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# **Vegetative and soil mesofaunal changes at boreal peatland field sites from produced water spills:**

## **Implications for the environmental assessment and remediation of upstream oil and gas sites**

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July 2011

**Project No: 60113925**

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Dear John:

**Re: Vegetative and soil mesofaunal changes at boreal peatland field sites from produced water spills**

AECOM is pleased to provide this final version of this report for the SGRP-funded project on environmental risk-based approaches for managing saline releases to boreal peatlands. We very much appreciate the opportunity to assist with this research and development initiative. If you have any questions, please contact the undersigned.

Sincerely,  
**AECOM Canada Ltd.**



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## Abstract

Moderately to highly saline groundwater co-occurs with most petroleum oil and natural gas deposits. Releases of saline “produced water” potentially affect peatland-forming wetlands (bogs and fens), particularly in temperate to sub-arctic circumpolar environments. This study quantifies vegetative and soil faunal responses to salinization of western Canadian boreal peatlands, towards the development of ecological risk-based remediation guidance.

Field data on the abundance (or percent cover) of vascular plants, bryophytes, and soil mesofauna were obtained in the summer of 2008 and 2009 from nine produced water release sites in Alberta and British Columbia. Research plots (1 m<sup>2</sup> quadrat and peat core sample sites) were established along salinity gradients within fen (n=5), bog (n=3) and marsh (n=1) ecosystems arising from recent spills (mostly <5 years old). The diversity of vascular plants and bryophytes, measured as species richness, exhibited a significant decrease linearly in peatlands and other wetlands in relation to the log<sub>10</sub> of salinity (measured as electrical conductivity) of either wetland soils or interstitial water. The particulars of the salt concentration – biotic response relationship were very similar for seven of nine sites (predominantly fen sites), while a statistically significant relationship between species richness and the log<sub>10</sub> of salt levels was different for a large marsh site in central Alberta. Marshes generally exhibit higher plant diversity than peatlands: Plots at the marsh site exhibited higher plant diversity at a given salinity than the fen sites, but the slope of the best-fit line describing the species richness – salt concentration relationship was similar to that of the fen sites. The overall presence-absence data provide a clear indication of the salinity tolerance ranges of 31 dominant vascular plant and bryophytes species, including three commonly occurring species of sphagnum moss, rough bentgrass, black spruce, dwarf birch, Labrador tea, and dwarf bog cranberry. Some plants such as fireweed, Labrador tea, dwarf birch or aquatic sedge were observed to persist at soil salinities in excess of 6 - 8 mS/cm, while some species such as *Sphagnum girgeneshoni* or common strawberry were not observed at soil salinities in excess of 3 to 4 mS/cm.

The vast majority of observed plants and bryophytes (101 of 162 observed species) were observed in only one or a few of the 128 plots total. The salinity tolerances of these more rare species cannot be defined without use of an alternative methodology, since presence in any plot for rare species is ascribed firstly to ‘chance’ encounters and secondarily to the salt levels in the co-located soils and interstitial water.

No appreciable relationship could be discerned between soil or interstitial salt levels and the abundance or composition of soil invertebrates (mesofauna) extracted from peat cores obtained from the same plots used to assess vegetative ecology. The absence of a discernible influence of plot salinity on mesofaunal abundance or composition is hypothesized to have resulted from microenvironmental variability in near-surface environments (both laterally and horizontally) that was not adequately captured by the sampling methods, and identification of mesofaunal taxa to only the family level or higher.

Occurrence data for each plant and bryophyte species that were observed in eight or more of the 127 plots assessed at field sites allow for estimation of frequency of occurrence statistics for each taxon along a (log<sub>10</sub>) gradient of increasing salinity. The field data for Alberta peatland environments provide evidence of salinity ranges at which various plants may persist, at least for shorter time periods of approximately a half decade or less. Among the commonly occurring peatland sphagnidae, *S. magellanicum* was observed to be relatively salt tolerant, while *S. girgeneshonii* and *S. fuscum* are relatively salt intolerant. Black spruce (*P. mariana*) is poorly tolerant to salinization; therefore, at historical release sites, it can be expected that the majority of other peatland vegetation can potentially recolonize the disturbance site once *P. mariana* seedlings start to appear.

# Table of Contents

Statement of Qualifications and Limitations  
 Letter of Transmittal  
 Distribution List  
 Abstract

	page
<b>1. Introduction .....</b>	<b>1</b>
<b>2. Methods .....</b>	<b>3</b>
2.1 Soil sampling .....	4
2.2 Water sampling.....	4
2.3 Plant and bryophyte assessments.....	4
2.4 Soil invertebrate sampling.....	5
2.5 Aquatic invertebrates in open water .....	5
2.6 Quality assurance/quality control.....	5
2.7 Statistical analyses – relationships between dependent and independent variables.....	5
2.8 Data analyses – plant/bryophyte community associations .....	6
<b>3. Results.....</b>	<b>7</b>
3.1 Chemical characteristics of study sites.....	7
3.2 Vegetative characteristics .....	7
3.3 Soil mesofauna.....	7
3.4 Aquatic invertebrates in open water .....	8
<b>4. Discussion.....</b>	<b>9</b>
4.1 Influence of salinization on plant/bryophyte species richness.....	9
4.2 Multivariate community responses .....	10
4.3 Species sensitivity distributions for boreal peatland salinization.....	10
4.4 Major conclusions.....	12
<b>5. References .....</b>	<b>14</b>

## List of Tables

- Table 1 - Summary of field study sites
- Table 2 - Soil and water chemistry at the field sites
- Table 3 - Co-Variations among different measures of peatland salinization
- Table 4 - Summary of plant and bryophyte taxa observed
- Table 5 - Summary of chemistry and zooplankton data for five samples from three standing water areas, 2009.
- Table 6 - Least-squares linear regression estimates for salt concentration - vegetation response relationships at produced water release sites in peatland environments
- Table 7 - Soil EC extirpation threshold estimates for commonly encountered boreal peatland vegetation (EC concentrations and SSD values expressed in mS/cm)



## List of Figures

- Figure 1 - Study site locations
- Figure 2 - Frequency distribution of salinity levels in uppermost soils in sampling quadrats. (a) Ranks percentile follows a log-linear distribution without undue heteroscedasticity. (b) Frequency distribution of Log10 transformed soil EC data
- Figure 3 - Chloride in peatland water samples was highly correlated with EC. The strength of the correlation was greater at higher chloride concentrations.
- Figure 4 - Salinity data from surface soil samples were similar to data for deeper samples. The 2008 Sites were selected so as to minimize vertical gradients associated with post-release hydrodynamic processes (recent release sites; high water table).
- Figure 5 - Bryophyte and vascular plant taxa observed in >10% of quadrats
- Figure 6 - Species rarefaction. The majority of taxa encountered were rare, being encountered in only a small percentage of plots and sites.
- Figure 7- Dominant mesofauna in peat samples included mites and, to a lesser extent, collembola. Nematodes, earthworms and various other groups were poorly represented
- Figure 8 - Mesofaunal activity was concentrated in soil microenvironments within fen soils. Minor variations in between-plot sampling (including depth sampled) likely contributed to appreciable variability that was not directly related to salt contamination.
- Figure 9 - Summary of chloride concentrations and diversity of taxa sampled from the water column at three 2009 Sites.
- Figure 10 - There was no apparent relationship between measures of salinity and abundance of any mesofaunal group
- Figure 11 - Vegetation species richness was negatively correlated with soil salinity measured as the log(10) of EC.
- Figure 12 - Overall relationship between soil salinity, as EC, and plant/bryophyte species richness.
- Figure 14 - Sensitivity of individual woody shrubs and trees or smaller vascular plants to soil EC.
- Figure 15 - Sensitivity of individual grass, sedge, and moss species to soil EC
- Figure 16 - Occurrence of salinity and of plants is better approximated by log-linear than linear frequency distribution.
- Figure 17 - Examples of single species sensitivity distributions based on presence-absence observations.
- Figure 18 - Multi-species sensitivity distribution for boreal wetland vegetation based on extirpation potential associated with salinization
- Figure 19 - New information on relative sensitivity of different taxa is directly relevant for assessing degree of site impairment and recovery
- Figure 20 - Plant or bryophyte abundance data were generally not conducive to the development of synoptic concentration - response curves.

# 1. Introduction

Produced water, defined as water extracted from subterranean formations that host petroleum and natural gas deposits during oil and gas (O&G) exploration and extraction, comprises the largest single waste stream by volume of upstream O&G activities: Produced water is generated at approximately seven to ten times the volume of oil or gas that is co-produced (Santos and Wiesner, 1997; Benko and Drewes, 2008). The major inorganic composition of most produced water is similar to seawater (mainly sodium and chloride, with lesser amounts of sulphate, calcium, and other major ions). Salt concentration, however, can vary substantially between production fields from less than a few parts per thousand to more than 250 parts per thousand ( $^{\circ}/_{\infty}$ ) (>250,000 mg/L) (Sauer *et al.*, 1997; Benko and Drewes, 2008). Subsurface injection is the primary means of produced water from on-land O&G operations; however, environmental releases often occur prior to re-injection, especially as a result of the corrosion and rupture of emulsion and produced water pipelines. In northern Alberta, Canada, alone, greater than 6,000 oil effluent (emulsion, oil and produced water) pipelines and approximately 1,900 produced water pipelines are registered.

Barrett (2002) documented through aerial photo interpretation the characteristics of landscape-level scarring and similar changes in vegetation communities in an Arkansas, United States, hydrocarbon production field that came into production in 1922. Many persistent drainage scars are evident from past releases of produced water, characterized by either strong reductions in vegetation growth or domination by salt-tolerant plant species. Wang *et al.* (2007) demonstrated watershed-scale changes in water quality as a result of produced water releases from coal bed methane production in a United States Rocky Mountain watershed, based especially on changes in sodium compared with other major cations.

Peatland environments coincide with several major O&G operational areas, and O&G-related salt releases to peatland ecosystems are very common in temperate and subarctic regions of western Canada, including the Province of Alberta. Alberta contains about 100,000 km<sup>2</sup> of peatland, which is approximately 16.3% of the land base. Salinization of peatlands often arises from the accidental releases of produced water. Unperturbed boreal plain wetlands may also exhibit a wide salinity range: for example, Trites and Bayley (2009) observed salinities in western Canadian boreal wetlands, expressed as electrical conductivity, in the range of 0.5 to 28 mS/cm. More typically, however, boreal plain peatlands are freshwaters systems with EC < 0.4 mS/cm (Zoltai and Vitt, 1995).

Environmental issues arising from the release of sodium chloride and other salt ions can be exceedingly complex in spite of the apparent simplicity of the substances released. The resulting soil and water contamination is often addressed – based on a lack of understanding of the longer term consequences - by excavation and landfilling of contaminated soil, extraction and off-site disposal of large volumes of surficial water, or other forms of large scale soil disturbance. The results of such remedial efforts, however, can be highly counterproductive relative to ecosystem restoration goals. According to Gorham and Rochefort (2003), peatland ecosystem restoration requires provision of the appropriate hydrological regime, manipulation of surface elevation, improving microclimate, adding appropriate sphagnum peat diaspores, manipulating base ions where necessary, applying fertilizers at some sites, dealing with opportunistic and invasive plant species, as well as monitoring and managing through at least one long-term flood – drought cycle (UMA, 2008). Attaining effective peatland restoration after profound disturbance is thereof challenging.

The cumulative loss of peatlands, or conversion of land area from peatlands to other types of wetlands such as marshes or swamps, is undesirable for at least four reasons: (i) the new ecosystem type, which may persist over long time periods, is incongruous with the surrounding landscape and may disrupt wildlife habitat; (ii) loss of peatlands may be accompanied by loss of associated flora and fauna that are well adapted to such habitats; (iii) peatlands represent large carbon sinks, and conversion to other ecosystem types can result in substantially enhanced CO<sub>2</sub> releases to the atmosphere (Strack, 2008); and (iv) peatlands function to filter water as it moves from higher to lower elevation areas within watersheds and to maintain water quality.

There are a range of wetland types found within temperate to sub-arctic boreal and taiga ecozones, which vary in geochemistry, microbial ecology, role of soil-associated fauna, plant productivity, rates of nutrient turn over, and biodiversity (Zoltai and Vitt, 1995). Four major categories of wetland capture the vast majority of ecosystem types that might potentially be influenced by oil and gas activities. These include (i) marshes - non-peat-forming wetlands dominated by sedges (Cyperacea) and other monocotyledonous plants; (ii) swamps - forested or shrubby, non-peaty

wetlands, also with a poorly developed bryophyte layer; (iii) fens - peat forming wetland dominated by bryophytes and influenced by the chemistry of the surrounding mineral soils; and (iv) bogs - ombrogenous peatlands that obtain surface water inputs only from precipitation and snowmelt, and have restricted water flows. Bogs are naturally acidic, nutrient poor, and exhibiting low plant diversity. This study focused on primarily on peatland (bog and fen) ecosystems.

Contaminant assessment and remediation guidelines that are of direct relevance to the major portion of western Canadian wetlands (especially peatlands) do not exist. Guidelines specific to peatlands and other wetland types would be invaluable in allowing for the standardization and streamlining of contaminated sites work at salt-affected northern boreal and sub-arctic peatland sites. Such guidance is important for assisting with spill responses. Furthermore, the remediation of contaminated soils and water is a necessary pre-requisite to the achievement of scientific and regulatory vegetative reclamation goals for upstream oil and gas sites.

The overall objective of the research reported here was to obtain sufficient scientific data from field studies on salinity responses in peatlands to allow for the development of ecological risk-based remediation guidance. Specific objectives were to (i) characterize the abundance or percent cover of vascular plant and bryophyte species across salinity gradients identified in both peaty soil and interstitial water; (ii) assess the abundance of soil invertebrate (mesofaunal) taxa across the same natural salinity gradients; (iii) quantitatively define soil/water salinity – ecological response relationships using a regression type design; and (iv) characterize the range of tolerance of individual soil associated flora and fauna to salt ions in wetland systems. An understanding of salinity tolerances of important boreal peatland taxa is a pre-requisite to the further reconstruction of vegetation and other multiple species sensitivity distributions (SSDs).

## 2. Methods

A field-based approach was selected over laboratory-based or greenhouse experiments, since routine culturing/manipulation techniques have not yet been developed for the vast majority of biota of interest. The intent of field sampling was to obtain co-located data on soil or water chemistry, vegetative characteristics and mesofauna across a range of salinities. Field sites were selected in each of 2008 and 2009 from recent produced water release sites in predominantly peatland ecosystems. In order to optimize the potential for observing salinity – biotic response relationships, selection criteria were developed for sites with the intent of minimizing the degree of natural variability across the site. Candidate spill sites were selected for inclusion in the field study using the following criteria: (i) area salinized was relatively large (>3 ha) and there existed a gradient of salt contamination within the active rooting zone with a concentration range of more than 4 to 5 orders of magnitude; (ii) there was an immediately adjacent uncontaminated area that adequately represented the reference state; (iii) the site had adequately high residual salt concentration within the rooting zone that vegetative and faunal effects were likely to occur (i.e.,  $[Cl^-]$  in soil > 3000 mg/kg or in interstitial water > 1,000 mg/L;  $[Na^+]$  > 1,500 mg/kg or > 500 mg/L ; EC (electrical conductivity) >10 mS/cm); and (iv) site was not unduly influenced by confounding variation across the available study area, including but not necessarily limited to significant changes in elevation or depth of the seasonal water table, presence of natural or constructed ditches that have resulted in alterations in site hydrology, other physical disturbance, presence at appreciable levels of co-contaminants such as petroleum hydrocarbons, or transitions between wetland types (e.g. strong transitional changes from bogs to marshes and more open water systems).

Four sites were selected from eight candidate spill sites for study in the summer of 2008 (sampled between August 6th and 16th). An additional five sites were selected from eleven candidate sites in 2009 (sampled between July 27th and August 8th). Figure 1 shows the nine site locations while Table 1 provides a brief summary of the sites. The nine sites span a latitudinal distance of approximately 395 km.

Selection of plot locations at each site was guided by initial site reconnaissance observations. First, the concentration of salinized soil and water in the near-surface environment was roughly mapped by excavating shallow pits in the wetland surface and measuring the electrical conductivity of standing water in the pit using a handheld EC meter (Oakton Instruments ECTester 11). Second, visual evidence of bryophyte and/or vegetative impairment was used as preliminary evidence of impact thresholds. Based on these observations, a series of linear transects was established, with one end of each transect located in most contaminated zone; i.e. within the area of immediate influence of the spill event. The other end of the transect was located beyond the inferred zone of influence of the salt spill, in an area intended to reflect background or reference conditions. The transects were located within a single vegetative ecotype to the extent possible, so as to limit variability associated with potentially confounding influences such as site hydrological and/or elevation characteristics along the entire length. Once a transect was established, an average of five plots were established along the transect line, from the most contaminated point to a reference location, with three or more plots established at intermediate salt concentrations, along an approximate log series of EC levels. This procedure was repeated to establish between three and eight transects at each site, each with four to eight plots. Fewer transects were established at Site 2-12, since the site was small and the range of observed field EC concentrations were relatively narrow (Table 2). At each plot, UTM coordinates (NAD83) were recorded using a hand held Garmin 76CSX GPS unit.

A custom-designed, 60 cm long peat corer was used to excavate a plug of peat, and depth to the water table was measured with a tape measure. A photograph was taken of each plot and additional photographs of vegetation and soil cores were taken.

In 2009, site selection and methods were modified to obtain observations from a larger range of peatland sites, located over broader geographic area, and the level of sampling effort at each site was reduced to accommodate this.

Individual plots are herein designated using their abbreviated Site name, Transect number, and Plot number (e.g. S1-21 T1P2).

## 2.1 Soil sampling

Soil samples were collected from every plot using a stainless steel, custom-built soil/peat corer (inside diameter approximately 4 cm), or a stainless steel narrow-nosed shovel depending on site conditions. Up to three different soil layers were sampled in 2008 depending on the presence of particular soil layers and depth to water table. Where possible soil samples were collected from three layers: the fibric/humic layer (FH), 10 cm above the water table (AWS) and 10 cm below the water table (BWS). In most instances there was no distinct fibric/humic layer in soil cores. The fibric/humic interface may not have been encountered within the maximum depth of soil sampling (up to 60 cm below ground surface). There was typically no discrete transitional zone between fibric and humic layers, as was expected for bogs and fens undergoing rapid peat accumulation. At plots where the water table was at or near the surface, no AWS layer could be sampled and in such cases only BWS samples were collected. In 2009, only AWS samples were collected.

Five samples from each layer (if present) were collected from cores located throughout the 1 m<sup>2</sup> plots, to produce one composite sample for each depth, which was retained for laboratory analysis. Soil samples were stored and transported in coolers packed with ice to a commercial analytical laboratory, Bodycote Testing Group in Edmonton, for analysis. Soil samples were analyzed for pH, electrical conductivity (EC), and major anion and cation concentrations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, sulphate-S) by standardized saturated paste methods (Carter, 1993; McKeague, 1978). Nutrients in soil (nitrate-N, phosphorus) were analyzed based on the modified Kelowna soil test (Ashworth and Mrazek, 1995; Havlin et al. 1999).

## 2.2 Water sampling

Groundwater samples from each plot were collected using 1.5 m length PVC drive-points with 0.6 m slotted sections and HDPE weighted bailers. Drive-points were manually installed within the plot, most often into a pilot hole created with the peat corer. The drive-points were purged by withdrawing  $\geq 3$  times the volume within the piezometer, before the samples were transferred directly into pre-cleaned, analytical grade polyethylene bottles. All samples were stored in chilled coolers ( $\sim 4^{\circ}\text{C}$ ) and nutrient samples were preserved immediately with 2 mL sulphuric acid by acidifying to pH < 2, to prevent microbial activity. Coolers were shipped at the end of each sampling day, or every two days, to Bodycote Testing Group in Edmonton, AB. The analyses included nutrients (nitrate-N, phosphorus, orthophosphate) and salinity descriptors (EC and major ions: calcium, magnesium, sodium, potassium, chloride and sulphate). Analyses were conducted by APHA (2005) standardized test methods (pH and EC by conductivity: Method 2510; anions by ion chromatography: Method 4110 B; chloride by automated ferricyanide method: 4500-Cl-E; Kjeldahl N and Total P by automated ascorbic acid reduction method: 3120 B; orthophosphate-P by automated ascorbic acid reduction method: 4500-P-F; metals and cations by inductively coupled plasma: 3120 B).

## 2.3 Plant and bryophyte assessments

Vegetation data were collected using a 1 m<sup>2</sup> quadrat frame, which was further divided into quarters. Vascular plants and bryophytes found within the plots were identified to the extent possible to genus and species based on identification guides written by Johnson *et al.* (1995) and Vitt *et al.* (1988). The percent cover and individual counts (no./m<sup>2</sup>) were recorded for each species identified. In the case of bryophytes, only percent cover was recorded, since it is challenging to count individual moss plants. Additional notes were collected opportunistically such as the percentage of dead vegetation or the number of stressed individuals. Voucher specimens of unknown species were collected and placed into paper (bryophytes) or plastic bags (vascular plants) and kept in chilled coolers on site. At the end of each field day, unidentified vascular plants were placed into a plant press for drying and preservation. Unidentified mosses were air dried indoors and placed into dry paper bags for preservation. Vegetation samples were shipped to the AECOM Victoria office for identification at the end of the field program.

In 2009, there was a desire to expand the range of biological endpoints at the study sites relative to the 2008 field work. A reduced set of commonly occurring plants was selected for assessment of total wet biomass in survey plots. In particular, biomass estimates were provided for *Carex aquatilis* at Site 1-21 and some plots at Site 16-29. Other species selected for biomass estimates in addition to abundance or percent cover included *Carex utriculata* (S1-21 T1P2), *Rubus chamaemorus* (S15-08 T1P3, T1P8 & T1P13), *Clintonia uniflora* (S15-08T1P11; S16-29 T1P3, T1P4 & T1P8), and *Cornus Canadensis* (SA-82-G T1P3 & T1P5).

## 2.4 Soil invertebrate sampling

Soil invertebrate (mesofauna) samples were collected from every plot for each soil layer sampled in 2008 using the stainless steel soil corer. A five centimetre length soil core sample from each soil layer present (FH, AWS, and BWS) was placed in plastic ziplock bags and stored in chilled coolers for transport to Paragon Environmental Consultants (Edmonton) for processing. Soil samples were extracted into isopropyl alcohol using a series of Berlese funnels. Samples were then sorted to the family level or higher level of taxonomic order and counted, under a hand lens and dissecting scope. Mesofauna were identified only to the order or family level or similar: i.e. soil mites (prostigmatids, orobatids, mesostigmatids, astigmatids), collembolla (hypogasturidae, isotomidae, onychiuridae, neelidae, poduridae, sminthuridae), thrips, pscoptera, coleopteran, diptera, lepidoptera, homoptera, nemoatoda, copepod, other hymenoptera, pseudoscorpions, lumbriculiae, enchytraeidae, aranea, and entombryidae.

## 2.5 Aquatic invertebrates in open water

In 2009, limited effort was directed at characterizing the composition and abundance of various aquatic invertebrates (juvenile insects and other taxa) in small to moderate sized pools of open water that were observed at a subset of peatland study sites. There was limited opportunity for sampling similar open water habitat over the range of salinity concentrations that might occur at produced water peatland release sites. A total of 6 ponds were sampled in 2009 at three of the five sites (1-21, 16-29, A-82-G). Pelagic invertebrate samples were collected from these six ponds and in addition benthic samples were collected from three ponds.

Pelagic, drift fauna, epibenthos and benthos recovered from water samples were identified to the species level for rotifer, most cladoceran families, cyclopoid copepods, and amphipods. The other major taxonomic groups were generally identified to the genus or family level.

## 2.6 Quality assurance/quality control

Quality control was maintained through the field sampling program by:

- Ensuring all sampling bottles received were clean, free from defects and sealed.
- Using a new pair of clean nitrile gloves for soil and water samples collected at every plot.
- Cleaning soil and water sampling equipment between plots and sites.
- Preserving nutrient and silicon/silica water samples immediately after collection.
- Maintaining sample temperature by storing samples in coolers packed with ice.
- Contracting a CAEAL-certified laboratory for sample analysis (which provided analytical QC data for laboratory replicates, blanks, and reference materials).
- Shipping samples to the analytical laboratory within the recommended holding times.
- Using pre-made field forms to ensure all data were collected at every plot in a similar manner.

In addition, the chemistry data were reviewed for completeness and accuracy. Water analytical results were further evaluated through the collection of seven field duplicates and one process/travelling blank. Voucher specimens of bryophytes were examined by Karen Golinski, Ph.D. (Karen Golinski Ecological Consulting, Tennessee) to assist with refining species identification. Additional training on bryophyte taxonomy was also provided to AECOM by Dr. Golinski.

## 2.7 Statistical analyses – relationships between dependent and independent variables

A major objective was to evaluate the presence of and strength of relationships between soil or water chemistry (especially salinity), the vegetative status of plots (species richness, presence-absence of individual taxa, individual taxon abundance, biomass) and soil mesofaunal diversity or abundance. The inherently correlational nature of the study design was accommodated through completion of routine bivariate correlations (Pearson R) and simple linear (or log-linear) regressions, using Systat 6.0, for those analyses where both the dependent variable (e.g. soil

electrical conductivity) and independent variable (e.g., taxon richness) are continuous or semi-continuous as opposed to binary. Bivariate normality was examined visually, and to the extent possible, the bivariate analyses were conducted on either the original or log-transformed data, which ever best approximated a Gaussian distribution and minimized heteroscedasticity. The statistical methods used are either not dependent on (calculation of a Pearson R) or generally robust against more minor departures from normality, provided that the distribution of residuals is symmetric with small to moderate variance (Miller, 1998).

## **2.8 Data analyses – plant/bryophyte community associations**

The degree of co-occurrence of different vascular plant and bryophyte species in the 127 individual quadrats and nine sites was evaluated using multivariate ordination techniques. In particular, a principal components analysis (PCA) was conducted using Systat 6.0. The multivariate community assessments were based on plant density and bryophyte percent cover in each of the 127 quadrats, rather than on presence-absence data. The PCA was run using the correlation (variance of all variables equals one) rather than co-variance matrix to remove the influence differences in absolute values between species, so that the first principal component described the direction of maximum variance. The data set contained two disparate data types: i.e., recorded densities for most vascular plants with values in the range from 0 to 360 plants/m<sup>2</sup>, and recorded percent cover for bryophytes, with values in the range from 0 to 100%. As a further check against any undue influence of disparate data ranges, the vascular plant density data were converted to a range similar to that of the bryophyte percent cover data by converting each result to a percentage of the maximum observed density in any of the field quadrats. This resulted in a maximum value for any individual vascular plant species of 100, and is functionally similar to (but not identical to) mean centering the data for each individual attribute.

Density or percent cover data were developed for a total of 142 taxa by 127 individual plots. The number of taxa were further reduced to 48 by removing any plants or bryophytes that were not observed in five or more of the 127 quadrats (i.e., the multivariate analyses focused on relatively abundant, as opposed to rare, taxa). This was intended to produce a set of solutions that were not over-specified relative to the input data, and more amenable to generalizability of the results. The PCA models were run orthogonally (without rotation), and up to six factors identified as containing the most useful (primarily non-stochastic) variance in the larger data set for pattern analysis. This was based primarily on a scree test. Based on the unrotated solution, the first six factors respectively accounted for 7.7%, 6.3%, 5.9%, 4.9%, 4.6% and 4.2% of the total variance (total of 33.6%). An additional model was run with varimax rotation; however, no further insights regarding underlying patterns in the data were revealed.

## 3. Results

### 3.1 Chemical characteristics of study sites

The salinity range encountered in soils or near-surface water at the nine field sites is summarized in Table 2. The maximum observed soil salinity, measured as EC, was 17 mS/cm. Four sites exhibited maximum observed soil salinities > 10 mS/cm (1-20, 4-18, B-39-J, 14-36) while the maximum observed result at the five other sites was 4.2 mS/cm or lower. An additional four sites were screened out as candidate field sites in 2008 and a further six sites screened out in 2009 primarily owing to the high degree of physical disturbance that has occurred as a result of spill response activities and/or because of the relatively low residual salt concentrations. Overall, the range of soil salinity values represented by the plot samples was constrained by the availability of adequately contaminated (but otherwise minimally physically disturbed) study sites and availability of suitable areas for vegetative and associated studies within each site.

It was intended that the 127 plots sampled in 2008 and 2009 would adequately reflect a geometric mean distribution of soil salinities, in light of the desire to define toxicological thresholds. Figure 2 presents the cumulative frequency distribution of soil EC for the 127 plots. The concentration is based on the upper-most depth of measurement for the 2008 plots where chemical characteristics of the peatland were measured at two or more depths. The 127 plots fall along a long-normal distribution with adequate representation of soil salt concentrations across the spectrum of 0.13 to 16.9 mS/cm. As shown in Figure 2(a), there was no clear separation between EC levels in reference plots and those affected by produced water releases.

The degree of salinization of exposure media (both near-surface or surficial water and peatland soils) within the plots was measured in several ways: using field and laboratory EC measurements, and as individual ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^{2-}$ ), measured in soil using saturated paste techniques. All salinity measurements were significantly inter-correlated (Table 4 and Figure 3). Furthermore, the Pearson correlation co-efficient for co-variations between EC and individual ions in soil was greater than 0.70 for all ions except  $\text{K}^+$  ( $r = 0.31$ ,  $p = 0.0004$ ),  $\text{SO}_4^{2-}$  ( $r = 0.29$ ,  $p = 0.0001$ ), and  $\text{NO}_3^{2-}$  (majority of samples did not exhibit a detectable concentration). Therefore, concentration-response relationships are discussed herein based on EC data. The equivalent concentrations of individual salt ions can be estimated from the linear regression equations provided in Table 3. Correlations between EC and individual salt ions were generally stronger in water than in soil (Table 3).

The chemistry of peatland soils was characterized at multiple depths in 2008, relative to the depth to the water surface. Only a minimal difference in the soil EC or of individual salt ions was observed between the different soil depths (Figure 4). The upper-most soil salinity levels, therefore, adequately reflect biotic exposure potential throughout the biologically active portion of peatland soils. This may reflect the fact that all spill sites examined in 2008 were recent. Differential groundwater and surface water mediated transport of anions and cations over time would be expected to alter vertical distribution profiles.

### 3.2 Vegetative characteristics

A total of 162 different bryophyte or vascular plant taxa were observed in the 127 1.0 m<sup>2</sup> plots sampled in 2008 and 2009. Considerable effort was expended in 2009 and early 2010 to improve the accuracy and consistency of taxonomic identifications. Two vascular plant specimens and 13 bryophyte specimens could not be identified to the family or genus level. A detailed list of vegetative taxa observed is provided in Table 4. Figure 5 illustrates the most commonly encountered taxa, which are all commonly occurring species within boreal peatlands.

Twenty one (21) taxa were observed in more than 10% of all plots. In contrast, 101 individual taxa were observed in three or fewer of the 127 plots. The relatively high proportion of relatively rare taxa encountered is illustrated in Figure 6.

### 3.3 Soil mesofauna

Peat cores were collected in 2008 for the enumeration of soil mesofauna (small-bodied invertebrate fauna) as described in the methods. A total of 139 discrete soil samples were enumerated, comprising samples collected from



both above and below the water surface, as identified by the water depth in the hole left in the peatland after core extraction. Mites and collembola were encountered in many of the plots, while the other taxonomic groups were rare or absent from the suite of samples collected (Figure 7).

The mesofauna were far more abundant in the partially saturated, fibric layer soils than in soil core samples obtained from below the water table, regardless of the level of soil salinization. Samples from the fibric horizon (FH), just above the water surface (AWS), and just below the water surface (BWS) were evaluated for a small number of peat cores initially, after which it was decided that there was little value in quantifying mesofauna within the saturated zone. For site 1-20, the per sample abundance of mesofauna collected from the FH, AWS and BWS zones was 37, 3 and 0, respectively. Figure 8 illustrates the abundance in peat core samples of oribatid mites from several plots at Site 1-20, based on samples collected in the upper most fibric horizon or lower down within 10 cm of the water surface in the wetland: these mites were the most abundantly observed mesofauna. This observation further illustrates that mesofauna were relatively rare not just below the water table, but also in partially saturated zones below the active growing layer in these Sphagnum dominated ecosystems.

### 3.4 Aquatic invertebrates in open water

In 2009, open pools of standing water at three sites (1-21, 16-29 and A-82-G) provided an opportunity to characterize zooplankton abundance and species composition in relation to degree of salinization. The magnitude of salinization in the standing water was relatively low, and salt concentrations were relatively uniform between the sites (Table 5). Dominant taxa in the standing water pool from Site 1-21 included unidentified harpacticoid copepods, unidentified cyclopoid copepods, unidentified ostracods, the cladocerans *Acroperus harpae*, *Chydorus sphaericus*, and *Graptoleberis testudinaria*. Dominant taxa in the pool at Site 16-29 included the daphnid *Ceriodaphnia dubia* and the rotifer *Brachionus* sp. The dominant taxa within all three pools from Site A-82-G were similar, including the daphnid *Scapholeberis ramneri* and unidentified cyclopoid copepods.

The limited data preclude a detailed quantitative evaluation of the data. There was an apparent sigmoidal association between the taxon richness and chloride concentration in the water (Figure 9). An EC50 concentration for taxon richness estimated from a Boltzman-type sigmoidal curve fit through the limited data was 262 mg/L Cl<sup>-</sup>. This is comparable to the USEPA Final Chronic Value (FCV) water guideline of 210 mg/L. It is important to note, however, that all three samples with low taxon richness came from the same site. The data, therefore, do not reflect general conditions across produced water release sites. The total abundance (Table 5) was not significantly associated with the degree of salinization, either as measured based on EC or individual ion concentrations (e.g. for Cl<sup>-</sup>).

Soft-bottom benthic invertebrates were sampled from the bottom of the pond at Site 1-21 (two replicates: Cl<sup>-</sup> in overlying water was 2.6 mg/L) and 16-29 (one sample: measured Cl<sup>-</sup> concentration in the overlying water was 248 mg/L). The taxon richness in all three samples was similar: 44 taxa, 38 taxa and 29 taxa respectively. The total macroinvertebrate abundance in all three samples was less similar: 1,680 organisms, 848 organisms, and 6,624 organisms respectively. Several phyletic groups were well represented in the samples, including mollusks (gastropods: several families; sphaerid bivalves), annelids (oligochaetes), acari, crustacea (calanoids, cyclopoids, ostracods, cladocerans, amphipods), and insects (ephemeroptera, odonata, hemiptera, trichoptera, coleopteran, diptera).

## 4. Discussion

The major objective of this study was to better define salinity concentration – biotic response relationships for important boreal peatland flora and fauna. No discernible relationship could be found between salt levels in peatland soils or water and the abundance, diversity or composition of soil mesofauna (Figure 10). This is probably attributable to the scale at which mesofaunal communities are structured within peatland ecosystems. In particular, it is expected that mesofauna would occupy microenvironments especially within the peaty soils and in the active bryophyte growing zone. Chemical characterization within the study plots would not capture this micro-environmental variation. The results do suggest, however, that mesofaunal communities within boreal peatlands at depths below the fibric horizon are relatively depauperate.

The responses of mesofaunal assemblages to environmental stressors are likely to be complex, revealing either relative species sensitivities or synoptic community level changes only for those studies that incorporate a high degree of replication and controls on other sources of variability in abundance or presence-absence. Khalil *et al.* (2009) studied oribatid mite responses in soil cores across gradients of metal pollution. Across nine sites in the Netherlands, Belgium, France and Germany, there was no evidence of an effect of metal levels in the soil on species richness, densities, or the dominance structure of the mite community. The densities of one oribatid mites species was observed to be negatively correlated with litter cadmium concentration while densities of a second species was observed to be strongly positively correlated with cadmium. One conclusion from the study is that possible effects of soil metal contamination across sites may have been obscured by site differences in vegetation and humus types, along with broad geographic trends.

Limited sampling was completed in 2009 of aquatic biota within pools of standing water at produced water release sites. The data are considered very preliminary. The sampling techniques and sites selected for sampling do not adequately address potential confounding influences on salinity – biotic response relationships or between-sample variability over relatively small scales. The benthic and pelagic communities in these types of habitats are generally poorly understood by the scientific community even in the absence of anthropogenic stressors. The results nonetheless illustrate that pelagic, epibenthic and benthic invertebrate assemblages in boreal wetland pools can exhibit a relatively high degree of diversity, and - in the case of the sites sampled in 2009 - include taxa that are commonly observed in other types of aquatic habitats. An adequate description of salt concentration – biotic responses for aquatic invertebrates inhabiting standing water habitats in boreal peatlands would require a more focused study. An outstanding question is the extent to which aquatic invertebrate assemblages in western Canadian boreal peatlands vary across peatland types (rich fens, poor fens, bogs) with or without the additional influence of salinization.

### 4.1 Influence of salinization on plant/bryophyte species richness

The primary focus of this investigation was on the elucidation of salt concentration – response relationships for peatland bryophytes and vascular plants. The ability of a site to support dominant species of sphagnidae (for example, *S. augustifolium*, *S. magellanicum*, *S. jensenii* and *S. majus*; Vitt and Chee, 1990) is fundamentally important to the development and basic structure of peatlands. Furthermore, it is likely that the vegetative status of peatlands substantially facilitate the presence and functioning of other ecosystem components such as mesofauna, amphibians, reptiles, birds and mammals.

There was a significant negative relationship between the diversity (richness) of vegetation, including both bryophytes and vascular plants, and the degree of soil salinization at four of the nine sites (Figure 11). The relationship was not significant at the other five sites, largely because of the small number of plots established (e.g. for the 2009 sites) and/or the limited range of soil salinity encountered (e.g. for site 2-12, sampled in 2008). Nonetheless, there was a significant negative relationship between vegetation taxon richness and soil salinity (measured as EC) for the data from all sites combined (Figure 12).

The relationship between soil salinization and vegetation taxon richness was similar for most sites (Figure 11) with the exception of Site 14-36. This is a large site comprising a mosaic of bog, fen and especially marshland habitat. Marshes typically exhibit greater plant species diversity than peatlands. The position of the best-fit line in Figure 11 above the generalized linear regression line for all data is consistent with an expectation of greater diversity under

low salinity conditions. The slope of the linear regression for the Site 14-36 data was similar to the other sites, unlike the y-intercept (expected taxon richness in the absence of salt contamination). This finding suggests that a subset of ten or more salt-tolerant species may persist even at relatively high soil salt concentrations ( $EC > 10$  mS/cm).

## 4.2 Multivariate community responses

The degree of co-occurrence of different bryophyte and vascular plant taxa might reflect similar habitat conditions, as well as degree of tolerance to substrate salinity. The 127 quadrats sampled at the nine sites reflects a range of wetland ecosystem types even in the absence of salinity influences, and the multivariate community composition (based on abundance or percent cover estimates: Figure 13) above all reflected affinity of specific taxa for different wetland or upland conditions; i.e., preference for conditions along a gradient of wetland conditions from bogs to fens to open marsh, and from pallustrine conditions with hygric soils to more upland conditions within boreal plain ecosystems. The plant and bryophyte species associated highlighted by the PCA exhibited only limited similarities to fen communities characterized by Vitt and Chee (1990) for 23 sites in central to northern Alberta. Taxa that plot to the right of the principal components plot in Figure 13 have similarities to poor fen hummock and carpet or pool species identified by Vitt and Chee, including *Sphagnum angustifolium*, *S. magellanicum*, *S. fuscum*, *Betula glandulosa*, *Ledum groenlandicum*, *Picea mariana*, *Rubus chamaemorus*, and *Vaccinium vitis-idaea*. Conversely, taxa that plot to the left of the plot are more similar to Vitt and Chee's moderate-rich fen species (e.g., *Aulacomnium palustre*, *Potentilla palustris*). Any greater similarity in composition would not be expected given differences in study objectives and methodology between the Vitt and Chee study and this study. In particular, Vitt and Chee partially restricted their observations to fens as opposed to other boreal plain wetland types by selecting 14 of the 23 sites using the following criteria:  $5.5 < \text{pH} < 7.0$ ; were not *Sphagnum* dominated; *Scorpidium scorpioides* and *Drepanocladus revolvens* absent.

The PCA did not reveal any appreciable co-variation between any aspect of vegetative community structure and degree of salinization. In particular, there was no correlation between factor loadings on any of the first six principal components and any chemical measure of the degree of salinization. This was contrary to our expectations that vegetation community composition would reflect elimination of salt intolerant species in favour of more tolerant taxa. It is possible that such shifts would dominate the observed community structure for older historical release sites as opposed to the recent release sites that were the focus of this study.

Trites and Bayley (2009) observed a correlation between wetland (primarily marsh) community composition and electrical conductivity over a range of 0.4 to 27 mS/cm, pH and water depth. In particular, the first principal component in their PCA model, accounting for 29% of the total variance in composition of non-rare vegetation, was significantly correlated with EC. More salt tolerant wetland plants included *Schoenoplectus tabernaemontani*, *Triglochin maritima*, *Scholochloa festucaceae*, and *S. maritimus*. These are broadly recognized by various researchers (Adams, 1977; Howatt, 2000) as being obligate to facultative halophytes. The sites examined by Trites and Bayley were generally more similar to prairie marshes than boreal peatlands, and therefore the indicator species and communities identified are very different than those described herein. Of note is that Trites and Bayley described indicator sedges and grasses which clearly reflected the dominance of salt tolerant taxa in northern Alberta marshes that are naturally saline. This perhaps underscores the importance of long-term, multi-generational exposure conditions for observations of community shifts towards salt tolerant vegetation.

## 4.3 Species sensitivity distributions for boreal peatland salinization

An estimate of the range of soil salinities that peatland bryophytes or vascular plants can tolerate is provided by observations of presence in specific plots. In particular, the measured salinity concentration in plots where a plant or bryophyte was observed is within the overall range of tolerance for that species. Provided that enough observations are made across a range of salinities and natural site conditions, the documented salinity levels provide an indication of the relative sensitivity of a species to peatland salinization.

Figures 14 (woody and smaller vascular plants) and 15 (bryophytes, sedges, grasses) are plots of the range of soil salinities associated with the presence of specific taxa. Taxa observed in fewer than eight plots of the 127 total (6.3% or more) were not evaluated further, since the absence of a relatively rare species within any measured

salinity range is probably a reflection of autecological characteristics or habitat factors other than the degree of salt contamination. As illustrated in Figure 6, only 23 taxa were observed in 10% or more (>14) of the 127 plots.

The soil salinity concentration in Figures 14 and 15 are plotted as EC using a linear scale. These plots appear to have limited data in the salinity range greater than 4 mS/cm. When the taxon presence data, however, are plotted on a log<sub>10</sub> EC scale, it can be seen that the number of observed occurrences across soil salinity sub-ranges approximates a normal distribution. This point is illustrated for the Labrador tea (*Ledum groenlandicum*) presence data (Figure 16).

Figure 17 provides several examples of idealized single species salt sensitivity ranges based on presence– absence data. The probability of encountering a species in a soil plot at a produced water release site will decrease as the concentration approaches the upper tolerance limit for that species, particularly if it is an otherwise common species that is well suited to the habitat. An upper limit of the salinity range in which a species was encountered, therefore, (e.g. the 90<sup>th</sup> or 95<sup>th</sup> percentile concentration) provides a good indication about salinity levels that would exclude the species from the area of interest.

The use of field data for development of species sensitivity distributions (SSDs) and in turn for the development of environmental quality criteria has gained increased acceptance over the last decade (Cormier *et al.*, 2009), particularly in cases that do not lend themselves to conventional toxicity testing approaches. The field data for Alberta peatland environments provide evidence of salinity ranges at which various plants may persist, at least for shorter time periods of approximately a half decade or less. Assuming that the data are adequately reflective of upper end of the sensitivity range for individual species, it is reasonable to conclude that extended exposures in peatland environments to salinities in excess of the observed upper range for plots where a species is observed would result in localized extirpation of that species. The upper limit of the observed salinity range for non-rare species, therefore, can be considered as an extirpation threshold. Table 7 presents extirpation threshold data for 31 different species based on a least squares linear regression estimate of the 50<sup>th</sup>, 75<sup>th</sup>, 90<sup>th</sup> and 95<sup>th</sup> %ile EC concentration in plots similar to those illustrated in Figure 17. Given the number of field observations made (i.e., the number of plots 'n' in which a taxon was encountered), the estimated 90<sup>th</sup> %ile of the idealized range was generally lower than the maximum EC concentration at which a species was encountered, but the 95<sup>th</sup> %ile estimate was higher in some cases (e.g. for *Epilobium angustifolium*).

The collated data in Table 7 were subsequently used to develop multi-species extirpation SSDs for relevant to the larger suite of non-rare plant and bryophyte taxa in boreal peatlands. Figure 18 illustrates the re-constructed multi-species SSDs for wetland species salinity responses, based on use of either a 90<sup>th</sup> %ile or 95<sup>th</sup> %ile concentration as the estimate of a single-species extirpation threshold. Based on a 90<sup>th</sup> %ile concentration estimate, a peatland soil concentration of approximately 5 mS/cm would be predicted to result in loss of approximately 50% of all non-rare vegetation species, while a 95<sup>th</sup> %ile concentration estimate would lead to a loss of approximately 50% of all non-rare species at a peatland soil concentration of approximately 8 mS/cm.

The specific value of a salinization extirpation threshold for each taxon depends on various types of uncertainty, including whether a 90<sup>th</sup> %ile or 95<sup>th</sup> %ile concentration estimate is assumed as an extirpation threshold, and the amount of available field-based or other co-occurring soil chemistry and taxon data. The absolute values, therefore, of environmental management thresholds based on multi-species extirpation SSDs would probably be the subject of some scientific debate. On the other hand, the re-constructed multi-species extirpation SSDs provide a relatively unambiguous picture of the relative sensitivities of individual taxa. This is illustrated in Figure 19. Among the commonly occurring peatland sphagnidae, *S. magellanicum* was observed to be relatively salt tolerant, while *S. girgenшонii* is relatively salt intolerant. *S. fuscum* (Table 7) appears to be even less salt tolerant than *S. magellanicum*. One of the most visually obvious evidence of produced water impacts in treed fens and bogs is the die off of black spruce (*Picea mariana*). Our results further suggest that *P. mariana* is poorly tolerant to salinization. Such information is useful for assessing site reclamation potential following a produced water release. At historical release sites, it can be expected that the majority of other peatland vegetation can potentially recolonize the disturbance site once *P. mariana* seedlings start to appear.

Based on the values presented in Table 7, commonly occurring peatland plants and bryophytes can be categorized according to their sensitivity to salinization, as follows:

Salt-tolerant (extirpation thresholds  $\geq 8$  mS/cm):

*Epilobium angustifolium*  
*Sphagnum magellanicum*  
*Carex tenuiflora*  
*Carex aquatilis*  
*Galium trifidum*

Moderately salt-tolerant (extirpation thresholds between 4 and 8 mS/cm):

*Chamaedaphne calyculata*  
*Calliergon* sp. Unident.  
*Sphagnum angustifolium*  
*Vaccinium vitis-idaea*  
*Aulacomnium palustre*  
*Carex utriculata*  
 Arrowleaf sp. Unident.  
*Clintonia uniflora*  
*Polytrichum strictum*  
*Andromeda polifolia*  
*Smilacina trifolia*  
*Scutellaria galericulata*  
*Rubus chamaemorus*  
*Ledum groenlandicum*

Salt intolerant (extirpation thresholds  $\leq 4$  mS/cm):

*Betula glandulosa*  
*Pleurozium schreberi*  
*Comarum palustre*  
*Salix maccalliana*  
*Fragaria virginiana*  
*Sphagnum girgensohnii*  
*Oxycoccus oxycoccus*  
*Picea mariana*  
*Sphagnum fuscum*  
*Agrostis scabra*  
*Tomentypnum nitens*

The concentration – response data can also be quantified from field studies using the abundance data (as opposed to presence-absence data, as discussed above). This approach for developing environmental quality guidelines is presented by Leung *et al.* (2005). These researchers used co-occurrence data for contaminants in marine sediments and the abundance of benthic macroinvertebrates to develop an EC50 estimate for each of a large number of species. The effects endpoints for individual species were then combined into an overall species sensitivity distribution. The use of soil concentration – abundance data from the nine wetland produced water release sites was examined. For the majority of vegetation species observed, the data did not produce a linear or curvilinear fit for the bivariate relationship. Examples of abundance data from the plots are provided in Figure 20, for *P. mariana*, *C. utriculata*, and *R. chamaemorus*.

#### 4.4 Major conclusions

Overall, field observations of the vegetative status and soil/water chemistry in plots established along gradients of produced water contamination have provided a means of defining salinity tolerance range for several boreal peatland species. The data provide a clear indication of the salinity tolerance ranges of 31 non-rare vascular plant

and bryophytes species, including four commonly occurring species of sphagnum moss, rough bentgrass, black spruce, dwarf birch, Labrador tea, and dwarf bog cranberry. Some plants such as fireweed, Labrador tea, dwarf birch or aquatic sedge were observed to persist at soil salinities in excess of 6 - 8 mS/cm, while some species such as *Sphagnum girgenshoni* or common strawberry were not observed at soil salinities in excess of 3 – 4 mS/cm.

The intent at each site was to enumerate the biological status of plots that spanned the range of contamination from background reference conditions to levels of salinization expected to be associated with profound effects on bryophytes, vascular plants, and soil invertebrates (provisionally thought to be at soil EC levels of 10 mS/cm and above). At all nine field sites except A-82-G, prior attempts have been made to recover the released produced water, and to limit the magnitude as well as spatial extent of contamination within the peatland. This has generally been accomplished at these sites by direct recovery of salinized surface and shallow groundwater by pumping from one or more “bell holes” and disposal offsite. At five sites, additional trenching was completed in an attempt to limit the outward flow of salinized water to adjacent portions of the wetland. In addition, physical excavation, removal and landfilling of highly salinized soil within the immediate vicinity of the point of release was carried out shortly after the release was discovered. Anecdotal observations during the field visits, along with discussions with Alberta Environment managers, strongly suggest that some of the wetland produced water spill response activities that were attempted over the last decade and more may be counter-productive. This is not discussed herein, but a more detailed analysis and guidance on optimized remedial strategies is currently in preparation.

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