Acute and chronic effects of oil sands process water components on the mayfly

*Hexagenia* and field-collected aquatic macroinvertebrate communities

by

Julia Howland

Bachelor of Science, University of New Brunswick, 2014

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

**Master of Science**

in the Graduate Academic Unit of Biology

Supervisor: Alexa Alexander-Trusiak, Ph.D., Environment and Climate Change Canada
Joseph Culp, Ph.D., Wilfrid Laurier University and Environment and Climate Change Canada

Examining Board: Les Cwynar, Ph.D., Department of Biology
Amy Parachnowitsch, Department of Biology
Michelle Gray, Ph.D., Faculty of Forestry and Environmental Management

This thesis is accepted by the
Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK

August, 2019

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ABSTRACT

Tailings ponds in northeastern Alberta, Canada contain over one trillion litres of oil sands process water (OSPW) that cannot currently be released due to toxicity of some components. Limited space and the need for reclamation of oil sands operation sites necessitates release of OSPW in the near future. Knowledge of the composition and toxicity of OSPW is often lacking yet is crucial for both risk assessment and management planning. This thesis examines the acute and chronic toxicity of environmentally relevant mixtures of two process water components, naphthenic acid and sodium naphthenate, with and without the added stress of polycyclic aromatic hydrocarbon spiked sediment. We assess the effects of these simplified oil sands process water (OSPW) mixtures under planned and un-planned tailings release scenarios using traditional and novel bioindicators for aquatic invertebrate taxa. The results of this study demonstrate the significant negative effects of OSPW contaminants on aquatic communities.
DEDICATION

For those making a difference.

“One individual cannot possibly make a difference, alone. It is individual efforts, collectively, that makes a noticeable difference—all the difference in the world!”

— Dr. Jane Goodall
ACKNOWLEDGEMENTS

I would like to express my gratitude to Drs. A. C. Alexander, J. M. Culp and D. J. Baird for supervising my thesis research and Drs. M. A. Gray and A. L. Parachnowitsch for participating in my thesis examination. I would like to thank everyone in the Environment and Climate Change Canada laboratory in Fredericton who offered indispensable guidance and assistance and made my time there an absolute pleasure.

This work could not have been completed without the expertise of Kerry Peru, Danielle Milani and Donna Giberson. I am so grateful to have such kind and loving family and friends that have encouraged and supported me throughout this process. Thank you.
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List of Symbols, Nomenclature or Abbreviations

ANOVA: analysis of variance

NA: naphthenic acid

NA mixture: commercial 10:1 naphthenic acid and sodium naphthenate mixture

OSPW: oil sands process water

PAH: polycyclic aromatic hydrocarbon

PCA: principal component analysis
1. INTRODUCTION

1.1 Background

Canada’s oil sands region is located in northeastern Alberta, extending into Saskatchewan, and is one of the largest crude oil deposits in the world (CAPP, 2017; Figure 1.1). Oil sands are a semi-solid mixture of bitumen, sand, clay, and water which is mined, crushed and transported to a processing plant for bitumen extraction (Schramm, 2000). A key challenge for the oil sands industry is the large quantity of tailings produced during the hot-water extraction process. This process requires the use of substantial amounts of freshwater, sourced mostly from the Athabasca River for the Athabasca oil deposit (Figure 1.1). The tailings mixture is then pumped to on-site tailings ponds, where the solid components (e.g., clay, sand, residual bitumen) are expected to settle and consolidate (Natural Resources Canada, 2013a), ultimately facilitating land reclamation. The aqueous portion of tailings, referred to as oil sands process-affected water (OSPW), can be reused in the extraction process. However, after each use the water accumulates dissolved chemicals. This increasing chemical burden within the process water can reduce extraction efficiency and cause fouling of processing equipment. At present OSPW and tailings cannot be released back to the environment without further treatment (Natural Resources Canada, 2013b). More than one trillion litres of OSPW is estimated to be contained in tailings ponds across Canada’s oil sands region (McNeill and Lothian, 2017).

The proximity of large tailings ponds adjacent to the Athabasca River is an ongoing cause for concern, as components of OSPW are known to have both lethal and
sublethal toxic effects on aquatic species (e.g. Scarlett et al., 2013; Brown and Ulrich, 2015; Bartlett et al., 2017; Gagné et al., 2017; McQueen et al., 2017). OSPW has the potential to enter the Athabasca River in the event of a tailings-pond rupture, or through a planned release of treated industrial waters to the environment (Barton, 1970; Dibike et al., 2018; Tanna et al., 2019). Planned release of OSPW will likely be necessary in the future due to increasingly limited tailings storage space, unsustainable water management practices, and the ongoing need for reclamation of mining areas (Allen, 2008; Van den Huevel, 2015; Tanna et al., 2019). Unfortunately, there is much uncertainty concerning the ecological effects of OSPW on aquatic environments due to the complexity of the tailings mixtures, proprietary nature of many of the substances used in the extraction process and lack of guidelines for many of these chemical compounds. Knowledge of the composition and toxicity of OSPW is crucial for risk assessment and management planning.

Naphthenic acids (NA) are a main component of OSPW and have been long associated with toxicity (Mackinnon and Boerger, 1986). However, it has not been conclusively demonstrated that NAs are responsible for observed impacts of OSPW mixtures (Van den Heuvel, 2015). NAs are natural constituents of bitumen; they also often comprise the organic components of surfactants used in the extraction of oil from bitumen. This process leads to the solubilization and concentration of these carboxylic acids in OSPW (Headley and McMartin, 2004). NAs are toxic to aquatic species and are of concern due to their solubility and widespread persistence in waterways (Mackinnon and Boerger, 1986; Frank et al., 2014). NAs readily ionize during oil sands processing to form metallic salts, most commonly, sodium naphthenate (Howland et al., 2019).
There is little toxicological information on the toxicity of sodium naphthenate, despite its high production volume and potential for pollution.

A recent workshop – which included representation from First Nations, industry, academia, and governments – aimed to review existing science and identify necessary steps to inform the safe return of OSPW to the environment, while protecting the ecosystem and human health. Chemistry and characterization of OSPW, toxicity of OSPW, environmental effects monitoring, and characterization of the receiving environment were identified as major informational gaps (Tanna et al., 2019). The following work addresses toxicological characterization and acute and chronic toxicity of OSPW mixtures. My objectives were to establish environmental thresholds for toxic components of OSPW and to examine the possible outcomes of release of these mixtures. This research will provide information to stakeholders in the oil sands industry regarding the potential consequences of OSPW release and management.

1.2 Potential risk

Assessing the toxicity of two key compounds present in OSPW – naphthenic acid and sodium naphthenate – is important due to the potential risks to the environment such as loss of biodiversity and abundance of both aquatic and terrestrial species (see review: Mahaffey and Dubé, 2017; Tanna et al., 2019) and human health (see review: Kindzierski et al., 2012). An environmentally-relevant naphthenic acid mixture was tested. This thesis focuses on examining the potential for safe levels of dilution and release of OSPW in the future. Observed concentrations of NA in Alberta’s tailings ponds range from 50 to 128 mg/L, with previously proposed release targets of 7 to 35
mg/L (based on hypothetical treatment objectives for environmental discharge of oil sands process water [Allen, 2008]). The concentrations of NA used to evaluate chronic toxicity are much lower than what is observed in Alberta’s tailings ponds, while also covering a concentration range above and below the previously proposed targets. Even with decades of research, the treatment options currently available have not yet been successful in reducing toxicity of OSPW to a level safe for aquatic communities. Here I show that the release of OSPW at the proposed target concentrations has the potential to cause extreme loss of aquatic invertebrate abundance and richness. Additional losses due to factors outside the scope of my research may also occur due to unmanaged synergies with other stressors (e.g., climate change, drought, habitat degradation). These types of effects could result in extirpation of some taxa, further decoupling aquatic food webs and disrupting the function of downstream aquatic communities (reviewed in Brook et al., 2008).

In addition to the imminent prospect of a planned OSPW release, there is also potential for an unplanned release if a tailings pond rupture or breach was to occur. A recent study by Dibike et al., (2018) considers potential effects of tailings pond breach on the water and sediment quality of the Lower Athabasca River and hypothesizes how sediment and chemicals deposited during a breach could have long-term effects on the water quality and aquatic ecosystem of the river and the downstream delta. The estimated concentration of NAs in a tailings pond breach outflow would be almost 40 mg/L, which could have a devastating impact on the aquatic communities in the region. Moreover, combine these toxicity effects with other factors associated with an unplanned release, including increased flow, higher sediment load, and additional
toxicity caused by other oil sands components, then the environmental damage caused by a tailings breach could indeed be catastrophic.

The potential risks of OSPW release are assessed in the following two chapters and are grouped by acute and chronic exposures. Acute toxicity of NA was examined by exposing nymphs of the mayfly *Hexagenia* spp. to a concentration gradient in (48- and 72-h) laboratory tests, as well as exposing a field-collected macroinvertebrate community in an artificial stream setting, both with lethal endpoints. Chronic toxicity of NA was examined in a laboratory test with multiple stressors and endpoints. *Hexagenia* spp. nymphs were exposed to a concentration gradient of the NA mixture, with and without the added stress of contaminated sediments over a 21-d period.

### 1.3 Test Species

Relatively few studies have been published on the toxicity of OSPW to invertebrates in comparison to studies examining effects on fish (Mahaffey and Dubé, 2017). Invertebrates are appropriate for toxicity testing because they occupy key positions in aquatic ecosystems and have been shown to vary in sensitivity to chemical stressors. Invertebrates typically have shorter lifespans than fish and can be useful early-indicators of ecosystem deterioration (Lagadic and Caquet, 1998). Aquatic invertebrates are also ecologically important as they often provide the energy link between primary producers and secondary consumers in the food web and contribute to critical ecosystem services such as nutrient cycling (Covich et al., 1999). In aquatic ecosystems, sediment can accumulate contaminants; consequently, benthic invertebrates are often the first organisms to experience toxic effects (Baun et al., 2008). In particular, burrowing
invertebrates such as *Hexagenia* spp. (Ephemeroptera: Ephemeridae) can be at high risk for exposure to contaminants in both the sediment and water, which has the potential to produce additively toxic effects.

Appropriate species for toxicity tests must be chosen based on their sensitivity, relevance to the potentially affected community and availability/ease of maintenance and culture (USEPA, 1994). *Hexagenia* has been identified as a useful species for toxicity testing because it is ubiquitous, sensitive, and easy to culture in the laboratory (Harwood et al., 2014; Watson-Leung et al., 2015). *Hexagenia* are also present in the Athabasca River, the proposed receiving environment for release of OSPW (Day and Reynoldson, 1995). *Hexagenia* spp. spend most of their life cycle in the nymphal stage, molting up to 30 times over a period of one to four years before they emerge from the water as subimagos. Nymphs are detritivores; they dig burrows in soft sediment and rely on gill movement to filter water and transport food particles. *Hexagenia* eggs can be cooled to induce diapause, making them easy to store until needed for use in laboratory cultures (Giberson and Rosenberg, 1994). There is a growing body of work that demonstrates the effectiveness of *Hexagenia* in toxicity testing (Ort et al., 1995; Bargar & Fisher 1997; Milani et al., 2003; Bartlett et al., 2018; Cadmus et al., 2018).

In order to understand the broader ecological impacts of a substance, it is important to also examine effects on invertebrate assemblages containing genera with different functional traits (Thorp, 2015). The acute effects of NA on *Hexagenia* and on an invertebrate assemblage are examined in Chapter 2. Future work will aim to assess chronic effects of NA on assemblages as well, to build upon the results of the single-species chronic exposure discussed in Chapter 3.
1.4 Aims and objectives

This thesis is structured in article format. Chapter 1 contains a general introduction to the thesis. Chapters 2 and 3 describe the detailed aims, objectives and results of the research. Chapter 4 contains the general discussion, conclusions and recommendations.

Chapter 2 has been previously published as:


My contribution to this research included designing the research proposal, carrying out the experiments, analyzing the data and preparing the manuscript, except for the historical data and mesocosm experiment.

Chapter 3 has been previously published as:


My contribution to this research included designing the research proposal, carrying out the experiments, analyzing the data and preparing the manuscript.

1.4.1 Acute toxicity of NA

The objective of Chapter 2 was to characterize and examine the impact of environmentally-relevant concentrations of naphthenate mixtures on the survival of Hexagenia nymphs as well as on the composition of benthic macroinvertebrate communities using artificial stream mesocosms. These experiments included 10:1 mixtures of naphthenic acids and sodium naphthenate that are similar to those present in
OSPW (Howland et al., 2019). Ecological effects were measured using a holistic approach combining both traditional toxicological methods (median lethal concentration [LC$_{50}$] using *Hexagenia*) with stream mesocosm studies.

### 1.4.2 Chronic toxicity of NA

The chronic effects of OSPW contaminants, including combined aqueous and sediment exposures, on a relevant aquatic species (mayfly *Hexagenia* spp.) are explored in Chapter 3. This 21-d experiment and analysis investigates the combined effects of mixtures of contaminants commonly found in the oil sands region (NA and Polycyclic Aromatic Hydrocarbons (PAHs)) for the mayfly *Hexagenia* spp. Treatment levels of NA were tested on mayflies either with or without the added stress of a PAH-spiked sediment. Endpoints examined included a combination of toxicological (e.g., lethal) and functional (e.g., growth) responses.

### 1.5 Overall significance

There is uncertainty of the toxicity of OSPW mixtures and a lack of guidelines for many OSPW contaminants. This work addresses this knowledge gap concerning the ecological effects of OSPW contaminants on aquatic invertebrate assemblages. A combined approach was used to elucidate the acute and chronic effects of NA on survival of mayflies and other aquatic invertebrates, as well as sublethal effects of NA and PAH-spiked sediment on *Hexagenia* spp. Discerning the possible ecological effects of OSPW release on aquatic communities is crucial for the ecological risk assessment and management planning of OSPW containment and release (Tanna et al., 2019).
1.6 References


1.7 Figures

**Figure 1.1.** Map of Alberta, Canada depicting boreal forest, oil sands surface minable area, oil sands minable area and the Peace River, Athabasca and Cold Lake oil sands (Government of Alberta, 2017).
2. Effects of oil sands process water mixtures on the mayfly Hexagenia and field-collected aquatic macroinvertebrate communities

2.1 Abstract

Extraction of Canada’s oil sands has created over one trillion litres of tailings, which are stored in on-site tailings ponds. Due to limited storage capacity, the planned release of tailings to the surrounding environment may be required. This represents an environmental management challenge, as the tailings contain contaminants that are known toxins to aquatic communities. Of particular concern are naphthenic acids and their metallic counterparts, as they are correlated with toxicity of tailings, are relatively soluble, and persistent in aquatic environments. This study examines the acute toxicity of environmentally relevant 10:1 mixtures of two process water components, naphthenic acid and sodium naphthenate. We assess the effects of these simplified oil sands process water (OSPW) mixtures under planned and unplanned tailings release scenarios using traditional and cutting-edge bioindicators for aquatic invertebrate taxa. We found that safe concentrations to mayflies and other aquatic macroinvertebrates may be less than 1 mg/L, as no mayfly taxa survived exposure to this dose in either the 48-h or 72-h acute toxicity test. In the 72-h test, no mayflies survived treatment levels greater than 0.5 mg/L. In the pilot mesocosm study even a 90% dilution of the OSPW contaminant mixture was not sufficient to protect sensitive macroinvertebrate communities. The results of this study provide information to help develop a management plan for oil sands tailings ponds, which will provide insight into the potential for process water
release into the surrounding environment while conserving unique ecosystems downstream of development in the oil sands region.

2.2 Introduction

Canada’s zero-discharge policy has led to the accumulation of over one trillion litres of oil sands process-affected water (OSPW) and solid tailings in northeastern Alberta (McNeill and Lothian, 2017). OSPW, which is wastewater formed during bitumen mining or upgrading, contains a mixture of residual bitumen (a semi-solid form of petroleum), as well as dissolved salts (e.g. sodium), trace metals (e.g. aluminum) and organic compounds (e.g. naphthenic acids). These components vary in concentration between tailings ponds due to differences in mining practices and the composition of the ore body (Mahaffey and Dubé, 2017). Naphthenic acids are considered the principal toxic components of OSPW and range in concentration from 50-128 mg/L (Mackinnon and Boerger, 1986; Allen, 2008; Frank et al., 2014; McQueen et al., 2017).

OSPW is often recycled during processing and thus tends to have highly ionized naphthenic acids due to the accumulation of cations and fine solids at the petroleum-water interface (Schramm et al., 2000). Naphthenic acids are also ionized in OSPW due to hot water or sodium hydroxide (NaOH) addition as part of the extraction of bitumen (Schramm et al., 2000). Collectively, these processes form mixtures of highly mobile, anionic carboxylates that are natural surfactants that can be highly corrosive to mine processing equipment. As such, some of the components of common surfactants (Na+) ions have been carefully monitored by industry for decades. Some early estimates suggest that $69 \pm 27\%$ of the naphthenic acids in OSPW could be present as mixtures
containing metallic naphthenates (predominantly sodium naphthenate), depending on the grade of bitumen and the processing technique (e.g., FTFC 1995).

Relatively few studies have been published on the toxicity of OSPW to invertebrates (e.g., in comparison to fish, Mahaffey and Dubé, 2017). Even less is known about mixtures containing both naphthenic acids and organo-metallic salts such as sodium naphthenate (e.g., C_{10}H_{17}NaO_2, 192.234 g/mol, CAS# 61790-13-4). Other metallic (e.g., Al, Mg, Ca, Ba, Co, Cu, Pb, Mn, Ni, V, and Zn) naphthenates are used extensively as highly effective pesticides, wood preservatives, thickening agents, and synthetic detergents (USEPA Ecotox database, https://cfpub.epa.gov/ecotox/). Further, studies such as this one, which combine standard toxicity testing approaches and outdoor field experiments, are particularly relevant, as laboratory studies tend to underestimate the ecological effects of chemicals (Culp et al., 2017). Lack of information on the toxicity of these mixtures to invertebrates is particularly troublesome, because, in the event of the release of OSPW, invertebrates would likely be among the species most affected: These organisms are widely distributed and highly sensitive to the increased salinity associated with metallic naphthenates (Lagadic and Caquet, 1998; Scheibener et al., 2016; Nowghani et al., 2019). Aquatic invertebrates, such as mayflies, are also ecologically important, providing a vital link between primary producers (algae) and secondary consumers (fish) in the aquatic food web. Additionally, they perform ecosystem services such as nutrient cycling (Covich et al., 1999). This study focuses on the cosmopolitan mayfly *Hexagenia* spp. (Ephemeroptera: Ephemeridae) that has been previously identified as a key species for toxicity testing, because it is ubiquitous,
sensitive, and easy to culture in the laboratory (Harwood et al., 2014; Watson-Leung et al., 2015).

The objective of the present study was to characterize and examine the impact of environmentally relevant concentrations of commercial naphthenate mixtures (10:1 of naphthenic acids and sodium naphthenate) similar to those present in OSPW on the survival of Hexagenia nymphs and on the composition of benthic macroinvertebrate communities in artificial stream mesocosms. Effects were measured using a two-tiered approach, combining traditional toxicological methods with outdoor mesocosm studies. The lethality tests focused on the impact of low concentrations (< 1 mg/L) over time periods associated with the short-term planned release of OSPW mixture components, whereas the mesocosm studies will allow observation of ecological changes within a simplified experimental community due to planned or unplanned release scenarios. This work addresses a key knowledge gap concerning the ecological effects of OSPW contaminants on aquatic communities, which exists due to the uncertainty of the toxicity of OSPW mixtures and lack of guidelines for many of the contaminants. Bridging this knowledge gap is crucial for the ecological risk assessment and management planning of OSPW containment and release. Further, the following is, to our knowledge, the first such effort to synthesize historical data and test for the toxic effects of OSPW mixtures on benthic macroinvertebrate communities.
2.3 Materials & methods

2.3.1 Study species

*Hexagenia* spp. spend most of their life cycle in the nymphal stage, molting up to 30 times over a period of one to four years before emerging from the water as subimagos. *Hexagenia* nymphs are detritivores, digging burrows in soft sediment and relying on gill movement to filter water and transport food particles (Giberson and Rosenberg, 1994). *Hexagenia* were chosen for this study because in aquatic ecosystems sediments can accumulate contaminants, and consequently, sensitive invertebrates such as burrowing mayflies are at high risk for exposure to contaminants in both sediment and water, which has the potential to produce additive toxic effects (Baun et al., 2008).

Culturing protocols were adapted from well-established methods of Fremling (1980), Friesen (1981) and Giberson and Rosenberg (1992a). Fertilized *Hexagenia* eggs were collected in June 2017 from Lake St. Clair in Windsor, Ontario using the method of Hanes and Ciborowski (1992). Hatched nymphs were transferred to 6-L glass culture tanks held in the growth chambers at 22 ± 3°C with a 16:8-h light:dark photoperiod. Each tank contained autoclaved sediment collected from Magaguadavic Lake, New Brunswick, to a depth of approximately 5 cm. Tanks were filled with de-chlorinated water and constant aeration was provided. Mayfly densities were 5 to 6 nymphs per cm² of the bottom surface area of each tank. Each tank received 0.1 g/L of ground NutraFin Bug Bites fish food (Rolf C. Hagen Inc., Baie d’Urfé, QC) twice per week and was topped up with de-chlorinated water as needed. Every 4-6 weeks, a 50% water
exchange (pH, 8.1 ± 0.1; conductivity, 261 ± 5 μS/cm) from each tank was replaced with aerated de-chlorinated water (model 20-36 dechlorinator; Culligan, NB, Canada).

In contrast, the mesocosms were inoculated with a field-collected community of periphyton and benthic macroinvertebrates from the upper reaches of Corbett Brook (45°55'11.8"N 66°38'46.3"W). Corbett Brook is a small stream running through wetlands and forested areas in the University of New Brunswick woodlot near Fredericton, NB, Canada. Aquatic communities collected in Corbett Brook included a range of genera at multiple trophic levels (consumers and predators) as well as both sensitive and tolerant macroinvertebrate taxa (e.g., Ephemeroptera vs. Oligochaeta; Merritt and Cummins, 1996). To create a similar aquatic assemblage in each of the four replicate streams per treatment (control, 10, 50 and 100% OSPW), we inoculated each artificial stream with a subsampled portion of the same benthic macroinvertebrate community. Substratum (coarse and fine) as well as benthic invertebrates (<5 cm in size) were also collected and subsampled (into four equal portions, one per treatment level). Each replicate subsample was placed into one of the four artificial stream treatments and was dosed with the process water mixture (see Alexander et al., 2013, 2016 for detailed descriptions of subsampling procedure). At the end of the experiment, all 16 replicate streams were dismantled, and the invertebrates were collected and identified to the lowest practical level (typically genus).

2.3.2 Chemical analyses

Mixture concentrations to simulate OSPW were prepared using a commercial 10:1 preparation of naphthenic acids and sodium naphthenate (lot # DLODE of NO397,
TCI America, Portland, OR). For the sake of simplicity, the concentration of this mixture will be referred to by the NA concentration alone, unless otherwise stated. Two approaches were used to analyze and characterize the composition of the commercial preparation. First, naphthenic acids were compared to known fluorescence signatures. We used samples from Syncrude Canada Ltd. (Pond 9) and Suncor Energy Inc. (Suncor – MFTS) tailings ponds to assess how comparable the tested substances are to reported field conditions (Figure 2.1). These chemical analyses of OSPW samples were conducted using the scanning synchronous fluorescence spectroscopy (SFS) method of Kavanaugh et al., (2009) on an Aquamate spectrophotometer with deuterium and tungsten lamp attachments (Thermo Spectronic, Rochester, NY). Second, to confirm the above SFS findings and fully characterize the naphthenic acids from the commercial preparation, samples from the same lot number of the commercially prepared stock were also analyzed at the Environment and Climate Change Canada laboratory in Saskatoon, SK by high resolution mass spectrometry (Orbitrap) with electrospray ionization (see proof of concept: Marshall and Hendrickson 2008, methodology: Headley et al., 2016, Ajaero et al., 2018).

2.3.3 Background toxicity information

Using data sets from the scientific literature, the USEPA Ecotox database (https://cfpub.epa.gov/ecotox/), and publicly available industry reports (Syncrude and Suncor, FTFC 1995), we compiled a database consisting of the toxicological and chemical measurements of naphthenic acids and three metallic naphthenates (Na, Cu and tributyl Sn; Figure 2.2) for aquatic species including bacteria, plants, invertebrates
and fish. The compiled database contained 93 toxicological observations and 39 observations of a suite of more than 25 water quality variables associated with bitumen content, naphthenic acid concentration and a suite of metals and major ions. These data were evaluated using conventional statistical approaches to determine patterns within the dataset that could improve our understanding of the toxicity of different components in the OSPW mixture.

2.3.4 Acute toxicity tests (48-h and 72-h)

Acute toxicity tests included a 72-h test (27-30 September, 2016) and a subsequent 48-h test (19-21 October, 2016). *Hexagenia* nymphs were exposed to process water mixtures in glass Pyrex Petri dishes (diameter 100 x 20 mm, Corning Inc., Fisher Scientific #08747D) under the environmental conditions described above. Ten early instar *Hexagenia* mayflies of similar total body length were exposed in each of five replicate treatments levels during each test period (72-h: 5370 ± 288 µm total length vs. 48-h: 6716 ± 86 µm total length). Both tests were conducted with a concentration gradient of a 10:1 mixture of naphthenic acid and sodium naphthenate (see Table 2.1), using standard acute-toxicity test techniques (USEPA 2000; Milani et al., 2003). Observations were taken every 24-h, at which time a static renewal of the aqueous process water mixture was completed. Nymphs that appeared immobile were stimulated using gentle aeration from a pipette. If the stimulation did not cause movement nymphs were considered to be dead and were removed from the treatment.
2.3.5 Outdoor mesocosm study

Outdoor mesocosms were deployed from 12 to 26 of September 2016 at the Environment and Climate Change Canada mesocosm facility located at Agriculture and Agri-Foods Canada, adjacent to the Saint John River, approximately 10 km southeast of Fredericton, New Brunswick, Canada (45°55’36.92”N, 66°36’55.56”W). This study was designed to simulate lotic habitats while examining benthic macroinvertebrate community responses to OSPW contaminant mixtures at 4 treatment levels (control, 10, 50 and 100% OSPW). In brief, each treatment level contained 4 replicate streams with an inoculum of the same, subsampled, benthic macroinvertebrate assemblage (see Alexander et al., 2016). The stream mesocosm method of Culp et al., (2017) was modified to a 14-d study that included a 96-h exposure followed by a 10-d recovery period. Each replicate stream was cylindrical and had a planar area of 0.065 m² and a 10-L volume. Each stream was connected to a reservoir to enable the partial renewal of treatment water and to distribute water at uniform flow rates similar to 3rd-4th order streams. The volume of treated water was controlled using positive displacements pumps (Viking pumps, Pulsefeeder 25-H duplex pump, Cedar Falls, IA, U.S.A.). Water was fed at a constant rate to each replicate stream and was completely exchanged every 7 minutes.

2.3.6 Statistical analyses

Principal component analysis (PCA) was applied to subsets of historical concentration data assembled from industry sources to evaluate correlations between naphthenic acid concentration, toxicity, and different major ions (e.g., Na⁺). Survival data from the standard toxicity tests were assessed to determine the concentration that
would be lethal to 50% of *Hexagenia* nymphs during a 48-h exposure using a traditional LC$_{50}$ probit analysis (r package: binomial family of GLM). Probit analysis is a type of regression used for binomial response variables that can be applied in toxicity testing as a means of transforming sigmoid dose-response curves to linear relationships for regression analysis (Finney, 1952). For each OSPW treatment of the mesocosm study, relative abundance and richness were calculated as the average of the observed taxa per replicate stream normalized for the average number of taxa observed in the initial community subsamples collected at Corbett Brook. One-way analysis of variance (ANOVA) tested for differences between treatment levels in the mesocosm study. Post hoc comparisons, where applicable, were conducted using Tukey’s HSD test ($\alpha = 0.05$).

2.4 Results

2.4.1 Chemistry

Naphthenic acids in the OSPW mixtures were compared to known fluorescence signatures of oil sands tailing water using the scanning synchronous fluorescence spectroscopy (SFS) method (Figure 2.1). The 10:1 commercial preparation used in this study was found to overlap the characteristic fluorescence signature of Syncrude Canada Ltd. and Suncor Energy Inc. first reported by Kavanaugh et al., (2009) (Figure 2.1b). Further analysis by high resolution mass spectrometry with negative ion electrospray ionization revealed that the naphthenic acid composition in the mixture samples were 99.8% O$_2$ species, indicating the presence of mostly classical naphthenic acids (Figure 2.1c). The range of carbon numbers (8 to 22, z=1 to z=6 O$_2$ double bonds equivalent) found in the commercial mixture samples was also typical of oil sands process water
extracts as opposed to the typical commercial preparations (Figure 2.1c). Ratios of these constituents vary by tailings pond, but in two ponds on the Syncrude property in 2017 the ratios were 9:1 for total hydrocarbons vs. C\textsubscript{10} (naphthenate) (unpub. data). Results from the laboratory yielded correlations of $r^2 \geq 0.99$ between nominal and actual values for the sodium naphthenate in the 72-h lethality tests ($y_{[\text{conc}]} = 145071x_{[\text{ABS at 390 nm}]} - 560.88$) and in the 48-h lethality tests ($y_{[\text{conc}]} = 15879x_{[\text{ABS at 390 nm}]} - 87.646$). Table 2.1 includes the actual concentrations of both components of the commercial process water mixture, namely naphthenic acid and sodium naphthenate, for each acute toxicity test. Given this high laboratory performance, concentrations are reported as the nominal concentrations throughout.

2.4.2 Background toxicity information

Similar ranges of toxic responses to naphthenic acids (2.4 to 80.5 mg/L) and sodium naphthenate (1.4 to 75 mg/L) were reported in the USEPA Ecotox database (Figure 2.2). Concentrations of naphthenic acids and sodium naphthenate that affected 50% of the reported taxa were 11.80 ± 3.45 mg/L and 19.50 ± 7.85 mg/L, respectively (Figure 2.2a). Only fish toxicity data was available for the sodium naphthenate tests. For other metallic naphthenates, such as copper (Cu\textsuperscript{2+}, 2.00 ± 0.53 mg/L) and tributyl Sn (TBT\textsuperscript{2-}, 0.01 ± 0.04 mg/L), more than 80% of the taxa tested were severely impacted at much lower concentrations ($LC_{80} < 2$ mg/L) (Figure 2.2a).

Background toxicity information was further investigated using industry-collected data (FTFC 1995). A PCA analysis compared the concentration of major ions, metals, and nutrients in tailings porewater to responses in the standard marine bacterial
test, *Vibrio fischeri* (Microtox) (Figure 2.2c). Overall, the PCA captured > 60% of the variation in the dataset with the toxicity to the marine alga, *A. fischeri*, strongly associated with the concentration of naphthenic acid as well as Na\(^+\) and Cl\(^-\) ions (Figure 2.2c). Specifically, Microtox responses were strongly negatively correlated to the concentration of both Na\(^+\) (*F* \(_{1,20} = 15.45, r^2 = 0.41, p < 0.01\)) and Cl\(^-\) (*F* \(_{1,21} = 36.38, r^2 = 0.62, p < 0.01\)) (Figure 2d). In contrast, Microtox responses showed no relationship to naphthenic acid concentration (*F* \(_{1,15} = 2.66, r^2 = 0.09, p = 0.12\)) (Figure 2.2d).

### 2.4.2 Acute toxicity tests (48-h and 72-h)

Two series of lethality tests were conducted with the standard test mayfly, *Hexagenia*, under conditions of a 10:1 naphthenic acid and sodium naphthenate mixture for either a 48-h or 72-h duration (Figure 2.3). The 72-h lethality tests indicated the median lethal concentration was 0.25 ± 0.10 SE mg sodium naphthenate/L (2.5 mg naphthenic acid/L) (Probit: \(\alpha = -1.1144 \pm 0.6223, \beta = 1.3710 \pm 1.2676, p = 0.22\)). In the 48-h tests, results were similar with a 48-h LC\(_{50}\) of 0.43 ± 0.34 mg sodium naphthenate/L (4.3 mg naphthenic acid/L) (Probit: \(\alpha = -1.288 \pm -1.045, \beta = 7.934 \pm 6.498, p = 0.28\)). In neither the 72-h nor the 48-h test series was there survival in treatments with concentrations of 1 mg/L or greater of the metallic naphthenate mixture (Figure 2.3). For the 72-h test, there was no survival in treatment levels greater than 0.5 mg sodium naphthenate/L.

### 2.4.3 Outdoor mesocosm study

Community responses to treatments compared the impact of an OSPW mixture gradient at four different treatment levels (control, 10, 50 and 100% OSPW, see Table
2.1) on a field-collected stream community in outdoor mesocosms (Figure 2.4). Each stream received 871 ± 62 SE individual macroinvertebrates from Corbett Brook. Sensitive taxa were predominant (58 ± 1%: Ephemeroptera, Plecoptera, Trichoptera). The richness of the initial stream community was 32 ± 0.6 insect families and 40 insect genera per replicate subsample. By the end of the experiment, aquatic invertebrate abundance of sensitive taxa was reduced by ≥ 69 ± 5 % compared to control abundance levels and ≥ 80 ± 6% compared to the initial Corbett Brook stream community in all of the OSPW concentrations tested (Figure 2.4a). Similar reductions were observed in the individual abundance of invertebrates in ‘pollution-tolerant’ orders, such as the Oligochaetes, Coleoptera, and Diptera, (≥ 65 ± 8% to controls, ≥ 66 ± 8% to Corbett Brook) (Figure 4a). Richness of sensitive taxa was also significantly depleted (p < 0.05, Tukey’s), particularly in the 50 and 100% OSPW treatment levels (≥ 32% and ≥ 69% respectively), whereas richness was but 13% lower (e.g., s = 16.5 ± 0.9) in the 10% OSPW treatment (Figure 2.4b). In contrast, richness of tolerant taxa only decreased (63% lower, p < 0.05) in the highest concentrations tested (100% OSPW) (Figure 4b). Only 4 ± 0.4 genera were found, on average, in the highest treatment level tested and included Optioservus sp. (Coleoptera, Elmidae, Tolerance = 4), Helicopsyche sp. (Trichoptera, Helicopsychidae, Tolerance = 3), Chironomus (Diptera, Chironomidae, Tolerance = 7), and Hydropsyche (Trichoptera, Hydropsychidae, Tolerance = 4) (Merritt and Cummins, 1996).
2.5 Discussion

Conventional acute toxicity tests and an outdoor mesocosm study were conducted to provide insight into the effects of 10:1 mixtures of naphthenic acids and sodium naphthenate. In the acute toxicity tests, survival of Hexagenia mayflies was reduced at mixture concentrations orders of magnitude lower than levels reported in the literature (e.g., 48-h LC50 4.3 ± 6.2 mg naphthenic acid/L; 128 mg naphthenic acid/L (Allen, 2008)). Similar results were found for sodium naphthenate, the other component of the OSPW mixtures tested, with Hexagenia mayflies immobilized at concentrations of 0.25 to 0.43 mg/L. Further, in the mesocosm study > 99% of the benthic assemblage was eliminated in the scenario simulating a catastrophic, unplanned tailings pond breach (e.g., 100% OSPW, or 70 to 127 mg naphthenic acid/L, Table 2.1), and more than half of the benthos were eliminated at concentrations in the 6.27 to 34.97 mg naphthenic acid/L range (Figure 2.4). These results are much lower than values reported in the published literature. The reason for this may be two-fold. First, different species have often been used for previous toxicity testing. Specifically, for sodium naphthenate, the few responses reported were all for fish (LC50 ~ 19.50 ± 7.85 mg/L) (Figure 2.2) or for diatoms (Nitzschia linearis 96-h EC50 of 43.1 mg/L), whereas, for naphthenic acid, the bulk of the published literature (LC50 ~ 11.80 ± 3.45 mg/L) and historical data in industry reports (LC50 ~ 40 ± 11 mg/L) focused on tolerant freshwater biota, or even marine, organisms (e.g., Vibrio fischeri) (Syncrude and Suncor, FTFC 1995; Leung et al., 2001; USEPA Ecotox, 2019). Second, OSPW components have previously been treated individually, and their potential joint toxicity has been ignored. Either approach could easily find that much higher concentrations of OSPW contaminants were not
associated with toxicity. As such, this study is unique in that new, sensitive phyla were
tested (mayflies, benthic macroinvertebrates) and in that a realistic, process-water
mixture, rather than individual components, were examined (10:1 mixture of naphthenic
acid and sodium naphthenate).

2.5.1 Chemistry

Chemical characterization was important to understand how environmentally-
relevant the findings of this study could be in the context of tailings management (see
also West et al., 2011; Mahaffey and Dube, 2017). Naphthenic acids are considered the
primary source of tailings toxicity (MacKinnon and Boerger, 1986); however, these
acids are also among the most poorly understood components of OSPW (see Grewer et
al., 2010; Brown and Ulrich, 2015). Commercial and OSPW-derived naphthenic acids
are often heterogenous mixtures, containing other compounds, some of which also have
the potential to increase toxicity (West et al., 2011). Indeed, the environmental relevance
of some previous studies has been questioned due to these differences (see West et al.,
2011; Bartlett et al., 2017). In this study, two methods were used to characterize and
confirm the constituents in the commercial OSPW mixture (SFS and high-resolution
mass spectrometry). The SFS signature of the commercial OSPW mixture was found to
overlap the tailings pond OSPW samples (Figure 2.1b). The findings also confirmed the
presence of mostly classical naphthenic acids (99.8% O₂), as is typically found in
OSPW (Bartlett et al., 2017). Thus, the OSPW mixture tested in this study is both
homogeneous and comparable to OSPW naphthenic acid mixtures in tailings ponds in
Canada’s oil sands region (Figure 2.1).
2.5.2 Background toxicity information

Both lethal (LC$_{50}$: 2.4 to 42 mg/L) and sublethal effects (e.g., inhibition of diatom growth 260 to 805 mg/L) of naphthenic acid on aquatic taxa have been reported previously (Scarlett et al., 2013; Brown and Ulrich, 2015; Gagné et al., 2017), albeit at levels higher than those reported in this study (Figure 2.2). However, there remains uncertainty as to the ecological effects of OSPW on invertebrates: particularly with respect to mixtures of metals and naphthenic acids (Mahaffey and Dubé, 2017). Previous research found that metal-PAH mixtures are likely to lead to more than additive, if not synergistic, toxicity outcomes in laboratory trials (Gauthier et al., 2014). Organo-metallic mixtures such as the metallic naphthenates tested here are, unfortunately, very poorly represented in the scientific literature. For instance, only three studies have examined the effect of these compounds on invertebrates—all of which are focused on molluscs. This information gap is problematic because sodium naphthenate is routinely generated as part of multiple oil sands processing techniques and merits further study (see Schramm et al., 2000, COSIA 2014). To our knowledge, no studies have used field-collected aquatic communities or mayfly taxa.

Given conflicting evidence and reports questioning the chemical composition of moieties reported as ‘naphthenic acids’ (see Grewer et al., 2010, Brown and Ulrich, 2015), it appears possible that complex mixtures, possibly also containing organo-metallic salts, are also routinely being tested. This could explain some of the discrepancies in the toxicity literature for naphthenic acid. Historical use by industry of Microtox bioassays (e.g., FTFC 1995) further highlights the importance of salinity, and by extension, the likely contribution of organo-metallic salts, as the inhibition of this
alga was strongly correlated to the sodium ion content in the tailings porewater ($R^2 = 0.41$, $p < 0.01$) but showed no relationship to naphthenic acid concentration ($R^2 = 0.09$, $p = 0.12$). However, caution over the interpretation of this Microtox data (e.g., $IC_{50} = 40$ mg/L) is warranted as the chemical analysis of naphthenic acids has vastly improved since the 1990s. Moreover, these marine algae may not be as sensitive to organo-metallic salt mixtures as freshwater taxa.

2.5.3 Acute toxicity tests (48-h and 72-h)

Results of the 48-h toxicity test indicate that unplanned release of OSPW into aquatic environments could be potentially devastating to sensitive freshwater taxa such as mayflies. OSPW in tailings ponds can contain concentrations of naphthenic acids as high as 128 mg/L (Allen, 2008), well above the 48-h $LC_{50}$ of 4.3 mg naphthenic acid/L ($0.43 \pm 0.34$ mg sodium naphthenate/L) and the 72-h $LC_{50}$ of 2.5 mg naphthenic acid/L ($0.25 \pm 0.10$ mg sodium naphthenate/L). There was no survival at concentrations of $\geq 10$ mg naphthenic acid/L in either toxicity test (Figure 2.3). These findings are troubling given that an unplanned release of OSPW could occur at any time. The controlled release of OSPW to the environment is also a likely scenario in the near future, as tailings ponds containing OSPW must be reclaimed as part of mining lease agreements (Government of Alberta, 2017). Although it is unlikely that aquatic communities would be exposed to undiluted OSPW, acute exposure results from this study suggest that OSPW mixture components will require significant treatment and dilution before planned release to the environment can be considered.
2.5.4 Outdoor mesocosm study

Results from the mesocosm study support the acute toxicity test findings in that the exposure scenario simulating an unplanned, catastrophic release of tailings into the freshwater receiving environments showed significant reduction in the abundance of both sensitive and tolerant taxa. Indeed, there was a systemic removal of all but four genera. Our findings overlap with the only proposed release targets in the published literature (e.g. 7-35 mg naphthenic acid/L, Allen et al., 2008). Our experimental evidence of the toxicity of these mixtures suggests these proposed targets will not be sufficiently protective as 7 and 35 mg naphthenic acid/L reduced the abundance of the macroinvertebrate community by more than half (≥ 65 ± 8%). The proposed naphthenic acid concentrations do not appear to be suitable targets of for the planned release of OSPW. Further, such extreme loss of abundance and richness could lead to unmanaged synergies with other stressors (e.g., climate change, drought, habitat degradation) that could result in extirpation of some taxa, further decoupling aquatic food webs and disrupting the function of downstream aquatic communities (reviewed in Brook et al., 2008). The resilience of actual aquatic communities to chronic low dose exposure to dilute OSPW in the event of planned release scenarios is, at present, unknown.

2.5.5 Implications for guidance

Compared to other contaminants, sodium naphthenate is > 10x more toxic (e.g., 72-h LC50 = 0.25 ± 0.10 mg sodium naphthenate/L) than the neonicotinoid insecticide, acetamiprid to Hexagenia (96-h LC50 = 0.78 mg/L; Bartlett et al., 2018). Guidelines for this class of insecticide have recently been re-evaluated (acute and chronic HC5 [concentration at which five percent of the species in the species sensitivity data exhibit
an effect] of 0.36 and 0.041 μg a.i./L respectively) (Health Canada 2016). At present there is insufficient data to make such determinations for OSPW mixtures. However, if a similar process of identifying safe concentrations was applied to our results, safe levels would be orders of magnitude less than 1 mg/L as no mayfly taxa survived exposure to this dose in either toxicity test. In the 72-h test, no mayflies survived treatment levels greater than 0.5 mg sodium naphthenate/L. Further, our mesocosm findings strongly suggest that previous proposed targets (naphthenic acid ~35 mg/L, 50% OSPW) are not sufficient to protect macroinvertebrate communities. In addition, because characterization of OSPW mixtures tends to focus on naphthenic acids and not on metallic salts commonly associated with these highly ionized carboxylic acids, dose-dependent effects of mixtures of organo-metallic salts may be prevalent. Further study that combines environmentally relevant mixtures of process water components and field-collected macroinvertebrate communities is warranted.

2.6 Conclusion

Tailings ponds in the oil sands region contain complex mixtures of the remnants of bitumen extraction, including hydrocarbons, metals and fine silt. Oil sands tailings also tend to have a high salt content because 80 to 95% of the water used in bitumen processing is recycled, and bitumen is extracted using hot water and NaOH. As baseline information on the effects and mixture toxicity of these complex industrial waters is largely unknown for aquatic macroinvertebrate communities, preliminary experiments were required to identify safe levels of these OSPW constituents for environmental protection and guideline development. The main objective of this work was to explicitly
examine the toxicity of a common mixture of commercial naphthenic acid and sodium naphthenate to simulate OSPW. The use of commercial mixtures in toxicity tests has been previously questioned, which warranted the inclusion of chemical characterization and comparison to tailings pond OSPW mixtures in this study to ensure similarity. Our findings strongly suggest that safe concentrations of these compounds may be far lower than previously supposed. In this study, exposure to 10:1 mixtures of two OSPW constituents (naphthenic acid and sodium naphthenate) resulted in toxic effects in the mayfly, *Hexagenia*, and more generally to field-collected benthic macroinvertebrate communities at concentrations routinely measured in tailings ponds, and also for concentrations that have been proposed for potential OSPW planned-release concentrations.
2.7 References


of the Total Environment 408: 5997-6010. https://doi.org/10.1016/j.scitotenv.2010.08.013


2.8 Tables

**Table 2.1** Comparison of actual (mean ± standard error [SE]) versus nominal concentrations of 10:1 mixture of total naphthenic acids and sodium naphthenate as determined by scanning SFS analysis (Kavanaugh et al., 2009). Chemical concentrations are in mg/L unless indicated (* in μg/L). All solutions were prepared using serial dilution of stock concentrations of a commercial source representative of oil sands process water (OSPW).

*a) 72-h lethality tests and mesocosm† community-level tests*

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*b) 48-h lethality tests*

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ND‡ No sodium naphthenate was detected in the 0.0001 μg/L treatment.
† Treatment levels also used in mesocosm study
2.9 Figures

Figure 2.1. Chemical analysis and characterization of commercial 10:1 mixture of naphthenic acids and sodium naphthenate. The process water extracts match the fluorescence (b), and the molecular weight (c) of Syncrude and Suncor tailings ponds in northeastern Alberta (a). Specifically, (b) fluorescence signatures match those originally reported by Kavanaugh et al., (2009) for Pond 9 (Syncrude) and MFT-S (Suncor). Further characterization of (c) the relative abundance of a range of molecular weights of the commercial 10:1 naphthenic acid mixture were also determined by Orbitrap high resolution mass spectrometry, using negative ion electrospray ionization. See text.
Figure 2.2. Summary of existing toxicity information for aquatic invertebrates exposed to naphthenic acid and metallic naphthenates. The effect of different concentrations of naphthenic acids and three metallic naphthenates (Na, Cu and tributyl Sn) on the potentially affected fraction (PAF) of all available species, using previously published laboratory bioassay tests of plants, invertebrates, and fish from the USEPA Ecotox database (a and b); Principal component analysis (PCA) of the physico-chemical parameters in Suncor and Syncrude tailings pore water and industry collected data (see FTFC 1995) using the standardized marine bacteria test, *Allivibrio fischeri* (Microtox, formerly *Vibrio fischeri*) (c); and comparison of individual correlations for four parameters of interest (NA, Cl, Na+ and Microtox IC50) (d).
Figure 2.3. Acute toxicity test results on the survivorship of Hexagenia spp. nymphs exposed to 10:1 OSPW mixture of naphthenic acid and sodium naphthenate during repeated lethality tests (72-h or 48-h). Probit analysis determined that the 48-h LC$_{50}$ was $0.43 \pm 0.34$ mg sodium naphthenate/L and the 72-h LC$_{50}$ was $0.25 \pm 0.10$ mg sodium naphthenate/L, respectively.
Figure 2.4. Outdoor mesocosm study. (a) Corbett Brook invertebrate community field collection site. (b) Outdoor artificial streams during initial substrate inoculation. Comparison of the responses of field-collected aquatic invertebrate communities to treatment with a 10:1 OSPW mixture of naphthenic acid and sodium naphthenate (c) and (d). (c) Relative abundance (total number of organisms, ‘N’) and (d) richness (total number of genera, ‘s’) of tolerant versus sensitive aquatic macroinvertebrate assemblages (based on tolerance values from Merritt and Cummins 1996).
3. Risks of mixtures of oil sands contaminants to a sensitive mayfly sentinel, Hexagenia.

3.1 Abstract

Tailings ponds in northeastern Alberta, Canada contain large quantities of oil sands process water (OSPW) that cannot currently be released safely due to toxicity of some components. Limited space and the need for reclamation of oil sands operation sites requires release of OSPW if oil sands mining continues. Knowledge of the composition and toxicity of OSPW is crucial for both risk assessment and management planning. This study examines chronic toxicity of a mixture of OSPW components sodium naphthenate and naphthenic acid (NA) to nymphs of the mayfly Hexagenia spp. in control and polycyclic aromatic hydrocarbon (PAH)-spiked sediment. The objective of this study was to determine whether the addition of the PAH-spiked sediment significantly contributed to or masked responses in these sensitive mayflies to a mixture of NA. Mean survival in nymphs exposed to NA and PAH-spiked sediment treatments was reduced by 48% compared to those exposed to the NA mixture alone. Lethal responses to treatment were observed in all of the PAH-spiked sediment treatments. However, within PAH and control sediment treatments, there was no significant difference in nymph survival due to NA concentration, indicating that changes in survivorship were predominantly a reflection of increased mortality associated with sediment PAHs and not to the NA mixture treatment. Sublethal effects on body segment sizes suggest that mayflies exposed to NA and PAH-spiked sediment, as well as those exposed to the highest NA concentration tested (1 mg/L) and control sediment made...
developmental trade-offs in order to emerge faster. These results reveal that release of OSPW to the surrounding environment could cause a reduction in mayfly populations. Mayflies provide important ecosystem services (e.g. bioturbation, bioirrigation, decomposition, nutrient cycling) and are an important food source for higher trophic levels in both the aquatic and terrestrial communities.

3.2 Introduction

Canada’s oil sands in northeastern Alberta are among the largest crude oil deposits in the world (CAPP, 2017). Oil sands is a complex mixture of bitumen, sand, clay, and water that must be extracted and upgraded, often using hot water and proprietary surfactants (Natural Resources Canada, 2013). The hot water extraction process used for Canada’s oil sands requires the use of substantial amounts of fresh water, much of which is recycled, but is originally sourced from the Athabasca River or its tributaries. After use in extraction, the residual water contains a complex mixture of left-over bitumen, dissolved salts, trace metals, and organic compounds such as surfactants. The aqueous and solid components of this mixture, collectively referred to as tailings, is then pumped to on-site tailings ponds, where the solid components settle (Natural Resources Canada, 2013). The aqueous portion, commonly referred to as oil sands process water (OSPW), can be recycled but tends to accumulate becoming increasingly saline over time (Schramm et al., 2000).

At present there is a zero-discharge policy for oil sands tailings in Canada (Natural Resources Canada, 2013). However, due to limited storage capacity the treatment and release of treated OSPW to the surrounding environment may be required
in the future (Allen, 2008; Brown and Ulrich, 2015). Knowledge of the composition and toxicity of OSPW is crucial for both risk assessment and management planning (Mahaffey and Dubé, 2017). OSPW varies between tailings ponds due to differences in mining practices such as the application of different surfactants such as metallic naphthenates (Mahaffey and Dubé, 2017). Assessing risk is further challenged by the considerable uncertainty concerning the ecological effects of OSPW on aquatic environments due to the complexity of these process water mixtures and the lack of guidelines even for many of the more common chemicals used in the extraction process such as sodium naphthenate.

Naphthenic acids (NA) have been identified as important components of OSPW and high concentrations of NA have been correlated with toxicity (van den Heuvel, 2015). These carboxylic acids are the organic components of many common surfactants used in the extraction of oil from bitumen (Mackinnon and Boerger, 1986; Allen, 2008). NA are toxic to aquatic species and are of concern due to their solubility and widespread persistence in waterways (Frank et al., 2014). In a prior study, mixtures of NA and a common metallic surfactant, sodium naphthenate, were found to be acutely toxic to sensitive aquatic communities, and mayflies in particular (Chapter 2). This study further examines this trend by investigating the same aqueous NA mixtures in the presence of a suite of sediment-borne PAHs.

Polycyclic aromatic hydrocarbons (PAH) are another common contaminant in the Athabasca oil sands region, with the levels of these compounds increasing in the lower Athabasca watershed since the oil sands industry boom of the 1960s (Kurek et al., 2012). PAHs can be toxic to both fish and invertebrates (e.g., De Hoop et al., 2017).
Effects of mixtures of NA mixtures in combination with other contaminants present in OSPW are largely unknown but may be additively toxic (Gauthier et al., 2014). Many tributaries of the lower Athabasca River are prone to slumping such that significant influx of sediments and contaminants is not uncommon (Ferrel et al., 2018). A recent study on PAH signatures in sediment found the presence of naphthalenes, phenanthrenes, fluoranthens, pyrenes, as well as benzo(a)pyrenes in sediments from two tributaries of the lower Athabasca River (Droppo et al., 2019). These environmentally-relevant PAHs were among the 17 examined in the sediment testing portion in this study.

My aim was to explore the combined effects of environmentally-relevant mixtures of co-occurring contaminants commonly from the oil sands region (naphthenic acids, sodium naphthenate and a mixture of 17 PAHs) on an environmentally-relevant aquatic insect species. *Hexagenia* burrowing mayflies were selected as the test organism as such sediment-dwelling organisms are often at a heightened risk of exposure to mixtures of contaminants due to prolonged contact with both sediment and water (Baun et al., 2008). Treatments levels of NA mixtures were tested on *Hexagenia* spp. mayflies either with or without the added stress of PAH-spiked sediment. Endpoints examined included a combination of toxicological (e.g., lethal) and functional (e.g., growth) responses. We hypothesized that co-exposure to both NA and PAH mixtures would be more toxic than either substance individually.
3.3 Methods

3.3.1 Study species

*Hexagenia*, a burrowing mayfly, was chosen for this study because burrowing invertebrates have a higher risk of exposure to contaminants in both sediment and water, which has the potential to produce additive or even synergistic toxic effects (Baun et al., 2008). Fertilized *Hexagenia* eggs were collected in June 2017 from Lake St. Clair in Windsor, Ontario using the method of Hanes and Ciborowski (1992). Culturing protocols were adapted from methods of Fremling (1980), Friesen (1981) and Giberson and Rosenberg (1992a). In brief, hatched nymphs were transferred to glass culture tanks held in the growth chambers at 22 ± 3°C with a 16:8 light to dark cycle. Tanks contained approximately 5 cm of sediment topped with de-chlorinated water and all received constant aeration and feeding twice per week. See Chapter 2 for more detail.

3.3.2 Establishment of treatments

In this study, two sediment treatments (control and PAH-spiked [Table 3.2]) were examined in a 21-d chronic exposure experiment (10 April – 3 May, 2018). In each sediment level, five concentrations of a 10:1 mixture of naphthenic acids and sodium naphthenate were reproduced (see Table 3.1 for concentrations). Each treatment level contained 3 replicates. Each replicate also received ten late instar *Hexagenia* nymphs (average total length 6.7 mm). Mixture concentrations were prepared using a commercial preparation (lot # DLODE of NO397, TCI America, Portland, OR) that has previously been chemically characterized as similar to tailings pond water and acutely toxic to mayflies at the 48-h LC50 of 0.43 ± 0.34 mg sodium naphthenate/L (4.3 mg
naphthenic acid/L) (see Chapter 2). For the sake of simplicity, the concentration of the aqueous 10:1 naphthenic acid / sodium naphthenate solution will be referred to by the NA concentration alone, unless otherwise stated. Sediment treatments included a PAH-spiked sediment from Sigma Aldrich (CAS# CRM104-50G, lot# LRAB5247) (Table 1, as above), and an unspiked natural sediment of similar <1 mm particle size collected from Magaguayavic Lake, New Brunswick (45° 47.62’N, 67° 13.48’W, see Chapter 2). Wet sediment was fine sieved to 1 mm and 30 g of control (unspiked), wet sediment was added to each replicate 300-mL beaker (Kimble, KIMAX™ tall form beakers, Fisher Sci., USA). For PAH treatments, 10 g of PAH-spiked sediment was also added. Each replicate then received 0.15 g of ground NutraFin Bug Bites fish food (Rolf C. Hagen Inc., Baie d’Urfé, QC). Beakers were then filled to the 300 mL mark with the appropriate NA solution and gently mixed to ensure integration of NA and ground NutraFin into the sediment pore water. Plastic lids with a hole drilled in the center for aeration were placed on top and sealed to prevent evaporation. Constant aeration was provided using an air pump, tubing and glass pipettes fed through plastic lids sealed to the top of each beaker (Figure 3.1). The beakers were stored in Percival® (Percival Scientific, Boone, IA, USA) environmental chambers at 22±3°C with a light-dark cycle of 16:8 hours. The beakers were allowed to settle for 24 hours before mayflies were added to the treatments.

3.3.3 Chemical analyses

The chemical analyses are described in detail elsewhere (Chapter 2). In brief, NA mixture concentrations were prepared using a commercial 10:1 preparation of
naphthenic acids and sodium naphthenate (lot # DLODE of NO397, TCI America, Portland, OR). Two approaches were used to analyze and characterize the composition of the NA commercial preparation. Firstly, these tests were conducted using the scanning synchronous fluorescence spectroscopy (SFS) method of Kavanaugh et al., (2009) on an Aquamate spectrophotometer with deuterium and tungsten lamp attachments (Thermo Spectronic, Rochester, NY). Secondly, to fully characterize the naphthenic acids from the commercial preparation, samples from the same lot number of the commercially prepared stock were also analyzed at the Environment and Climate Change Canada laboratory in Saskatoon, SK by high resolution mass spectrometry (Orbitrap) with electrospray ionization (see Chapter 2).

PAH sediments were prepared using a certified reference material (Lot # LRAB5247, CRM104-50G, Sigma-Aldrich). Sediments were weighed on an OHAUS® Navigator™ scale to the closest 0.01 g and then added to the un-spiked sediment as described previously. These reference sediments are certified for round-robin testing of laboratories seeking ISO 8100, 8270, 8310 or equivalent certification (https://www.iso.org/home.html). Sediments in each beaker were unfortunately insufficient for individual testing, however, a series of reference materials and 300-g composite samples prepared using the same methods as those in the test beakers were also sent to a local commercial laboratory with ISO certification for validation (RPC Fredericton). All results from the whole and composite samples were found to be within acceptable ISO certification limits. Actual concentrations for these standard materials are reported in Table 3.2.
3.3.4 Biological response variables

At the end of the 21-day experiment, replicate beakers were dismantled and the contents collected. Sediment was sieved to remove nymphs and the number surviving from each beaker was recorded. Mayfly nymphs were then collected and preserved in 95% ethanol for subsequent measurement and weighing. Total body length, thorax length, thorax width, head length and head width were measured using the Auto-Montage® imaging system (Syncroscopy, Synoptics Inc., Frederick, ND, USA) with a Leica© digital camera and dissecting microscope (Leica© Microsystems Ltd., Cambridge, UK). At the onset of the experiment, a subsample of the same-sized nymphs were also collected, measured and weighed in order to determine the size at the onset of the experiment (time zero). Over the course of the 21-d experiment each replicate was fed 0.15 g of NutraFin biweekly and at which time solutions were also statically renewed. Wing pad development was noted, and each nymph was also subsequently weighed. Finally, nymphs were dried for 48 hours in a 60°C drying oven and re-weighed.

3.3.5 Statistical analyses

A split-plot ANOVA design (Winer et al., 1991) was used to analyze the data. Shapiro-Wilks test was used to test normality. The predictor variables were NA concentration and sediment type (blocking variable) and the response variables were the nymph measurements (e.g., length, wet weight). A principal component analysis (PCA) was used to visualize and independently evaluate the relative importance of naphthenic acids, sodium naphthenate, and the 17 PAHs tested on Hexagenia body size measurements. PCA is a multivariate technique that can be used to confirm the strength
of relationships among different factors (principal components). Nymph survival between sediment types was compared using Welch’s t-test, as the assumption of homogeneity of variances was not met when tested by Levene’s Test for Homogeneity of Variance (p = 0.008). Nymph survival between NA solution treatments was compared using one-way ANOVA. All tests were conducted using R (v. 3.1).

3.4 Results

3.4.1 Lethal responses to treatment

Mayfly survival in the combined NA and PAH-spiked sediment treatments was significantly reduced, by 48% on average, compared to responses in nymphs exposed to the NA mixture alone (p ≤ 0.05, t = 3.31, t-crit = 1.86) (Figure 3.2). Lethal responses to treatment were observed in all of the spiked sediment treatments. However, within PAH and control sediment treatments, there was no significant difference in nymph survival due to NA concentration (PAH sediment: p = 0.36, F = 1.22, F-crit = 4.07; control sediment: p = 0.70, F = 0.49, F-crit = 4.07). Therefore, changes in survivorship were predominantly a reflection of increased mortality associated with sediment PAHs and not to the aqueous naphthenic acid mixture treatment.

3.4.2 Sublethal responses to treatment

The PCA biplot highlighted the two distinct groupings dependent on exposure to PAH or control sediment that cumulatively accounted for 91.3% of the variation in the nymph body size dataset (Figure 3.3). Factor 1 explained 66.3% of the variation (x-axis, Figure 3.3) and was strongly positively correlated to the presence of PAHs in the spiked sediment. Factor 2 explained 25.0% of the variation and was modestly positively
correlated to NA concentration and strongly negatively correlated to the body size measurements (Figure 3.3).

Mayflies exposed to NA and control sediments exhibited significant \( p < 0.05 \) growth over the course of the 21-d experiment with the exception of head length (Figure 3.4). In contrast, thorax length (43.1 ± 1.9%: Figure 3.4a), thorax width (25.5 ± 2.0%; Figure 3.4b), and wet weight (105.5 ± 9.8%; Figure 3.4d) were uniformly larger (34.7 ± 1.7%) irrespective of NA treatment by the end of the experiment. Overall, head length was reduced in all NA treatments compared to control \( (p = 6.995e-06) \). Head length was particularly reduced in the 0.001 and 0.1 mg/L NA treatments which were 13.3% and 17.1% lower than the control head lengths respectively. In contrast, treatments with PAH-spiked sediments significantly \( p < 0.05 \) reduced the size of some body segments in the mayflies (Figure 3.4). For instance, thorax length \( (p = 0.004) \) and thorax width \( (p = 0.031) \) were both reduced 7.7% and 10.2% respectively in mayfly survivors exposed to PAH-spiked sediment treatments compared to control (Figure 3.4a & b). Although not significant at the \( p < 0.05 \) level, wet weight of nymphs in PAH sediment treatments were also reduced by 14.2% \( (p = 0.103, \text{Figure } 3.4d) \) while total length was reduced by 3.8% compared to controls \( (p = 0.094) \).

Further, in PAH sediments, surviving mayflies typically grew 33.7 ± 2.6% in thorax length over the course of the 21-d experiment compared to time zero (Figure 3.4a). However, growth pattern responses within PAH sediment treatment levels were NA concentration dependent (Figure 3.4). Notably, the thorax (Figure 3.4a), head length (Figure 3.4c) and wet weight (Figure 3.4d) of mayflies were unchanged \( (P > 0.05) \) compared to time zero in the combined PAH sediment and lowest concentration of
NA mixture tested (0.001 mg/L). A similar pattern was also observed for mayflies in the combined PAH sediment and NA treatments at the 0.1 mg/L level for thorax width (Figure 3.4b) and wet weight which was particularly variable (e.g., CV = 72.3%; Figure 3.4d).

As above, total length to thorax length ratios also significantly differed due to the sediment treatment type but seldom responded to changes in NA concentration (Figure 3.5). In particular, total length to thorax length ratios were significantly different (p = 0.0006) between the PAH sediment and control sediment groups. Total length was favoured (ratios < 0.3) in mayflies in the control sediment and thorax length was favoured (ratios > 0.3) in mayflies in the PAH sediment. Thorax length favoured ratios were also apparent (p = 0.072) in the highest concentration (1 mg/L) NA control sediment treatment compared to the 0 mg/L NA control sediment treatment (Figure 3.5).

3.5 Discussion

It is probable that in the event of planned release of OSPW into the Athabasca River or other freshwater rivers mayflies and other members of the aquatic community macroinvertebrates will be exposed to low levels of tailings contaminants long-term. Chronic exposure to even very low concentrations of toxins can cause both lethal and sublethal effects in mayflies (Lowell et al., 1995; Alexander, 2008). Sublethal effects on growth and development in response to stress can have an impact on reproductive success of adult mayflies (Atkinson 1995; Scrimgeour et al., 1994). This study documents the lethal and sublethal response of *Hexagenia* nymphs due to exposure to
environmentally-relevant concentrations of aqueous NA mixtures with control versus PAH spiked-sediments.

3.5.1 Lethal responses to treatment

The reduction in nymph survival was predominantly due exposure to the PAH sediment treatment (Figure 3.2, 3.3). This effect is of concern because there has been a striking increase in PAH levels in the Athabasca area sediment since the oil sands boom of the 1970s (Kurek et al., 2012, Evans et al., 2016, Raine et al., 2017b). Total sediment PAH concentrations of over 6000 ng/g have been measured in the Athabasca region, with higher concentrations associated with samples closer to development sites (Evans et al., 2016). Although levels tested in this study were 50X lower than concentrations measured near oil sands developments (Table 3.1), and significant effects were observed, nonetheless. It is possible that local populations of *Hexagenia* have adapted tolerance to PAHs.

In this study, no differences in survival were detected in control sediment treatments due to NA concentration. However, it is worth noting that the greatest concentration of the NA mixture tested was but 1 mg/L, two orders of magnitude lower than concentrations reported in tailings ponds (128 mg/L, Allen 2008). At present, even the most effective methods of removing naphthenic acids, such as constructed wetlands, remove only up to 80% of the types of substances tested in this study (Ajaero et al., 2018). Thus, the concentrations of NA in treated OSPW could easily remain in the 8-24 mg/L range. In a previous study, acute toxicity of the same mixture of NA was also tested and found that NA could be acutely toxic with a 48-h LC₅₀ of 0.43 ± 0.34 mg
sodium naphthenate/L (4.3 mg naphthenic acid/L) (Chapter 2). It seems plausible that even a 100-fold dilution of concentrations of naphthenic acids and their metallic salts in contemporary tailings pond could be somewhat toxic to sensitive aquatic insects like mayflies.

Responses to sediments with trace metal concentrations are, at present, understudied and may offer further insights. Trace metal levels in the area are naturally elevated and can be further amplified by industrial activity (Headley et al., 2005; Baker et al., 2012). These effects paired with other stressors such as oil production emissions, and/or natural occurring PAHs and hydrocarbons, as well as climate change (Kurek et al., 2012), could have a devastating effect on aquatic biota. Aquatic macroinvertebrates such as Hexagenia provide significant contributions to ecosystem services in freshwater rivers that could create a cascading effect on the ecosystem if populations are diminished (Collier et al., 2016). Aquatic invertebrates provide prey for aquatic predators and are also consumed by terrestrial predators such as birds, reptiles and insects. Adult insects also transport aquatic material to terrestrial habitats upon emerging which is an important energy transfer between ecosystems (Thorp, 2015).

3.5.2 Sublethal responses to treatment

In control sediment treatments, a significant reduction in head length was observed in all NA treatments in comparison to the 0 mg/L control. This indicates that developmental trade-offs were made in order to reduce time to emergence in response to NA exposure. Reduced head length may also affect brain and eye size, which in turn could decrease success in foraging/or mate pairing. Although differences were not
significant, average wet weight displayed a similar pattern, excluding the 0.001 mg/L treatment, which was slightly larger than the 0 mg/L treatment. This may be due to a common sublethal stress response called ‘hormesis’ – a biphasic dose response to toxins that represents an apparently favourable response to low dose treatment (Stebbing 1982). Rather, the ability for organisms to compensate for exposure may be an important indicator of plasticity to these stressors and may also be useful for estimating the no observable effect concentration.

There were no significant differences in thorax width among treatments and there was less growth over time in comparison to thorax length. This again indicates that resources were used to develop the thorax and wings to expedite emergence, rather than reach an optimal body size for reproduction. Similar patterns have also been reported in other studies conducted by Scrimgeour et al., (1997) and Peckarsky et al., (2001) who suggested that mayflies can escape a stressful aquatic environment by favouring development over size. Similarly, in the PAH-spiked sediment treatments, there was an interesting pattern among thorax width, head length and wet weight responses: The 0.001 mg/L did not show significant growth compared to time zero, while almost all other treatments did – those that did not show a significant response still grew, but there was more variation leading to a non-significant result. This again supports the hypothesis that development trade-offs are made in order to escape exposure to stressors by emerging earlier. All NA treatments in the PAH sediment group, as well as the highest concentration NA control sediment group favoured a longer thorax in relation to total length. This again indicates advancing thorax and wing development as a trade-off for reaching larger size overall in response to stress.
When analyzing sublethal effects, it is imperative to remember that only the responses of surviving nymphs are measured. In the treatments with PAH-spiked sediment, there was considerable variation in survival, indicating possible differences in fitness between individuals within the population.

3.6 Conclusion

In the lower Athabasca River, it is important to consider other possible stressors when working on a management plan for OSPW release. Sediment and nutrient load, flow velocity and volume, pollution from emissions and groundwater and presence of other contaminants can all affect community responses. Survival in treatments with both NA and PAH sediment was reduced by 60%. This highlights the importance of considering additive and/or interactive effects from multiple stressors. Sublethal effects also indicate body size trade-offs to enable earlier emergence. While small changes may seem insignificant, long term effects on adult fecundity and/or survival could be detrimental to the ecosystem. In colder climates such as northern Alberta, Hexagenia spend from two to four years in their aquatic stage (Giberson and Rosenberg, 1994) making them susceptible to multiple exposures depending on the proposed OSPW release plan. Release of OSPW to the surrounding environment is not advised until current technologies can further reduce toxicity.

3.6.1 Future directions

Continued development of OSPW treatment technology and management planning is necessary. As new treatment options become available, continued study of the possible effects of an OSPW release or breach will be necessary. A mesocosm study
that exposes a field-collected community to chronic low-level NA or diluted OSPW would prove useful to help determine long term effects of OSPW release on community structure. Use of field-collected invertebrates from the Athabasca region would also account for differences in sensitivity among populations. In the event of a planned OSPW release there would also likely be other contaminants as well as suspended sediment depending on the treatment process. All of these factors should be taken into account when creating a management plan for release of OSPW in the Athabasca region.
3.7 References


### 3.8 Tables

**Table 3.1.** Comparison of actual (mean ± standard error [SE]) versus nominal concentrations of 10:1 mixture of total naphthenic acids and sodium naphthenate as determined by scanning SFS analysis. All solutions were prepared using serial dilution of stock concentrations of a commercial source representative of oil sands process water (OSPW).

<table>
<thead>
<tr>
<th>% OSPW</th>
<th>Naphthenic acids (mg/L)</th>
<th>Sodium naphthenate (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nominal</td>
<td>Actual</td>
</tr>
<tr>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
<td>0.17</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1.45</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>14.47</td>
</tr>
</tbody>
</table>
Table 3.2. Comparison of estimated actual (mean ± standard error [SE]) concentrations of 17 PAHs present in spiked sediment test used in this study versus field levels reported by Droppo et al., (2019).

<table>
<thead>
<tr>
<th>PAH</th>
<th>This study</th>
<th>Droppo et al., 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/kg</td>
<td>AVG (ng/mg)</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>130.5</td>
<td>0.174 ± 0.009</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>88.75</td>
<td>0.118 ± 0.006</td>
</tr>
<tr>
<td>Anthracene</td>
<td>120</td>
<td>0.16 ± 0.008</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>25.75</td>
<td>0.034 ± 0.002</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>60</td>
<td>0.08 ± 0.004</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>76.25</td>
<td>0.102 ± 0.005</td>
</tr>
<tr>
<td>Benzo(b+k)fluoranthen</td>
<td>114.25</td>
<td>0.052 ± 0.008</td>
</tr>
<tr>
<td>Benzo(g,h,i)perylene</td>
<td>33.25</td>
<td>0.044 ± 0.002</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>47.25</td>
<td>0.063 ± 0.003</td>
</tr>
<tr>
<td>Chrysene</td>
<td>34.5</td>
<td>0.046 ± 0.002</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>19.05</td>
<td>0.025 ± 0.001</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>81.25</td>
<td>0.108 ± 0.005</td>
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<tr>
<td>Fluorene</td>
<td>56.5</td>
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</tr>
<tr>
<td>Indeno(1,2,3-cd)pyrene</td>
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<td>Naphthalene</td>
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<td>Phenanthrene</td>
<td>87</td>
<td>0.116 ± 0.006</td>
</tr>
<tr>
<td>Pyrene</td>
<td>47.75</td>
<td>0.064 ± 0.003</td>
</tr>
</tbody>
</table>
3.9 Figures

**Figure 3.1.** Laboratory setup for 21-d exposure to a commercial naphthenate (NA) mixture (0, 0.001, 0.1, 1 mg/L) and control sediment or 1:3 mixture of PAH-spiked and control sediment: (a) individual replicate and (b) treatment set up in environmental chamber.
**Figure 3.2.** Mean nymph survival ± SE, grouped by sediment treatment. Survival was significantly lower in the PAH sediment treatment group in comparison to the control sediment treatment group (denoted by “*”).
Figure 3.3. Principal component analysis (PCA) explaining 91.3% (66.3 + 25% explained variance) of the total variation in the body size measurements of *Hexagenia* nymphs exposed to a commercial naphthenate (NA) mixture gradient (0 – 1 mg/L) and sediment with or without PAHs (see Table 3.2 for PAH concentrations).
Figure 3.4. Boxplot comparison of sublethal changes (showing the maximum, 75<sup>th</sup> percentile, median, 25<sup>th</sup> percentile and minimum) in thorax length (a), thorax width (b), head length (c), and wet weight (d) in *Hexagenia* mayfly nymphs exposed to an NA mixture in either control (white, left) or PAH-spiked sediment treatments for a 21-day period. Significant differences from the control (0 mg/L NA and control sediment, far left bar) are indicated by “*” and time zero measurements are indicated by grey horizontal lines for comparison.
Figure 3.5. Boxplot comparison of total length to thorax length ratios (showing the maximum, 75th percentile, median, 25th percentile, and minimum) in total length to thorax length ratio in Hexagenia mayfly nymphs exposed to an NA mixture in either control (white, left) or PAH-spiked sediment treatments for a 21-day period. Significant differences from the control (0 mg/L NA and control sediment, far left bar) are indicated by "*" and the time zero measurements are indicated by a grey horizontal line for comparison.
4. SYNTHESIS AND RECOMMENDATIONS

My primary objective was to examine the impacts of mixtures of environmentally-relevant oil sands contaminants (i.e., sodium naphthenate, naphthenic acids and polycyclic aromatic hydrocarbons) on the survival and development of relevant aquatic species and communities. This is important because I observed both sublethal and lethal effects on aquatic invertebrates at contaminant concentration levels much lower than the previously proposed environmental guidelines. These results highlight the potential for negative environmental impacts if oil sands process water (OSPW) treatment and disposal is not properly managed.

In Chapter 2 I examined the acute effects of relevant concentrations of commercial naphthenate mixtures (10:1 of naphthenic acids and sodium naphthenate), using a chemical composition similar to those present in OSPW, on the survival of Hexagenia nymphs and on the composition of benthic macroinvertebrate communities in artificial stream mesocosms. I found that safe concentrations for mayflies and other aquatic macroinvertebrates were less than 1 mg/L, as no mayfly taxa survived exposure to this dose in either the 48-h or 72-h acute toxicity test. In the 72-h test, no mayflies survived treatment levels greater than 0.5 mg sodium naphthenate/L. In the mesocosm study, even a 90% dilution of the OSPW contaminant mixture was insufficient to protect sensitive macroinvertebrate communities.

In chapter three I explored the combined effects on Hexagenia spp. of mixtures of contaminants commonly found in the oil sands region (10:1 of naphthenic acids and sodium naphthenate (NA) and polycyclic aromatic hydrocarbons (PAHs)). Mean survival in nymphs exposed to both aqueous NA as well as PAH-spiked sediment
treatments were reduced by up to 63% compared to those exposed to the NA mixture alone. Further, lethal responses to treatment were observed in all of the PAH-spiked sediment treatments although these concentrations were lower than some reports of concentrations in the field (e.g., Droppo et al., 2019). In this study, sublethal changes in body segment sizes suggest that mayflies exposed to the combined NA and PAH treatments, as well as those exposed to the highest NA concentration tested (1 mg/L) made developmental trade-offs in order to emerge faster. In effect these insects risked obtaining smaller adult body size and being less fecund in order to reduce time exposed to these contaminants. Collectively, these results suggest that release of OSPW to the surrounding environment could cause a major reduction in mayfly populations.

In the following discussion I extrapolate my findings to examine potential, broader ecological impacts of OSPW contamination. In addition, I address the use of *Hexagenia* spp. as a standard toxicological test species and elaborate on the potential impacts of acute and chronic exposure to NA. The results of my thesis are already influencing management strategies for release of OSPW and will call attention to the importance of further study in this field. Finally, I give recommendations based on the conclusions of the two data chapters.

### 4.1 Study Species

*Hexagenia* spp. have been recommended as a standardized test species since 1980 (Fremling & Mauck, 1980) but it is only within the past decade that their use in toxicity testing has become more common (e.g. Nguyen et al., 2012, Besser et al., 2013, Harwood et al., 2014, Bartlett et al., 2018, Cadmus et al., 2018). Although seldom used
in toxicity testing compared to invertebrates such as *Hyalella* and *Chironomus* (Harwood et al., 2014). *Hexagenia* spp. is a standard and complementary test species that can be more sensitive to contaminants. The main advantage of this species in our particular field of research is the ability to test multiple stressor effects by exposure to contaminants in both sediment and water. The long aquatic stage is ideal for chronic testing. We would recommend test durations that exceed 21-d for future studies for the potential to observe greater differences in sublethal measurements. Other benefits of the use of mayflies in toxicity testing have been discussed at length elsewhere and include relatively large biomass, ease of collection and rearing, and a juvenile aquatic stage (Nguyen et al., 2012, Harwood et al., 2014).

### 4.2 Potential impacts

#### 4.2.1 Acute exposure to NA

In the 48-h and 72-h *Hexagenia* spp. exposure, we observed significant decreases in survival when exposed to concentrations of NA as low as 1 mg/L (48-h LC50 of 4.3 mg/L and 72-h LC50 of 2.5 mg/L), with no survival in concentrations ≥ 10 mg/L. In the outdoor mesocosm study the macroinvertebrate community abundance was reduced more than half (≥ 65 ± 8%) by acute exposure to 7 mg/L NA. The literature suggests that NA is often in the form of metallic salts such as sodium naphthenate, which we determined to be 10X more toxic than NA alone. It is clear from these results that the suggested release guidelines of 7-35 mg/L (Allen 2008) will not be sufficient to protect macroinvertebrate communities.
Preservation of the abundance of biodiversity of macroinvertebrate communities is intrinsically valuable, because of their importance to key ecosystem services including but not limited to biodiversity, nutrient cycling and food resources for fish. Aquatic macroinvertebrates affect an array of ecosystem functions such as nutrient cycling, primary productivity, decomposition and translocation of materials (Wallace & Webster, 1996; Jacobus et al., 2019). They also provide important food sources for other invertebrates, fish and terrestrial species (Covich et al., 1999). Loss of macroinvertebrate diversity and abundance in freshwater ecosystems are often the first sign of degradation and thus can be used as valuable indicators of overall ecosystem health (Wallace & Webster, 1996; Jacobus et al., 2019). The diversity of roles and functional traits associated with macroinvertebrate communities highlights the importance of their conservation.

As tailings ponds in the oil sands region of Alberta continue to grow in size without a viable remediation solution, the threat of a breach becomes increasingly likely (Kelly et al., 2010). In the event of a tailings pond breach the estimated concentration of NAs in an outflow would be almost 40 mg/L. This catastrophic release of contaminants would cause significant environmental damage, but would be exacerbated by the increased flow, substantial sediment load, and additional toxicity related to the release of other oil sands components during a tailings pond breach.

4.2.2 Chronic exposure to NA and PAHs

Lethal effects were seen in all treatments containing PAH sediment. PAH concentrations far lower than what is reported in the field had significant lethal and
sublethal effects on *Hexagenia* over the 21-d test period. It is possible that local populations have developed tolerance to this stressor, thus future work will incorporate the use of field-collected assemblages to mitigate this effect. These findings highlight the importance of considering contaminants already present in the Athabasca when planning release of OSPW to the environment.

Sublethal effects were observed in treatments with control sediment and 1 mg/L NA mixture as well as treatments with PAH sediments. Our test period of 21-d is relatively short in comparison to the two- to four-year aquatic life stage of *Hexagenia* suggesting that sublethal effects would likely be further pronounced with a longer period of exposure. Functional sublethal effects such as different ratios of body size measurements indicate that nymphs may be making developmental trade-offs in order to escape a stressful environment, which parallels results from Scrimgeour et al., (1997) and Peckarsky et al., (2001) who described this phenomenon under predation risk regimes. If nymphs are favouring wing development over body size, fecundity may be reduced, leading to further decline in abundance and potential reproductive success.

This result emphasizes the importance of development of new technologies to treat OSPW mixtures, as there has been no success in lowering NA concentrations by this magnitude to date. At present, even the most effective methods of removing naphthenic acids, such as constructed wetlands and petroleum coke adsorption, remove only up to 80% of the types of substances tested in this study (Zubot et al., 2012; Ajaero et al., 2018). Thus, the concentrations of NA remaining in treated OSPW could easily remain in the 8-24 mg/L range.
4.2.3 OSPW release

To support landscape reclamation, water management practices and industry environmental performance, OSPW must be treated and returned to the environment (Tanna et al., 2019). My results suggest that untreated OSPW is likely unsafe for release to the environment. Thus, new technologies will be required to sufficiently process this effluent in the event of a planned release scenario in order to ensure the protection of the environment and human health (e.g., see Zubot et al., 2012). At present, the release of OSPW containing NAs into the Athabasca River would likely place additional stress to an already impacted environment. The oil sands industry has significant responsibilities for managing the pollution in the Athabasca River and its tributaries. There have been significant increases in PAHs in lake sediments downstream of industrial oil sands development (Droppo et al., 2019, Kurek et al., 2013). Kelly et al., (2010) found that the concentration of 13 elements (Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, and Zn) identified as priority pollutants by the USEPA were greater in areas downstream compared to upstream of oil sands development. There have been significant changes in water quality and quantity in the Athabasca River basin due to climate warming and increased industrial, agricultural and urban development (Squires et al., 2009). These factors will likely act synergistically to drastically change the ecosystem.

There is potential for human health risk, but more research must be done before conclusive inference can be made on the matter (Kindzierski et al., 2012). The most plausible effect on humans of the proposed guidelines for NA would be pollution of drinking water and loss of food species. First Nations communities north of the oil sands
region rely on these resources and have previously reported deleterious health effects from environmental pollution in the area (Chen, 2009).

### 4.3 Recommendations for guidance

The primary conclusions of this research suggest that the planned or unplanned release of OSPW into the surrounding environment could cause substantial damage to the ecosystem. A significant decrease in mayfly survival occurred at concentrations of NA as low as 1 mg/L and sublethal effects on growth were observed at lower concentrations. These findings indicate that dilution of OSPW under the current, proposed scenarios will not be sufficient to avoid negative consequences of release on the river ecosystem. Considering the combined effects of NAs, PAHs, other environmental contaminants and climate warming, it would be unadvisable to allow release of OSPW into the surrounding environment given our current understanding of release impacts. Continuing to produce and store toxic OSPW in tailings ponds is also unsustainable and could potentially lead to a catastrophic breach. As extreme weather events increase in frequency and severity (Pink, 2018), the threat of a tailings breach becomes critical. There is now an urgent need for development of new treatment techniques to reduce toxicity of OSPW or to create more secure storage solutions.
4.4 References


https://doi.org/10.1146/annurev.en.41.010196.000555

Curriculum Vitae

Julia Rae Howland

BSc, University of New Brunswick, Fredericton, 2014

Publications:


Conference Presentations:

