ascot Beach (reduction of oiling by about 50%; Figure 7), but it did not entirely eliminate oiling on plants in these areas. Oil deposits were present on *S. tabernaemontani* plants in four of the seven sites, including the previously assessed “unaffected” reedbed of Ascot Beach and most prominently in the affected and untreated Grebe Reedbed (Figure 7). Mean oiling ratings were low (max. 2.9/10); however, in the sites still affected by oil, 6-98% of *S. tabernaemontani* plants had oil residue on their leaves (Ascot Beach, affected and treated – 20%; Ascot Beach, affected and untreated – 41%; Rizzie Beach, affected and treated – 6%; Grebe Reedbed, affected and untreated – 98%). Thus, treatment did reduce the amount of oil on the plants (based on the rating).





**Figure 7.** Oiling rating of *Schoenoplectus tabernaemontani* plants (1-10; means  $\pm$  SE) in lacustrine marshes in Wabamun Lake, AB. Different letters indicate significant differences among sites ( $p < 0.05$ ).



**Figure 8.** Correlations between *Schoenoplectus tabernaemontani* biomass and the proportion of *S. tabernaemontani* plants with seedheads and *S. tabernaemontani* plant density in Wabamun Lake, AB.

There were positive correlations between *S. tabernaemontani* biomass and the proportion of *S. tabernaemontani* plants with seedheads ( $p > 0.05$ ,  $R^2 = 0.435$ ) and *S. tabernaemontani* density ( $p > 0.05$ ,  $R^2 = 0.430$ ; Figure 8).

## **4.2. LACUSTRINE MARSH SEDIMENT, *SCHOENOPLECTUS TABERNAEMONTANI* RHIZOME, AND WATER CHEMISTRY**

### **4.2.1. LACUSTRINE MARSH SEDIMENT CHEMISTRY**

Concentrations of four of the ten measured PAHs were above detection limits in five of the seven sites (Table 1). Most prominently, concentrations of naphthalene, phenanthrene, pyrene, and benzo(a)anthracene ranged from 0.10-0.49 mg kg<sup>-1</sup> in the Grebe Reedbed (both sites), the area with the most PAHs above detection limits. Similarly, concentrations of phenanthrene and pyrene ranged from 0.50-2.70 mg kg<sup>-1</sup> at Rizzie Beach (both sites) and Ascot Beach (affected, untreated; Table 1). Concentrations of all PAHs were below detection limits at Ascot Beach (affected, treated) and the control site (Table 1). Post oil spill treatment results varied among sites, with concentrations of PAHs decreasing following treatment at Ascot Beach but remaining similar at Rizzie Beach. Interestingly, concentrations of benzo(a)anthracene were higher in the “unaffected” Grebe Reedbed than in the affected and untreated Grebe Reedbed (Table 1). None of the differences were statistically significant ( $p > 0.05$ ).

Concentrations of NAs in all sediment samples in all sites were below detection limits ( $< 5 \text{ mg kg}^{-1}$ ; Table 1).

### **4.2.2. *SCHOENOPLECTUS TABERNAEMONTANI* RHIZOME CHEMISTRY**

Concentrations of PAHs in *S. tabernaemontani* rhizomes were generally below detection limits ( $< 0.01 \text{ mg kg}^{-1}$ ). Only one rhizome sample had PAH concentrations above detection limits (0.03 mg kg<sup>-1</sup>; Rizzie Beach – unaffected; Table 2). None of the differences were statistically significant ( $p > 0.05$ ).

Concentrations of NAs in *S. tabernaemontani* rhizomes were also generally below detection limits ( $< 5 \text{ mg kg}^{-1}$ ), with only one sample having NA concentrations above detection limits (9 mg kg<sup>-1</sup>; Grebe Reedbed – affected, untreated; Table 2).

**Table 1.** Mean ( $\pm$  STDEV, where applicable) concentrations of polycyclic aromatic hydrocarbons (PAHs) and naphthenic acids (NAs) in lacustrine marsh sediments in Wabamun Lake, AB. n = 3 per site. Detection limits varied for PAHs (0.03-1.0 mg kg<sup>-1</sup>, dependent on moisture content of sample) and were 5 mg kg<sup>-1</sup> for NAs. Bold values indicate concentrations above detection limits.

Chemical variables	Control	Ascot Beach (affected, treated)	Ascot Beach (affected, untreated)	Rizzie Beach (unaffected)	Rizzie Beach (affected, treated)	Grebe reedbed (unaffected)	Grebe reedbed (affected, untreated)
Polycyclic aromatic hydrocarbons							
Naphthalene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	<b>0.13 (0.12)</b>	<b>0.16 (0.03)</b>
Quinoline (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	< 0.1	< 0.1
Phenanthrene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	<b>2.7 (4.9)</b>	<b>0.10 (0.05)</b>	<b>0.16 (0.07)</b>	<b>0.48 (0.35)</b>	<b>0.49 (0.17)</b>
Pyrene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	<b>&lt; 0.3-2.0</b>	<b>&lt; 0.03-0.05</b>	< 0.09	<b>0.29 (0.21)</b>	<b>0.22 (0.06)</b>
Benzo(a)anthracene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	<b>0.10 (0.07)</b>	< 0.1
Benzo(b)fluoranthene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	< 0.1	< 0.1
Benzo(k)fluoranthene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	< 0.1	< 0.1
Benzo(a)pyrene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	< 0.1	< 0.1
Indeno(1,2,3-cd)pyrene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	< 0.1	< 0.1
Dibenzo(a,h)anthracene (mg kg <sup>-1</sup> )	< 0.03	< 0.03	< 1	< 0.04	< 0.07	< 0.1	< 0.1
Naphthenic acids (mg kg <sup>-1</sup> )	< 5	< 5	< 5	< 5	< 5	< 5	< 5

**Table 2.** Concentrations of polycyclic aromatic hydrocarbons (PAHs) and naphthenic acids (NAs) in *Schoenoplectus tabernaemontani* rhizomes in lacustrine marshes in Wabamun Lake, AB. n = 3 per site. Detection limits for PAHs and NAs were 0.01 and 5 mg kg<sup>-1</sup>, respectively. Bold values indicate concentrations above detection limits.

Chemical variables	Control	Ascot Beach (affected, treated)	Ascot Beach (affected, untreated)	Rizzie Beach (unaffected)	Rizzie Beach (affected, treated)	Grebe reedbed (unaffected)	Grebe reedbed (affected, untreated)
Polycyclic aromatic hydrocarbons							
Naphthalene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Quinoline (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Phenanthrene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	<b>&lt; 0.01-0.03</b>	< 0.01	< 0.01	< 0.01
Pyrene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(a)anthracene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(b)fluoranthene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(k)fluoranthene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(a)pyrene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Indeno(1,2,3-cd)pyrene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dibenzo(a,h)anthracene (mg kg <sup>-1</sup> )	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Naphthenic acids (mg kg <sup>-1</sup> )	< 5	< 5	< 5	< 5	< 5	< 5	<b>&lt; 5-9</b>

#### 4.2.3. SURFACE WATER CHEMISTRY

Total phosphorus (TP), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) concentrations were similar among all sites (TP: 25.0-36.0  $\mu\text{g L}^{-1}$ ; TDP: 9.0-11.0  $\mu\text{g L}^{-1}$ ; SRP: 1.0  $\mu\text{g L}^{-1}$ ; Table 3). Similarly,  $\text{NO}_3^-/\text{NO}_2^-$  and  $\text{NH}_4^+$  concentrations showed little variation among the seven sites ( $\text{NO}_3^-/\text{NO}_2^-$ : 2.0-3.5  $\mu\text{g L}^{-1}$ ;  $\text{NH}_4^+$ : 7.5-11.0  $\mu\text{g L}^{-1}$ ; Table 3). Total nitrogen (TN) concentrations were lower at Ascot Beach (994.0-1010.0  $\mu\text{g L}^{-1}$ ) than in the other areas, where they were similar (1055.0-1115.0  $\mu\text{g L}^{-1}$ ; Table 3). Total dissolved nitrogen (TDN) concentrations were variable, with the lowest TDN concentrations in the control site (813  $\mu\text{g L}^{-1}$ ) and the highest in the Grebe Reedbed and at Rizzie Beach (unaffected) (873.0-880.0  $\mu\text{g L}^{-1}$ ). The remaining three sites had intermediate TDN concentrations (both Ascot Beach sites, Rizzie Beach – affected, untreated; 834.5-845.5  $\mu\text{g L}^{-1}$ ; Table 3). The pH of the seven sites was similar, ranging from 8.57-8.61 (Table 3).

#### 4.2.4. CHL. *a* CONCENTRATIONS

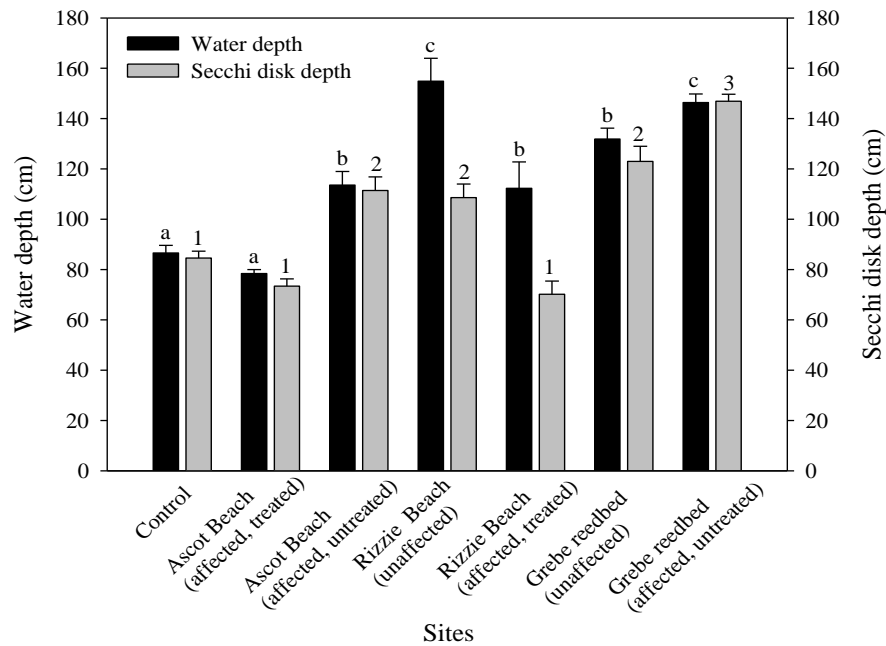
Chl. *a* concentrations, an indicator of algal productivity, ranged from 10.5-21.4  $\mu\text{g L}^{-1}$  (Table 3), with the control site having the highest chl. *a* concentrations and the sites of other four areas having similar concentrations (Ascot Beach: 11.1-13.6  $\mu\text{g L}^{-1}$ ; Rizzie Beach: 12.0-12.8  $\mu\text{g L}^{-1}$ ; Grebe Reedbed: 10.5-12.5  $\mu\text{g L}^{-1}$ ; Table 3).

**Table 3.** Surface water nutrient concentrations, pH, and chlorophyll *a* (chl. *a*) concentrations in lacustrine marshes in Wabamun Lake, AB, Canada. TP = total phosphorus, TDP = total dissolved phosphorus, SRP = soluble reactive phosphorus, TN = total nitrogen, TDN = total dissolved nitrogen.

Chemical variables	Control	Ascot Beach (affected, treated)	Ascot Beach (affected, untreated)	Rizzie Beach (unaffected)	Rizzie Beach (affected, treated)	Grebe Reedbed (unaffected)	Grebe Reedbed (affected, untreated)
TP ( $\mu\text{g L}^{-1}$ )	36.0	25.0	28.5	32.5	28.5	28.0	31.5
TDP ( $\mu\text{g L}^{-1}$ )	10.5	9.0	11.0	9.5	10.5	10.0	9.5
SRP ( $\mu\text{g L}^{-1}$ )	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$\text{NO}_2^-/\text{NO}_3^-$ ( $\mu\text{g L}^{-1}$ )	2.0	2.0	2.0	3.0	3.0	3.0	3.5
$\text{NH}_4^+$ ( $\mu\text{g L}^{-1}$ )	8.5	9.5	8.5	7.5	8.0	11.0	8.5
TN ( $\mu\text{g L}^{-1}$ )	1,100.0	994.0	1,010.0	1,055.0	1,115.0	1,115.0	1,065.0
TDN ( $\mu\text{g L}^{-1}$ )	813.0	845.5	834.5	873.0	842.5	880.0	873.5
pH	8.61	8.59	8.61	8.58	8.58	8.57	8.57
Chl. <i>a</i> ( $\mu\text{g L}^{-1}$ )	21.4	11.1	13.6	12.8	12.0	12.4	10.5

#### 4.2.5. WATER DEPTH

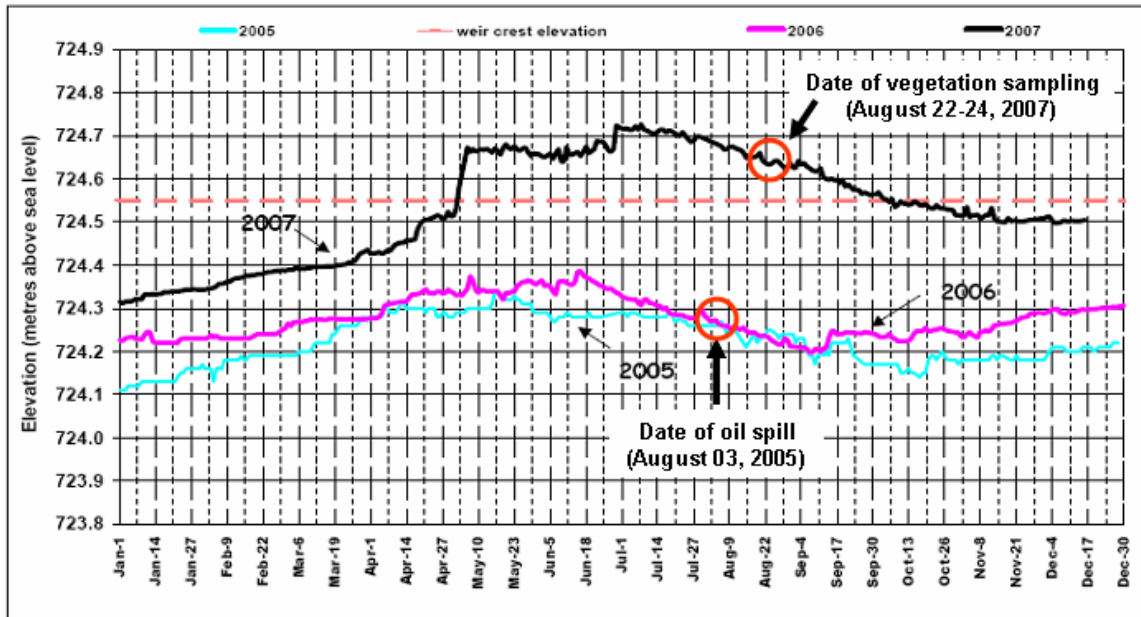
Water depth varied significantly among areas ( $F = 18.180$ ,  $d.f. = 3$ ,  $p < 0.001$ ) and sites ( $F = 28.588$ ,  $d.f. = 6$ ,  $p < 0.001$ ; Figure 9). On a marsh area basis, mean water depths were significantly lower at the control site (86 cm) and at Ascot Beach (mean of 96 cm) compared to Rizzie Beach (mean of 134 cm) and the Grebe Reedbed (mean of 139 cm). On a site basis, Rizzie Beach (unaffected) and the Grebe Reedbed (affected and untreated) had the deepest water (155 cm and 146 cm, respectively), while the control site and Ascot Beach (affected and treated) had the shallowest water (86 cm and 78 cm, respectively; Figure 9). Since the oil spill on August 03, 2005, water levels in Wabamun Lake have risen about 40 cm between April and September by 2007 (Figure 10).



**Figure 9.** Water and Secchi disk depths (means  $\pm$  SE) in lacustrine marshes in Wabamun Lake, AB. Different letters and numbers indicate significant differences in water depth and Secchi disk depth, respectively, among sites ( $p < 0.05$ ).

Secchi disk depth also varied significantly among areas ( $F = 17.674$ ,  $d.f. = 3$ ,  $p < 0.001$ ) and sites ( $F = 44.595$ ,  $d.f. = 6$ ,  $p < 0.001$ ; Figure 9). On average, the Grebe Reedbed had the greatest Secchi disk depth (135 cm) compared to the remaining three areas, which were similar to each other (control site – 85 cm; Ascot Beach – mean of 92 cm; Rizzie Beach – mean of 90 cm). The Grebe Reedbed (affected and untreated) had the greatest





**Figure 10.** Daily water levels in Wabamun Lake, AB, in 2005, 2006, and 2007 (Alberta Environment 2007).

Secchi disk depth (147 cm), and the control site, Ascot Beach, and Rizzie Beach (both affected and treated) had the lowest Secchi disk depths (85 cm, 73 cm, and 70 cm, respectively). The remaining sites had intermediate Secchi disk depths (109 cm to 123 cm; Figure 9). Secchi depths were similar to water depths in most sites ( $p > 0.05$ ), except at Rizzie Beach (unaffected), where the water depth was significantly greater than the Secchi disk depth (155 cm vs. 109 cm;  $t$ -statistic = 3.700, d.f. = 6,  $p = 0.010$ ; Figure 9).

#### 4.3. SAV SPECIES RICHNESS, PVI, AND COVER

Species richness of submersed aquatic vegetation at every site was low, with a maximum of five (at the Grebe Reedbed unaffected, affected and untreated) and a minimum of two SAV species per site (Ascot Beach affected and untreated and control; Table 4). The most common SAV species was *Utricularia macrorhiza* Le Conte (common bladderwort), which occurred in all sites. *Potamogeton* spp. (pondweed) was similarly common, occurring in six of the seven sites. Contrastingly, the floating leaf plant *Lemna minor* L. (duckweed) occurred at only two sites (Ascot Beach – affected and untreated; Grebe Reedbed – unaffected; Table 4).

**Table 4.** Submerged aquatic vegetation (SAV) and floating leaf plant species in lacustrine marshes in Wabamun Lake, AB.

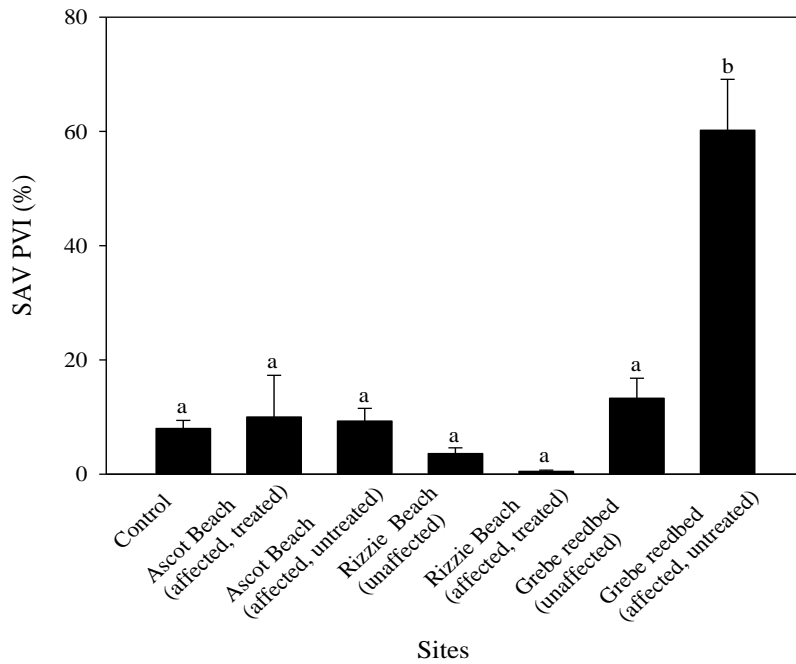
Sites	SAV and floating leaf plant species
Control	<i>Potamogeton richardsonii</i> (Benn.) Rydb., <i>Utricularia macrorhiza</i> Le Conte
Ascot Beach - affected, treated	<i>Ceratophyllum demersum</i> L., <i>P. richardsonii</i> , <i>U. macrorhiza</i>
Ascot Beach - affected, untreated	<i>Lemna minor</i> L., <i>U. macrorhiza</i>
Rizzie Beach - unaffected	<i>Myriophyllum sibiricum</i> Komarov, <i>Nuphar lutea</i> (L.) Sm. ssp. <i>variegata</i> (Dur.) E.O. Beal, <i>P. richardsonii</i> , <i>U. macrorhiza</i>
Rizzie Beach - affected, treated	<i>M. sibiricum</i> , <i>N. lutea</i> ssp. <i>variegata</i> , <i>P. richardsonii</i> , <i>U. macrorhiza</i>
Grebe Reedbed - unaffected	<i>C. demersum</i> , <i>L. minor</i> , <i>N. lutea</i> ssp. <i>variegata</i> , <i>Potamogeton pusillus</i> L., <i>U. macrorhiza</i>
Grebe Reedbed - affected, untreated	<i>C. demersum</i> , <i>N. lutea</i> ssp. <i>variegata</i> , <i>P. pusillus</i> , <i>Potamogeton zosteriformis</i> Fern., <i>U. macrorhiza</i>

**Table 5.** Algal growth forms in lacustrine marshes in Wabamun Lake, AB. SAV = submerged aquatic vegetation.

Sites	Algal growth forms
Control	Abundant epiphyton on <i>S. tabernaemontani</i> and SAV, abundant <i>Nostoc</i> sp., abundant plankton in water column (turbid)
Ascot Beach - affected, treated	Abundant epiphyton on <i>S. tabernaemontani</i> and SAV, abundant <i>Nostoc</i> sp.
Ascot Beach - affected, untreated	Abundant epiphyton on <i>S. tabernaemontani</i> and SAV, abundant <i>Nostoc</i> sp. uncommon
Rizzie Beach - unaffected	Abundant epiphyton on <i>S. tabernaemontani</i> and SAV
Rizzie Beach - affected, treated	Abundant epiphyton on <i>S. tabernaemontani</i> and SAV, <i>Nostoc</i> sp. uncommon
Grebe Reedbed - unaffected	Abundant epiphyton on <i>S. tabernaemontani</i> and SAV, abundant <i>Nostoc</i> sp.
Grebe Reedbed - affected, untreated	Abundant epiphyton on <i>S. tabernaemontani</i> and SAV

Various algal growth forms occurred in the lacustrine marshes of Wabamun Lake. Encrusting and filamentous epiphytic algae abundantly colonized emergent vegetation and SAV in all sites (not identified to genus or species). Also, free-floating *Nostoc* colonies (up to 3 cm in diameter) occurred abundantly in four of the seven sites (Table 5).

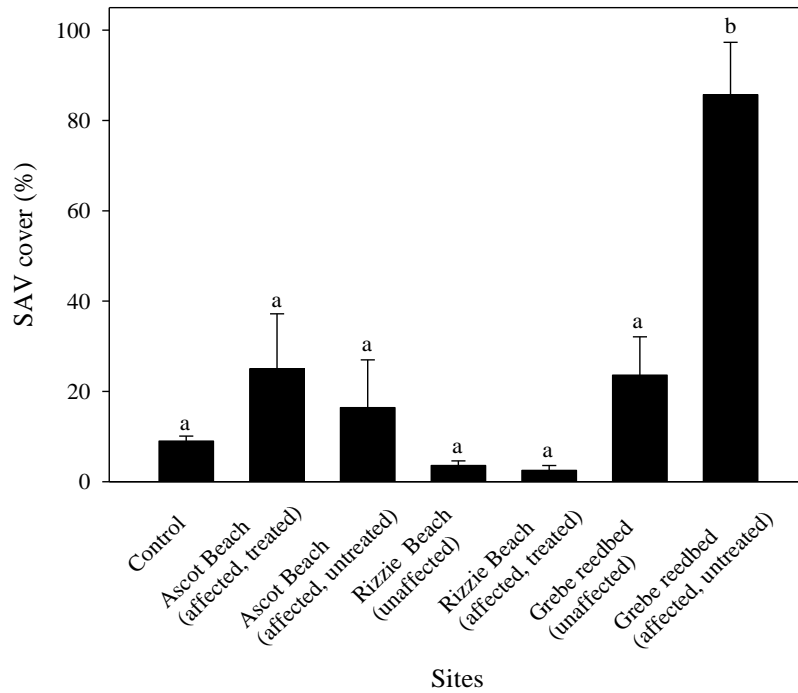
SAV PVI differed significantly among sites ( $F = 20.727$ , d.f. = 6,  $p = 0.001$ ), with the Grebe Reedbed (affected, untreated) having the highest SAV PVI (60%). The remaining sites had similar SAV PVI values (1-13%; Figure 11).



**Figure 11.** Percent volume inhabited (PVI; means  $\pm$  SE) of the water column by submerged aquatic vegetation (SAV) in lacustrine marshes in Wabamun Lake, AB. Different letters indicate significant differences in SAV PVI among sites ( $p < 0.05$ ).

SAV cover also differed significantly among sites ( $F = 13.034$ , d.f. = 6,  $p = 0.001$ ; Figure 12). Most prominently, Ascot Beach (16-25%) and the Grebe Reedbed (24-86%) had the highest SAV covers. Neither treatment nor presence of oil affected the SAV PVI or SAV cover. For example, in the affected and untreated Grebe Reedbed, SAV cover was 86%, the highest of any of the seven sites. Also, SAV cover was similar between previously

treated areas, e.g., at Ascot Beach and Rizzie Beach, and between the affected and treated and unaffected sites within the same area, e.g., at Rizzie Beach (Figure 12).



**Figure 12.** Cover of submerged aquatic vegetation (SAV; means  $\pm$  SE) in lacustrine marshes in Wabamun Lake, AB. Different letters indicate significant differences among sites ( $p < 0.05$ ).

## 5.0 DISCUSSION

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### 5.1. ECOLOGY AND NATURAL VARIABILITY OF *SCHOENOPLECTUS TABERNAEMONTANI* MARSHES

In the lacustrine marshes in Wabamun Lake, biomass of the dominant emergent macrophyte *S. tabernaemontani* differed significantly among the seven study sites. Biomass was significantly lower at Ascot Beach (affected and treated, 300 g m<sup>-2</sup>) and at Rizzie Beach (unaffected, 128 g m<sup>-2</sup>), intermediate at the control site (449 g m<sup>-2</sup>), and similarly higher in the remaining four sites (549-683 g m<sup>-2</sup>; Figure 2). The *Schoenoplectus* biomass values reported here generally fall within the range of biomass values reported for this genus in freshwater wetlands in central and western North America (van der Valk and Davis (1980): 391-486 g m<sup>-2</sup> in Iowa; Lieffers and Shay (1982a): 361 g m<sup>-2</sup> in Manitoba, Saskatchewan, Alberta; Karagatzides and Hutchinson (1991): 316-625 g m<sup>-2</sup> in B.C.). Only one site, Rizzie Beach (unaffected; 128 g m<sup>-2</sup>) fell well below this range, possibly due to the greater water depth (154 cm; Figure 8), which can reduce rates of *S. tabernaemontani* seed germination and plant production (van der Valk and Davis 1980, Kellogg et al. 2003).

Water levels and soil characteristics significantly affect the establishment and biomass of *S. tabernaemontani* in freshwater marshes. In an Iowa glacial marsh, van der Valk and Davis (1980) showed that *S. tabernaemontani* growth significantly decreased following 1+ years of elevated water levels, which ultimately eliminated this macrophyte from the marsh. A similar trend had previously been demonstrated by Weller and Spatcher (1965) and Weller and Frederickson (1974). This disappearance has been linked to the inability of *S. tabernaemontani* rhizomes to tolerate anoxic soil conditions, which may result from elevated water levels (Laing 1941). From a soil perspective, Barko and Smart (1978) determined that *S. tabernaemontani* grows better in fine-textured soils, such as silty clays and clays compared to sands *in vitro*. They attributed increased growth rates for above and below ground plant tissues to greater nutrient availability in fine-textured soils, i.e., nutrients adsorb more effectively to clays and silts than sands, thereby establishing a

potentially greater nutrient pool for the vegetation. Similarly, increased organic matter content of soils and sediments increases the available nutrient pool for plants.

Re-establishment of *S. tabernaemontani* following water drawdown in marshes depends on the survival of seeds in the seedbank and favourable conditions for germination. Increased aeration due to lower water levels facilitates seed germination, as does organic matter content of the soil. Both resulted in increased biomass of *S. tabernaemontani in vitro* (Kellogg et al. 2003). Sediment samples from Ascot Beach, Rizzie Beach, and the Grebe Reedbed in 2006-2007 revealed similar sand (45-48%), silt (36-46%), clay (9-16%), and organic matter (9-11%) content among the sites (Alberta Environment unpublished data). No sediment samples were collected at the control site; however, a previous study reported substantially higher sand (> 85%) and substantially lower silt (< 10%), clay (< 5%), and organic matter (< 2%) sediment content from the vicinity of the control site (sampling points 4.5 and 5.4 along the north shore west of Fallis Point; Anderson 2003) compared to the other areas. The similarity in sediment characteristics in three of the four areas partially explains the relatively small range of *S. tabernaemontani* biomass values we obtained in our study sites (range of area means = 492-573 g m<sup>-2</sup>). Interestingly, the low end of this range occurred at the control site (Figure 2), where sediment characteristics may have been less favourable for *S. tabernaemontani* growth (high sand and low clay and organic matter content, see above; Barko and Smart 1978).

In contrast to increased biomass at lower water levels, Seabloom et al. (1998) showed that some emergent macrophytes, including species of *Typha* and *Schoenoplectus*, had higher seed germination rates under flooded conditions; however, their “flooded” conditions represented a water depth of 7 cm. An only marginally higher water depth (10 cm) resulted in lower seed germination rates for *S. tabernaemontani* (van der Valk and Davis 1978) compared to those presented by Seabloom et al. (1998). Weisner et al. (1993) showed that seedlings of *Schoenoplectus acutus* (Muhl. ex Bigelow) A.& D. Löve var. *acutus* (previously *Scirpus lacustris* L.) were able to survive and grow in water up to 80 cm deep; however, they germinated their seeds under aerobic conditions and

transplanted established seedlings (3-5 cm tall) into pots which were then submerged to various depths. The lowest water depth at any of our sites was 78 cm (Ascot Beach – affected, treated; Figure 8), which is suitable for the growth of established plants of *S. tabernaemontani*, but likely not the germination of their seeds. Thus, we hypothesize that the establishment and expansion of *S. tabernaemontani* in the lacustrine marshes of Wabamun Lake depend primarily on vegetative propagules (rhizomes) rather than seeds due to low seed production and dispersal and low seedling vigor (USDA 2007).

*Schoenoplectus tabernaemontani* height varied significantly among our sites, ranging from < 2 m to nearly 3 m (Figure 4). There is no single environmental variable that determines macrophyte plant height and density, although water depth, stem density, soil/sediment chemistry, nutrient availability, and climatic conditions (e.g., temperature) have been implicated by van der Valk and Davis (1980), Lieffers and Shay (1982a, b), and Kellogg et al. (2003). Our plant heights are substantially greater than those reported by van der Valk and Davis (*S. tabernaemontani*; 144-162 cm; 1980) and Lieffers and Shay (*Scirpus maritimus* var. *paludosus* (A. Nels.) Kükenth.; 8-140 cm; 1982b), likely owing to different environmental conditions at Wabamun Lake.

The cover of *S. tabernaemontani* differed significantly among our sites, ranging from < 2% m<sup>-2</sup> to 19% m<sup>-2</sup> (Figure 2). We did not determine the number of stems m<sup>-2</sup>, but showed that *S. tabernaemontani* biomass was independent of stem density and fecundity (expressed as number of plants with seedheads m<sup>-2</sup>; Figure 7). van der Valk and Davis (1980) also reported no correlation between biomass and stem density. The number of plants with seedheads m<sup>-2</sup> was statistically similar in our sites, despite a large range in proportions (15-53% m<sup>-2</sup>). This resulted from great inter-plot variability, as shown by the large SEs associated with these data (Figure 5). Our proportion of *S. tabernaemontani* plants with seedheads (mean of 31% across all sites) was significantly lower than van der Valk and Davis' (100%; 1980) but similar to Lieffers and Shay's (34%; 1982b). The production of flowers and seeds in emergent macrophytes in any given year is partially dependent on whether or not an individual plant had produced both the previous year, i.e., the likelihood of the same plant producing flowers and seeds in successive years is small,

as Linde et al. (1976) showed for *Typha glauca* Godr. Moreover, disturbances, such as rapid changes in water levels, can reduce flower and seed production for up to 2 years, as van der Valk and Davis (1980) showed for *T. glauca* as well as *Schoenoplectus fluviatilis* (Torr.) M.T. Strong (previously *S. validus*) and *Sparganium eurycarpum* Engelm. ex Gary. We do not know if the proportion of *S. tabernaemontani* plants with seedheads in Wabamun Lake marshes were naturally higher or lower in previous years or if prior disturbances had any impacts on them.

Thus, water level is likely the most critical variable affecting the productivity, fecundity, and re-establishment success of *S. tabernaemontani* in lacustrine marshes in Wabamun Lake. This has significant implications for the degree and rate of recovery of this macrophyte following natural and anthropogenic disturbances, such as the oil spill and subsequent treatment regimes, in Wabamun Lake's lacustrine marshes.

## **5.2. IMPACTS OF OIL SPILL AND TREATMENT ON *SCHOENOPLECTUS TABERNAEMONTANI* MARSHES**

The train derailment on CN's primary route through northern Alberta released about 712,500 L of Bunker 'C' oil and about 88,000 L of Imperial Pole Treating oil (PTO) onto the ground in the community of Whitewood Sands on the north-shore of Wabamun Lake. An estimated 21% of the released Bunker 'C' oil and "trace" amounts (no estimates available) of PTO entered Wabamun Lake and subsequently dispersed along its northern, eastern, and southern shores. CN previously released a report on "macrophytes" in Wabamun Lake; however, that report addressed *L. minor*, which is a minute floating aquatic plant (frond size = 2-5 mm) and not a macrophytes *per se* (Chapter 5 – Macrophytes; CN Railway Co. 2007a). Since then, CN Railway Co. (2007b) has conducted a follow-up study to examine the impacts of the oil spill on true macrophyte communities; however, that study suffered from a series of methodological flaws (e.g., transect orientation, quadrat size, biomass processing, data reporting). These flaws obscured real differences in biomass production among marshes, likely underestimated the true impacts of the oil spill on lacustrine marshes in Wabamun Lake, and provided



only very limited data for proper comparative analyses to other studies. Its results should be viewed with caution.

The effects of oil spills have been examined most extensively in coastal marshes, e.g., along the Alaskan and Gulf of Mexico coasts (primarily Texas, Louisiana, Florida) (e.g., Cowell 1969, Baker 1973, Delaune et al. 1979, Mendelssohn et al. 1990, Peterson et al. 2003). Studies have shown that the effects of oil on vascular plants can cover a broad range. In the short-term, stomata (sites of gas exchange on leaf surfaces) can be blocked, which then disrupts plant gas exchange, transpiration, photosynthesis, and interrupts the O<sub>2</sub> transporting system vital to plant functioning. In the long-term, oiling of plant tissues can result in severe leaf damage and mortality (Baker 1970, Alexander and Webb 1985, Pezeshki and Delaune 1993, Pezeshki et al. 1995, Marwood et al. 2003). The extent of damage to plants depends on the species affected and their age, oil quality and quantity, time of impact, weather conditions, and soil characteristics (Burk 1977, Hershner and Moore 1977, Alexander and Webb 1985, 1987, Mendelssohn et al. 1990, IPIECA 1994, Pezeshki et al. 1998). In some species, e.g., certain species of *Typha*, *Schoenoplectus*, and *Sagittaria*, plant functioning and growth generally resume (Delaune et al. 1979, IPIECA 1994, Pezeshki et al. 1995, 1998); however, variation in biotic and abiotic variables have resulted in conflicting results, even for the same plant species.

The effects of the oil spill and subsequent treatments varied among the lacustrine marshes in Wabamun Lake. *Schoenoplectus tabernaemontani* biomass significantly decreased in the treated site at Ascot Beach (683 g m<sup>-2</sup> to 300 g m<sup>-2</sup>), while it significantly increased at Rizzie Beach (128 g m<sup>-2</sup> to 559 g m<sup>-2</sup>; Figure 2). The two sites differed significantly in water depth and recovery from treatment. Rizzie Beach had one of the greatest water depths, which likely negatively impacted *Schoenoplectus* seed germination, rhizome growth, and shoot production (Seabloom et al. 1998). The affected and subsequently treated area of this site had a lower water depth compared to the unaffected area (112 cm vs. 155 cm, respectively; Figure 9). Consequently, *Schoenoplectus* biomass was greater in the shallower treated area. Ascot Beach had a significantly lower water depth in the affected and treated area compared to the same area at Rizzie Beach (78 cm vs. 112 cm,

respectively; Figure 9). Thus, based on water depth alone, *Schoenoplectus* biomass should be substantially greater in the treated Ascot Beach site; however, it was significantly lower ( $300 \text{ g m}^{-2}$  vs.  $559 \text{ g m}^{-2}$ ; Figure 2), contrary to expectations. It appeared that the impact of treatment at Ascot Beach, a heavily affected area from the oil spill, was greater than at the moderately-affected Rizzie Beach. In addition, a significant proportion of the treated area remains open at Ascot Beach, i.e., with very low to no *Schoenoplectus* cover and substantially lower rhizome cover than in all unaffected or affected and treated sites (Figures 3, 4), although sporadic revegetation has occurred on the lake-side periphery of the lacustrine marsh. At Rizzie Beach, a uniform cover of *Schoenoplectus* has developed since treatment (Figure 3). In contrast, more aggressive treatment likely significantly reduced *Schoenoplectus* cover at Ascot Beach (Figure 3) as a result of significant disturbance and/or damage of *Schoenoplectus* rhizomes in the lake sediment (Figure 4). A similar reduction in *Schoenoplectus* cover following treatment was not apparent at Rizzie Beach, despite an even lower rhizome cover; however, that could be the result of different physical characteristics at this site. For example, Rizzie Beach may be more sheltered from wave and wind action than Ascot Beach, thereby providing a more favourable environment for emergent plant re-establishment and growth. Disturbance of the sediment from treatment may not have been as damaging to *S. tabernaemontani* rhizomes at Rizzie Beach; however, this remains speculative, since we did not assess rhizome health in any of the sites (we only assessed rhizome cover).

Treatment significantly reduced oiling on plants. This was particularly evident at Ascot Beach, where successful oil removal treatments have resulted in the reduction of oil on plants from a rating of 0.5 to a rating of 0.21 (reduction of oiling rating of 42%; Figure 7). While oiling ratings were low across all sites, i.e., there were generally only small, localized oil deposits on individual plants, in the sites still affected by oil, 6-98% of *S. tabernaemontani* plants had oil residue on their leaves (Ascot Beach, affected and treated – 20%, 20 of 100 plants; Ascot Beach, affected and untreated – 41%, 71 of 175 plants; Rizzie Beach, affected and treated – 6%, 10 of 175 plants; Grebe Reedbed, affected and untreated – 98%, 160 of 165 plants). These residue deposits likely occurred from oil sheen on the water surface rather than from sediment oil deposits, which would have

encrusted more than a 5-10 cm zone along the leaves of *S. tabernaemontani* plants as shoots emerged from rhizomes in the spring and grew through the sediment and water column. At the Grebe Reedbed, the highest oiling rating of any of the sites was obtained in the affected and untreated site (2.88/10; Figure 7), and 98% of all plants had oil residues on their leaves. This was likely the result of the absence of any form of treatment and the presence of a boom, which reduced dispersal of oil on the water surface. Nonetheless, the heavy oiling still persistent at this site did not seem to have had any negative effects on *S. tabernaemontani* biomass or cover. Lateral transport of floating and/or suspended tar balls due to wave action can result in the expansion of oil spill impacted areas (e.g., Fremling 1981). This was evident at Ascot Beach, where a previously classified oil spill “unaffected” area of the marsh had to be re-classified as “affected”, because of oil sheens on the water surface, oiling on plants, and PAHs at detectable concentrations in the sediment (Figure 7, Table 1). Lateral transport of oil residues cannot be effectively controlled and will likely result in the occurrence of oil residues in other lacustrine marshes in Wabamun Lake, which were thought to be unaffected. This problem is exacerbated by the longevity of oil residues in aquatic ecosystems (Prince et al. 2003, Foght 2006).

Neither plant height nor seedhead production varied significantly between treated and unaffected sites within areas (Ascot Beach and Rizzie Beach; Figures 5, 6). The only significant difference in plant height occurred at the Grebe Reedbed, where *S. tabernaemontani* height was significantly greater in the unaffected site than in the affected and untreated site (263 cm vs. 282 cm; Figure 5). The increased plant height is likely a response to the significantly greater water depth at the affected and untreated site at the Grebe Reedbed (132 cm vs. 146 cm; Figure 9).

The long-term prognosis of the treatment areas (where the rhizome and plant cover is still low) remains uncertain due to a suite of environmental variables that affect vegetation re-colonization of these areas, such as water levels.

### **5.3. LACUSTRINE MARSH SEDIMENT, *SCHOENOPLECTUS TABERNAEMONTANI* RHIZOME, AND WATER CHEMISTRY**

#### **5.3.1. POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS AND RHIZOMES**

Several PAHs were above detection limits in sediments in five of the seven sites, most prominently in the Grebe Reedbed and at Rizzie Beach (both sites) and Ascot Beach (affected, untreated; Table 1). Post oil spill treatment results varied among sites, with concentrations of PAHs decreasing following treatment at Ascot Beach but remaining similar at Rizzie Beach. Interestingly, concentrations of benzo(a)anthracene were higher in the “unaffected” Grebe Reedbed than in the affected and untreated Grebe Reedbed (Table 1). If these elevated PAHs have any effect on the plants is unknown. Given the wide range of natural variability in plant biomass and % cover and the range and intensity of treatments, it is unlikely that we could detect effects. In addition, PAHs were generally below detection limits in *S. tabernaemontani* rhizomes (Table 2).

The fate, behavior, and toxicity of PAHs in aquatic systems are influenced by a number of physical, chemical, and biological processes. While some of these processes, including photo-oxidation, hydrolysis, biotransformation, biodegradation, and mineralization, result in the transformation of PAHs into other substances, other physical processes, including adsorption, desorption, solubilization, volatilization, re-suspension, and bioaccumulation, are responsible for the cycling of these substances throughout the aquatic environment (CCME 2007). The relative importance of each of these processes depends on the characteristics of the sediments and on the properties of the individual PAH under consideration; however, considering that most PAHs are relatively non-volatile and poorly soluble in water, they generally adsorb to particulate matter in the water column and subsequently accumulate in sediments (Government of Canada 1994).

The effects of PAHs on aquatic plant species and communities are complex and vary among plant guilds (algae, SAV, emergent macrophytes), genera, and species, PAH chemistry (low vs. high molecular weight PAHs), water and sediment chemistry, water depth, seedbank characteristics, sediment composition (organic vs. mineral sediments and clay vs. silt vs. sand), and climatic conditions. This creates uncertainty in predicting the

trajectory of recovery of oil spill affected marshes; however, it appears that aquatic plants are resilient to light to moderate oil spills and do not show chronic exposure impacts.

The impacts of oil spills on individual marsh plants and marsh plant communities have been extensively studied in the past, e.g., Cowell (1969), Baker (1973), Delaune et al. (1979), Mendelsohn et al. (1990), and Peterson et al. (2003); however, the impacts of specific oil constituents, such as PAHs, on marsh plants have received less attention. For example, Kirso and Irha (1998) showed that certain marine algae, e.g., *Fucus* (a brown alga), are efficient bioaccumulators and degraders of benzo(a)pyrene, a carcinogenic PAH. In contrast, green algae (*Enteromorpha* and *Cladophora*) bioaccumulated and degraded less benzo(a)pyrene. No apparent negative effects on their biomass were noted by Kirso and Irha (1998). In a study examining the effects of creosote (about 85% PAHs) on *Myriophyllum spicatum* L., McCann et al. (2000) showed that concentrations as low as 1.5 mg L<sup>-1</sup> adversely affected this submerged aquatic plant. Marwood et al. (2003) later supported those findings. They demonstrated that some components of creosote had a stimulatory effect on plant growth, while others, at the same nominal concentration of creosote, had an inhibitory effect. They concluded that plant growth, as measured by shoot length, was a sensitive endpoint for determining adverse creosote effects. Instead, visual observations on pigmentation and effects on roots were the most sensitive endpoints (McCann et al. 2000). Emergent macrophytes can also bioaccumulate PAHs. Watts et al. (2006) showed that the salt marsh plant *Spartina alterniflora* Loisel. had elevated concentrations of PAHs following contact with PAH-contaminated soil and water, although tissue concentrations in leaves and roots were one to two orders lower than those in the soil or water column. They did not report any negative effects of the PAHs on plant growth; however, this is not uncommon, since some emergent plant species show no physiological or growth impacts (Delaune et al. 1979, IPIECA 1994, Pezeshki et al. 1995, 1998).

Confounding factors influencing PAH concentrations are the presence of power plants and other fossil fuel burning activities near the lake, which have caused significant increases in PAHs in the past (Anderson 2003, Donahue et al. 2006). Man-made sources

include coal mining and coal burning, creosote-treated wood structures in or near the lake (e.g., railway line, boat docks), and fossil fuel burning for the powering of boats, vehicles, trains, weed harvesters, and heating of homes. In addition, natural sources of PAHs include exposed coal seams in and near the lake and forest fires (Donahue et al. 2006). Our analysis of a limited number of PAH at relatively high detection limits provided only scant information regarding probable sources. Oil spilled during the CN Rail accident contained only traces of pyrene and benzo(a)anthracene compared with phenanthrene (Wang 2005), so the comparable concentrations of these three PAH in the unaffected Grebe Reedbed (Table 1) suggests sources other than (and possibly including) the spilled CN Rail oil. Consequently, it is difficult to link the detected PAH concentrations in the lacustrine marshes in 2007 specifically to the CN Rail oil spill in 2005 based on the PAH results presented here.

### **5.3.2. NAPHTHENIC ACIDS IN SEDIMENTS AND RHIZOMES**

Concentrations of NAs in sediments and *S. tabernaemontani* rhizomes were generally below detection limits in all sites (Tables 1, 2). Like PAHs, the solubility of NAs varies with the molecular weight of individual NAs, whereby the lowest molecular weight structures will have the greatest water solubility of all compounds in a complex mixture. Since NAs are water soluble, it is likely that they can persist for extended periods of time in the water column (Headley and McMartin 1992) before microbial biodegradation; however, biodegradation processes tend to be restricted due to the chemical structure of NAs (Headley and McMartin 1992) and other hydrocarbons associated with crude oil, temperature, oxygen concentrations, salinity, nutrients, pressure, and pH (Leahy and Colwell 1990, Foght 2006). Hence, the ultimate fate and behavior of NAs in aquatic systems is influenced by various physical, chemical, and biological processes. There are no guidelines for NA concentrations in any ecosystem in Canada (CCME 2007); however, the maximum permissible level of 0.15 mg L<sup>-1</sup> of Na-naphthenates in sea water established in the former Soviet Union (Fine Tailing Fundamentals Consortium 1995) is 10-fold lower than the threshold range of 6-19 mg L<sup>-1</sup> provided by Leung et al. (2001).

Information about the toxicity of NAs to aquatic plants is scarce (Patrick et al. 1968, CEATAG 1998, Yong and Ludwig 1988) and often report variable results (e.g., Alberta Environmental Protection 1996, Leung et al. 2001, 2003). Generally, NAs impact algal community composition due to variable toxicity levels of individual algal species (Patrick et al. 1968, Yong and Ludwig 1988). Leung et al. (2001) determined that the threshold for ecological effects on phytoplankton communities lies between 6 mg L<sup>-1</sup> and 19 mg L<sup>-1</sup> NA. This range is higher than the concentrations we detected in sediments and *S. tabernaemontani* rhizomes in the lacustrine marshes in Wabamun Lake (generally <5 mg kg<sup>-1</sup>; Tables 1, 2); however, chronic long-term exposure may have negative effects on phytoplankton communities, as has been shown in other aquatic organisms (e.g., Dokholyan and Magomedov 1983, Rogers et al. 2002). We could not find any data on toxicity levels of NAs on emergent macrophytes; however, NA toxicity has been shown in *Populus tremuloides* Michx. (trembling aspen) at concentrations ranging from 75-300 mg L<sup>-1</sup>, causing a reduction in growth, chlorophyll production, photosynthetic rates, root water flow, and root respiration (Kamaluddin and Zwiazek 2002). CN (2007) reported no toxicity effects of the oil spill on *L. minor* frond production and biomass.

### 5.3.3. WATER CHEMISTRY

Most of the surface water chemical variable showed little variation among the seven sites (pH, TP, TDP, SRP, NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup>), likely in response to the frequent mixing of the lake water and the substantially higher than average lake water level in 2007 (Figure 10). Only TN and TDN concentrations varied among the sites (Table 3).

It appears that the CN Rail oil spill had no impacts on N and P surface water variables in the lacustrine marshes of Wabamun Lake as one would expect. The range of concentrations of most nutrient variables was similar to historical values. For example, TP concentrations ranged from 28-38 µg L<sup>-1</sup> from 1982-2001 (Casey 2003) and averaged 30.0 µg L<sup>-1</sup> at all seven sites in our study (Table 3). Conversely, concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> were somewhat lower in our study compared to previous records (NH<sub>4</sub><sup>+</sup>: 8.8 µg L<sup>-1</sup> vs. 5-28 µg L<sup>-1</sup> from 1982-2001; NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup>: 2.6 µg L<sup>-1</sup> vs. < 4.0 µg L<sup>-1</sup> from 1982-2001; Casey 2003), while TN values were higher in our study compared to previous

records (1,065  $\mu\text{g L}^{-1}$  vs. 770-995  $\mu\text{g L}^{-1}$  from 1982-2001; Casey 2003). Differences are attributable to environmental conditions at the time of sampling, e.g., water levels, as well as sampling time, location, and depth within the lake. Nutrients within reedbeds tend to be higher than in deeper lakes due to the higher amounts of organic matter and decomposing plant matter.

Based on TP concentrations (25-36  $\mu\text{g L}^{-1}$ ; Table 3), Wabamun Lake is mesotrophic, which is characteristic for nearly one third of lakes in Alberta (Mitchell and Prepas 1991). Chl. *a* concentrations, an indicator of algal productivity, ranged from 11-21  $\mu\text{g L}^{-1}$  in our study and compared favourably with previous data from Wabamun Lake (9-14  $\mu\text{g L}^{-1}$  from 1982-1998; Casey 2003). The highest chl. *a* concentration occurred at the control site (21  $\mu\text{g L}^{-1}$ ; Table 3), which resulted from extensive phytoplankton growth in this lacustrine marsh. Concomitantly, this site also had the highest turbidity and one of the lowest Secchi disk depths (85 cm; Figure 8). While the Secchi disk depths at the affected and treated sites at Ascot Beach and Rizzie Beach were similarly low (73 and 70 cm, respectively; Figure 8), the turbidity at these two sites was much lower as a reflection of substantially lower chl. *a* values (11 and 12  $\mu\text{g L}^{-1}$ , respectively; Table 3). The lower turbidity at the affected and treated sites at Ascot Beach and Rizzie Beach was caused primarily by epiphyton as opposed to phytoplankton at the control site.

There were no trends apparent for the pH or any of the surface water nutrient and chl. *a* concentrations with respect to treatment regimes following the oil spill. It is likely that treatment regimes (a form of disturbance), e.g., flushing and/or vacuuming, caused a sudden release of nutrients, particular TP and SRP, from lake sediments (Hamilton and Mitchell 1988, Kristensen et al. 1992, S ndergaard et al. 1992, Reddy et al. 1999) due to altered sediment redox potentials and adsorption-desorption dynamics. These nutrient flushes would not be detectable shortly after the disturbance due to the rapid assimilation of the nutrients by aquatic vegetation (algae, SAV, emergent vegetation) and microbes (mostly bacteria; Riber et al. 1983, Hansson 1989, Reddy et al. 1999). We cannot comment on the effects of these nutrient flushes in the lacustrine marshes in Wabamun Lake due to the absence of plant community data preceding our study, although we



hypothesize that an increase in algal biomass could have occurred during that growing season. In any event, nutrient concentrations two years following the oil spill and various subsequent treatment regimes were within the range of historical values.

#### **5.4. SUBMERGED AQUATIC VEGETATION**

Species richness of SAV at each of the seven sites was low, with a maximum of five (at the Grebe Reedbed unaffected, affected and untreated) and a minimum of two SAV species per site (Ascot Beach affected and untreated and control; Table 4). SAV PVI differed significantly among sites, with the Grebe Reedbed (affected, untreated) having the highest SAV PVI (60%). The remaining sites had similar SAV PVI values (1-13%; Figure 10). SAV cover also differed significantly among sites (Figure 10). Ascot Beach (16-25%) and the Grebe Reedbed (24-86%) had the highest SAV covers.

The dominance of *U. macrorhiza* and *Potamogeton* spp. in shallow lakes and lacustrine marshes is not uncommon in southern boreal Alberta (Mitchell and Prepas 1991). Particularly *Potamogeton* spp. are present in nearly all of the lakes for which plant surveys have been conducted in continental western Canada (e.g., Haag and Gorham 1977, Pip 1979, 1987, Mitchell and Prepas 1991, Taylor and Helwig 1995). *Potamogeton* spp. generally prefer to grow in substrata with high sand/gravel but low clay and organic matter content (Pip 1979). Conversely, the similarly ubiquitous *U. macrorhiza* prefers substrata with substantially lower sand/gravel and substantially higher clay and organic matter content (Pip 1979). The third-most frequently encountered SAV species in Wabamun Lake was *C. demersum*, which has similar substrate preferences to *Potamogeton* spp. (elevated sand/gravel and low clay and organic matter content; Pip 1979). Since substrate quality was very similar in our study sites (see above; Alberta Environment unpublished data), it was not surprising to encounter these SAV species in nearly all of our sites. We did not examine SAV species community dynamics along the control – Grebe Reedbed transect; however, in a previous study of SAV communities in Wabamun Lake, Haag and Gorham (1979) showed that dominant SAV species in exposed areas included stonewort (*Chara* sp.) and *M. exalbescens*. In areas of less exposure and finer-grained sediments, *Stuckenia vaginata* (Turcz.) Holub. (previously

*Potamogeton vaginatus* Turcz.) and *P. richardsonii* dominated the SAV community. Persistent populations of *Stuckenia pectinata* (L.) Böerner (previously *Potamogeton pectinatus* L.) were confined to more fine-textured sediments. In general, sheltered areas had higher total plant cover and greater species diversity than exposed areas (Haag and Gorham 1979).

Neither presence of oil nor treatment appeared to have affected the SAV PVI or cover in any of the sites, as SAV cover was greatest in the most heavily oil spill-affected site (Grebe Reedbed, affected and untreated) and similar in affected but untreated and unaffected sites of the same area (Rizzie Beach; Figures 9, 10). These observations support previous studies that showed that many SAV and floating leaf plant species, including *C. demersum*, *L. minor*, and *N. lutea* ssp. *variegata*, were apparently unaffected by oil spills or other forms of pollution (Lind and Cottam 1969, McCombie and Wile 1971, Stuckey 1971, Burk 1977, Brown and Goodman 1989, CN Railway Co. 2007). Contrastingly, McCann et al. (2000) and Marwood et al. (2001, 2003) showed that creosote significantly reduced growth rates of *Lemna gibba* L. and *M. spicatum*, which they attributed to reduced rates of photosynthesis; however, these negative impacts may be the result of high concentrations of creosote applied to their plants (up to 100 mg L<sup>-1</sup>) and in some cases multiple applications of creosote over a short time frame.

The resilience of many SAV and floating leaf plant species has been attributed to their growth form and life history. Burk (1977) showed that perennial plants were more resilient than annual plants to oil spills, because annuals grow from seeds every year, and seedlings have shown poor recovery from oiling. In contrast, perennials do not have this requirement. All SAV and floating leaf plant species we encountered in the seven sites (Table 4) were perennials and would be less affected by the oil spill than annuals.

##### **5.5. DECISION FACED IN ECOSYSTEMS MANAGEMENT FOLLOWING AN OIL SPILL**

The most important activity to be completed prior to any oil recovery program is the development of defined semi-quantitative endpoints. Responders must formulate consensus targets using systematic reasoning and technical knowledge of the impacted

ecosystem with consideration given to socio-economic sensitivities and limitations. The creation of a multi-disciplinary expert group is required to support the creation of defensible and achievable environmental outcomes.

A thorough geographical assessment and species inventory of the impacted area is required to describe accurately the treatment reaches and quantify the amount of oil. This activity is often iterative due to the dynamic nature of oil products in freshwater environments with low energy and experiencing temperate climates. With an accurate geographic inventory of oiling conditions, priority matrixes can be created to guide the selection of appropriate treatment actions to improve risk management at specific locations.

An effective remediation program must attempt to balance the net environmental benefit equation. The balance point of the hypothetical equation can be visualized as the transect point of two mirrored and exponentially increasing lines that simultaneously approach infinity. One line represents the treatment effort and the opposing curve is the effects on the environment due to treatment. A small deviation above the transect point of the two hyperbolic lines will significantly increase the effort required to recover residual product with a corresponding increase in cost, technological improvements and manpower requirements. Additional remedial efforts will reduce the exposure of environmental receptors of concern to remaining product and in the process deteriorate near-shore habitat. Describing and then attaining the net environmental balance is the goal of any remediation effort; however, this activity is often strongly influenced by competing social and economic considerations.

## 6.0 RECOMMENDATIONS

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Treating oil spill affected lacustrine marshes clearly reduced oiling on emergent macrophytes; however, treatment also significantly reduced *S. tabernaemontani* cover and biomass at selected sites. Water depth appeared to play a significant role in the recovery process of the lacustrine marshes from treatment. Our data showed clear interactions among treatment regime and intensity, water depth, and recolonization success by *S. tabernaemontani*. Intense and highly disruptive treatment regimes can significantly disrupt lake sediments and damage the rhizomes of emergent plants, thereby decreasing rates of recolonization of treated areas, plant cover, and plant biomass. Recolonization of treated lacustrine marshes was also dependent on water depth, with recolonization rates reduced in deeper water.

Therefore, prior to any treatment regime, the treatment intensity and water depth need to be considered to assure successful re-establishment and growth of emergent plant species, which provide habitat for waterfowl and aquatic animals communities, including fish. Any treatment regime that significantly disturbs lake sediments, and hence emergent macrophyte rhizomes, could have long-term impacts on lacustrine marsh re-establishment, thereby resulting in a significant reduction in crucial wildlife habitat.

We recommend the following initiatives:

1. Examine plant community dynamics in the recently treated sites at the Grebe Reedbed to gain a better understanding of post-treatment recovery processes in this lacustrine marsh site;
2. Continue monitoring of macrophyte plant and rhizome densities in the previously treated areas (Ascot Beach and Rizzie Beach);
3. Continue monitoring of sediment PAH concentrations, since they were elevated even two years after the oil spill in two sites;
4. Monitor plant community and sediment characteristics in previously classified unaffected areas, one of which was proven this study to have been affected by the oil spill in (Ascot Beach – affected and untreated);

5. The best approach to remove oil from vegetation (and the lake) is to cut plants at the end of the growing season and dispose of the oiled plants outside of the marsh, when risk to animals and lake users are systematically evaluated and found to be minimal;
6. Limit treatment regimes that significantly disturb lacustrine marsh sediments, particularly in marshes with deep water;
7. Establishment of a randomly stratified sampling design and permanent sampling plots to monitor short- and long-term impacts of treatment regimes and intensities; and
8. Development and implementation of less-intensive treatment technologies and/or techniques to minimize ecosystem impacts and maximize recovery of oil.

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**APPENDIX – GPS COORDINATES OF SAMPLING LOCATIONS**

Site	Treatment	Plot	N	W	Treatment	Plot	N	W
Control		1	53° 34.533'	114° 39.521'				
		2	53° 34.529'	114° 39.547'				
		3	53° 34.538'	114° 39.580'				
		4	53° 34.538'	114° 39.587'				
		5	53° 34.524'	114° 39.641'				
		6	53° 34.547'	114° 39.674'				
		7	53° 34.555'	114° 39.695'				
Rizzie Beach	unaffected	1	53° 33.242'	114° 31.691'	affected, treated	1	53° 33.250'	114° 31.625'
		2	53° 33.240'	114° 31.714'		2	53° 33.253'	114° 31.627'
		3	53° 33.209'	114° 31.714'		3	53° 33.262'	114° 31.637'
		4	53° 33.237'	114° 31.731'		4	53° 33.270'	114° 31.639'
		5	53° 33.242'	114° 31.755'		5	53° 33.269'	114° 31.649'
		6	53° 33.243'	114° 31.760'		6	53° 33.256'	114° 31.645'
		7	53° 33.247'	114° 31.783'				
Ascot Beach	affected, untreated	1	53° 33.516'	114° 32.656'	affected, treated	1	53° 33.490'	114° 32.603'
		2	53° 33.511'	114° 32.644'		2	53° 33.487'	114° 32.619'
		3	53° 33.505'	114° 32.627'		3	53° 33.466'	114° 32.648'
		4	53° 33.499'	114° 32.612'		4	53° 33.473'	114° 32.671'
		5	53° 33.493'	114° 32.595'		5	53° 33.482'	114° 33.695'
		6	53° 33.499'	114° 32.612'		6	53° 33.502'	114° 32.706'
		7	53° 33.435	114° 32.573'		7	53° 33.529'	114° 32.708'
Grebe Reedbed	unaffected	1	53° 33.201'	114° 30.150'	affected, untreated	1	53° 33.207'	114° 30.273'
		2	53° 33.215'	114° 30.146'		2	53° 33.209'	114° 30.237'
		3	53° 33.224'	114° 30.164'		3	53° 33.206'	114° 30.253'
		4	53° 33.231'	114° 30.191'		4	53° 33.214'	114° 30.269'
		5	53° 33.234'	114° 30.216'		5	53° 33.219'	114° 30.234'
		6	53° 33.246'	114° 30.246'		6	53° 33.219'	114° 30.300'
		7	53° 33.235'	114° 30.278'		7	53° 33.217'	114° 30.317'