CHARACTERIZING ANNUAL CHANGES IN THE CHEMISTRY AND TOXICITY

OF SURFACE WATER FROM BASE MINE LAKE,

AN ALBERTA OIL SANDS END PIT LAKE

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ABSTRACT

Base Mine Lake (BML) is an end pit lake (EPL) located within the Alberta oil sands containing fluid fine tailings (FFT) capped with oil sands process-affected water (OSPW) and freshwater. EPLs have been proposed as a method for the long-term reclamation of oil sands surface mining activity, however, they contain a complex mixture of dissolved organic acids, metals, and salts that are toxic to aquatic organisms. The purpose of this thesis was to characterize annual changes in the inorganic chemistry and toxicity of BML surface water to more adequately assess the sustainability and environmental risks of EPLs, and to predict the time required to reach acceptable surface water quality necessary for the development of a functional aquatic ecosystem.

Annual changes in inorganic surface water concentrations (2014 to 2016) were strongly correlated with competing processes previously described in BML: influx from FFT pore water into the overlying surface water via advective-diffusive transport offset by efflux via continuous manual pumping by Syncrude (freshwater in, process water out). Toxicological risk was assessed using a variety of benchmarks and was consistent with the consensus that salinity (as Na^+, Cl^-, HCO_3^-) is the primary driver of inorganic toxicity to sensitive aquatic organisms, while dissolved metals are of negligible concern. This was confirmed by toxicity tests using the freshwater invertebrates *Chironomus dilutus* and *Ceriodaphnia dubia* which indicated limited effects on survival, growth, development, and reproduction even after chronic exposure to whole BML surface water, and the discovery of wild *Daphnia pulex* in August 2016 surface water.

Although BML will continue to pose risk to some aquatic organisms, current surface water quality conditions are now sufficient to support certain salt-tolerant freshwater aquatic organisms. As long as Syncrude continues pumping water, these studies indicate that surface water quality will continue to slowly improve over time, promoting colonization and ecological succession of BML into a functional aquatic ecosystem likely dominated by brackish conditions. However, if water pumping were to be reduced or stopped entirely, the concentrations of many inorganic constituents within BML surface water would likely begin to rise again, leading to increased toxicity and reduced surface water quality.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

Acronyms

AEMERA	Alberta Environmental Monitoring, Evaluation and Reporting Agency
AEP	Alberta Environment and Parks
AER	Alberta Energy Regulator
AERSD	Alberta Environment and Sustainable Resource Development
ANOVA	Analysis of variance
ARW	Athabasca River water
BML	Base Mine Lake
CAPP	Canadian Association of Petroleum Producers
CCME	Canadian Council of Ministers of the Environment
COSIA	Canadian Oil Sands Innovation Alliance
CWQG	Canadian Water Quality Guideline (for the protection of freshwater aquatic life)
DOC	Dissolved organic carbon
EPEA	(Alberta) Environmental Protection and Enhancement Act
EPL	End pit lake
ERCB	(Alberta) Energy Resources Conservation Board
FFT	Fluid fine tailings
HQ	Hazard quotient
JOSM	Joint (Canada Alberta Implementation Plan for) Oil Sands Monitoring
MHRW	Moderately hard reconstituted water
NAs	Naphthenic acids
OSPW	Oil sands process-affected water
RAMP	Regional Aquatics Monitoring Program
RO	Reverse osmosis
SSD	Species sensitivity distribution
TDS	Total dissolved solids
TMF	Tailings Management Framework (for the Mineable Athabasca Oil Sands)

TSS	Total suspended solids
US EPA	United States Environmental Protection Agency
WCT	Water-capped tailings
WIP	West In-Pit

Units

°C	Degrees Celsius
d	Days
g	Grams
h	Hours
IC_p	Inhibition concentration (where $p = 1$ to 99%)
km^2	Square kilometres
L	Litres
LC_{50}	Median lethal concentration
m	Metres
meq	Milliequivalents
mg	Milligrams
mL	Millilitres
mmol	Millimoles
mV	Millivolts
n	Sample size
n	Carbon number
NTU	Nephelometric Turbidity Unit
µg	Micrograms
µL	Microlitre
µm	Micrometres
µS/cm	MicroSiemens per centimeter (measure of electrical conductivity)
x	Factor (1x = 100%)
z	Degree of unsaturation (associated with ring structures and double bonds)

CHAPTER 1 – GENERAL INTRODUCTION

1.1   The Alberta oil sands

Despite environmental concerns over fossil fuels in recent decades and the push towards more sustainable and renewable energy sources, more than 30% of total global primary energy consumption is still provided by oil (CAPP, 2015). After Saudi Arabia and Venezuela, Canada has the third largest proven crude oil reserves in the world at 173 billion barrels and currently exports more than 3 million barrels/day to the Unites States alone (Government of Alberta, 2016). Over 97% of these oil reserves (168 billion barrels) are present as bitumen and underlie 142 000 km^2 of land within three large deposits – Athabasca, Peace River and Cold Lake – which comprise Alberta's oil sands region (Government of Alberta, 2016). Bitumen is a viscous mixture of hydrocarbons containing high levels of sulfur and nitrogen that typically occurs naturally at 10% per weight of oil sands (Government of Alberta, 2016). While raw bitumen extracted from oil sands has some uses, it is generally upgraded to synthetic crude oil before being transported and refined into fuels and other petroleum products.

Although development of the Alberta oil sands has been ongoing since the late 1960s, the region remains largely untapped with less than 10 billion of the 168 billion barrels of bitumen having been extracted as of 2015 (AER, 2015a). However, as conventional global oil sources continue to be depleted, non-conventional oil sources such as Alberta's oil sands are gaining increased international attention and interest from China and India – the countries with the fastest growing demand for crude oil (CAPP, 2015; Government of Alberta, 2016). Due to this growing interest and demand, Canada's total oil production is expected to increase from 3.7 million barrels/day in 2014 to 5.3 million barrels/day by 2030 (CAPP, 2015). As the source of most of Canada's crude oil reserves, Alberta oil sands development is expected to be the primary driver for this growth with projected bitumen production increasing from 2.2 million barrels/day in 2014 to nearly 4.0 million barrels/day by 2030 (CAPP, 2015). As oil sands development increases, so too will scrutiny of bitumen extraction processes and associated environmental impacts.

1.1.1   Bitumen extraction

There are currently two major extraction processes used within the Alberta oil sands: surface mining is used where bitumen deposits are present at a depth less than 75 m, while *in situ* drilling is used where deposits are located deeper than 75 m (Government of Alberta, 2016).

Surface mining is only possible in a small portion of the North Athabasca oil sands region which contains <20% of Alberta's 168 billion barrels; the remaining oil reserves must be extracted via *in situ* methods (CAPP, 2015; Government of Alberta, 2016). Although large-scale development of the Alberta oil sands began with surface mining techniques in the late 1960s and 1970s, *in situ* methods have become increasingly prevalent in recent years. As of 2014, surface mining activity produced nearly 1 million barrels/day while *in situ* produced 1.2 million barrels/day (58% of total production) (CAPP, 2015; Government of Alberta, 2016). To date there are 6 active surface mining operations within the Athabasca region and over 25 thermal *in situ* operations across the three Alberta oil sands regions (Government of Alberta, 2016).

It should be noted that the total oil sands reserves of 168 billion barrels only accounts for resources which can be feasibly extracted with current technology; the total reserves within the Alberta oil sands is estimated to be 1.7 to 2.5 trillion barrels (Government of Alberta, 2016). As new technology and extraction processes are developed, the total economically viable bitumen reserves will increase. Although several experimental *in situ* methods currently exist in various stages of development, most large-scale operations use thermal recovery – either steam assisted gravity drainage (SAGD) or cyclic steam simulation (CSS). In SAGD operations, two horizontal wells are drilled into a bitumen deposit – one at the top and one at the bottom. High pressure steam is injected into the top well which heats the bitumen, reducing its viscosity and causing it to flow into the lower well via gravity where it is pumped out to the surface. CSS uses a similar technique with a single well and cycles of steam injection and soaking to decrease the viscosity of bitumen, which is then pumped back out using the same well (Government of Alberta, 2016).

1.1.2 Environmental impacts of surface mining

Despite the growing prevalence of *in situ* bitumen extraction methods, surface mining operations remain active within the Athabasca deposit and have resulted in a number of legacy environmental issues over the past four decades. The human footprint from oil sands development within the mineable area of the Athabasca deposit is currently 21% (AEMERA, 2015) with a total industry footprint of 933 km^2 (AEP, 2015d). Compared to *in situ* operations which have minimal surface impact, mining operations result in a significant amount of cleared and disturbed land following the removal of overburden (the layer of vegetation, soil, gravel, and shale overlying mineable bitumen deposits) and the creation of pits and associated structures at the surface through

which oil sands ore is mined. As previously discussed, bitumen is naturally present at only 10% per weight of ore and must first be separated from the remaining sand, silt, clay, and residual water within the oil sands. Although a number of proprietary variations exist, all mine operators currently use some form of thermal liquid separation based on the original Clark hot water extraction process: 1) mined oil sand ore is crushed and mixed with hot water and caustic soda to reduce bitumen viscosity and allow it to separate from the sand (particle size >44 µm) which settles out and is removed as coarse tailings; 2) the resulting suspension is vigorously mixed and aerated causing the bitumen to coat air bubbles and float to the surface where it is removed as froth; and 3) the bitumen froth is treated with chemicals and solvents to further reduce viscosity and accelerate the removal of remaining impurities and is then skimmed off the top for further processing and upgrading (Dai and Chung, 1996). The residual mineral solids (clays, or 'fines', particle size <44 µm) and water removed during these froth treatment processes are referred to as tailings and are stored on site in tailings ponds either above ground behind dikes, or below ground in mined-out pits (ERCB, 2009; Government of Alberta, 2015). While most of the sand and larger particles settle out relatively quickly, the remaining fines take decades to settle leading to stratification within tailings ponds – coarse tailings on the bottom covered by a layer of fines in suspension, or 'fluid fine tailings' (FFT) (ERCB, 2009; Government of Alberta, 2015). After 40 years of mining activity and development, Alberta oil sands mine operators have accumulated nearly 1 trillion L of 'legacy' fluid tailings (AEP, 2015a) which are currently stored in tailings ponds covering over 88 km^2 of the Athabasca region (AEP, 2015b). Syncrude's Mildred Lake Mine is the largest surface mine operation within the Athabasca region accounting for 24% of the total industry footprint (222 km^2; AEP, 2015d), but storing over 40% of the total mining industry's fluid tailings (423 billion L; AEP, 2015a). Due to this disparity in facility size and tailings storage, Syncrude is currently leading the oil sands mining industry's first large-scale demonstration of an experimental tailings reclamation method which serves as the basis for this thesis (see Section 1.2).

In addition to the land impact caused by mining and tailings storage, oil sands mine operators also consume significant amounts of fresh water during the bitumen separation and extraction processes compared to that consumed at *in situ* operations. For example, Syncrude's Mildred Lake mine consumed 3.3 barrels of fresh water per barrel of oil produced in 2014 whereas Suncor's MacKay River – an *in situ* operation located to the west of Mildred Lake in the Athabasca region – consumed just 0.2 barrels of fresh water per barrel of oil produced the same year (AEP, 2015f).

Furthermore, in 2012 the Alberta oil sands mining industry consumed 176 billion L of fresh water (AEP, 2015e) of which 66% was diverted from the nearby Athabasca River (116 billion L; AEP, 2015c). A summary of all land and water impacts by the Alberta oil sands mining industry, as well as Syncrude's Mildred Lake Mine, is provided in Table 1.1.

Table 1.1 - Current land and water impacts of bitumen surface mining activity within the Athabasca oil sands region (Oil Sands Information Portal, Alberta Environment & Parks).

	Surface mining industry total	Syncrude's Mildred Lake Mine	Percentage of industry total
Total oil sands operation footprint (Cumulative land cleared or disturbed)	933 km^2 2013 (AEP, 2015d)	222 km^2 2013 (AEP, 2015d)	24%
Total tailings footprint (Includes dikes, beaches, DDAs, etc.)	220 km^2 2013 (AEP, 2015b)	*Not available*	-
Total tailings pond footprint (Wet tailings surface area only)	88 km^2 2013 (AEP, 2015b)	*Not available*	-
Total stored fluid tailings volume	976 billion L 2013 (AEP, 2015a)	423 billion L 2013 (AEP, 2015a)	43%
Annual fresh water use	176 billion L 2012 (AEP, 2015e)	50 billion L 2012 (AEP, 2015e)	28%
Annual Athabasca River water use	116 billion L 2012 (AEP, 2015c)	40 billion L 2012 (AEP, 2015c)	34%

In order to reduce water use and their impact on local watersheds, oil sands mine operators recycle as much as 80 to 90% of their fresh water causing residual bitumen, hydrocarbons, salts and trace metals released during the bitumen separation process to become increasingly concentrated in both the water and FFT (AEMERA, 2016). This water is referred to as oil sands process-affected water (OSPW) and is disposed of and stored in tailings ponds – often with FFT – when it cannot be recycled any further. The trace organics, metals, and salts present within OSPW and FFT are toxic and known to cause adverse effects on aquatic organisms (see Section 1.3). The toxicity of OSPW and constant accumulation of fluid tailings within the Alberta oil sands confounds current reclamation strategies and makes the development of robust monitoring and regulatory approaches for environmental management within the region essential.

1.1.3 Environmental monitoring of Alberta oil sands activity

In the past, environmental monitoring in the Alberta oil sands region was performed by both federal and provincial governments as well as industry, however, these activities were often performed independent of one another with limited communication of results among groups (Government of Alberta, 2012). In order to more effectively monitor and regulate oil sands development, the federal and provincial government implemented the three-year Joint Canada Alberta Implementation Plan for Oil Sands Monitoring (JOSM) in effect from April 1, 2012 through March 31, 2015 (Government of Alberta, 2012). The purpose of JOSM was to implement a single, government-led monitoring program, which focused on improving scientific integrity, accessibility and transparency of results from ambient environmental monitoring activities in the oil sands region (Government of Alberta, 2012; AEMERA, 2016).

On April 28, 2014 – during the implementation of JOSM – the Alberta provincial government introduced the *Protecting Alberta's Environment Act* which led to the establishment of the Alberta Environmental Monitoring, Evaluation and Reporting Agency (AEMERA), a provincial monitoring agency responsible for measuring, assessing, and communicating the status of Alberta's environment (AEMERA, 2015a). AEMERA took over the previous management of environmental monitoring activities from Alberta Environment and Sustainable Resource Development (AESRD, now Alberta Environment and Parks, AEP) and "[operated] at arm's length from government to separate the monitoring, evaluation and reporting of environmental data and information from environmental policy development and regulatory decision-making" (AEMERA, 2015a). As of July 1, 2016, AEMERA's core functions were returned to the provincial government with the creation of the AEP Environmental Monitoring and Science division which is now solely responsible for all environmental monitoring, evaluation and reporting activities in the Alberta oil sands region.

As previously discussed, many of the chemicals of concern associated with oil sands development are already naturally present in the environment, but can be concentrated via bitumen extraction and processing activities. Monitoring activities within the Alberta oil sands region cover air, surface water, groundwater, snow, sediment, and biota, and track environmental concentrations of metals, polycyclic aromatic hydrocarbons, particulate matter, naphthenic acids (NAs), and nutrients (AEMERA, 2016). Based on the results of these ongoing monitoring activities, there

exists consistent evidence of low-level cumulative changes to the ambient environment of the Alberta oil sands region (AEMERA, 2016). While these effects are generally low and of no immediate concern, AEMERA stressed that continued monitoring "is essential for identifying potential long-term impacts with sufficient time for corrective actions to be effective" (AEMERA, 2016). Corrective environmental actions arising from monitoring concerns within the Alberta oil sands area are based on a variety of provincial and industry-specific regulations and generally enforced by Alberta's Energy Regulator (AER).

1.1.4 Environmental regulation of Alberta oil sands activity

Historically the regulation of Alberta oil sands development and activity was enforced by two separate provincial organizations: the Alberta Energy Resources and Conservation Board (ERCB) regulated the technical aspects of the development of energy resources in the province while AESRD was responsible for their environmental management and regulation (AER, 2015a). However, in June 2013 the Alberta government introduced the *Responsible Energy Development Act* which dissolved the ERCB and created the AER in its place (AER, 2015a). The AER was granted autonomy and authority to operate at 'arm's length' from the provincial government, while also taking on the environmental regulatory functions of AESRD (AER, 2015a). The AER is now the sole regulator for all hydrocarbon resources (oil, oil sands, natural gas, and coal) in the province of Alberta and is responsible for enforcing both federal and provincial legislation governing the development of these resources.

In March of 2014, the AER was granted additional authority to enforce Alberta's *Environmental Protection and Enhancement Act* (EPEA) which governs reclamation and remediation activities within the province. Under the EPEA, companies operating within the Alberta oil sands are legally required to return disturbed land to a functionally equivalent capacity upon completion of the project; the land must be able to support the uses that existed before oil sands development began (AER, 2015a). As a result, the AER is responsible for evaluating reclamation plans submitted by operators for new oil sands projects, as well as oversight and certification of existing land in various stages of reclamation. It should be noted that the AER enforces several additional policies and legislation that govern oil sands development (e.g. air quality, land and water use, etc.), however, only those pertaining to the regulation and reclamation of tailings resulting from oil sands mining activity will be discussed herein.

1.1.5 Environmental regulation of oil sands surface mine tailings

In 2009, Alberta's ERCB introduced a new directive for Tailings Performance Criteria and Requirements for Oil Sands Mining Schemes (Directive 074) in an attempt to more effectively manage and regulate the growing land impact of tailings arising from mining activity in the Athabasca oil sands region. Directive 074 enforced three major criteria relating to fines:

> Increased capture of fines (50% of total production) from oil sands mining operations; the formation and management of dedicated disposal areas (DDAs) for the deposition of captured fines; and requirements for DDAs to be ready for reclamation within 5 years following active deposition and have a trafficable surface layer with a minimum undrained shear strength of 10 kPa.
> - ERCB, 2009

After six years of limited success, Directive 074 was indefinitely suspended with the introduction of the Government of Alberta's new Tailings Management Framework for the Mineable Athabasca Oil Sands (TMF) in March of 2015 (Government of Alberta, 2015). The TMF is a more robust management plan for long-term fluid tailings accumulation and reclamation that complements existing provincial legislature (e.g. the EPEA) on the governance of oil sands mining activity (Government of Alberta, 2015). The goal of the TMF is to manage the long-term liability and environmental risk of fluid tailings by lowering volumes and minimizing accumulation while focusing on cumulative effects and the progressive treatment of both new and legacy tailings over the full lifecycle of mining operations (Government of Alberta, 2015):

> Fluid tailings accumulation is minimized by ensuring that fluid tailings are treated and reclaimed progressively during the life of a project and all fluid tailings associated with a project are ready-to-reclaim within 10 years of the end of mine life of that project. The objective will be achieved while balancing environmental, social and economic needs.
> - TMF (Government of Alberta, 2015)

In order to achieve this objective, the TMF provides the following new elements and indicators to the existing management framework for Athabasca oil sands mining operations:

A trigger and limit on the volume of fluid tailings accumulation for each oil sands mining project; a trigger on the deviation of fluid tailings volume growth from each oil sands mining project's approved fluid tailings volume profile; a management response when thresholds (i.e. triggers and limits) are exceeded; a target of all fluid tailings in a ready-to-reclaim state within 10 years of end of mine life for all oil sands mining projects; and a requirement to have all legacy tailings in a ready-to-reclaim state at the end of mine life.

- TMF (Government of Alberta, 2015)

While the TMF provides the framework, it is still the AER's responsibility to establish and enforce the site/project-specific triggers, thresholds, limits and end of mine life target for each oil sands mining operation. The initial phase of the TMF implementation will be focused on slowing and then stabilizing the accumulation of fluid tailings at oil sands mining operations, until ultimately a point at which more tailings are being reclaimed than being produced is reached; this process is expected to take more than 40 years (see Figure 1.1) (Government of Alberta, 2015).
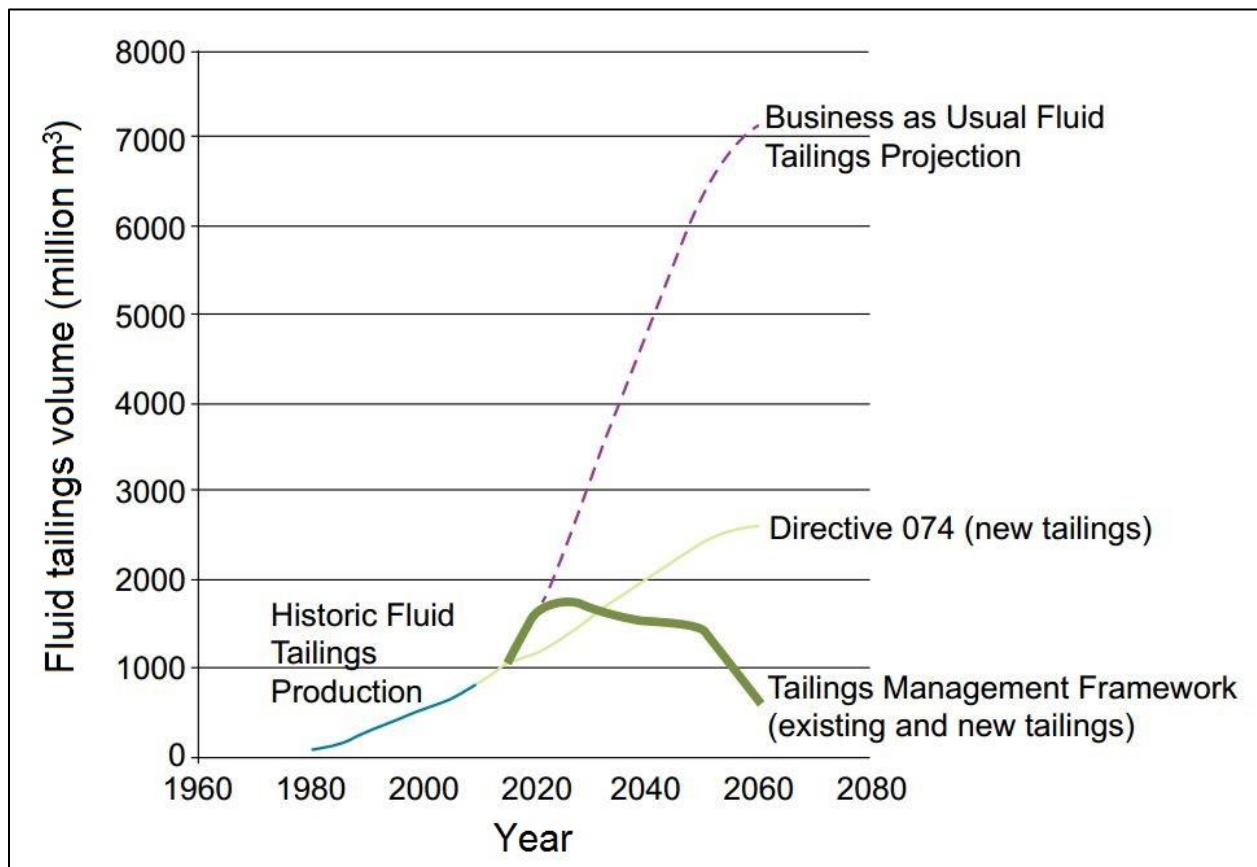
Figure 1.1 – Projected impact of TMF implementation on fluid tailings accumulation in the Athabasca mineable oil sands region over time (modified from Government of Alberta, 2015).

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Despite the advances in oil sands fluid tailings management regulation in the past five years, there remains a distinct lack of oil sands-specific regulations governing the management of waste water generated from oil sands mining operations and activities. The entire TMF only briefly discusses water management and states:

> Where preferred approaches are found to be insufficient to manage all of the [tailings]-liberated [OSPW] generated and stored on site, regulatory applications that seek the return of new wastewater streams to the environment may be considered…subject to supplemental requirements, which may include enhanced wastewater characterization (i.e., physical, chemical and toxicological evaluation), additional criteria and guidelines for acceptable quality of release, wastewater treatment performance standards, more comprehensive in-stream modelling, and more stringent environmental effects monitoring requirements.
>   – TMF (Government of Alberta, 2015)

As a result, many oil sands mine operators will aim to solve their wastewater storage problems by seeking to release their OSPW to the environment (in this case the Athabasca River). If this is the case, the TMF acknowledges that a significant number of supplementary requirements will need to be adhered to and enforced beyond the existing provincial and federal water use regulations. Following the initial implementation of the TMF, the Alberta government and AER will develop these supplemental requirements and criteria necessary for regulating oil sands tailings water release (Government of Alberta, 2015).

1.1.6 Current reclamation efforts

As of 2014, only 9.5% of the oil sands mining industry's 933 km^2 footprint (88.4 km^2) is in some stage of reclamation, and only 1 km^2 of land has been certified as reclaimed (AEP, 2015d). Despite having the single largest mine site footprint and accumulation of fluid tailings in the Athabasca mineable oil sands, Syncrude's Mildred Lake mine is currently the industry leader in land reclamation. Syncrude is responsible for 54% of the total industry reclaimed land with 21.4% of its 222 km^2 mine site (47.5 km^2) in various stages of reclamation, including 11 km^2 of permanent wetlands and aquatics, and 21.5 km^2 of permanent terrestrial reclaimed land (AEP, 2015d). Even though the TMF was designed to more effectively manage the reclamation of new and legacy tailings, it is important to note that it does not actually provide guidance on how this reclamation should be performed. As previously discussed, while many tailings treatment methods have been used by oil sands mine operators in the past, they have had limited success in reducing the total

volumes and rate of accumulation of fluid tailings (Government of Alberta, 2015). Although several experimental treatment methods currently exist, most are small-scale and still in development or field trial stages; the establishment of performance measures for tailings technologies and reclamation criteria are key policies highlighted for future development (Government of Alberta, 2015).

For the time being, the TMF allows that – contrary to the EPEA requirements – the land use following reclamation of oil sands mining activities may be different than its pre-development use (e.g. formation of artificial lakes where previously there were none), as long as the following criteria are still met:

> Tailings deposits are integrated into the closure landscapes that have functional drainage systems with acceptable water quality in shallow soil and runoff; tailings landforms are geotechnically stable and have a natural appearance consistent with the region; tailings structures will remain stable under a natural disturbance regime typical for their location; regional groundwater criteria, as established outside of this Framework, are adhered to; reclaimed landforms on tailings deposits have soil and soil process development consistent with boreal forest soils; and/or ecological communities on reclaimed tailings have functional and structural aspects consistent with the regional boreal forest.
> – TMF (Government of Alberta, 2015)

This amendment to the provincial EPEA reclamation requirements likely arises due to the increasing popularity and promise of water-capped tailings technology and end pit lakes among oil sands mine operators as a method for the long-term reclamation and storage of tailings.

1.2 Water-capped tailings and end pit lakes in oil sands reclamation

As previously discussed, FFT are a liquid suspension of oil sands fines in water which can be further classified and defined based on solids content and sands-to-fines ratio. FFT deposited into tailings ponds are generally >2% solids per weight, however, initial dewatering and settling of sand and coarse silt occurs rapidly over a few days leading to the formation of intermediary thin fine tailings with a solids content of 15 to 30% (COSIA, 2012). As FFT dewater, a liquid interface referred to as the 'mudline' begins to form between the liberated water above and the fines in suspension below. After 1 to 2 years, thin fine tailings dewater and densify further to mature fine tailings (MFT) with a solids content >30%. Further dewatering of MFT occurs significantly slower and may take centuries before a solid clay layer forms (COSIA, 2012). Several process methods based on coagulation, flocculation, and centrifugation can be used to accelerate FFT dewatering, however, MFT remain relatively recalcitrant to further densification and are responsible for many of the legacy problems faced by the oil sands mining industry today.

Although a number of new experimental reclamation techniques exist in varying stages of development, the oil sands mining industry currently employs four main commercial-scale methods for the long term storage and treatment of FFT: 1) *Thin fines-dominated deposits* rely on natural environmental processes such as evaporation and freeze-thaw cycles for dewatering; 2) *Deep fines-dominated deposits* instead rely on self-weight consolidation for further dewatering; 3) *Fines-enriched sand deposits* also use self-weight consolidation though dewatering occurs more rapidly due to higher permeability than fines-dominated deposits; and 4) *Water-capped fines deposits (water-capped tailings, WCT)* are composed of FFT stored at the bottom of mined-out bitumen ore pits (end pits) and are capped with a surface layer of water (COSIA, 2012). Unlike the other FFT management methods, the performance of WCT is assessed on surface water quality.

The WCT method has many distinct advantages over other reclamation techniques in that it solves the problems of both tailings accumulation and storage, as well as the land impact of end pits by using readily available waste products (FFT and OSPW) as low cost and energy-efficient backfill materials (COSIA, 2012). Water-capped tailings implementation generally results in the formation of structures which resemble artificial lakes, thus they are often referred to as end pit lakes (EPLs). The establishment of littoral and wetland habitats surrounding EPLs can further accelerate reclamation of surface water quality as well as ecological development (COSIA, 2012).

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Despite the advantages of WCT and EPLs, several key concerns still exist: 1) the stability of the pond layers (mixing/resuspension of overlying water and underlying FFT); 2) interactions with groundwater and the surrounding watershed; 3) chemical flux across the water-FFT interface (mudline); and 4) the toxicity of surface water and potential impacts on ecological development (COSIA, 2012). As one of the biggest proponents for WCT and EPLs within the Alberta oil sands, Syncrude has been performing small-scale field and laboratory testing for over two decades, however, certain aspects of WCT and EPL implementation can only be studied and confirmed on a much larger scale (COSIA, 2012). To address these concerns, Syncrude initiated the Base Mine Lake (BML) experiment in December of 2012 – the first large-scale demonstration of WCT EPLs.

1.2.1 The Base Mine Lake demonstration

Base Mine Lake is an 8 km^2 EPL located on Syncrude's Mildred Lake mine site that contains 186 billion L of FFT capped with approximately 52 billion L of mixed fresh water and OSPW (Dompierre *et al.*, 2016). In order to create BML, Syncrude filled an old mined-out pit – formerly known as West In-Pit (WIP) – with FFT from the Mildred Lake Settling Basin over the course of 17 years (1995 to 2012) and then capped it with a mixture of OSPW and fresh water until December 2012 at which point all FFT and OSPW inputs ceased and BML was created. The purpose of the BML experiment is to demonstrate the WCT method on a much larger scale in order to improve industry understanding of EPL design and operation – specifically the time required to reach acceptable surface water quality and development into a functional aquatic ecosystem (Syncrude, 2013). Over time, liberated pore water will increase the volume of the overlying water as the FFT densifies and the mudline level will decrease. As a result of this process, the surface water depth of BML is expected to increase from 5 m to over 20 m, while the depth of FFT at the bottom of the pit is expected to densify from 50 m to less than 35 m (see Figure 1.2; Syncrude, 2013). As reclamation progresses and surface water quality improves, BML's initial 4.5 km^2 watershed will be connected to and incorporated into the surrounding landscape; the final BML watershed is expected to cover 430 km^2 (Syncrude, 2013).
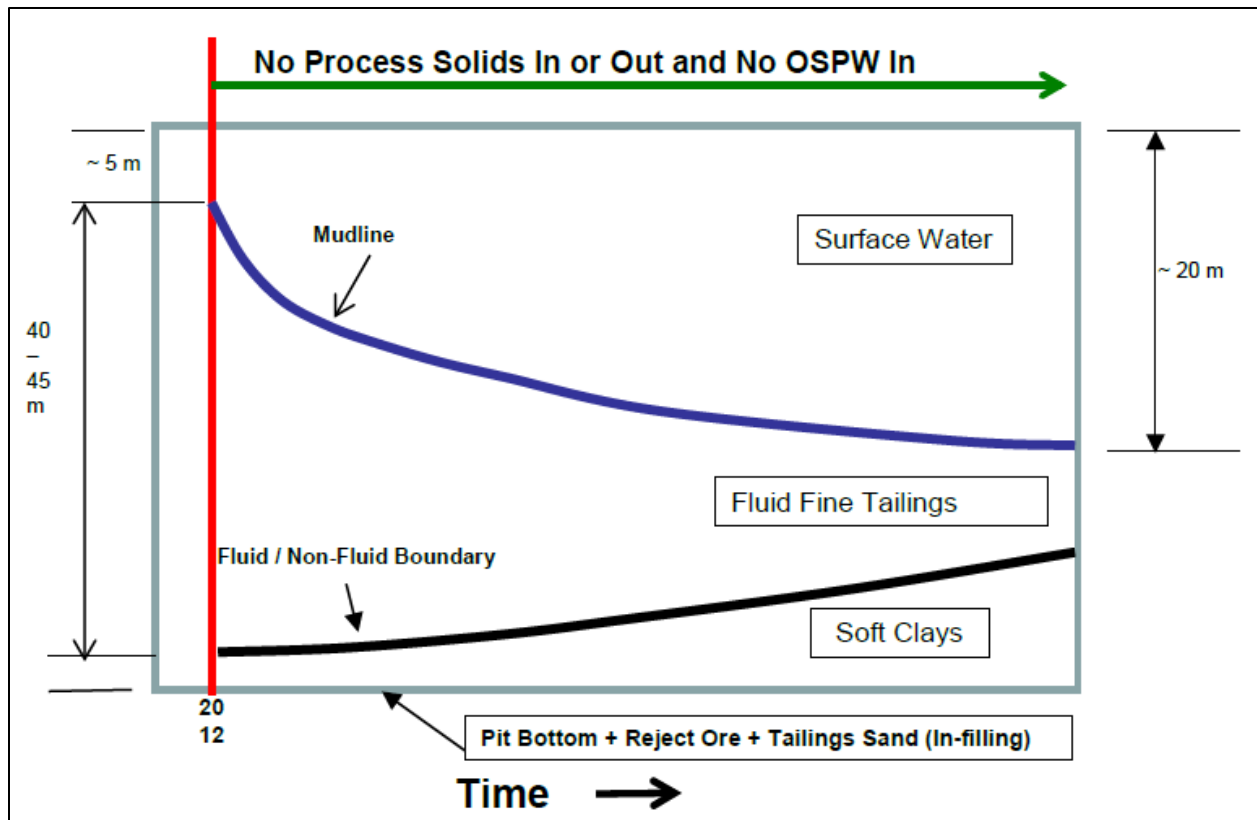
Figure 1.2 - Timeline of FFT densification and dewatering within BML. As FFT densifies the mudline height decreases and liberated pore water causes an increase in the surface water volume (Syncrude, 2013).

Syncrude acknowledges that it will take decades for the BML watershed to reach full maturity and has therefore divided the implementation and monitoring plan into three phases based on projected physical, chemical, and biological characteristics: 1) *Start-Up Phase* (1 to 4 years, 2013 to 2016) – period of relatively rapid and large changes in physical and chemical processes, biological activity is not expected due to poor quality and toxicity of surface water; 2) *Stabilization Phase* (5 to 10 years, 2017 to 2022) – rate of change slows as physical and chemical processes begin to reach equilibrium, lower trophic level organisms (algae and zooplankton) begin to populate the aquatic ecosystem; 3) *Maturation Phase* (15 to 25+ years, ~2025 to 2035+) – physical and chemical properties have reached equilibrium, significant ecological development occurs with the appearance of higher trophic levels (aquatic vertebrates, e.g. fish).

1.2.2 Base Mine Lake surface water quality and chemical performance monitoring

As the chemical and physical processes within BML begin to reach equilibrium (during the Stabilization and Maturation Phases), Syncrude will place increasing focus on monitoring biological development. In the Start-Up Phase, however, current monitoring activities are focused on chemical performance, as the ability to track changes in surface water quality is essential to understanding the initial function of BML and predicting future changes (Syncrude, 2013). The chemical parameters measured within the BML monitoring plan are summarized in Table 1.2.

Table 1.2 – Chemical parameters measured as part of Syncrude's Base Mine Lake surface water chemical performance monitoring plan (Syncrude, 2013).

	Measured parameters
Water quality	Temperature, pH, dissolved oxygen, alkalinity, hardness, salinity/conductivity/total dissolved solids, total suspended solids/turbidity, chemical oxygen demand
Major ions and nutrients	Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, S^{2-}, F^-, NH_3, NO_3^-, NO_2^-, total Kjeldahl nitrogen, PO_4^{3-}, total and dissolved P
Biological	Biological oxygen demand, chlorophyll a, phenophytin a
Total and dissolved metals	Al, Sb, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Li, Mn, Hg, Mo, Ni, Se, Ag, Tl, Sn, Ti, U, V, Zn
Organics	Total organic carbon, dissolved organic carbon, naphthenic acids, polycyclic aromatic hydrocarbons, benzene toluene ethylbenzene and xylene, total phenolics, total recoverable hydrocarbons

The desired outcome of the chemical performance monitoring plan is the reduction of acute and chronic toxicity, as well as the concentrations of chemicals of concern in BML surface water. Once the Stabilization Phase has been reached, Syncrude will use the monitoring data to determine chemical and mass balances in order to develop models that describe the future trajectories for the key chemicals of concern. The ultimate goal will be for surface water quality and toxicity to reach acceptable levels in the Maturation Phase necessary to support a healthy aquatic ecosystem and for potential release to the environment (Syncrude, 2013).

1.3 Aquatic toxicity and chemistry of oil sands tailings

The aquatic toxicity of whole OSPW has been well-characterized over the past decade with numerous studies investigating both *in vitro* and *in vivo* effects in fish and invertebrates including reduced survival (He *et al.*, 2011; Anderson *et al.*, 2012a; McQueen *et al.*, 2017) and growth (Anderson *et al.*, 2012a; Wiseman *et al.*, 2013a); impaired reproduction (Kavanagh *et al.*, 2011; McQueen, *et al.*, 2017); altered endocrine function (He *et al.*, 2010; He *et al.*, 2011; Kavanagh *et al.*, 2011; He *et al.*, 2012b; van den Heuvel *et al.*, 2012; Wiseman *et al.*, 2013a); effects on development such as lifecycle timing and success (Anderson *et* al., 2012a; He *et al.*, 2012a) and malformation (He *et al.*, 2012a); and effects on immune response such as altered lymphocyte and antibody production, and histological changes in lymphatic tissue size and condition (McNeil *et al.*, 2012; Leclair *et al.*, 2013). In most cases the exact mechanisms for these effects remain unclear, although many have recently been attributed to oxidative stress and apoptosis (He *et al.*, 2012a; Wiseman *et at*., 2013a; Wiseman *et al.*, 2013b). It should be noted that OSPW does not define an exact chemical or group of compounds, but rather is a catch-all term used to describe bitumen processing wastewater. Therefore, the exact chemical composition and toxicity of OSPW can vary greatly depending on the process method and location where it is produced, as well as its age and treatment prior to sampling. Although BML research is relatively new, several of these toxicity tests were conducted using fresh OSPW from WIP and thus the chemical profile of BML should be similar, albeit aged and diluted.

1.3.1 Organic constituents

In many aquatic tests using whole OSPW, observed toxicity has been mitigated or eliminated following the reduction or removal of the dissolved organic fraction via physical and chemical treatments such as ozonation (He *et al.*, 2010; He *et al.*, 2011; He *et al.*, 2012a; Anderson *et* al., 2012a; Wiseman *et al.*, 2013b; McQueen *et al.*, 2017) and activated charcoal (Anderson *et al.*, 2012a; He *et al.*, 2012a; McQueen *et al.*, 2017). Thus, the dissolved organic fraction of OSPW is now generally accepted as being the primary driver of aquatic toxicity. Historically this has been attributed to the high concentrations of naphthenic acids (NAs) found in OSPW, a group of cyclic carboxylic acids described by the general formula $C_nH_{2n+z}O_2$, where n is the number of carbon atoms and z is the degree of unsaturation associated with ring structures or double bonds. Several toxicity studies using extracted NA fractions have observed the same effects as those seen in whole OSPW including reduced survival (Morandi *et al.*, 2015; Alharbi *et al.*, 2016a); changes in

lifecycle timing and increased malformation (Alharbi *et al*., 2016b); reduced reproduction (Kavanagh *et al*., 2012); altered endocrine function (Leclair *et al*., 2015); and impaired immune response (MacDonald *et al*., 2013). However, recent studies have shown that NAs (O2$^-$ compounds) comprise only a portion of the total organic acid fraction which also includes O$^-$ and SO2$^-$ compounds, and that polar neutral compounds such as the O2$^+$, NO$^+$, and SO$^+$ classes also contribute to the toxicity of OSPW (Morandi *et al*., 2015; Morandi *et al*., 2016; Alharbi *et al*., 2016a). These chemical classes are largely uncharacterized and their contribution to the effects of chronic exposure to OSPW remains unclear.

It is important to note that although the toxicity of OSPW has also been shown to be mitigated from aging due to natural degradation and transformation of these compounds (Anderson *et al*., 2012a; Wiseman *et al*., 2013a), several of the previously discussed studies were conducted using aged OSPW taken from tailings ponds. In a year-long wetland microcosm study, Toor *et al*. (2013a) showed that although NA concentrations naturally decreased over time leading to reduced acute toxicity, some residual fraction of NAs were recalcitrant and associated with persistent chronic toxicity. It was discovered that natural in situ degradation was dependent upon structure, with small (C11-16) and slightly unsaturated (z = -2, -4) NAs degrading significantly faster (half-life of 5 to 7 months) than large (C17-20) and highly unsaturated (z = -6 to -12) NAs (half-life up to one year) (Toor *et al*., 2013b). Thus, the use of natural biodegradation processes within reclamation tailings ponds will likely be insufficient to completely remove dissolved organic-associated toxicity from OSPW in a timely manner (Anderson *et al*., 2012b; Toor *et al*., 2013b).

1.3.2 Inorganic constituents

Since the dissolved organic fraction of OSPW has been found to be the primary driver of aquatic toxicity, toxicological characterization of the inorganic components – specifically major ions and metals – has been largely ignored. Furthermore, full chemical characterization of OSPW, including dissolved metal concentrations, is almost never reported. This is likely because most studies have focused on fish (e.g. *Pimephales promelas*) and invertebrates (e.g. *Chironomus dilutus*) which are sensitive to NAs, but generally tolerant of salinity and metals at the concentrations found within OSPW (see Chapter 2). However, as previously discussed, vertebrates such as fish will be the last organisms to appear in EPLs such as BML as they cannot be sustained

without first establishing a functioning zooplankton community – organisms which are often much more sensitive to salinity and metals.

Most OSPW toxicity studies with fish acknowledge elevated concentrations of major ions such as sodium (Na^+), chloride (Cl^-), sulfate (SO_4^{2-}), and bicarbonate (HCO_3^-), but rarely discuss their influence on observed effects. The only studies which have specifically examined the effects of OSPW salinity were solely focused on algae and plants (Leung *et al*., 2001; Leung *et al*., 2003; Trites and Bayley, 2009; Pouliot *et al*., 2013). It should be noted that in addition to any direct effects on toxicity, the elevated salinity will also indirectly affect the activity of the other components. The influence of salinity on metal toxicity is relatively well characterized and is based on changes in speciation and complexation, as well as altered uptake due to competition (see Chapter 2). Conversely, the influence of salinity on the toxicity of the organic fraction within OSPW has only briefly been investigated. Nero *et al*. (2006) found that co-exposure of an NA extract with 1 g/L of Na_2SO_4 significantly reduced mortality, but increased inflammation and gill deterioration in yellow perch. The exact mechanism for this response was unclear, but reduced mortality was attributed to decreased NA uptake due to reduced gill surface area resulting from the inflammation. Similarly, Kavanagh *et al*. (2012) found that co-exposure of an NA extract with 700 mg/L $NaHCO_3$ significantly reduced embryo and larval mortality in fathead minnow, and ameliorated observed decreases in reproduction and testosterone levels. These effects were also attributed to reduced NA uptake. Interestingly, no effects were observed when NaCl or Na_2SO_4 were used instead of $NaHCO_3$. This may have been partly due to altered pH, however, the exact reasons for this difference are unknown and have not been further investigated. An antagonistic interaction between salinity and NAs has in fact previously been observed to decrease the toxicity of OSPW by both Turcotte *et al*. (2009) and Kennedy (2012), however, these studies were preliminary and never published in the peer-reviewed literature.

As for the specific toxicity of OSPW-associated metals, the results are even more limited. In a critical review of oil sands water toxicity (Natural Resources Canada, 2010), Al, As, B, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Se, and V were all identified as being of interest based on Canadian Water Quality Guidelines (CWQGs) for the long-term protection of freshwater aquatic life, and concentrations found within OSPW as well as sediments and surface waters within the oil sands region. However, until recently, the only studies which had assessed the toxicity of oil sands-

associated metals had been derived from coke leachate, a solid by-product of bitumen upgrading, not OSPW. Although the metals identified as being of concern fall within the same list (Al, Mn, Mo, Ni, and V), the concentrations within coke leachate are much higher than those quantified in OSPW (Puttaswamy *et al*., 2010; Puttaswamy *et al*., 2011). It is worth nothing that although the concentrations and sources may be different, results of studies which investigate the influence of salinity on the toxicity of metals from coke leachates (Puttaswamy *et al*., 2012) can still be used to infer effects that may occur within OSPW. Recently, McQueen *et al*. (2017) conducted an experiment with whole OSPW containing elevated Na^+, Cl^-, SO_4^{2-}, and HCO_3^- concentrations, and identified Al, B, Cu, Fe, Ni, Se, and Zn all as metals of potential concern based on comparisons to CWQGs and toxicity data from *Ceriodaphnia dubia*. However, while toxicity testing of the whole OSPW resulted in significant decreases in *C. dubia* survival and reproduction, treatment of the OSPW with EDTA – intended to chelate metal cations – had no effect; conversely, both ozonation and activated charcoal treatments eliminated all observed toxicity. This suggests that, as previously discussed, the observed toxicity was due entirely to the dissolved organic fraction while the salinity and dissolved metals had no effect – even on a sensitive zooplankton species.

Even though the dissolved organic fraction within OSPW may be the primary driver for toxicity observed in these relatively short-term studies, in extremely long reclamation strategies in which OSPW will age for decades (such as EPLs), the contribution of the organic fraction is expected to slowly diminish over time due to natural aging and biodegradation processes. The inorganic constituents, however, will never be naturally removed and will therefore continue to persist in these systems indefinitely. This is of particular concern for BML, as the high concentrations of major ions and dissolved metals within surface water are expected to pose some toxicological risk to freshwater organisms, especially those involved in the initial colonization and ecological succession of aquatic ecosystems.

1.4   Project rationale and research objectives

Surface mining activity within the Alberta oil sands region over the past five decades has resulted in the accumulation of a significant volume of tailings and process water that is currently stored on site in vast tailings ponds. Due to the elevated concentrations of dissolved organics, salts, and metals, current reclamation efforts are extremely slow which is concerning as oil sands development continues to increase. Oil sands mine operators such as Syncrude have proposed water-capped tailings and end-pit lakes as a method for reclamation, however, their long-term efficacy remains unclear. The purpose of this thesis was to evaluate Syncrude's Base Mine Lake experiment in order to more adequately assess the sustainability and environmental risks of water-capped tailings and end pit lakes for the reclamation of Alberta oil sands surface mining activity. This is crucial, as the results of the BML demonstration will have a profound impact on future oil sands development within Canada.

The specific research objectives were as follows:

1) a. Identify inorganic constituents of concern within BML surface water (specifically metals and major ions) and track annual changes in their concentrations over the course of three years (2014 to 2016) to establish trends and predict future concentrations [Chapter 2]

    b. Evaluate how annual changes in surface water chemistry (specifically metals and major ions) affect toxicity and potential risk to aquatic organisms to estimate the time required to reach acceptable surface water quality [Chapter 2]

2) Characterize the toxicity of BML surface water to apical endpoints of freshwater species representative of those involved in the colonization and succession of aquatic ecosystems
    a. Assess the survival, growth, and development of the invertebrate *Chironomus dilutus* exposed to 2014 BML surface water for most of its lifecycle (30 to 40 d) [Chapter 3]
    b. Assess the survival and reproduction of the sensitive zooplankton *Ceriodaphnia dubia* exposed to both 2014 and 2015 BML surface water samples for 8 d [Chapter 4]

CHAPTER 2 – EFFECTS OF ANNUAL CHANGES IN BASE MINE LAKE SURFACE WATER CHEMISTRY ON TOXICOLOGICAL RISK TO AN EARLY AQUATIC ECOSYSTEM COLONIZER, *CERIODAPHNIA DUBIA*

2.1   Introduction

End pit lakes (EPLs) containing water-capped tailings (WCT) have been proposed as a method for the long-term reclamation of oil sands surface mining activity. By incorporating fluid fine tailings (FFT) and oil sands process-affected water (OSPW) directly into the reclamation landscape, oil sands operators can use natural physical and geochemical processes to aid in remediation. Unfortunately, the FFT and OSPW contained within these EPLs contain high concentrations of dissolved organic, metal, and salt compounds which reduce surface water quality and impede the colonization and ecological succession of EPLs into functional aquatic ecosystems. To address these concerns, Syncrude developed Base Mine Lake (BML), the Alberta oil sands first large-scale demonstration of the WCT and EPL method.

Surface water was sampled from BML and characterized over the course of three years (2014 to 2016) to identify key constituents of concern and track how the concentrations within surface water changed over time. These concentrations were also compared to known benchmarks, such as natural background Athabasca River water (ARW) concentrations, Canadian water quality guidelines (CWQGs) for the long-term protection of freshwater aquatic life, and chronic toxicity values to freshwater species representative of those involved in the colonization and succession of new aquatic ecosystems. The ultimate goal was to use these trends in surface water chemistry and toxicological risk to predict the rate and time required for BML surface water quality to reach an acceptable level necessary to support a functional aquatic ecosystem.

While changes in the dissolved organic fraction over time will be discussed briefly, detailed characterization and toxicological assessment of these compounds was performed by other groups within the University of Saskatchewan Toxicology Centre (Saskatoon, SK) and the University of Alberta (Edmonton, AB). As a result, this assessment largely focused on the less-studied inorganic constituents, specifically salinity (major ions) and dissolved metals.

2.2 Methods

2.2.1 Sample collection

Surface water samples were collected from the top 2 m of BML by Syncrude in October 2014, August 2015, and August 2016 (five 20-L buckets, two 1000-L tanks, and one 20-L bucket, respectively) and shipped to the University of Saskatchewan Toxicology Centre (Saskatoon, SK). The 2014 and 2016 sample buckets were held in cold storage (4°C) after their arrival, however, the 2015 sample tanks were stored at room temperature in the loading bay due to their size. To preserve the water and keep analysis consistent, subsamples of the 2015 surface water were taken from the tanks and stored in 20 L buckets at 4°C alongside the other samples. While the 2014 and 2015 samples were of sufficient volumes to run toxicity tests, the single 2016 sample was used solely for chemical analysis to strengthen predicted trends in BML surface water chemistry.

2.2.2 Characterization of surface water chemistry

Chemical analyses were performed based on those outlined in Syncrude's own BML chemical performance monitoring plan, with some modifications (see Tables 1.2 and 2.1). Conventional water quality analyses (pH, Eh, alkalinity, total hardness, conductivity, and turbidity) were performed in-house at the University of Saskatchewan Toxicology Centre. Conductivity and turbidity were used as preliminary indicators of total dissolved solids (TDS) and total suspended solids (TSS), respectively. Redox potential (Eh) was also measured to assist in modeling metal speciation and complexation.

Table 2.1 – Chemical parameters measured in BML surface water samples

	Measured parameters
Water quality	pH, Eh, alkalinity, hardness, ammonia, conductivity/total dissolved solids, turbidity/total suspended solids
Major ions	Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, HCO_3^-/CO_3^{2-}, NO_3^-, NO_2^-
Dissolved metals	Al, Sb, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Se, Ag, Tl, Sn, Ti, U, V, Zn
Organics	Dissolved organic carbon

2.2.2.1 Salinity and major ions

Although salinity and TDS cannot be directly measured in-house, conductivity can be used as a site-specific indicator of TDS; both increase proportionally, however, the proportion itself is dependent on location and ion content. Based on results from both 2014 and 2015 samples, the TDS (mg/L) of BML surface water is approximately equal to conductivity (µS/cm) x 0.57. Salinity can also be estimated by the sum of all major ions in the sample (e.g. Na^+, Cl^-, SO_4^{2-}, and HCO_3^-), however, this method proved to be less accurate than the conductivity conversion for BML surface water samples. Although direct carbonate measurement was not included in Syncrude's original chemical performance monitoring plan, HCO_3^- was found to be the single most concentrated ion in all surface water samples (~700 mg/L) and has a significant impact on pH and metal speciation.

All BML water samples for specific major ion analysis were collected in duplicate, syringe-filtered through 0.45 µm polyethersulfone membranes into 8 mL HDPE Nalgene bottles, and stored at 4°C. All multi-ion analyses were outsourced and performed by Maxxam Analytics (Calgary, AB): Na, K, Ca, and Mg via inductively coupled plasma optical emission spectrometry (ICP-OES); Cl^- and SO_4^{2-} via automated colourimetry; NO_3^- and NO_2^- via ion chromatography (IC); HCO_3^-, CO_3^{2-}, and OH^- via titration. Although Na, K, Ca, and Mg are reported as cations throughout this thesis, ICP-OES only detects the total concentration within the water sample, not the specific ionic state. All analyses were performed with matrix spikes, spiked blanks, and method blanks; percent recovery for all analytes fell within quality control limits (80 to 120%).

2.2.2.2 Dissolved metals

All BML water samples (5 mL) for dissolved metals analysis were collected in duplicate, syringe-filtered through 0.45 µm polyethersulfone membrane filters into 8 mL HDPE Nalgene bottles, acidified to a pH of ≤2 with 145 µL of high purity nitric acid (HNO_3), and stored at 4°C. All multi-element analyses were performed in-house via inductively coupled plasma mass spectrometry (ICP-MS) using an 8800 Triple Quadrupole ICP-MS (Agilent Technologies, Santa Clara, CA). To verify instrumental accuracy, all BML samples were run in tandem with method blanks as well as a natural water standard reference material (1640a, National Institute of Standards & Technology, Gaithersburg, MD) and a river water standard reference material (SLRS-5, National Research Council, Ottawa, ON).

As previously discussed, detailed characterization of the dissolved organic fraction within BML surface water was performed by a separate group at the University of Saskatchewan Toxicology Centre, as well as at the University of Alberta. However, it was still necessary to quantify the dissolved organic carbon (DOC) content within the BML surface water samples as this organic fraction affects the complexation and bioavailability of certain dissolved metals of concern and is therefore necessary for accurate modeling. Similar to the major ion analyses, BML samples were outsourced to Maxxam Analytics for DOC analysis performed via automated colourimetry. It should be noted that DOC analysis for all samples was not conducted until 2016. Thus, although the 2016 sample was fresh, the 2014 and 2015 samples were two- and one-year-old, respectively, at the time of analysis. While all samples should have been relatively well preserved in the dark at 4°C, it is possible that the organic fractions would have degraded to some degree during storage and therefore the actual DOC concentrations in the 2014 and 2015 surface water may have been different at the original time of sampling.

Although it falls outside the scope of this thesis, the naphthenic acid/acid-extractable fraction and profile were characterized and quantified for the original 2014 BML surface water sample using a liquid-liquid extraction method and performed in-house at the University of Saskatchewan Toxicology Centre using Orbitrap high-resolution mass spectrometry and high-performance liquid chromatography (see Appendix A for more information).

2.2.3 Speciation and complexation modeling

In addition to chemical characterization, aqueous speciation and complexation of quantified dissolved metals was modeled using Visual MINTEQ software, version 3.1 (KTH Royal Institute of Technology, Stockholm, Sweden) and default thermodynamic values from the National Institute of Standards and Technology (NIST) database. Modeling was performed using water quality, dissolved metal, and major ion concentrations quantified in Section 2.2.2 for the most recent 2016 sample. The oxidation states of redox-active metals within BML surface water (e.g. As and Cr) were estimated based on measured pH and Eh values, and equilibria reactions and standard electrode potentials summarized by Evans (2012). One of the challenges in assessing BML geochemistry was that pH gradually drifted once water samples had been removed from the lake; *in situ* pH values were approximately 8 while sampled water drifted to 9 if left open to the

atmosphere. Metal speciation and complexation were therefore modeled at pH values of 8.0, 8.5, and 9.0. Furthermore, as a result of this drift, bicarbonate/carbonate equilibrium within BML water samples also shifts depending on the pH at which it is measured. Thus, carbonate complexation was modeled using total alkalinity (as mg $CaCO_3$/L) instead of the individual measured concentrations of HCO_3^- and CO_3^{2-}. The final challenge was how to handle DOC and organic complexation within the model. For the sake of simplicity, organic complexation was modeled using the default Gaussian DOM (dissolved organic matter) model parameters. Even though the specific composition of DOC within BML surface water is extremely complex, the total dissolved organic fraction is comprised of naphthenic acids which structurally resemble some natural humic/fulvic acid fractions. As a result, the default model may in fact adequately represent the reactions occurring in BML, however, all organic complexation results should still be interpreted with caution.

2.2.4 Risk assessment parameters

The toxicological risk assessment followed a screening level approach using hazard quotients (HQs) based on a variety of water quality benchmarks including background ARW concentrations, CWQGs for the long-term protection of freshwater aquatic life, and chronic toxicity values for reproduction inhibition in *Ceriodaphnia dubia*, a sensitive freshwater species. All HQs were calculated using measured major ion and dissolved metal concentrations in 2014, 2015, and 2016 BML surface water. Although full multi-element analyses were performed on all samples, published chronic toxicity data for several metals present within BML surface water were poor (e.g. Sb, Ba, Sr, Ti) which made assessing the risk of long-term exposure to these elements difficult. Thus, only metals with CWQGs were included in this risk assessment. It is important to note, however, that even though some of these metals may be relatively non-toxic, their exclusion does not mean they inherently pose no risk.

2.2.4.1 Athabasca River water

Athabasca River water chemistry values were obtained from the Regional Aquatics Monitoring Program water quality database (RAMP, 2014; 2015) and were expressed as an average of monitoring data taken from several different sites along the Athabasca River (East, Central, and West) and at various times during the year (May, July, and September) corresponding with BML sampling times (winter monitoring values were not included in the averages). Due to

differences in the availability of data for certain parameters, both 2014 and 2015 averages are shown in the tables, however, only 2014 values were used in the HQ calculations (e.g. 2014, 2015, and 2016 BML surface water concentrations all compared to 2014 ARW concentrations only).

2.2.4.2 Canadian Water Quality Guidelines for the protection of aquatic life

Benchmark values for dissolved metals, Cl^-, and NO_3^- were based on the established CWQGs. While some of the newer CWQGs are derived using a species sensitivity distribution (SSD) approach (e.g. Cl, U, V), several of the older metal CWQGs are simply based on results from a few toxicity tests and incorporate safety factors. Furthermore, while many are set values, some are site-specific and dependent upon the pH or hardness of the water (e.g. Al, Cd, Cu, Pb). In these cases, all CWQGs were calculated based on a pH of 8.0 and a water hardness of 100 mg $CaCO_3$/L to reflect the chemistry of BML surface water. See Appendix C for the full list of CWQG values used for HQ calculations in this assessment.

2.2.4.3 Chronic toxicity values

Although the CWQGs reflect the values that would be used for surface water quality regulation of BML, it was also of interest to look at the specific toxicity of these chemicals to a sensitive aquatic organism. The freshwater zooplankton *Ceriodaphnia dubia* was chosen for this purpose (see Section 4.1 for more detailed selection reasoning). Where appropriate, chronic toxicity values were obtained directly from the CWQGs; for CWQGs which did not contain chronic toxicity data for *C. dubia*, values were instead obtained from querying the US EPA ECOTOX database (US EPA, 2016). The preferred endpoint was three-brood ($\leq$8 d exposure) inhibition of reproduction. For metals with multiple data points, preference was given to concentrations which were measured (instead of nominal) and from toxicity tests with similar water chemistry parameters to BML (e.g. pH of 8, hardness of 100 mg/L). See Appendix C for the full list of chronic toxicity values used for HQ calculations in this assessment.

2.3 Results

2.3.1 Annual changes in Base Mine Lake surface water chemistry

The water quality of BML surface water did not change drastically over the three-year course of this study (2014 to 2016) (Table 2.2). Both water hardness and alkalinity remained essentially unchanged at 100 and 600 mg $CaCO_3$/L, respectively, although hardness did increase slightly in 2016. Similarly, the pH of the system appears to have remained relatively unchanged around 8.5, however, pH values tended to fluctuate and vary greatly once samples were removed from the lake itself (*in situ* measurements of pH were closer to 8 while laboratory samples drifted up to almost 9 if left open to the atmosphere for 24 h). Even though redox potential was not measured in the original 2014 sample, values from 2015 and 2016 suggest that water at the surface of BML may gradually become more oxidized as the lake continues to age. Perhaps the most significant observed change as BML ages is the drastic decrease in suspended solids (measured as turbidity). The original 2014 sample contained a significant amount of fine clays and colloidal matter which made the water completely opaque and required months to completely settle out. By 2015 the water only contained a small amount of suspended solids, and as of 2016 the sampled surface water appeared almost completely clear. Despite this, the salinity of BML surface water remained quite elevated. In 2014, the conductivity and TDS of BML surface water were 3000 µS/cm and 1700 mg/L, respectively; by 2016, the values had only decreased approximately 10% to 2700 µS/cm and 1550 mg/L. The reason for this is apparent when examining the concentrations of specific major ions. While certain major ions such as Na^+ and Cl^- steadily decreased from 2014 to 2016 (630 to 540 mg/L and 460 to 405 mg/L, respectively), others such as SO_4^{2-} and HCO_3^-/CO_3^{2-} remained relatively unchanged (approximately 200 and 720 mg/L, respectively). Other ions such as K^+, Ca^{2+}, and Mg^{2+} also remained unchanged, while the concentration of NO_3^- decreased significantly from 10 mg/L in 2014 to 3.1 mg/L in 2016. Although detailed characterization of specific organic fractions within BML surface water was conducted by other groups, the DOC increased from 23 mg/L in 2015 to 35 mg/L in 2016.

Annual trends in the concentrations of dissolved metals within BML surface water were much less clear and varied depending on the specific metal (Table 2.3). Some metals such as Cd, Mo, Se, Tl, and V showed clear year-to-year decreases, while others such as Al, Fe, and Mn increased from 2015 to 2016. However, most concentrations (Sb, As, Ba, B, Cr, Co, Cu, Pb, Ag, Sr, Ti, U, and Zn) fluctuated somewhat year-to-year but remained relatively unchanged.

Table 2.2 – Water quality and major ion characterization of 2014, 2015, and 2016 Base Mine Lake surface water

Water Quality		WIP OSPW Summer 2009 (Pre-BML)†	BML Surface Water (Age)			Athabasca River Water 2014 Average‡	Athabasca River Water 2015 Average‡
			Oct 2014 (22 months)	Aug 2015 (32 months)	Aug 2016 (44 months)		
Conductivity	(µS/cm)	3929 ± 180	3000	2800 ± 0	2700 ± 0	255 ± 38	280 ± 38
pH		8.7 ± 0.4	8.4	8.5 ± 0.0	8.7 ± 0.0	8.0 ± 0.2	8.1 ± 0.1
Eh	(mV)	-	-	+120	+240	-	-
Hardness (mg CaCO$_3$/L)		86 ± 5	100	100 ± 0	115 ± 7	102 ± 11	113 ± 14
Alkalinity (mg CaCO$_3$/L)		497 ± 29	600	600 ± 14	610 ± 0	95 ± 10	100 ± 10
Turbidity	(NTU)	-	85	23	-	-	16 ± 17
Ammonia	(mg NH$_3$/L)	-	0.54 ± 0.05	<0.05 ± 0.05	0.24 ± 0.05	<0.05 ± 0	-
Dissolved Organic Carbon	(mg/L)	-	24	23 ± 0	35 ± 1	9.9 ± 3.9	5.7 ± 2.0
Total Dissolved Solids	(mg/L)	-	1700	1600 ± 0	1550 ± 71	158 ± 23	173 ± 26
Cation Sum	(meq/L)	-	29	27 ± 0	26 ± 0	-	2.9 ± 0.4
Anion Sum	(meq/L)	-	30	28 ± 1	28 ± 0	-	3.0 ± 0.4
Major Ions	**(mg/L)**						
Sodium	Na$^+$	1000 ± 55	630	580 ± 0	540 ± 0	12.2 ± 2.6	12.2 ± 3.1
Potassium	K$^+$	20 ± 1	9.1	8.7 ± 0.1	9.4 ± 0.3	1.35 ± 0.56	0.9 ± 0.1
Calcium	Ca^{2+}	15 ± 1	23	23 ± 0	27 ± 1	28.6 ± 3.4	31.2 ± 5.2
Magnesium	Mg^{2+}	15 ± 0	10	11 ± 1	12 ± 1	7.32 ± 0.78	8.9 ± 1.0
Chloride	Cl$^-$	620 ± 33	460	415 ± 7	405 ± 7	7.68 ± 3.20	9.4 ± 2.1
Sulfate	SO$_4^{2-}$	530 ± 30	210	185 ± 7	200 ± 0	21.5 ± 4.4	-
Bicarbonate	HCO$_3^-$	-	720	710 ± 14	695 ± 7	116 ± 13	-
Carbonate	CO$_3^{2-}$	-	9.5	11 ± 1	23 ± 1	<5 ± 0	-
Nitrate	NO$_3^-$	94 ± 3	10	6.4 ± 0	3.1 ± 0	<0.05 ± 0	<0.03 ± 0.05
Nitrite	NO2-	-	<0.033	<0.033	0.12 ± 1	<0.02 ± 0	<0.003 ± 0.000
Phosphate	PO$_4^{3-}$	-	-	0.016	-	0.039 ± 0.027	<0.014 ± 0.006

- Chemical parameter not measured

† West In-Pit oil sands process-affected water chemistry (WIP OSPW) represents fresh, active tailings before dilution and the creation of BML (Anderson *et al.*, 2012)

‡ Averages of Athabasca River monitoring data taken from multiple sites (east, central, and west) at various times (May, July, September) (RAMP, 2014; 2015)

Table 2.3 – Dissolved metals characterization of 2014, 2015, and 2016 Base Mine Lake surface water (all concentrations in µg/L)

Dissolved Metals		WIP OSPW Summer 2009 (Pre-BML)†	BML Surface Water (Age)			Athabasca River Water 2014 Average‡	Athabasca River Water 2015 Average‡
			Oct 2014 (22 months)	Aug 2015 (32 months)	Aug 2016 (44 months)		
Arsenic	As	7.7 ± 1.6	2.45 ± 0.28	2.17 ± 0.03	2.71 ± 0.03	0.50 ± 0.11	0.40 ± 0.08
Boron	B	-	2040.69 ± 21.68	1896.90 ± 14.08	2008.66 ± 8.88	27.35 ± 5.43	23.03 ± 2.33
Cadmium	Cd	0.5 ± 0.1	0.044 ± 0.002	0.029 ± 0.003	0.025 ± 0.010	0.022 ± 0.017	0.011 ± 0.002
Chromium	Cr	-	0.70 ± 0.10	0.66 ± 0.05	1.72 ± 0.15	<0.3 ± 0.2	<0.2 ± 0.1
Cobalt	Co	2.6 ± 0.1	0.86 ± 0.05	0.38 ± 0.04	0.81 ± 0.02	0.08 ± 0.05	<0.05 ± 0.03
Copper	Cu	-	1.62 ± 1.31	3.73 ± 0.22	0.81 ± 0.02	1.39 ± 0.69	0.88 ± 0.14
Molybdenum	Mo	270 ± 8	40.58 ± 0.95	33.12 ± 0.04	31.63 ± 0.19	0.60 ± 0.10	0.69 ± 0.07
Nickel	Ni	8.5 ± 0.3	6.42 ± 0.41	5.36 ± 0.01	6.63 ± 0.38	1.02 ± 0.84	0.60 ± 0.35
Selenium	Se	3.8 ± 0.1	0.64 ± 0.04	0.51 ± 0.11	0.46 ± 0.00	0.15 ± 0.05	0.14 ± 0.04
Silver	Ag	-	0.008 ± 0.001	0.007 ± 0.001	0.008 ± 0.000	<0.002 ± 0.001	<0.003 ± 0.003
Thallium	Tl	-	0.008 ± 0.001	0.010 ± 0.001	<0.004 ± 0.000	0.006 ± 0.003	0.012 ± 0.008
Uranium	U	29 ± 2	3.78 ± 0.02	3.22 ± 0.06	4.80 ± 0.42	0.30 ± 0.02	0.34 ± 0.04
Vanadium	V	12 ± 0	4.76 ± 0.17	4.05 ± 0.11	3.85 ± 0.00	0.35 ± 0.12	0.23 ± 0.05
Zinc	Zn	-	3.02 ± 0.60	3.84 ± 1.64	3.09 ± 0.05	1.79 ± 0.97	0.85 ± 0.38
Aluminum	Al	70 ± 4	3.12 ± 2.14	1.85 ± 0.39	11.23 ± 9.19	23.59 ± 17.92	11.93 ± 6.21
Antimony	Sb	-	0.56 ± 0.02	0.54 ± 0.01	0.58 ± 0.01	0.08 ± 0.02	0.06 ± 0.02
Barium	Ba	-	283.66 ± 0.73	260.10 ± 1.46	307.58 ± 1.38	39.2 ± 1.7	44.6 ± 4.4
Iron	Fe	-	6.46 ± 1.92	3.30 ± 1.01	11.12 ± 0.44	138.4 ± 112.0	52.7 ± 12.0
Lead	Pb	0.06 ± 0.02	0.022 ± 0.004	0.010 ± 0.003	0.062 ± 0.092	0.076 ± 0.060	0.031 ± 0.008
Manganese	Mn	0.7 ± 0.4	6.88 ± 9.37	2.72 ± 3.41	18.64 ± 0.03	1.45 ± 1.14	0.90 ± 0.28
Strontium	Sr	2700 ± 50	595.62 ± 2.33	577.53 ± 4.02	631.04 ± 0.68	155 ± 21	230 ± 29
Titanium	Ti	-	0.23 ± 0.04	0.17 ± 0.17	0.20 ± 0.03	2.45 ± 2.17	0.97 ± 0.37

- Chemical parameter not measured

† West In-Pit oil sands process-affected water chemistry (WIP OSPW) represents fresh, active tailings before dilution and the creation of BML (Anderson *et al.*, 2012)

‡ Averages of Athabasca River monitoring data taken from multiple sites (east, central, and west) at various times (May, July, September) (RAMP, 2014; 2015)

2.3.2 Risk assessment

2.3.2.1 Athabasca River water

The first benchmark used to assess the risk of BML surface water chemistry to potential aquatic life was the background concentrations of major ions and dissolved metals within the nearby Athabasca River. Due to gaps in the 2015 RAMP monitoring dataset and a lack of 2016 data, all BML concentrations (2014, 2015, and 2016) were compared to 2014 ARW and expressed as factors (Figure 2.1). It is important to note that elevation above background concentrations does not necessarily imply risk, however, major ions and metals present within BML below background ARW concentrations were deemed to be of 'no concern'. NH_3

The salinity of BML surface water (measured as conductivity and TDS) was approximately 10x higher than that of ARW, however, when examining individual major ions, Na^+ and Cl^- ions were 44x and 53x higher, respectively, as of 2016. NO_3^- was found to be the most significantly elevated major ion in 2014 BML surface water with a concentration approximately 200x higher than in ARW. Despite significant reductions over time, as of 2016 NO_3^- within BML surface water was still 62x higher than in ARW. The remaining major ions – K^+, SO_4^{2-}, and HCO_3^- – were 7x, 9x, and 6x higher than in ARW, respectively, as of 2016. The concentrations of Ca^{2+} and Mg^{2+} were nearly identical to those in ARW (similar water hardness) and were therefore deemed to be of no concern.

Four of the dissolved metals within BML – Al, Fe, Pb, and Ti – were present at levels below ARW concentrations and were therefore deemed to be of no concern as well. Of all the dissolved metals, only B and Mo were significantly elevated above ARW concentrations (73x and 52x, respectively). The next highest metals were Co, U, and V at 11x, 16x, and 11x higher, respectively. The remaining metals all fell somewhere below 10x. It should again be noted that Sb, Ba, Mn, and Sr all lack CWQGs and were extremely data poor, thus they were excluded from subsequent rounds of the assessment despite their elevation above ARW concentrations.
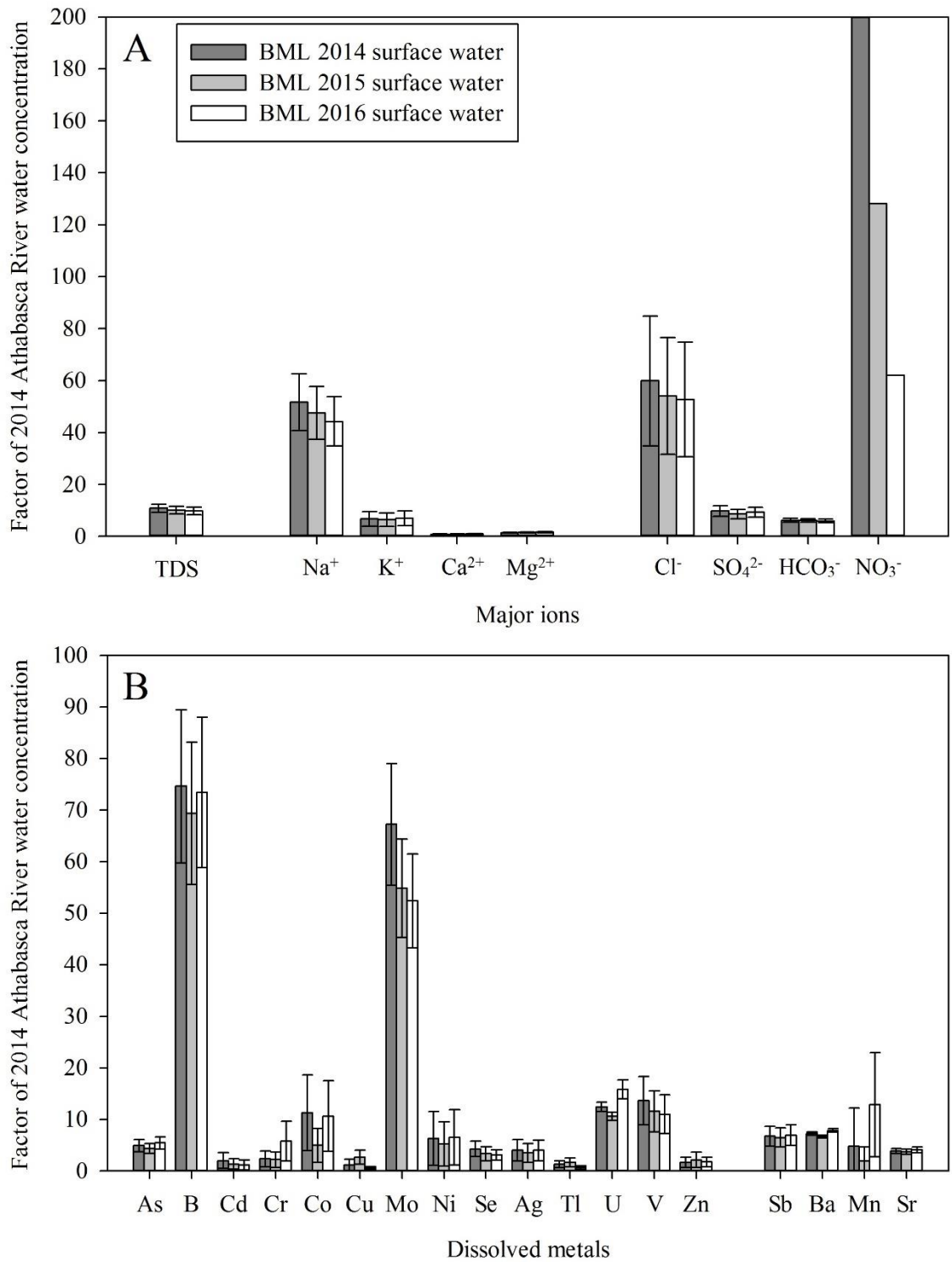
Figure 2.1 – Elevation of major ions and dissolved metals within 2014, 2015, and 2016 BML surface water compared to 2014 Athabasca River water concentrations (RAMP, 2014). Al, Fe, Pb and Ti were all deemed to be of no concern (not shown). Sb, Ba, Mn, and Sr lack Canadian Water Quality Guidelines and sufficient toxicity data and were thus removed from further assessment.

2.3.2.2 Canadian Water Quality Guidelines for the protection of aquatic life

After initial screening against background ARW concentrations, the remaining major ions and metals within BML surface water were compared CWQGs as a conservative measure of toxicological risk (Figure 2.2A). Comparisons were expressed as unitless HQs, with values greater than 1 indicating potential risk. Of the 15 metals with available CWQGs, only B consistently exceeded an HQ of 1 every year. Cu exceeded in 2015 but decreased to an acceptable concentration in 2016. As of 2016, Cr(VI) was also in exceedance of its CWQG, however, based on the water chemistry (Eh-pH) of BML surface water, Cr should be present as Cr(III) which fell well below its CWQG value. As of 2016, all other metals had an HQ ≤ 0.5. For the major ions, NO_3^- fell well below its CWQG with a 2016 HQ of 0.24, however, Cl^- significantly exceeded its guideline with a 2016 HQ of 3.3.

2.3.2.3 *Ceriodaphnia dubia* chronic toxicity values

In addition to the CWQGs, major ions and dissolved metal concentrations were also compared to chronic toxicity values for *C. dubia* (Figure 2.2B), a sensitive aquatic species used in toxicity testing (Chapter 4). The most sensitive endpoints available were chosen for this purpose, generally 7-d IC_{10} reproduction values. Compared to the CWQGs, concentrations for the majority of dissolved metals within BML surface water fell well below chronic toxicity values (all HQs < 0.2). However, the 2016 HQ for Ni was >1, and the 2016 HQ for Cl^- was very close at 0.9.

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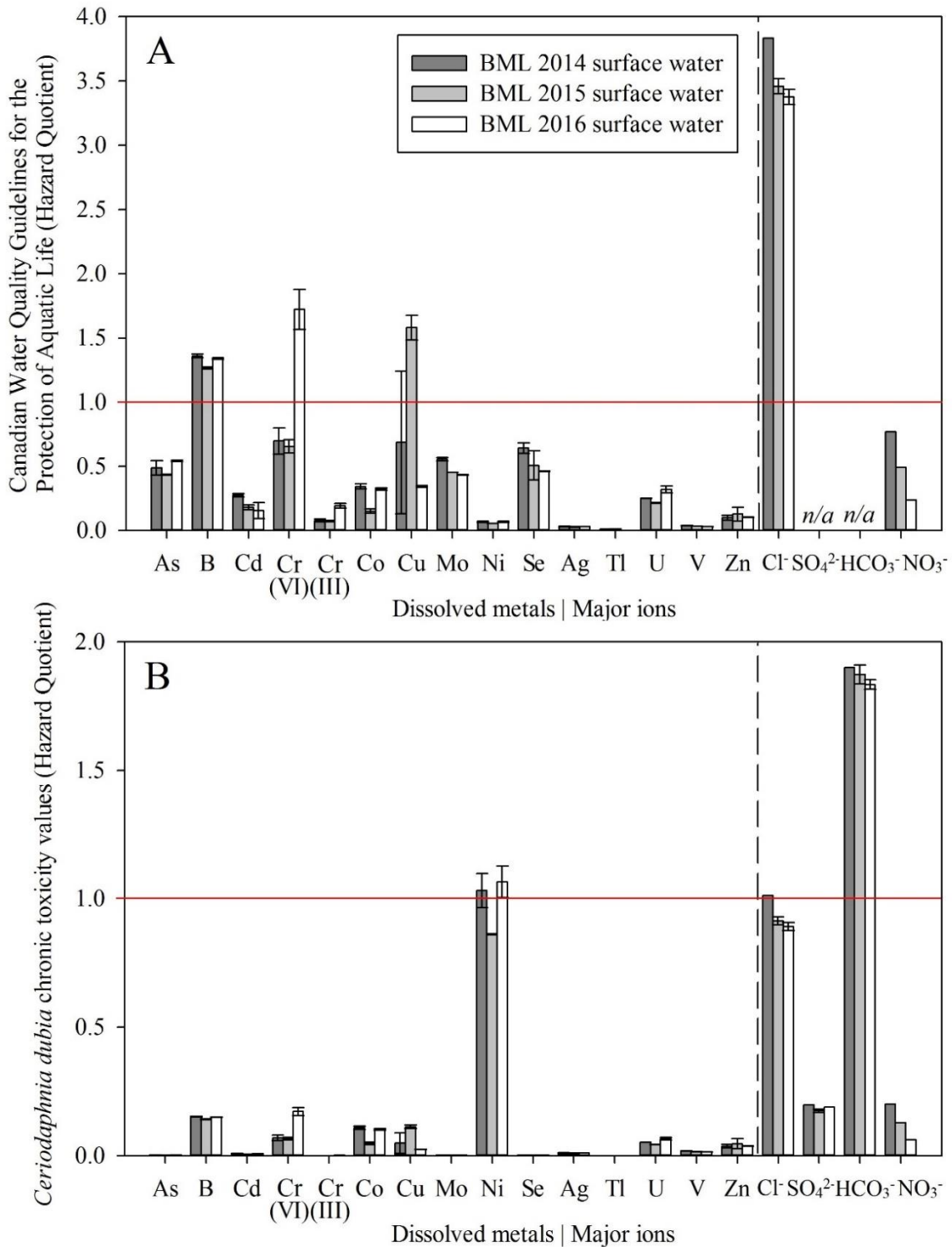
Figure 2.2 – Comparison of 2014, 2015, and 2016 BML surface water concentrations of dissolved metals and major ions to long-term Canadian Water Quality Guidelines (A), and chronic toxicity values for *Ceriodaphnia dubia* (B). Hazard quotients greater than 1.0 (horizontal line) indicate potential risk (where the surface water concentration exceeds the benchmark value).

2.3.2.4 Metal speciation and complexation modelling

The final component for predicting the toxicological risk of dissolved metals within BML surface water was to determine speciation and complexation which affect both the toxicity and fate of dissolved metals within aquatic systems. Due to the relatively high pH of BML surface water, as well as high concentrations of DOC and HCO_3^-/CO_3^{2-}, only a very small proportion of most cationic metals were predicted to exist in their free ion state (Table 2.4). Although Cl^- and SO_4^{2-} concentrations were also highly elevated, they had relatively little influence on the majority of the cationic metals within BML surface water; DOC- and HCO_3^-/CO_3^{2-}-complexes were predicted to be predominant. Similarly, although the range of modeled pH (8.0 to 9.0) did affect the predominance of specific complexes, the effects on the overall risk estimates were negligible. As expected, the predominance of carbonate complexes of the cationic metals decreased with decreasing pH, corresponding to an increase in the predominance of the more toxic free ions. However, free ion predominance still remained low at pH 8.0 compared to organic and carbonate complexes (see Appendix B for complete metal speciation modeling results).

Table 2.4 – Summary of modeled speciation and complexation of dissolved metals of potential concern in 2016 BML surface water (calculated at pH 8.5 using Visual MINTEQ).

Metal Species/Complex [Ligand] Predominance						
Metal Cations	**Free Ion**	**-[DOC]**	**-[OH]$_{(x)}$**	**-[Cl]$_{(x)}$**	**-[SO$_4$]$_{(x)}$**	**-[(H)CO$_3$]$_{(x)}$**
Cadmium Cd^{2+}	17.4%	28.4%	0.3%	10.3%	2.3%	41.1%
Chromium Cr^{3+}	-	-	100.0%	-	-	-
Cobalt Co^{2+}	32.3%	-	1.6%	0.1%	3.5%	62.5%
Copper Cu^{2+}	0.1%	7.5%	1.1%	-	-	91.2%
Nickel Ni^{2+}	15.7%	25.5%	0.5%	-	1.7%	56.5%
Silver Ag^+	2.9%	-	-	97.0%	0.1%	-
Thallium Tl^+	94.8%	-	-	2.9%	2.3%	-
Uranium UO^{2+}	-	-	-	-	-	100.0%
Zinc Zn^{2+}	10.4%	26.8%	9.1%	0.2%	1.3%	52.3%
Metal Anions	**Free Ion**	**[H]$_{(x)}$-**	**[Na]$_{(x)}$-**	**[K]$_{(x)}$-**	**[Ca]$_{(x)}$-**	**[Mg]$_{(x)}$-**
Arsenic AsO_4^{3-}	0.1%	99.9%	-	-	-	-
Boron H_3BO_3	-	99.5%	0.5%	-	-	-
Molybdenum MoO_4^{2-}	98.6%	-	-	-	0.4%	0.9%
Selenium SeO_3^{2-}	66.9%	33.1%	-	-	-	-
Vanadium VO_4^{3-}	-	100.0%	-	-	-	-

2.4   Discussion

2.4.1   Annual changes in water chemistry

The annual changes observed in the water chemistry parameters of BML surface water were generally consistent with conclusions drawn from recent characterizations of the geochemical and hydrological processes occurring within the lake (Dompierre & Barbour, 2016; Dompierre *et al.*, 2016; Dompierre, 2017). Beginning in May 2013, Syncrude began continuously pumping clean freshwater into BML from the nearby Beaver Creek Reservoir and pumping out BML surface water for re-use in bitumen processing. While surface runoff, groundwater leaching, precipitation and evaporation all affect the water balance of normal lakes, the effects of these processes are low to negligible in BML (Dompierre *et al.*, 2016); pumping activity is responsible for the majority of changes in the water and mass balance in BML and has led to the gradual dilution of surface water over time (Dompierre, 2017). Based on volume and flow measurements reported by Dompierre and Barbour (2016), BML surface water is diluted by approximately 5 to 10% annually which corresponds with the year-to-year reductions in BML TDS observed in this study. It is crucial to note, however, that this dilution is an oversimplification of the processes occurring within BML – in addition to pumping activity there is a significant volume of water and chemical mass that enters BML surface water from the underlying FFT below.

As previously discussed, settlement of the FFT underlying BML surface water occurs slowly via self-weight consolidation – as FFT densifies, pore water is liberated and flows upwards (via advection) to the overlying surface water carrying with it high concentrations of chemicals of concern (Dompierre and Barbour, 2016). For example, Dompierre (2017) measured similar concentrations of Cl^- in BML surface water as those measured in this study (450 mg/L and 410 mg/L in 2014 and 2015, respectively), however, the concentration within FFT pore water remained as high as it was in the initial surface water of BML in May 2013 prior to the first freshwater addition (approximately 640 mg/L). Thus, while the concentrations of major ions and dissolved metals are slowly decreasing within BML surface water due to dilution, the concentrations within the underlying FFT pore water remain unchanged. As this concentration gradient across the FFT-water interface increases, and as the settlement and dewatering of FFT slows, chemical flux from FFT pore water to the overlying water will begin to slow and become increasingly dominated by diffusion (Dompierre and Barbour, 2016). It is worth noting that in addition to this advective-diffusive mass transport, some chemical mass enters the overlying surface water as a result of

shallow mixing in the top 1 m of FFT due to weather events and seasonal lake turnover (Dompierre and Barbour, 2016). While the chemical flux from these mixing events is minimal in comparison to the constant advective-diffusive transport, they do contribute to the sustained high turbidity and resuspension of TSS within BML surface water (Dompierre and Barbour, 2016). This is likely the reason why the turbidity/TSS observed in this study was significantly higher in the October 2014 sample than the August 2015 and 2016 samples – BML surface water is strongly stratified from June to August (no mixing) while fall turnover occurs from September to November (significant mixing).

Currently, the advective-diffusive transport regime within BML results in an annual pore water flux of 6 to 7 m^3/m^2 of FFT corresponding to approximately 0.75 m of increased surface water depth and an estimated average release of 287 g Cl^-/m^2 from FFT into overlying surface water (Dompierre, 2017). Based on surface areas and volumes reported by Dompierre (2017), the chemical mass flux of Cl^- from FFT to surface water was 1.81 million kg in 2015 which should correspond to an annual *increase* in BML surface water Cl^- concentration of 31.2 mg/L. However, when the mass of Cl^- removed from the system via pumping-out activity (Dompierre, 2017) is included in the equation, the net annual change in the concentration of Cl^- in BML surface water is instead a 13.8 mg/L *decrease*, consistent with the values measured by Dompierre (2017) and in this study. This demonstrates how crucial Syncrude's pumping activity is to offset the mass flux of chemicals of concern across the FFT-water interface into BML surface water. The Dompierre (2017) study focused specifically on Cl^- as it is relatively geochemically inert, making it a suitable tracer for characterizing chemical movement through BML and the effects of dilution. Na^+ is similar in this regard and is strongly correlated with Cl^- concentrations within BML (Dompierre *et al*., 2016). Thus, as observed in this study, the annual change in the concentration of Na^+ within BML surface water is similar to that of Cl^- (predominantly controlled by dilution). K^+ is another major ion that has relatively few geochemical controls, however, it is only weakly correlated with Cl^- concentrations within BML and is therefore likely involved in certain in situ reactions such as ion exchange at clay surfaces (Dompierre *et al*., 2016). Even still, it would be expected to decrease to some degree based on the constant dilution of BML surface water, however, the measured concentrations remained relatively constant across the three years in this study. The exact reason for this is unclear, though the concentration is nearly two orders of magnitude smaller than Na^+ and Cl^-, and is similar to that of the pumped-in freshwater from Beaver Creek Reservoir (2.3 mg/L;

Kavanagh *et al*., 2009). Thus, changes due to dilution may simply be less apparent, especially when comparing single samples.

The movement and fate of the other major ions (HCO_3^-, SO_4^{2-}, NO_3^-, Ca^{2+}, and Mg^{2+}) within BML surface and pore water are controlled by additional processes and are much more complex. The pH, total hardness (Ca^{2+} and Mg^{2+}), alkalinity, and HCO_3^- within BML are all closely interlinked and are predominantly controlled by anaerobic biogeochemical processes (e.g. methanogenesis) occurring within the FFT (Siddique *et al*., 2014a; Dompierre *et al*., 2016). Microbial metabolism of dissolved organics within the FFT results in the production of methane and carbon dioxide causing a decrease in pH and increase in alkalinity which subsequently results in the dissolution of carbonate minerals culminating in an increase in dissolved concentrations of Ca^{2+}, Mg^{2+}, and HCO_3^- in pore water (Siddique *et al*., 2014a; Dompierre *et al*., 2016). Although dolomite ($CaMg(CO_3)_2$) was likely the predominant carbonate mineral within BML responsible for this phenomenon, it appears to have been completely depleted as of 2013 (Dompierre *et al*., 2016). Furthermore, the concentrations of Ca^{2+} and Mg^{2+} measured within FFT pore water (Dompierre *et al*., 2016) are approximately equivalent to both the concentrations within the pumped-in water from Beaver Creek Reservoir (Kavanagh *et al*., 2009), as well as BML surface water measured in this study. This suggests that the total water hardness of BML surface water should now remain relatively constant, consistent with the observations of this study. While carbonate mineral dissolution may no longer be occurring, the pH and alkalinity of BML surface water will continue to be in disequilibrium as long as the biogenic production of CO_2 continues within the FFT. This poses a problem for laboratory measurements, as field collected samples of BML surface water will degas CO_2 when left open to the atmosphere, resulting in a gradual increase in pH as high as 9 and a resultant shift in the HCO_3^-/CO_3^{2-} equilibrium (see Table 2.2). Without direct field measurements of BML surface water, it is difficult to assess these parameters with confidence.

Similar to the aforementioned ions, SO_4^{2-} is indirectly controlled by methanogenesis within FFT pore water via a secondary pathway involving the reduction of Fe(III) to Fe(II) (Siddique *et al*., 2014b). A substantial and rapid decrease in SO_4^{2-} concentration was observed across the BML FFT-water interface due to reduction to H_2S and the subsequent reaction with Fe(II) to form the insoluble mineral iron sulfide ($FeS_{(s)}$) (Siddique *et al.*, 2014b; Dompierre *et al*., 2016). The

presence of organic acids within the FFT pore water likely improves the iron and sulfur reducing conditions necessary for these reactions to occur, however, they are expected to decrease over time as the overlying water continues to be diluted and as the capacity for mineral precipitation becomes saturated (Dompierre *et al*., 2016). If SO_4^{2-} contributions from the pore water to overlying water were initially small due to this reduction, it may explain why concentrations in BML surface water initially declined 10% from 2014 to 2015 (due to dilution) but have since begun to increase again.

The final major ion of concern in BML, NO_3^-, is also perhaps the least understood. Historically, characterization and treatment of nitrogen within OSPW has focused on its presence as ammonia, thus there has been little discussion of nitrate/nitrite (Allen, 2008a; 2008b). While the ammonia concentration within BML surface water was initially elevated (6 mg/L in May 2013), as of 2016 it was only 0.2 mg/L. Furthermore, the concentration of NO_3^- was 10 mg/L in 2014, but had decreased to 3 mg/L by 2016. Based on the relative changes observed in the other ions, dilution due to pumping activity alone would be insufficient to account for this decrease. Thus, there must be additional processes within BML affecting the movement and fate of nitrogen within the system. Due to neutral pH and reducing conditions, Dompierre *et al*. (2016) observed elevated concentrations of NH_4^+ in FFT pore water (approximately 10 mg/L) attributed to desorption or ammonification of organic-bound nitrogen and coinciding with a complete absence of NO_3^-/NO_2^-. As NH_4^+ moves into the more oxic overlying water aerobic nitrification will likely occur, though this would result in a greater concentration of NO_3^-, which is inconsistent with the observations in this study. However, early research suggests that certain conditions within BML may be conducive to denitrification (Matthew Lindsay, personal communication) which would provide another possible explanation for the loss of nitrogen within the system (as $N_{2(g)}$).

As previously discussed, characterization of trace metals within OSPW has been limited. Furthermore, no published studies examining the geochemistry of FFT within oil sands EPLs have included trace metal concentrations. Based on the processes controlling major ions, it is difficult to adequately explain trends in trace metal concentrations within BML surface water without concomitant pore water concentrations. Some general predictions can be made, as the effect of dilution due to pumping activity would likely have the same effects on trace metal concentrations as on major ions. However, while the advective-diffusive transport of water from FFT to the surface would contain trace metals in addition to major ions, the rate at which the transport

occurred would not necessarily be the same as that modelled for Cl$^-$ – it would likely vary depending on the metal based on differences in diffusion coefficients as well as speciation/complexation. In general, despite some year-to-year fluctuations, most metal concentrations remained relatively constant which suggests that the annual rate of movement from FFT pore water to surface water is approximately equivalent to the rate of dilution (assuming concentrations within the FFT pore water are similar to that of the surface water). It is also important to note that all reported concentrations are from a single annual sample which makes it even more difficult to identify trends as the water chemistry within BML has been shown to vary both spatially and seasonally (Dompierre *et al.*, 2016).

Syncrude's pumping activity and consequent dilution has played a critical role in maintaining and reducing the concentrations of certain major ions and trace metals within BML surface water over time. For now, surface water quality and risk to aquatic organisms can be assessed assuming pumping activity remains constant, however, further geochemical characterization of chemical flux across the FFT-water interface is needed to more accurately predict future changes in surface water chemistry. Furthermore, if the pumping activity were to be reduced or stopped entirely, it is extremely likely that concentrations would begin to increase back to the pre-dilution 2013 levels still present within the FFT pore water (Dompierre *et al.*, 2016).

2.4.2   Toxicological risk to aquatic organisms

Toxicological risk of each individual major ion and dissolved metal within BML surface water was assessed based on three benchmarks: background ARW concentrations, CWQGs, and chronic toxicity to the sensitive freshwater zooplankton, *C. dubia*. In general, the ARW benchmark represented the most conservative risk benchmark followed by CWQG then chronic toxicity. Overall risk was heavily weighted towards the CWQG risk outcome as it represents the basis for future water quality regulation of BML, however, some exceptions were made depending on the risk outcomes of the other two benchmarks (see Table 2.5).

Table 2.5 – Risk assessment summary of individual major ions and metals within 2016 BML surface water

Major Ions		Risk Assessment Benchmarks			Overall Individual Risk
		Athabasca River Water (2014)	Canadian Water Quality Guideline	*Ceriodaphnia dubia* Chronic Toxicity	
Chloride	Cl⁻	Very high	Very high	High	Very high
Bicarbonate	HCO_3^-	Moderate	*n/a*	Very high	Very high
Sodium	Na^+	Very high	*n/a*	*n/a*	†Very high
Nitrate	NO_3^-	Very high	Low	Very low	Low
Sulfate	SO_4^{2-}	High	*n/a*	Low	Low
Potassium	K^+	Moderate	*n/a*	*n/a*	†Low
Magnesium	Mg^{2+}	Low	*n/a*	*n/a*	†Low
Calcium	Ca^{2+}	Very low	*n/a*	*n/a*	†Very low
Dissolved Metals					
Boron	B	Very high	Very high	Low	Very high
Nickel	Ni	Moderate	Very low	Very high	Very high
Arsenic	As	Moderate	Moderate	Very low	Moderate
Molybdenum	Mo	Very high	Moderate	Very low	Moderate
Selenium	Se	Low	Moderate	Very low	Moderate
Cadmium	Cd	Low	Low	Very low	Low
Chromium	Cr(III)	Moderate	Low	Very low	Low
Cobalt	Co	Very high	Low	Low	Low
Copper	Cu	Very low	Low	Very low	Low
Uranium	U	Very high	Low	Very low	Low
Zinc	Zn	Low	Low	Very low	Low
Aluminum	Al	Very low	Low	Very low	Very low
Iron	Fe	Very low	Very low	Very low	Very low
Lead	Pb	Very low	Very low	Very low	Very low
Silver	Ag	Moderate	Very low	Very low	Very low
Thallium	Tl	Very low	Very low	Very low	Very low
Titanium	Ti	Very low	*n/a*	*n/a*	Very low
Vanadium	V	Very high	Very low	Very low	Very low
Antimony	Sb	Moderate	*n/a*	*n/a*	*Unknown*
Barium	Ba	High	*n/a*	*n/a*	*Unknown*
Manganese	Mn	Very high	*n/a*	*n/a*	*Unknown*
Strontium	Sr	Moderate	*n/a*	*n/a*	*Unknown*

n/a: Data not available (risk cannot be determined, '*Unknown*')
Canadian Water Quality Guideline (CWQG) and *C. dubia* risk based on hazard quotient calculations:
    Very high (>1.0), High (0.70-0.99), Moderate (0.40-0.69), Low (0.10-0.39), Very low (<0.10)
Athabasca River risk based on factor calculations:
    Very high (>10x), High (7.0x-9.9x), Moderate (4.0-6.9x), Low(1.0x-3.9x), Very low (<1.0x)
† Overall individual risk based on acute *C. dubia* toxicity data and conclusions from Mount *et al.* (2016).
Refer to Appendix B for more detailed information on risk calculations.

As previously discussed, elevation above background ARW concentration is not necessarily a concern, thus 'high' risk outcomes for this benchmark were given minimal consideration if the risk outcomes of the other two biological benchmarks were low. On the other hand, major ions or metals that were present at concentrations within BML below those found in ARW were considered 'very low' risk, regardless of the other two risk outcomes (although in these cases the risk outcomes were no higher than 'low'). Furthermore, certain major ions and metals lacked CWQGs and thus the risk outcome was instead primarily based on toxicity to *C. dubia*.

2.4.2.1 Major ions

Consistent with previous discussion and characterization of surface water chemistry, major ions present a significant impediment for potential aquatic organisms within BML, with Na^+, Cl^-, and HCO_3^- all classified as being 'very high' risk. This was unsurprising, as the concentrations of these three ions alone account for approximately 85% of the elevated TDS within BML surface water. Unfortunately, accurate assessment of the risk major ions pose is still difficult due to limited studies and research within this field resulting in a lack of CWQGs and chronic toxicity data for many major ions (especially cations). For the past two decades, assessments have largely been based on the original findings of Mount *et al*. (1997) who surmised that the toxicity of salinity in natural systems was primarily dependent on anions, not cations, and established a toxicity ranking of: $K^+ > HCO_3^- = Mg^{2+} > Cl^- > SO_4^{2-}$ (Na^+ and Ca^{2+} were considered non-toxic to aquatic organisms). However, more recent studies with *C. dubia* have begun to show that – while the ranking still generally holds true – significant interactions exist between these ions in solution (Lasier and Hardin, 2010; Mount *et al*., 2016; Erickson *et al*., 2017). Lasier and Hardin (2010) found that the chronic toxicity of anion mixtures to *C. dubia* reproduction is generally described by the additive effects of Cl^-, HCO_3^-, and SO_4^{2-}, with increasing water hardness significantly reducing the toxicity of both Cl^- and SO_4^{2-}, but having no significant effect on HCO_3^-. Similar effects of water hardness reducing toxicity have also been observed in studies with cations (Mount *et al*., 2016). To account for the potential effects of water hardness on major ion toxicity in BML, where possible, all *C. dubia* toxicity values used in the risk assessment were taken from tests which had been conducted in moderately hard water (approximately 100 mg/L $CaCO_3$) similar to BML. The toxicity of Cl^- was also found to be significantly reduced with increasing concentrations of Na^+ and alkalinity, while HCO_3^- toxicity may be reduced with increasing concentrations of DOC

(Lasier and Hardin, 2010). These interactions suggest that while the individual risk of Cl^- and HCO_3^- may be 'very high', when BML surface water is assessed as a whole mixture the toxicity and risk of these anions may in fact be significantly lower.

While risk outcomes for Cl^-, HCO_3^-, SO_4^{2-}, and NO_3^- were all determined based on CWQGs and chronic toxicity to *C. dubia* reproduction, cations were much more poorly characterized, thus risk outcomes for Na^+, K^+, Ca^{2+}, and Mg^{2+} could only be determined using acute *C. dubia* survival toxicity data and observations by Mount *et al*. (2016) (denoted by † in Table 2.5). Consistent with their original 1997 findings, Mount *et al*. (2016) found Ca^{2+} to be relatively non-toxic, with both $CaSO_4$ and $CaCO_3$ salts having no effect on survival (up to their solubility limits), while $CaCl_2$ resulted in a 48-h LC_{50} of 719 mg Ca^{2+}/L, likely due to the Cl^- ions. The concentration within 2016 BML surface water (27 mg/L) was significantly lower than both this value and the concentration within ARW, thus Ca^{2+} was deemed to be of 'very low' risk. Toxicity of both K^+ and Mg^{2+} varied somewhat based on what salt (anion) was tested, however, average 48-h LC_{50} values reported by Mount *et al*. (2016) (231 mg K^+/L and 282 mg Mg^{2+}/L) were both well above BML surface water concentrations. Furthermore, the toxicity of K^+ was found to be significantly reduced in the presence of 300 mg Na^+/L (Mount *et al*., 2016), thus the risk within BML is 'low'. The risk of Na^+ was the most difficult to determine individually, as toxicity appears to be primarily dependent on what salt (anion) is tested. Mount *et al*. (2016) reported *C. dubia* 48-h LC_{50} values for $NaHCO_3$, $NaCl$, and Na_2SO_4 equivalent to 441, 788, and 1055 mg Na^+/L, respectively, which are similar in magnitude to the concentration within BML. These results are consistent with previous studies which suggest that the toxicity of Na-salts is dependent on the anion, in the same order of toxicity ($HCO_3^- > Cl^- > SO_4^{2-}$) (Mount *et al*., 1997; Lasier and Hardin, 2010). However, while Na^+ itself may not have a specific mechanism of action, high concentrations resulting from high concentrations of associated anions (such as Cl^- and HCO_3^- in BML) correspond to increases in the total osmolarity which in turn has been strongly correlated with nonspecific ion toxicity and osmotic stress (Mount *et al*., 2016; Erickson *et al*., 2017). Due to this, as well as the significant elevation above background ARW concentration, Na^+ in BML surface water was deemed a 'very high' risk in conjunction with Cl^- and HCO_3^-. Even though only acute data were available for the cations, it is worth noting that the acute-to-chronic ratios for the toxicity of the major anions (Cl^-, HCO_3^-, SO_4^{2-}) to *C. dubia* are all very low (4.5, 2.7, and 2.3, respectively) (Lasier and Hardin,

2010). This, in addition to a relatively steep dose-response, suggests that chronic toxicity values for these cations may not be significantly lower than the reported acute values.

2.4.2.2 Dissolved metals

With respect to metals, the overall individual risk outcomes were largely expected and indicated that most dissolved metals within BML are of little concern to aquatic organisms ('low' or 'very low' risk), consistent with previous discussion. The only two metals which were identified as being of 'very high' risk (B and Ni) both had significant discrepancies between the risk outcome based on CWQGs compared to that of *C. dubia* chronic toxicity, albeit for opposite reasons – CWQG-risk for B was much greater than *C. dubia*-risk, while *C. dubia*-risk for Ni was much greater than CWQG-risk. The CWQG for B is based on a modern SSD approach which sets the value equal to the HC_5 (5th percentile), in this case 1.5 mg/L (CCME, 2009). However, unlike many metals which are most toxic to invertebrates, the most sensitive species to B are aquatic plants, followed by fish. In fact, *C. dubia* is the 13th most sensitive aquatic organism to B in the SSD (45th percentile) corresponding to a 14-d maximum acceptable toxicant concentration (MATC) of 13.4 mg/L (CCME, 2009). Boron is different from most metals in that it exists predominantly as the neutral boric acid species ($H_3BO_3^0$) in natural aquatic systems, although it may also be present as the borate anion ($H_2BO_3^-$) depending on pH (under BML conditions the ratio is approximately 4:1, Visual MINTEQ). As a result, B toxicity is largely unaffected by common toxicity-modifying water chemistry parameters such as the elevated hardness, alkalinity, Na^+, and Cl^- found within BML (Dethloff, 2009). Thus, even though the elevated concentration of B within BML surface water appears to be of little concern to invertebrates, other species integral to aquatic ecosystem development such as aquatic plants may still be at risk. Conversely, the CWQG for Ni (CCME, 2008) is based on the nearly 40-year-old US EPA water quality guideline which is primarily derived from LC_{50} values for *Daphnia magna* at three different levels of water hardness (chronic $LC_{50} = 123$ µg Ni/L at a hardness of 105 mg/L as $CaCO_3$) (US EPA, 1980). Not only is *D. magna* generally less sensitive to metals than *C. dubia*, the LC_{50} (50% mortality) is also a significantly less sensitive toxicity endpoint than the IC_{10} (10% reproduction inhibition) endpoint used in the *C. dubia* risk assessment (6 µg Ni/L, Zuiderveen and Birge, 1997). This difference in test species and toxicity endpoint between the two assessments provides an explanation for why the CWQG-risk was determined to be 'very low' while the *C. dubia* risk is 'very high'. Although

this value suggests a significant risk to *C. dubia*, it should be noted that due to the high pH, alkalinity, and DOC within BML surface water, only 16% of the total dissolved Ni within the system should be present as the highly toxic free Ni^{2+} cation; DOC- and HCO_3^-/CO_3^{2-}-complexes are predicted to be predominant (Visual MINTEQ). As a result, the risk of Ni to *C. dubia* within the BML surface water is likely significantly reduced. Furthermore, the concentration is unlikely to be of concern to less sensitive species such as other invertebrates and fish (US EPA, 1980).

In addition to B and Ni, the total dissolved concentration of Cr within 2016 BML surface water was also in exceedance of its CWQG, but only when assessed as Cr(VI). However, based on the 2016 water chemistry conditions within BML surface water, Cr should have been present as >99% $Cr(OH)_{3(aq)}$ at pH 8.5 and Eh +240 mV (Visual MINTEQ). As a result, the risk assessment was based solely on the significantly less toxic Cr(III) state (Zuiderveen and Birge, 1997). Although redox potential was only measured in 2015 and 2016, the trend seems to suggest that the upper layer of the surface water (epilimnion) is becoming more oxic over time. If this continues, some of the Cr may become oxidized to Cr(VI) and pose some risk in the future.

Aside from B and Ni, only three other metals – As, Mo, and Se – were assessed as having greater than 'low' risk (all 'moderate') based on the CWQG risk outcomes. However, similar to Ni, the CWQGs for these three metals are in some cases 30 years old and were all established using a much less robust method which simply took the single most sensitive toxicity value available at the time and applied a safety factor of 0.1x. As a result, the individual risk outcomes for these metals should be interpreted with caution. Based on chronic reproductive toxicity to *C. dubia*, the toxicity ranking of these metals is Se > As >>> Mo corresponding to $IC_{12.5}$ values of 0.51, 1.02, and 34.0 mg/L, respectively, tested in moderately hard water (120 mg/L as $CaCO_3$) similar to BML (Naddy *et al*., 1995). These toxicity values are nearly three orders of magnitude higher than the concentrations found within BML surface water, thus the risk to invertebrate species is likely 'very low'. It is worth noting that all three of these metals are present in natural systems as anions and are required micronutrients for aquatic life (Naddy *et al*., 1995). The trace concentrations of As, Mo, and Se found within BML surface water may therefore actually promote aquatic ecosystem health at current concentrations, however, the range of this hormetic effect is relatively narrow.

The remaining metals included in this assessment – Al, Cd, Co, Cu, Fe, Pb, Ag, Tl, Ti, U, V, and Zn – were all deemed to be of either 'low' or 'very low' risk, and will not be discussed further. Finally, as previously discussed, Sb, Ba, Mn, and Sr all lacked CWQGs and chronic toxicity data for *C. dubia* and thus risk outcomes could not be assessed. However, their exclusion from this study does not imply a lack of risk, merely that it could not be adequately determined within the parameters of this study. Although the risk outcome of each metal was assessed on an individual basis, it is crucial to note that significant interactions will exist between many of the metals within BML surface water, similar to those among the major ions. There is a large body of recent work that covers how these interactions affect the toxicity of metals to *C. dubia*, including divalent cations such as Cd, Cu, Ni, Pb, and Zn (Naddy *et al*., 2015; Nys *et al*., 2016; Nys *et al*., 2017a; Nys *et al*., 2017b) and the previously mentioned As, Mo, and Se anions (Naddy *et al*., 1995). However, due to the generally low risk and concentrations of most dissolved metals within BML surface water, a more in-depth discussion of these mixture effects was deemed unnecessary and beyond the scope of this assessment.

With the exception of Na^+ and Ni, it is interesting that all of the ions and metals identified as posing the greatest risk to potential aquatic life within BML surface water are anions. Of the nine total anions characterized in this study, only NO_3^- and V (present as approximately 1:1 $H_2VO_4^-$ and HVO_4^{2-}, Visual MINTEQ) were assigned a risk value of 'Low' or less. The reason for the disproportionate risk of anions in BML surface water compared to cations is unclear, though it is again important to note that these individual risk outcomes were based solely on benchmark values and do not incorporate effects of mixtures or background water chemistry. A similarly designed risk assessment by McQueen *et al*. (2017) identified many of the same constituents of concern in OSPW from a different oil sands mine source, including Cl^-, B, and Ni, although the concentrations of Na^+ and HCO_3^- were much lower than those measured in BML and less sensitive toxicity endpoints were used. This suggests that the results of Syncrude's BML experiment and this study are applicable to other Alberta oil sands surface mine operators, especially those aiming to establish similar EPLs containing WCT.

2.4.3 Conclusion

This study was the first detailed investigation of inorganic surface water quality and toxicological risk in Base Mine Lake, as well as the first characterization of annual changes in surface water chemistry of a large-scale oil sands end pit lake. Based on the most recent 2016 water chemistry data, concentrations of the major ions Na^+, Cl^-, and HCO_3^-, as well as the metals B and Ni, are all expected to pose 'very high' risk to potential aquatic organisms within BML surface water due to exceedances of Canadian Water Quality Guidelines for the long-term protection of freshwater aquatic life, and/or chronic toxicity values for the inhibition of reproduction in the sensitive zooplankton species, *Ceriodaphnia dubia*. Over the three-year course of this study (2014 to 2016), the concentrations of these major ions and metals within BML surface water either remained relatively constant, or slightly declined. Current freshwater pumping activity by Syncrude – and the resulting dilution of BML surface water – has been identified as the primary driver for these changes in water chemistry, and offsets the impact of the continuous advective-diffusive mass flux of these constituents from within the underlying FFT pore water into the overlying surface water. However, if Syncrude were to reduce or stop this pumping activity, it is very likely that concentrations of these inorganic constituents of concern would begin to increase back to pre-dilution levels resulting in a significant increase in toxicity and reduction in surface water quality. Despite these concerns, while inorganic constituents within BML surface water will continue to pose some risk to sensitive aquatic organisms, as long as the pumping activity of Syncrude continues unabated surface water quality will continue to slowly improve over time and may be sufficient to allow for the establishment of a functional aquatic ecosystem with low diversity, primarily consisting of salt-tolerant freshwater species.

CHAPTER 3 – PARTIAL LIFECYCLE ASSESSMENT OF BASE MINE LAKE SURFACE WATER ON THE SURVIVAL, GROWTH, AND DEVELOPMENT OF THE FRESHWATER MIDGE, *CHIRONOMUS DILUTUS*

3.1 Introduction

Although the aquatic toxicity of oil sands process-affected water (OSPW) has already been well characterized, historically most of these studies have examined effects on higher trophic level organisms such as fish and focused on short exposures for release scenarios. However, in the case of Base Mine Lake (BML) and end pit lakes (EPLs) in general, it is more appropriate to examine the potential long-term effects on lower trophic level organisms such as the midge *Chironomus dilutus* which plays a vital role in colonization and ecological succession of aquatic ecosystems.

Chironomids are a species of freshwater non-biting flies, commonly referred to as midges, widespread throughout Canadian aquatic ecosystems (Environment Canada, 1997). They have four unique life stages: benthic egg, larval, and pupal stages, and then emergence into terrestrial adults. After mating, females oviposit a single egg mass onto a water's surface which serves as the primary route of introduction into new aquatic environments. The entire lifecycle spans approximately 25 to 40 days depending on environmental factors, although they spend most of their life as detrivorous sediment-dwelling larvae (Environment Canada, 1997). It should be noted that while BML does have a benthic zone around the shoreline, the lake itself does not actually contain sediment as there is no solid bottom, but rather a gel-like 'mudline' between the overlying surface water and underlying FFT (see Section 1.2.2). The larvae of *C. dilutus* comprise an important component of the diet of fish and waterfowl, and have therefore become a standardized test species in toxicity tests of contaminated sediment and water.

Recently there have been a few studies examining the effects of chronic OSPW exposure to *C. dilutus*, however they were performed using fresh OSPW taken from West In-Pit (WIP) which contained much higher concentrations of dissolved metals, salts, and organic compounds than found in BML (Anderson *et al*., 2012b). As previously discussed, while the source of OSPW is the same as WIP, the surface water in BML has been diluted with freshwater and had been aging for approximately 22 months at the time of the first sampling (October 2014). Thus, it was of interest to repeat similar chronic exposures with *C. dilutus* to determine whether the toxicity observed by Anderson *et al.* (2012a, 2012b) continued to persist in the surface water of BML.

Preliminary results of a standard 10 d toxicity test with BML 2014 surface water showed no effects on larval survival and only minimal effects on growth in 100% BML surface water (*results not included in thesis*). Due to these limited effects, a partial lifecycle test (approximately 40 d of exposure) was initiated in order to examine potential effects on more sensitive sublethal endpoints such as development and adult emergence.

3.2   Methods

3.2.1   Test organisms and culturing

The test was conducted in accordance with Environment Canada Biological Test Method EPS 1/RM/32 guidelines (1997) with some modifications for the increased exposure duration. *C. dilutus* used in the test were obtained from an in-house culture at the University of Saskatchewan Toxicology Centre (Saskatoon, SK) raised in 15-L aquaria containing a thin layer of silica sand as artificial sediment (particle size 200 to 400 µm) and aerated, carbon-filtered, bio-filtered municipal water from the City of Saskatoon. Aquaria were kept in an environmental chamber maintained at $23 \pm 1°C$ with a 16:8 h light:dark photoperiod, and were fed 15 mL of a slurry of Nutrafin fish flakes (Rolf C. Hagen Inc., Montreal, QC) three times a week with weekly water changes.

3.2.2   Exposure waters

Reconstituted control freshwater was prepared by diluting *C. dilutus* culture water with approximately 30% reverse osmosis (RO) water to mimic the general water quality characteristics (pH, conductivity, hardness, and alkalinity) of Athabasca River Water (ARW) (see Section 2.2 for more detailed chemical characterization). To account for the potential effects of the elevated salinity (as total dissolved solids, TDS) within BML surface water on *C. dilutus*, an additional batch of reconstituted control salt water was prepared to match the salinity and major ion concentrations measured within BML surface water by adding 666 mg/L sodium chloride (NaCl), 160 mg/L sodium sulfate (Na_2SO_4), and 960 mg/L sodium bicarbonate ($NaHCO_3$) to the original reconstituted control freshwater. All chemical salts were obtained from Fisher Scientific (Ottawa, ON). Water quality parameters and target values of both reconstituted control waters are summarized in Table 3.1. Surface water collected from BML in October 2014 was used as the final exposure water (see Section 2.2 for more detailed chemical characterization). All exposure waters were aerated for a minimum of 48 h prior to test initiation and water changes.

Table 3.1 – Water quality parameters of reconstituted ARW (freshwater control) and reconstituted BML (salt water control) compared to target ARW and BML values.

Water Quality		ARW 2014 (Target)	ARW Recon. (Fresh Control)	BML 2014 (Target)	BML Recon. (Salt Control)
Conductivity	(μS/cm)	260	360	2860	2580
pH		8.0	8.2	8.9	8.9
Hardness	(mg $CaCO_3$/L)	100	120	100	70
Alkalinity	(mg $CaCO_3$/L)	100	120	600	660
Major Ions	**(mg/L)**	**[Measured]**	**[Nominal]**	**[Measured]**	**[Nominal]**
Sodium	Na^+	12	16	630	590
Potassium	K^+	1.4	2.1	9.1	2.1
Calcium	Ca^{2+}	29	22	23	22
Magnesium	Mg^{2+}	7.3	12	10	12
Chloride	Cl^-	7.7	8.5	460	410
Sulfate	SO_4^{2-}	22	56	210	160
Bicarbonate	HCO_3^-	120	-	720	700
Nitrate	NO_3^-	<0.05	2.2	10	2.2

3.2.3 Experimental design

3.2.3.1 Days 0 to 11: Egg mass exposure

The standard procedure for *C. dilutus* toxicity testing is to set up experiments using second instar larvae (approximately 8 d old), however, as previously discussed, these organisms will naturally enter new water bodies via egg mass oviposition from adult females. Although *C. dilutus* egg masses have been found to be very resistant to contaminants, first instar larvae are the most sensitive with tolerance progressively increasing with age and instar (Gauss *et al*., 1985). Standard toxicity tests may therefore miss a crucially sensitive stage of exposure. In order to be as conservative and environmentally relevant as possible, the partial lifecycle test was initiated with three egg masses placed directly into each of three 15-L aquaria containing silica sand and one of the exposure waters (freshwater control, salt water control, and 100% BML 2014 surface water) [Day 0]. The three egg masses were all ≤24 h old, approximately the same size, and isolated from adults from a single in-house culture and breeding jar. The aquaria were periodically fed following normal culturing procedures and daily observations were made on the appearance of each egg mass. After 11 d of exposure, the second phase of the exposure was initiated with 100 larvae randomly isolated from each aquarium. An additional five groups of 10 larvae were randomly

selected and isolated from each aquarium then dried at 60°C for 72 h to determine average 11 d larval dry weights for each treatment.

3.2.3.2 Days 11 to 23: Larval exposure

The second phase of the exposure period followed the standard test procedure for *C. dilutus* toxicity testing with 100 second instar larvae randomly isolated from each exposure tank and transferred in groups of 10 into 300-mL lidded tall form glass beakers containing 50 mL of silica sand and 200 mL of the respective exposure water. Since the organisms had already been exposed for 11 d at this point in the test, they were transferred from the exposure tanks into the glass test vessels with their larval cases intact. In order to investigate both larval and adult endpoints, a double test was set up with 10 replicates per treatment (30 beakers total) so that half of the test vessels could be taken down prior to pupation while the rest continued the exposure through to adult emergence. Each beaker was continuously aerated and fed 60 µL of Nutrafin slurry daily; water changes (approximately 80 to 90% of volume, ~175 mL) and water chemistry (temperature, dissolved oxygen, pH, conductivity, total hardness, alkalinity, and total ammonia) were performed every other day. All test vessels were maintained under the same environmental conditions as previously described for the exposure tanks. Observations of survival as well as larval activity and behavior were recorded daily. Once pupation was observed after an additional 12 d of exposure (Day 23), test vessels #1 to #5 for each treatment were terminated – final observations of survival were recorded then all organisms were removed from their sediment cases and oven dried.

3.2.3.3 Days 23 to 39: Pupation and adult emergence

The remaining test vessels (#6 to #10) in each treatment continued with the exposure unchanged, however, daily observations were expanded to also take note of the timing of larval pupation and adult emergence. Adults which completely emerged from their exuvia after pupation and left the surface of the water to land on the sides or top of the test vessels were deemed to have successfully emerged (survival). All successfully emerged adults were collected and removed from test vessels to determine sex and then individually labelled and oven dried to determine adult dry weights. Organisms which did not successfully emerge were classified into four categories of mortality: 1) death during larval stage; 2) death during pupal stage; 3) incomplete emergence from exuvia (drowned under water or at surface); and 4) complete emergence but drowned at surface. Subsets of individual organisms from each category and treatment, as well as larval cases, were

collected from test vessels and stored in 70% ethanol if later morphological and structural characterization was deemed necessary.

3.2.4 Data and statistical analysis

Based on the lack of observed effects on survival or growth in the 25% and 50% reconstituted salt water and BML surface water treatments in the preliminary test, only the fully concentrated (100%) reconstituted salt water and BML surface water treatments were used in the partial lifecycle test. As a result, no dose-response trend could be determined. However, in order to determine whether exposure to the reconstituted salt control or BML surface water caused significant effects on the survival and growth of *C. dilutus* larvae and adults, mean survival and dry weights from each treatment were analyzed and compared using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests. If data did not pass the Shapiro-Wilk normality test, significant differences among treatments were instead determined using Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's post-hoc tests. All data and statistical analyses were performed and plotted with Sigmaplot v12.0 software (Systat Software Inc., San Jose, CA) and conducted with $\alpha = 0.05$.

3.3   Results

3.3.1   Larval survival and growth (11 d)

A significant difference in the time for egg masses to hatch/dissolve was observed with the freshwater control larvae appearing to hatch within the first 48 h while the salt water control larvae hatched after 72 h; the BML surface water larvae appeared significantly delayed as the egg mass was still visible and well-defined after 96 h while the other two had almost completely disappeared. After 11 d of exposure, larvae within the two control aquaria appeared relatively large, red, and visible in their cases under the sediment surface (second instar); larvae in the 100% BML aquarium were also visible, albeit paler and smaller. This was corroborated by significant differences observed in the average larval dry weights between the freshwater control, salt water control, and BML surface water treatments (0.322, 0.105, and 0.040 mg, respectively). Direct egg mass exposure to the salt control and BML surface water did not have any visible effect on hatching success or larval survival as >200 larvae were counted in each of the three aquaria.

3.3.2   Larval survival and growth (23 d)

When the first half of the test was taken down after 23 d of exposure, no statistically significant differences were observed in average larval survival among the three treatments. Average larval survival was ≥94% in all treatments (Figure 3.1A), and was ≥80% in all individual control test vessel replicates in accordance with the requirements for test validity (Environment Canada, 1997). Although significant differences were observed in average larval dry weights after the first 11 d, the differences were much less pronounced after an additional 12 d of exposure. By Day 23, average dry weights for the freshwater and salt water control treatments were 1.987 and 1.771 mg, respectively, while the BML-exposed larvae were significantly smaller at only 1.456 mg (Figure 3.1B).
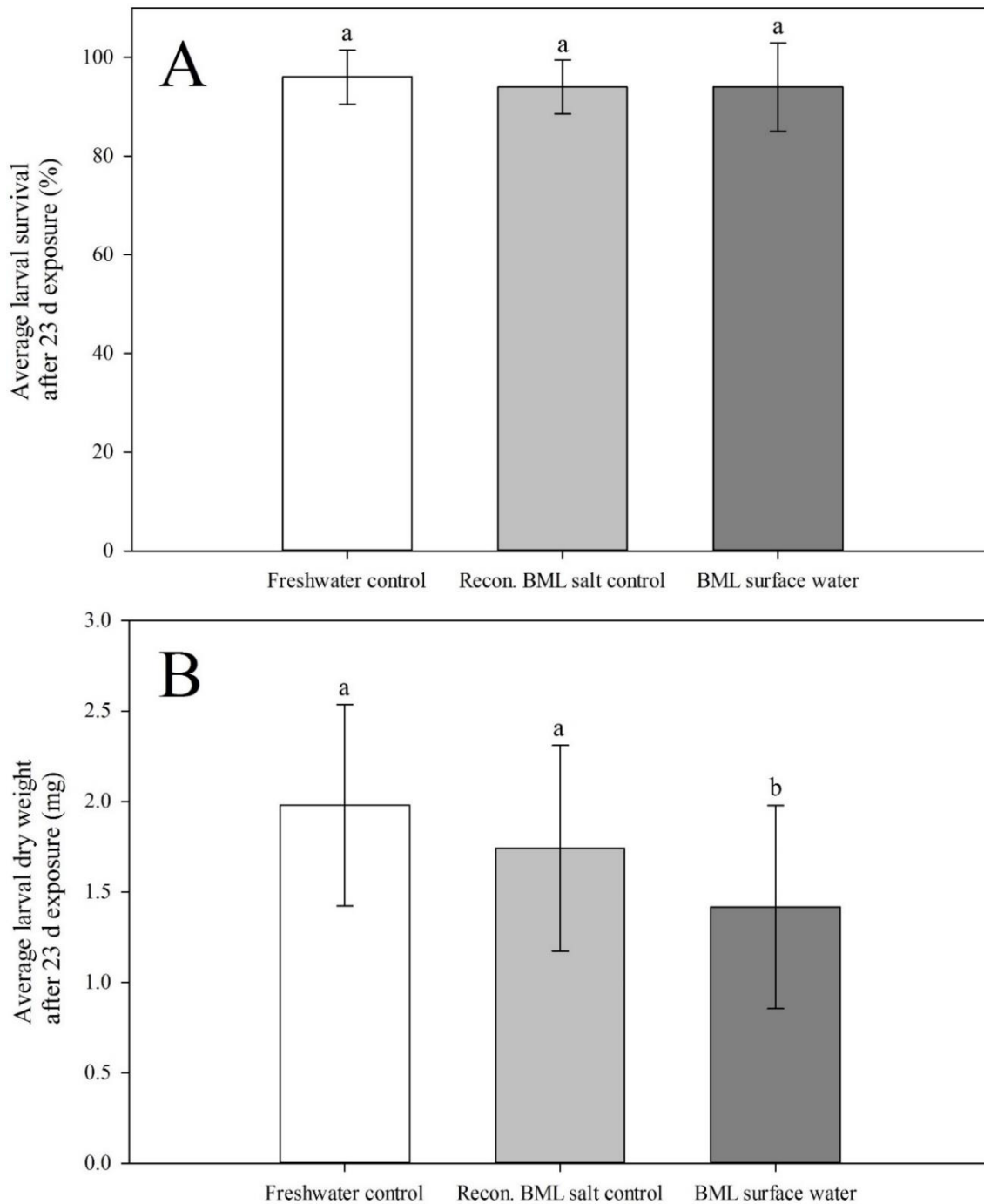
Figure 3.1 – Average survival (A) and dry weight (B) of *Chironomus dilutus* larvae after 23 d of exposure to freshwater control (reconstituted Athabasca River water), salt water control (reconstituted BML salt water), and 100% BML 2014 surface water. Error bars represent one standard deviation of the mean. Results did not pass Shapiro-Wilk normality test therefore significant differences among treatments were determined using Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's post-hoc test (n=5, α=0.05) and are denoted by different letters (a, b).

3.3.3   Adult growth, emergence timing and success (<40 d)

Despite initial differences in average larval dry weights, by test termination there were no longer any statistically significant differences among the three treatments once the sex of emerged adults was accounted for (Figure 3.2). Due to morphological differences, adult *C. dilutus* females are approximately 2.5x larger than males, however, the average size of adults of each sex were similar across all three treatments.
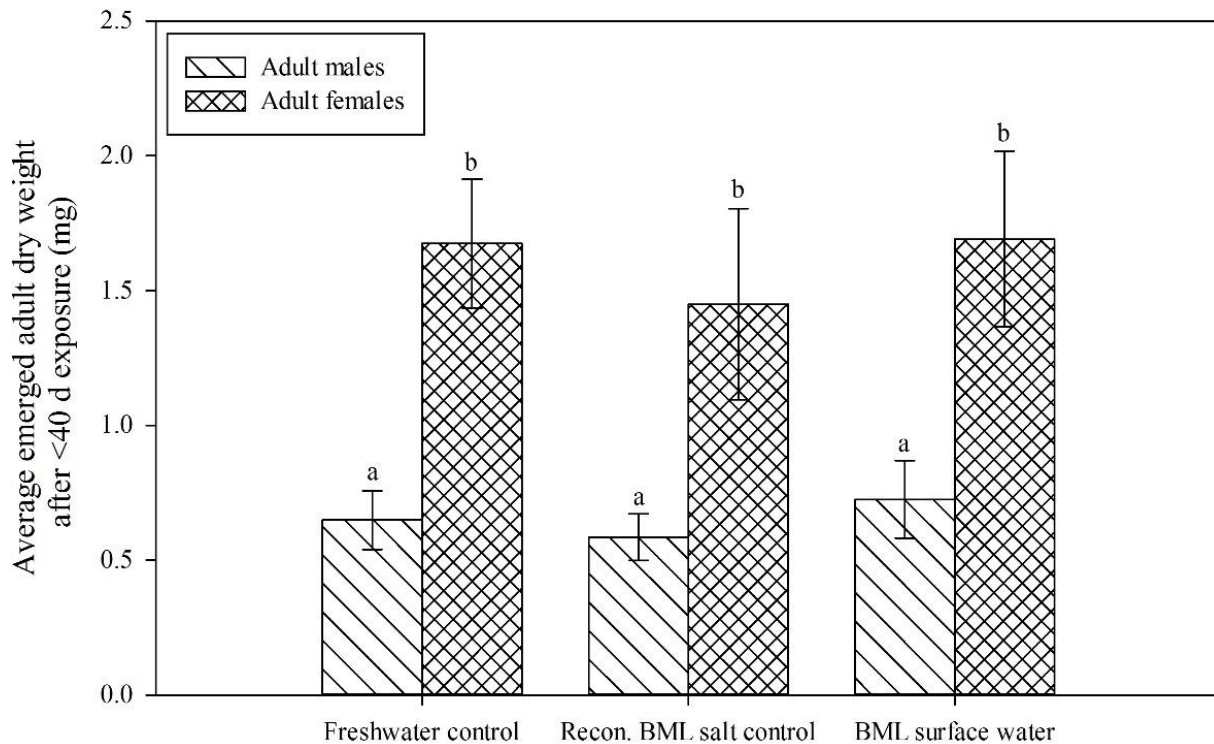
Figure 3.2 – Average dry weight of successfully emerged *Chironomus dilutus* adults after exposure to freshwater control (reconstituted Athabasca River Water), salt water control (reconstituted BML salt water), and 100% BML 2014 surface water. Exposure duration ranged from 23 to 39 d depending on time to emergence. Adults were separated into males and females based on morphological characteristics and were individually weighed. Error bars represent one standard deviation of the mean. Results did not pass Shapiro-Wilk normality test therefore significant differences among treatments were determined using Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's post-hoc test (n=5, α=0.05) and are denoted by different letters (a, b).

In the freshwater control, adult emergence began on Day 23 and ended on Day 34 after 60% of the organisms had successfully emerged; in the reconstituted BML salt water control, adult emergence began on Day 25 and ended on Day 36 after 64% of the organisms had successfully emerged; in the BML surface water treatment, adult emergence began on Day 27 and ended on Day 39 after only 36% of the organisms had successfully emerged (Figure 3.3). While adult emergence in the salt control treatment was delayed by two days, the rate of emergence followed a similar pattern to that of the freshwater control and resulted in a similar number of successfully emerged adults. Conversely, the adult emergence in the BML surface water treatment was much lower – reduced by 40% and delayed by 3 to 5 d compared to the controls.
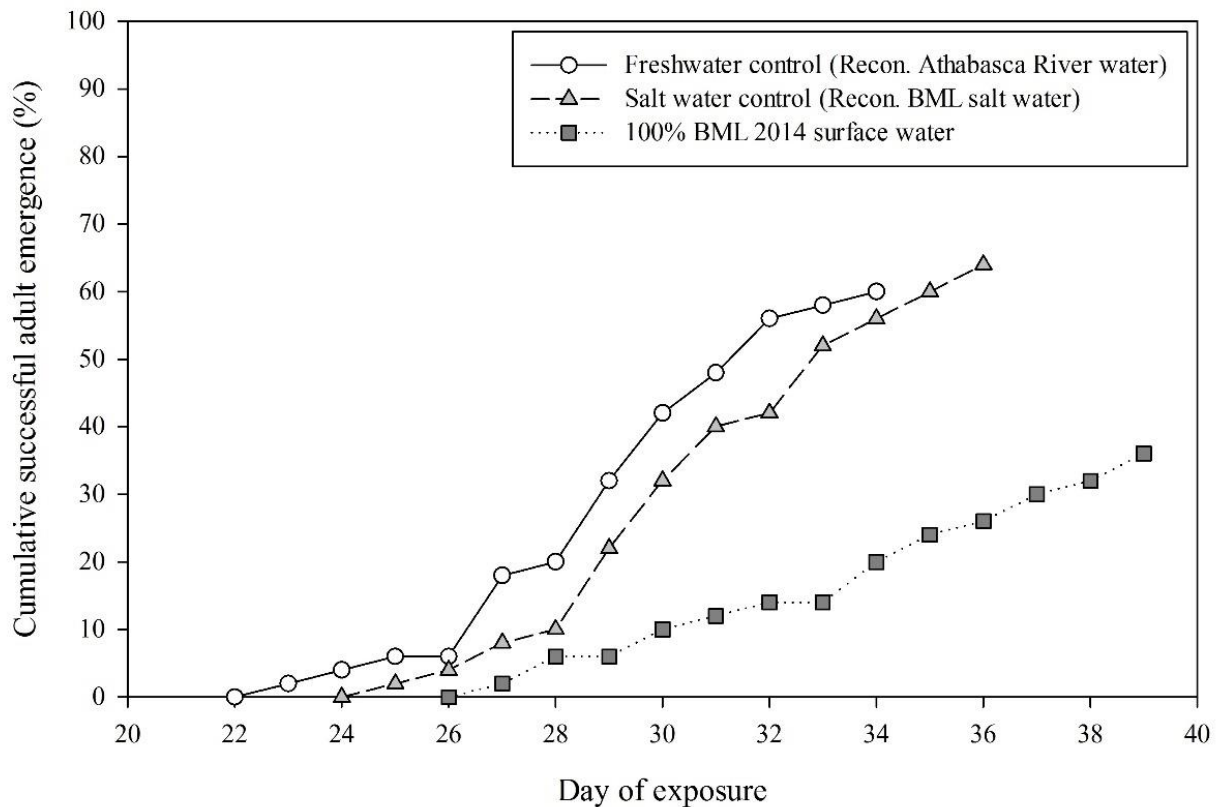
Figure 3.3 – Cumulative successful emergence timing of *Chironomus dilutus* adults after exposure to freshwater control (reconstituted Athabasca River Water), salt water control (reconstituted BML salt water), and 100% BML 2014 surface water. Emergence counts were conducted daily. Exposure duration ranged from 23 to 39 d depending on time to emergence.

As previously discussed, in order to more accurately identify effects on the survival and emergence of *C. dilutus* adults, organisms were separated into five distinct groups based on their survival at test termination: A) organisms which emerged completely from their exuvia and were able to leave the water's surface (survival, successful emergence); B) organisms which emerged completely from their exuvia but were unable to leave the water's surface and drowned (mortality); C) organisms which were unable to completely emerge from their exuvia and drowned (mortality); D) organisms which died as pupae (mortality); and E) organisms which died as larvae (mortality). Average percentages of organisms observed within each category of each treatment are shown in Figure 3.4.
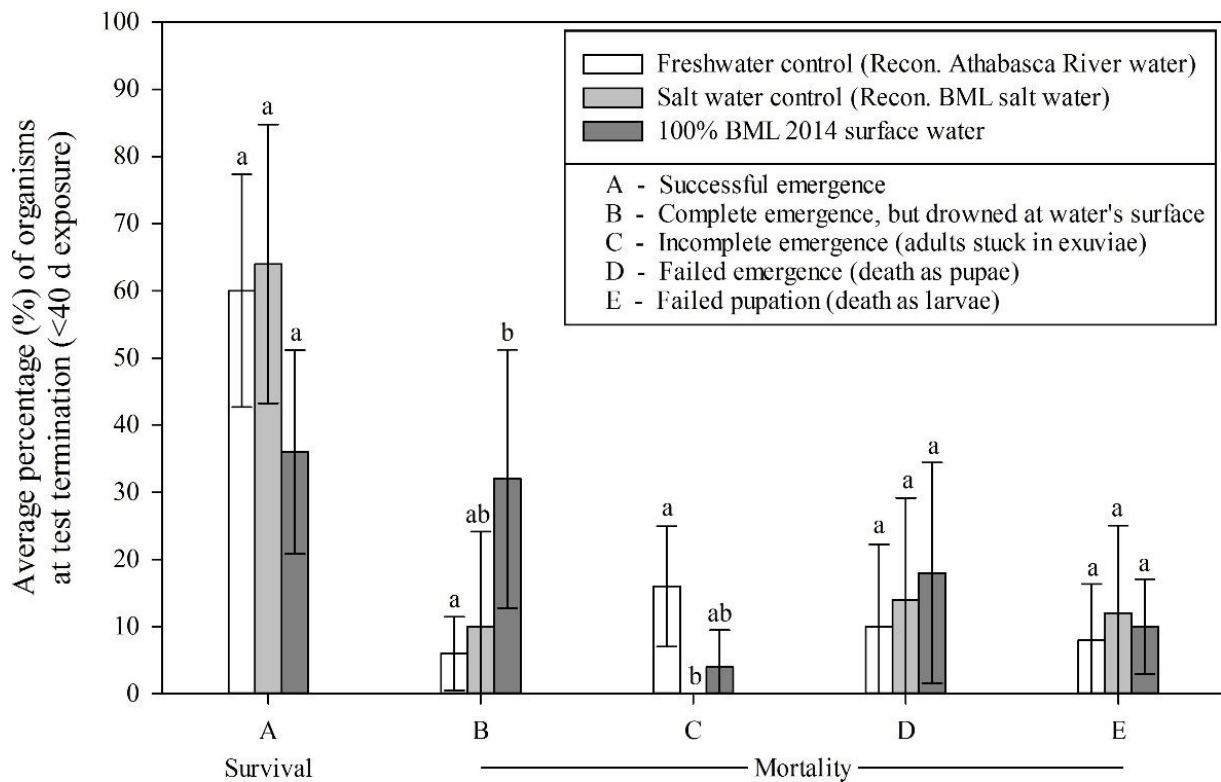
Figure 3.4 – Average percentage of *Chironomus dilutus* organisms observed within five defined categories of survival and mortality (A, B, C, D, E) after exposure to freshwater control (reconstituted Athabasca River water), salt water control (reconstituted BML salt water), and 100% BML 2014 surface water. Exposure duration ranged from 23 to 39 d depending on time to emergence (A, B, C). Error bars represent one standard deviation of the mean. Significant differences among treatments within each category were determined using one-way ANOVA followed by Tukey's post-hoc test (n=5, α=0.05) and are denoted by different letters (a, b).

Average successful emergence (category A) in the freshwater control, salt water control, and BML surface water were 60, 64, and 36%, respectively, as previously stated. Although the successful emergence in the BML surface water treatment appears to be much lower than that of the control treatments, no statistically significant difference was observed. It is important to note, however, that due to the small sample size resulting from taking down half of the test after 23 d (n=5), the statistical power of the test for category A was only 0.41, less than the desired power of 0.80. Thus, it is more difficult to detect a statistically significant difference (at α=0.05) even when one exists. If the test was repeated with the full number of replicates (n=10), it is possible that the decrease in successful emergence in the BML surface water treatment would be found as statistically significant. This is further corroborated by examining the other categories. Although there was a slight increase in pupal mortality (category D) in the BML surface water, the effect was not significant, with average mortality ranging from 10 to 18% across the three treatments. Similarly, the effect on average larval mortality (category E) was negligible, ranging from 8 to 12%. Interestingly, there was a significantly greater proportion of adults which died stuck in their exuvia (category C) in the freshwater control compared to the salt water control and BML surface water treatment (16, 0, and 4%, respectively). However, even combined, these three categories only explain 50% of the mortality observed in the BML surface water treatment, compared to 85% in the freshwater control, and 72% in the salt water control. The remaining 50% comes from a statistically significant increase in the average percentage of adults which emerged completely from their exuvia, but were unable to leave the water's surface (category B). The average mortality values for these organisms within the freshwater control, salt water control, and BML surface water treatments were 6, 10, and 32%, respectively.

3.3.4 Other sublethal endpoints

Although apical endpoints (survival and growth) were chosen as the primary focus for the partial lifecycle assessment, some additional sublethal endpoints were used to evaluate potential effects of exposure to BML surface water on the development of *C. dilutus* based on previous work done by Anderson *et al.* (2012a, 2012b). Quantitative analysis of the numbers and ratio of male to female emerged adults can be used as an indicator of stress in aquatic ecosystems. However, no statistically significant effects were observed between treatments or sexes – approximately equal proportions of male and female adults emerged successfully in all treatments (Figure 3.5). It should

be noted that similar to the survival and emergence data, due to the small number of replicates (n=5), the statistical power of the test was below the desired level (0.8).
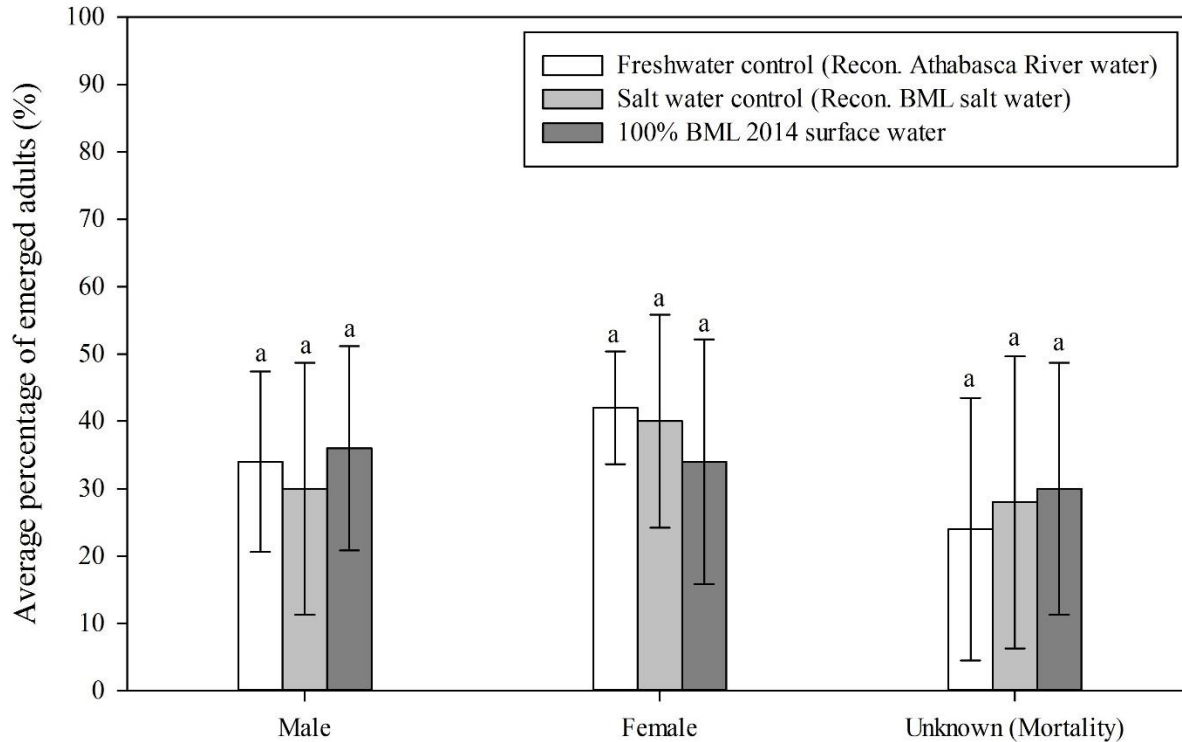
Figure 3.5 – Average percentage of emerged male and female *Chironomus dilutus* adults observed after exposure to freshwater control (reconstituted Athabasca River water), salt water control (reconstituted BML salt water), and 100% BML 2014 surface water. Exposure duration ranged from 23-39 d depending on time to emergence. Error bars represent one standard deviation of the mean. Significant differences among treatments within each category were determined using one-way ANOVA followed by Tukey's post-hoc test (n=5, α=0.05).

In addition to the quantitative measurements, qualitative observations were made on the behaviour of larvae in relation to their time spent within and outside their sediment cases, as well as the apparent structural integrity of the cases. In general, larvae in the BML surface water treatment appeared to be less active and spent more time within their cases than the larvae in the freshwater and salt water controls. Furthermore, the cases themselves within the BML treatment were more poorly constructed and fell apart when touched compared to the cases in the controls.

3.4 Discussion

As there have been very few studies of OSPW toxicity to aquatic invertebrates such as chironomids, this study was designed to complement previous work by Anderson *et al*. (2012a; 2012b) which examined the exposure of *C. dilutus* to a variety of fresh, treated, and aged Syncrude OSPW samples. As such, the test endpoints of this study correspond with those previously reported. Those tests used fresh WIP OSPW used in the initial creation of BML and containing a similar profile of contaminants, albeit less aged and dilute than those found in BML surface water. Furthermore, FE5 (FFT capped with OSPW, aged 20 years at the time of testing) and Big Pit (FFT capped with freshwater, aged 16 years at the time of testing) are two small-scale water-capped tailings (WCT) reclamation ponds, similar to BML, that can be used to predict the toxicity and chemistry of BML surface water now and into the future. Based on the findings of Anderson *et al*. (2012a; 2012b) the toxicity of 2014 BML surface water to *C. dilutus* was predicted to be significantly less than that of WIP, and possibly similar to that of the other reclamation pond water.

Across all test endpoints, exposure to reconstituted BML salt water had no statistically significant effect on *C. dilutus,* consistent with the observations from salt controls in the studies of Anderson *et al*. (2012a; 2012b). This apparent lack of salt toxicity was expected, as *C. dilutus* is generally much more tolerant of elevated major ion concentrations than more sensitive species such as *C. dubia* (20-d IC_{10} of 2316 mg Cl^- compared to 7-d IC_{25} of 454 mg Cl^-/L, respectively; Elphick *et al*., 2011). As such, all further discussion will focus solely on the 2014 BML surface water treatment.

3.4.1 Acute exposure – larvae

In this study, larvae were exposed directly to BML surface water as egg masses in order to mimic a realistic environmental exposure and to account for possible effects on the earliest life stage and initial case building activity. Despite this more conservative exposure scenario, there was no effect on larval survival, as observed in OSPW from other reclamation ponds (Anderson *et al*. 2012b) and in contrast to the significant 45% mortality observed in exposure to fresh WIP OSPW (Anderson *et al*., 2012a; 2012b). There was a small (27%) but significant reduction in larval dry mass after BML exposure similar to that observed in other reclamation ponds (approximately 20%; Anderson *et al*., 2012b), however, it was again significantly lower than the 64% reduction observed after exposure to fresh WIP OSPW (Anderson *et al*., 2012a; 2012b). Finally, although

no quantitative behavioural measurements were recorded in this study, general qualitative observations of slightly decreased larval activity and reduced size and structural integrity of cases were consistent with *C. dilutus* larvae exposed to waters from other reclamation ponds (Anderson *et al*., 2012b).

3.4.2 Chronic exposure – adults

Despite the initial reduction in larval biomass, by the time of emergence there was no longer any significant difference between the mass of the adults, once sex was accounted for. Furthermore, exposure to environmental stressors often leads to greater proportions of males in chironomid populations (Liber *et al*., 1996), however, no effects on sex ratio were observed in either this study or that of Anderson *et al*. (2012a). It is important to note that although there were no statistically significant differences in the final size of the adults, the time taken to reach this size varied depending on when emergence occurred. For example, adult emergence in the BML surface water exposure occurred approximately 4 to 5 d later than that of the freshwater control, meaning these organisms required nearly an additional week of growth to reach the same final mass. This delay in emergence timing, as well as the reduced larval biomass, is therefore most likely due to a decreased growth rate in BML-exposed organisms. A similar effect was observed in WIP-exposed organisms, although the delay in emergence was significantly longer (approximately 11 d), while, interestingly, no effect on emergence timing was observed in the FE5 or Big Pit treatments (Anderson *et al*., 2012a; 2012b). Of all tested endpoints, emergence success was found to be the most sensitive. There was a significant reduction in the BML surface water treatment with only 36% of adults emerging successfully. This successful emergence rate was almost identical to those of organisms exposed to FE5 and Big Pit treatments (approximately 35%; Anderson *et al*., 2012b), but still significantly higher than that of WIP-exposed organisms (13%; Anderson *et al*., 2012a). It should be noted that the successful emergence rate of the freshwater control in this study was only 60% and much lower than desired compared to 81% observed by Anderson *et al*. (2012b). The reason for this reduction may have been due to over-crowding of test vessels, especially during later stages of the exposure, as the studies by Anderson *et al*. (2012a; 2012b) purposely deviated from standard testing procedure by using larger 1-L beakers containing 500 mL of test solution compared to the 300-mL beakers and 200 mL of test solution used in this study. This suggests that improvements in test design may lead to an increase in the emergence success of organisms exposed to BML surface water. This is further supported by the fact that

while the successful emergence rate in BML was similar to those reported in FE5 and Big Pit (Anderson *et al*., 2012b), the successful pupation rate in BML was approximately 20% higher – equivalent to the observed difference in successful emergence rates of the controls.

3.4.3   Mechanisms of toxicity – organic acids and surface tension

Due to the lack of effects in the salt control treatment, as well as the significantly reduced toxicity in aged (FE5 and Big Pit), ozonated, and activated charcoal-treated OSPW samples, the toxicity of OSPW to *C. dilutus* has been attributed solely to the presence of dissolved organic compounds, specifically naphthenic acids (NAs) (Anderson *et al*., 2012a; 2012b; Wiseman *et al*., 2013a). This is further supported by the fact that the concentration of NAs in each of the discussed treatment waters was strongly correlated with toxicity and observed effects: WIP OSPW was significantly more toxic than all other treatments and contained 72 mg/L of NAs; the toxicities of all reclamation pond waters (BML, FE5, and Big Pit) were similar, significantly lower than WIP, and contained 25, 13, and 23 mg/L of NAs, respectively (Anderson *et al*., 2012b). See Appendix A for more detailed information on NAs within 2014 BML surface water. Although the results observed by Anderson *et al*. (2012a; 2012b) were attributed to NAs, no specific mechanism(s) of action were provided. However, based on the results of a previous study by Liber *et al*. (1996), Anderson *et al*. (2012b) suggested that the observed reductions in pupation and adult emergence were a result of reduced larval mass which in turn was possibly caused by stress or alterations in bioenergetics. The observed reductions in larval activity, and size and integrity of cases, were also attributed to stress and decreased energy stores (Anderson *et al*., 2012a). These conclusions are consistent with the results of this study which suggest that exposure to the dissolved organic fraction of BML surface water reduces the rate of growth of *C. dilutus* possibly due to stress and changes in energetics.

A later study by Wiseman *et al*. (2013a) examined more specific mechanisms of toxicity and found that exposure of *C. dilutus* to WIP OSPW caused increased abundances of the oxidative stress-related transcripts *gst*, *cat*, *gpx*, increased abundances of the endocrine disruption-related transcripts *usp, esr, err*, and increased lipid peroxidation. As a result, oxidative stress-induced apoptosis was proposed as the cause for decreased larval survival and growth via metabolism of organic compounds (e.g. NAs) in larvae resulting in the production of reactive oxygen species (Wiseman *et al*., 2013a). Additionally, reduced developmental effects (pupation and emergence)

were attributed to disruption of endocrine function, likely due to smaller organic acids with estrogen-like properties (Wiseman *et al*., 2013a). Similar effects of OSPW- and NA-induced oxidative stress and endocrine disruption have been observed in other aquatic species such as fish (see Section 1.3). Interestingly, these same effects were not observed in *C. dilutus* larvae exposed to aged FE5 OSPW which further supports the conclusion that aging reduces concentrations of NAs and their associated toxicity (Wiseman *et al*., 2013a). However, even though the toxicity of aged FE5 OSPW was significantly reduced compared to fresh WIP OSPW, it still contained an appreciable concentration of NAs and caused a significant reduction in the success of *C. dilutus* adult emergence. Studies have shown that smaller, slightly unsaturated NAs degrade significantly faster than larger, highly unsaturated NAs associated with persistent chronic toxicity in reclamation ponds (Toor *et al*., 2013a; 2013b). Anderson *et al*. (2012a) also found that ozonation of WIP OSPW significantly reduced the concentration of NAs and their associated toxicity, however, a small concentration and some effects remained. The results of Wiseman *et al*. (2013a) suggest that the smaller, more readily bioavailable and degradable NAs are responsible for these oxidative stress- and endocrine disruption-related mechanisms of toxicity to *C. dilutus* while the toxicity of aged OSPW in reclamation ponds is due to the larger, more recalcitrant NAs via some other mechanism.

The most interesting result of this study was that the reduction in emergence success of organisms exposed to BML surface water appeared to be due to a significant increase in the proportion of fully emerged adults that were unable to leave the surface of the water and drowned (see Figure 3.4). Unfortunately, Anderson *et al*. (2012a; 2012b) did not qualitatively assess adult emergence and therefore it is unknown whether a similar phenomenon occurred in their studies. Based on qualitative observations of water samples throughout the duration of this test, the surface tension of BML surface water was clearly lower than that of the freshwater and salt water controls, and may have been at least partly responsible for the effects on emergence. Studies characterizing OSPW and its dissolved organic fraction have frequently made note of the surfactant-like properties and structure of NAs (Schramm and Smith, 1985; Clemente and Fedorak, 2005; Frank *et al*., 2008; Pourrezai *et al*., 2011; Alharbi *et al*., 2016b), however, this surfactant effect in reducing surface water tension of OSPW and its impact on aquatic organisms has not been previously demonstrated. This is unsurprising, as this effect would have the most impact on insects, especially those that require surface tension to transition from an aquatic pupal stage to a terrestrial

adult stage (such as *C. dilutus*), which have only been briefly examined in the context of OSPW toxicity. It is also possible that this apparent inability to escape the surface of BML water may be a result of decreased energy, as previously discussed, or a combination of this effect with reduced surface tension.

3.4.4 Potential effects on population and ecosystem health

Unfortunately, neither the exposure in this study, nor those of Anderson *et al*. (2012a; 2012b), extended for the full lifecycle of *C. dilutus.* Without knowledge of the potential effects of OSPW on reproduction, it is difficult to predict population-level effects in the field. It has previously been shown that a reduction in larval growth can be associated with a decline in the reproductive output of female chironomids (Sibley *et al*., 1997), although the ovipositing success of females, and their second-generation offspring, may also be unaffected (Liber *et al*., 1996). Despite observed reductions in larval growth, the similar mass of all successfully emerged adults suggests that exposure to BML surface water should not significantly affect reproductive success. It has been proposed that delays in the emergence timing of *C. dilutus* adults, as well as altered behaviour and case structure, may increase predation of larvae and pupae (Sibley *et al*., 1997; Anderson *et al*., 2012b), however, this will only be a concern once BML surface water can sustain higher trophic level organisms such as fish, at which point the toxicity of BML surface water and prevalence of these effects will likely be further reduced. Delays in adult emergence may also affect mating success if they are sex-specific, such as those observed by Anderson *et al*. (2012a), although the lack of effect on sex ratio in successfully emerged adults in BML surface water suggests otherwise. Of all test endpoints, the reduced emergence success (36%) of adults exposed to BML surface water is therefore the most concerning with respect to long-term population and ecosystem health. Despite this reduction, adults that did successfully emerge appeared normal and healthy which may indicate that reproduction and population growth will still occur in BML, albeit at lower rates compared to natural aquatic ecosystems. This prediction is supported by the results of previous surveys of aquatic insect populations and succession in OSPW-affected constructed wetlands of the oil sands region. Whelly (1999) observed similar reductions in growth and survival of OSPW-exposed *Chironomus* larvae in these systems, but no significant effects on the rates of oviposition or total adults. However, a general trend of decreased taxonomic abundance and richness were observed by both Whelly (1999) and Leonhardt (2003) which indicated functional, but low diversity ecosystems.

3.4.5 Conclusion

Exposure to 2014 Base Mine Lake surface water caused significant reductions in the growth of *Chironomus dilutus* larvae, as well as delayed timing and reduced success of adult emergence. Due to a lack of effects in a salt water control, these effects were attributed to the presence of the dissolved organic fraction, specifically naphthenic acids, consistent with previous studies of *C. dilutus* exposed to oil sands process-affected waters (Anderson *et al*., 2012a; 2012b; Wiseman *et al*., 2013). The reduction in emergence success may have been due to the surfactant-like properties of NAs reducing the surface tension of BML surface water, the effect of which has not previously been discussed in aquatic organisms. The water chemistry and magnitude of toxicity to *C. dilutus* observed in BML surface water were similar to aged OSPW sampled from smaller-scale water-capped tailings reclamation ponds (Big Pit and FE5). However, it should be noted that at the time of sampling these reclamation ponds had been aging for 16 and 20 years, while BML surface water had been aging for less than two years. This disparity between the time required to reach similar water quality is likely due to the fact that Big Pit and FE5 have been naturally aging with no intervention, whereas BML is constantly being diluted as process water is pumped out and replaced with freshwater pumped in (as described in Chapter 2). As a result, surface water quality continued to improve over the course of this study and thus adverse effects in 2016 BML surface water will likely be further reduced. As long as Syncrude's pumping activity continues, the results of this study suggest that BML is a feasible reclamation method for oil sands tailings and that surface water is currently of sufficient quality to support some invertebrate populations. This trend should continue, especially as the littoral and benthic zones develop over time.

CHAPTER 4 – CHARACTERIZING THE CHRONIC TOXICITY OF BASE MINE LAKE SURFACE WATER ON THE SURVIVAL AND REPRODUCTION OF THE FRESHWATER MICROCRUSTACEAN, *CERIODAPHNIA DUBIA*

4.1 Introduction

Based on the limited toxicological effects observed in *Chironomus dilutus* exposed to 100% reconstituted salt water and 2014 Base Mine Lake surface water, additional toxicity testing was performed with a more sensitive species in order to be more conservative in the ecological risk assessment. To complement the work previously discussed in Chapter 2, *Ceriodaphnia dubia* was chosen for this purpose.

Ceriodaphnia dubia is a species of freshwater microcrustacean (zooplankton) ubiquitous throughout aquatic habitats in Canada, especially lakes and ponds. Along with other common zooplankton genera such as *Daphnia* and *Hyalella*, *Ceriodaphnia* fulfill a vital role in aquatic ecosystems by converting primary producers (phytoplankton and bacteria) into animal protein; they form an important link in aquatic food chains and comprise a significant portion of the diet of higher trophic level organisms such as juvenile fish (Environment Canada, 2007). *Ceriodaphnia dubia* is a standard test species used in aquatic toxicity testing and was specifically selected for this study due to its high sensitivity to a wide range of contaminants as well as its short life cycle and small size which allow for relatively short chronic reproduction studies (~7 days) with small requirements for test solution volumes (Environment Canada, 2007). Not only is *C. dubia* one of the most sensitive standard freshwater test species to many contaminants, it is also much more sensitive to salinity than *C. dilutus* (7-d reproduction IC_{25} = 454 mg Cl⁻/L vs 20-d growth IC_{10} = 2316 mg Cl⁻/L, respectively) (Elphick *et al*., 2011). Thus, it was a more suitable test species for assessing the toxicological risk of elevated salinity (as total dissolved solids, TDS) found within BML surface water.

At the time of testing, BML surface water samples were available from both 2014 and 2015. By performing toxicity tests with both samples, direct toxicological evidence of the effects of annual changes in BML surface water chemistry could be obtained. Before BML tests were performed, however, a standard sodium chloride (NaCl) reference toxicant test was performed to evaluate the sensitivity of in-house *C. dubia* cultures to salinity.

4.2 Methods

4.2.1 Test organisms and culturing

All tests were conducted in accordance with Environment Canada Biological Test Method EPS 1/RM/21 guidelines (2007) unless otherwise stated. Tests were conducted using healthy third-brood *C. dubia* neonates <24 h old taken from in-house mass cultures at the University of Saskatchewan Toxicology Centre (Saskatoon, SK). Mass cultures were held in 2-L glass jars containing approximately 80 organisms/L of moderately hard reconstituted water (MHRW) and kept in an environmental chamber maintained at $23 \pm 1°C$ with a 16:8 h light:dark photoperiod of <600 lux. Mass cultures were fed 15 mL of algae (*Pseudokirchneriella subcapitata*) and 4 mL of YCT (yeast, cerophyll, and trout chow) daily; water was changed at least three times per week.

4.2.2 Exposure waters

Freshwater control and NaCl reference toxicant

Moderately hard reconstituted water was used as the freshwater control and dilution water in all tests and was prepared by adding 96 mg/L sodium bicarbonate ($NaHCO_3$), 4 mg/L potassium chloride (KCl), 60 mg/L calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), and 60 mg/L magnesium sulfate anhydrous ($MgSO_4$) to reverse osmosis (RO) water. The reference toxicant test was performed using a 15.2 g/L stock solution of sodium chloride (NaCl) prepared in MHRW.

Reconstituted salt water and BML surface water

For the BML surface water exposures, reconstituted salt water control solutions were prepared similar to those used in the *C. dilutus* test (see Section 3.2.2) in order to account for the influence of salinity on *C. dubia* survival and reproduction. Since organisms were exposed to both 2014 and 2015 BML surface water, two separate reconstituted salt waters were prepared to mimic the water chemistry parameters and major ion concentrations in each BML surface water sample. Unfortunately, due to the high pH and HCO_3^-/CO_3^{2-} concentration within the reconstituted salt waters it was difficult to maintain the desired water hardness of 100 mg/L as $CaCO_3$ since the Ca and Mg appeared to precipitate out of solution over time. As a result, the water hardness of the reconstituted salt water in the first test ended up being only approximately 60 mg/L as $CaCO_3$ (henceforth referred to as the Hardness 60 test). A similar phenomenon was observed in the reconstituted salt water prepared in the *C. dilutus* test, although it had no effect on the results likely

due to the high tolerance of *C. dilutus* to salinity. However, water hardness has been shown to have a significant effect on mitigating the toxicity of salinity to sensitive freshwater organisms such as *C. dubia* (Lasier and Hardin, 2010; Elphick *et al*., 2011; Mount *et al*., 2016; Erickson *et al*., 2017). The test was therefore repeated with slightly altered recipes for MHRW and reconstituted salt waters for increased hardness, however, only 80 mg/L as $CaCO_3$ was achieved (henceforth referred to as the Hardness 80 test). A third and final test was performed at a water hardness of 100 mg/L as $CaCO_3$ with additional exposures of BML surface water treated with activated charcoal. Unfortunately, this test was unsuccessful and will not be discussed (see Appendix D for more information). Recipes and their respective water quality parameters and major ion concentrations for all reconstituted salt waters, in addition to their BML surface water targets, are summarized in Tables 4.1 and 4.2. All chemical salts were obtained from Fisher Scientific (Ottawa, ON). Surface water samples collected from BML in October 2014 and August 2015 were used as the final exposure waters (see Section 2.2 for more detailed chemical characterization).

Table 4.1 – Salts used to prepare each reconstituted exposure water (concentrations in mg/L).

Salts	Hardness 60 Test			Hardness 80 Test		
	MHRW Recon. Fresh	BML 2014 Recon. Salt	BML 2015 Recon. Salt	MHRW Recon. Fresh	BML 2014 Recon. Salt	BML 2015 Recon. Salt
$NaNO_3$	-	13.7	8.6	-	13.7	8.6
KCl	4	17.0	16.2	4	17.0	16.2
$CaCl_2$	-	58.2	58.2	-	-	-
$CaSO_4 \cdot 2H_2O$	60	-	-	86	111.6	111.6
$MgCl_2$	-	43.1	43.1	-	-	-
$MgSO_4$	60	-	-	-	-	-
$MgSO_4 \cdot 7H_2O$	-	-	-	172	141.4	141.4
Na_2SO_4	-	325.4	281.1	-	151.8	107.4
NaCl	-	593.7	545.0	-	711.5	662.8
$NaHCO_3$	96	939.8	921.1	96	976.3	957.7

Table 4.2 – Water quality and major ion characterization of all exposure waters used in *C. dubia* toxicity tests

Water Quality			Hardness 60 Test			Hardness 80 Test		
	BML 2014 Surface Water	BML 2015 Surface Water	MHRW Recon. Fresh	BML 2014 Recon. Salt	BML 2015 Recon. Salt	MHRW Recon. Fresh	BML 2014 Recon. Salt	BML 2015 Recon. Salt
Conductivity (µS/cm)	2970	2790	300	2750	2620	390	2800	2620
pH	8.9	8.7 – 8.9	8.0 – 8.3	8.9	8.8 – 8.9	8.0	8.4 - 8.9	8.6 – 8.9
Hardness (mg CaCO$_3$/L)	100 – 110	100 – 110	50 – 90	50 – 60	60	120	80	70 – 120
Alkalinity (mg CaCO$_3$/L)	580 – 600	600	60 – 80	440 – 520	480 – 540	60	520	480 – 520
Major Ions (mg/L)	[Measured]	[Measured]	[Measured]	[Measured]	[Measured]	[Nominal]	[Nominal]	[Nominal]
Sodium Na$^+$	600	560	28	570	540	28.6	600	560
Potassium K$^+$	8.9	8.5	2.0	8.4	7.6	2.1	8.9	8.5
Calcium Ca^{2+}	21	21	12	20	20	20	26	26
Magnesium Mg^{2+}	11	11	5.6*	9.6	9.5	17	14	14
Chloride Cl$^-$	440	410	2.9	420	390	2.3	440	410
Sulfate SO$_4$$^{2-}$	220	190	66*	210	180	116	220	190
Bicarbonate HCO$_3$$^-$	730	760	78	690	690	69.7	710	700
Carbonate CO$_3$$^{2-}$	33	<0.50	<0.50	9.5	17	-	-	-
Nitrate NO$_3$$^-$	10	6.3	0.3	12	7.6	0.2	10	6.3

*Lower than expected Mg^{2+} and SO$_4$$^{2-}$ concentrations likely due to human error in preparation (added together as MgSO$_4$)
MHRW Recon. Fresh: Moderately hard reconstituted water (freshwater control)
BML Recon. Salt: Reconstituted Base Mine Lake surface water (salt water control)

4.2.3 Experimental design

All tests were conducted in 25-mL borosilicate glass test tubes containing 15 mL of exposure water, 250 μL of algae (*P. subcapitata*), 25 μL of YCT, 1.2 μg of sodium selenate (Na_2SeO_4), 0.5 μg of Vitamin B12, and a single *C. dubia* neonate obtained from the in-house cultures. All exposure waters were aerated for at least 48 h prior to setup and were continuously aerated throughout the entire test duration. All tests were run with 10 replicates per treatment (10 organisms total) and held in test tube racks maintained under the same environmental conditions as the mass cultures. Test organisms were transferred to new, clean test tubes containing fresh exposure water, food, and nutrient solutions daily using transfer pipettes. During this process, the orientation and order of treatment groups was randomized in order to minimize potential effects of slight variations in light intensity. Observations on mortality, brood counts, and general appearance were recorded daily prior to water changes. Water quality parameters (temperature, dissolved oxygen, pH, conductivity, total hardness, alkalinity) were measured daily during water changes – new test solutions on Days 0, 2, 4, and 6; old test solutions on Days 1, 3, 5, and 7. Due to the small water volumes contained within each test tube, water quality for 'old' water was measured as a composite of all 10 replicates per treatment. The standard procedure is to terminate tests once ≥60% of the first-generation test organisms have produced three broods, or after 8 d, whichever comes first (Environment Canada, 2007). However, all tests were run for the full 8 d in order to standardize the test duration and collect as much data as possible. Survival is therefore based on 8 d of exposure, while reproduction endpoints are still based on time to produce three broods (≤8 d) in order to reduce variation among replicates.

NaCl reference toxicant test

The reference toxicant test was conducted by serial dilution of the stock solution to five concentrations: 0.475, 0.95, 1.9, 3.8, and 7.6 g NaCl/L using MHRW as the dilution water and control. All test solutions were prepared fresh daily.

Reconstituted salt and BML surface water tests

The tests with the reconstituted salt and BML exposure waters (for both 2014 and 2015, four exposure waters in total) were run at concentrations of 25, 50, and 100% using MHRW as the dilution water and control (see Figure 4.1 for experimental design). Despite the increased sensitivity of *C. dubia* compared to *C. dilutus*, no effects were predicted for concentrations of

either the reconstituted salt or BML surface water below 25% and were therefore not included in the test design. As previously discussed, the entire test (Hardness 60) was repeated a second time with different MHRW and reconstituted salt (2014 and 2015) recipes (Hardness 80) (refer to Tables 4.1 and 4.2). While the reconstituted salt waters differed between the two tests, the BML 2014 and 2015 exposure waters were identical and obtained from the same subsamples.
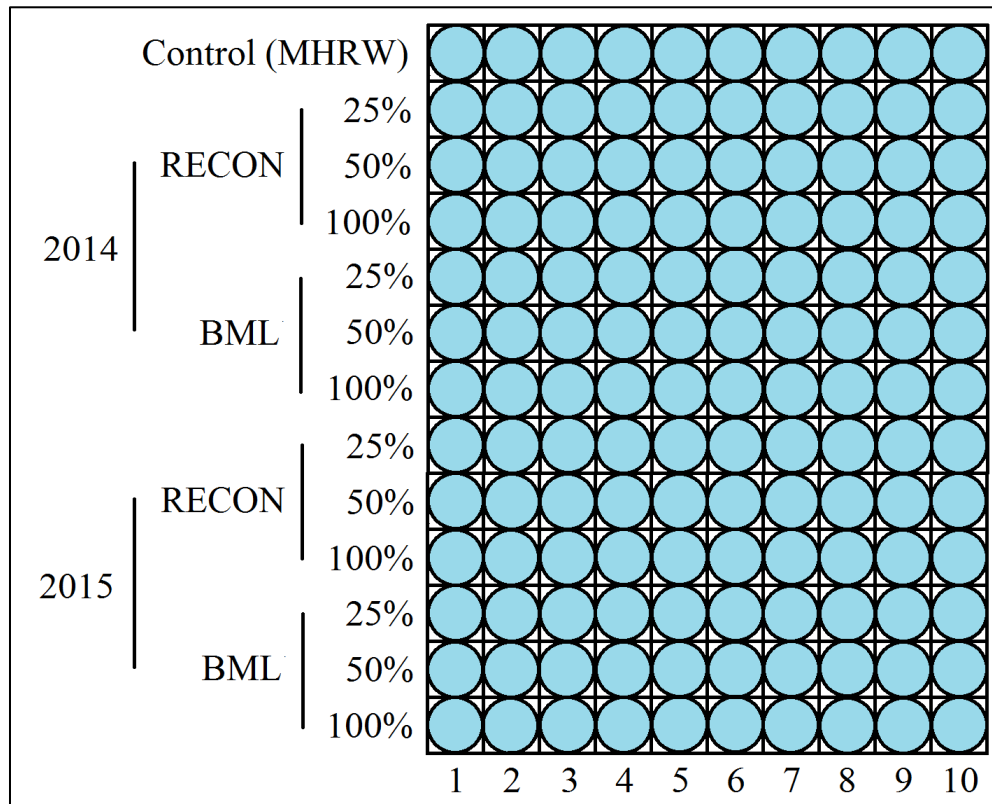
Figure 4.1 – Experimental design for chronic *Ceriodaphnia dubia* survival and reproduction tests. Each circle represents a test tube containing 15 mL of test solution and a single *Ceriodaphnia dubia* neonate. Each test contained 12 treatments consisting of four different exposure waters: 2014 and 2015 RECON (reconstituted Base Mine Lake salt water); and 2014 and 2015 BML (Base Mine Lake surface water) at three concentrations (25%, 50%, and 100%). Each treatment had 10 replicates. Moderately hard reconstituted water (MHRW) was used as the dilution water for all treatments as well as the control water. Treatments are shown in concentration order for clarity only – placement of test tubes in each treatment was randomized daily following water changes.

4.2.4 Data and statistical analysis

The toxicological endpoints for chronic *C. dubia* tests are survival expressed as a median lethal concentration (LC_{50}) and the reproduction inhibition concentration (IC_p). For the NaCl reference toxicant test, the 7-d LC_{50} value and its 95% confidence intervals were calculated using the trimmed Spearman-Karber method, version 1.5 software (US EPA, 1990). A probit analysis method requires ≥2 treatments with responses between 0 and 100% and therefore could not be used based on the observed results. The 3-brood IC_{50} value and associated 95% confidence intervals were calculated using the Inhibition Concentration (IC_p) approach, version 2.0 software (Norberg-King, 1993) based on the mean total number of neonates produced per test organism in their first three broods. Although the reconstituted salt and BML surface water tests involved four concentrations (0%, 25%, 50%, 100%) no direct dose-response endpoint was calculated. Instead, in order to compare the effects among the control, salt, and BML surface water treatments (as well as differences between 2014 and 2015) mean survival and reproduction from each treatment were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests. If results did not pass the Shapiro-Wilk normality test, significant differences among treatments were instead determined using Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's post-hoc tests. All data and statistical analyses were performed and plotted with Sigmaplot version 12.0 software (Systat Software Inc., San Jose, CA) and conducted with α = 0.05.

4.3 Results

4.3.1 NaCl reference toxicant test

Mortality of *C. dubia* neonates exposed for 7 d to NaCl followed a relatively steep dose-response curve. To predict the potential effects of BML salinity on survival of *C. dubia* neonates, results of the reference toxicant test were expressed as both Cl^- and TDS concentrations (Figure 4.2). Although the major ion composition of BML surface water is more complex than just NaCl, together Na^+ and Cl^- ions comprise most of the TDS within BML (36% and 26% as mg/L, respectively; 46% and 21% as mmol/L, respectively) and can thus be used as a surrogate for total salinity. The LC_{50} values were calculated for both parameters using the Spearman-Karber method yielding 937 mg Cl^-/L (95% confidence interval of 786 to 1116 mg Cl^-/L) and 1717 mg TDS/L (95% confidence interval of 1462 to 2017 mg TDS/L).
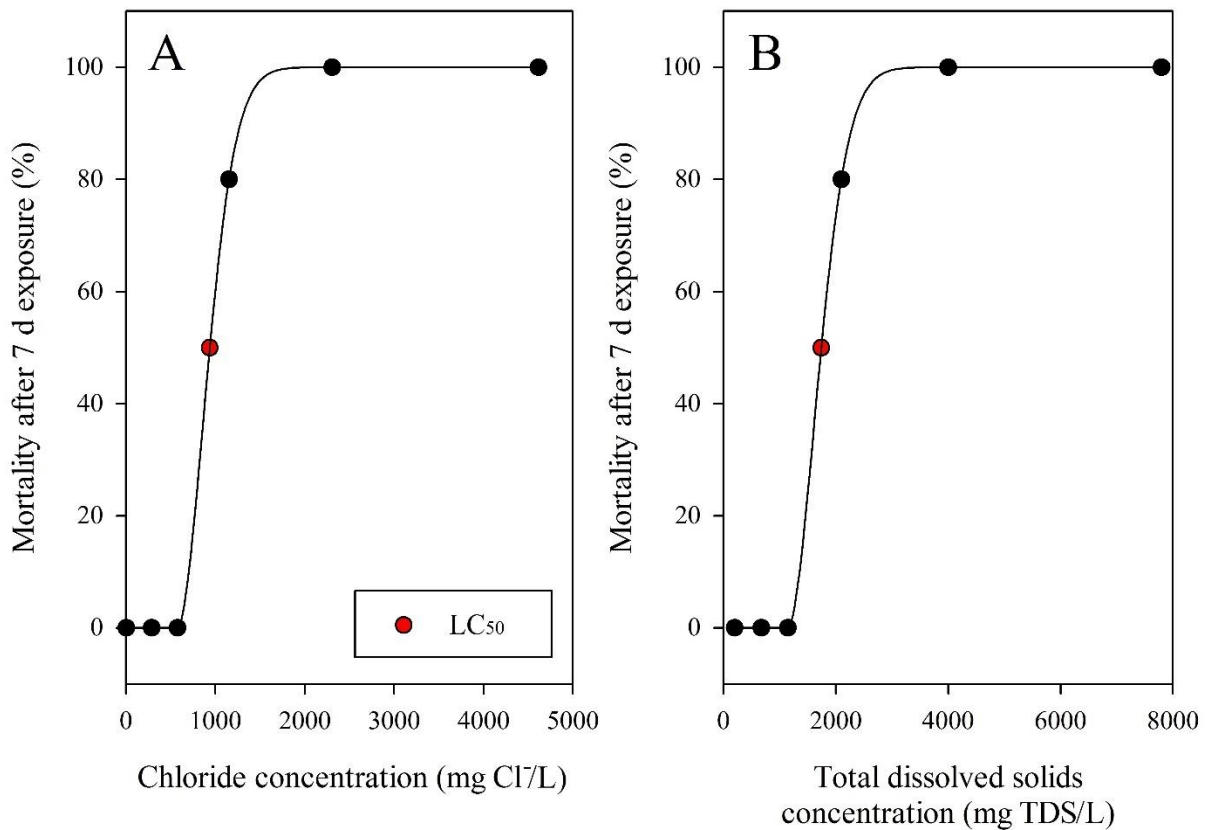
Figure 4.2 – Mortality dose-response curves for *Ceriodaphnia dubia* neonates exposed to sodium chloride (NaCl) in moderately hard reconstituted water for 7 d. Concentrations are expressed as both chloride (A) and total dissolved solids (B).

As expected, reproduction of *C. dubia* was found to be a more sensitive endpoint than mortality for NaCl exposure although it followed a similarly steep dose-response curve. Effects on reproduction were based on the total number of neonates produced per adult within their first three broods and used to calculate inhibition percentages in comparison to the control (Figure 4.3). Exact IC_{50} values corresponding to a 50% inhibition of reproduction were also calculated as 834 ± 26 mg Cl^-/L (95% confidence interval of 797 to 891 mg Cl^-/L) and 1534 ± 53 mg TDS/L (95% confidence interval of 1449 to 1654 mg TDS/L). More conservative IC_{10} values were also calculated as 406 ± 59 mg Cl^-/L (95% confidence interval of 289 to 529 mg Cl^-/L) and 832 ± 97 mg TDS/L (95% confidence interval of 591 to 987 mg TDS/L) to correspond with endpoints used in the derivation of Canadian Water Quality Guidelines (CWQGs) for the long-term protection of freshwater aquatic life, and the toxicological risk assessment in Chapter 2.

In addition to calculating reproduction IC_p values, the concentrations of Cl^- and TDS measured in 2014, 2015, and 2016 BML surface water were plotted against the reproduction inhibition dose-response curves. Based on Cl^- alone, the concentration within BML surface water was expected to only cause a 10% inhibition of reproduction to *C. dubia*. Furthermore, the CWQG value of 120 mg Cl^-/L (CCME, 2011) corresponds to approximately 2% inhibition of reproduction in *C. dubia*. Conversely, when examining TDS as a measure of total salinity, the concentrations in BML surface water fall along the steepest part of the dose-response curve. Thus, small changes in the salinity of BML surface water may have a significant impact on the reproduction of *C. dubia*. For example, the BML 2014 TDS of 1700 mg/L corresponds to approximately 70% inhibition of reproduction, while the BML 2016 TDS of 1550 mg/L corresponds to approximately 50% inhibition of reproduction. These results suggested that the salinity within BML surface water would have a significant effect on the reproduction of *C. dubia* and that salt controls were merited in subsequent toxicity testing.
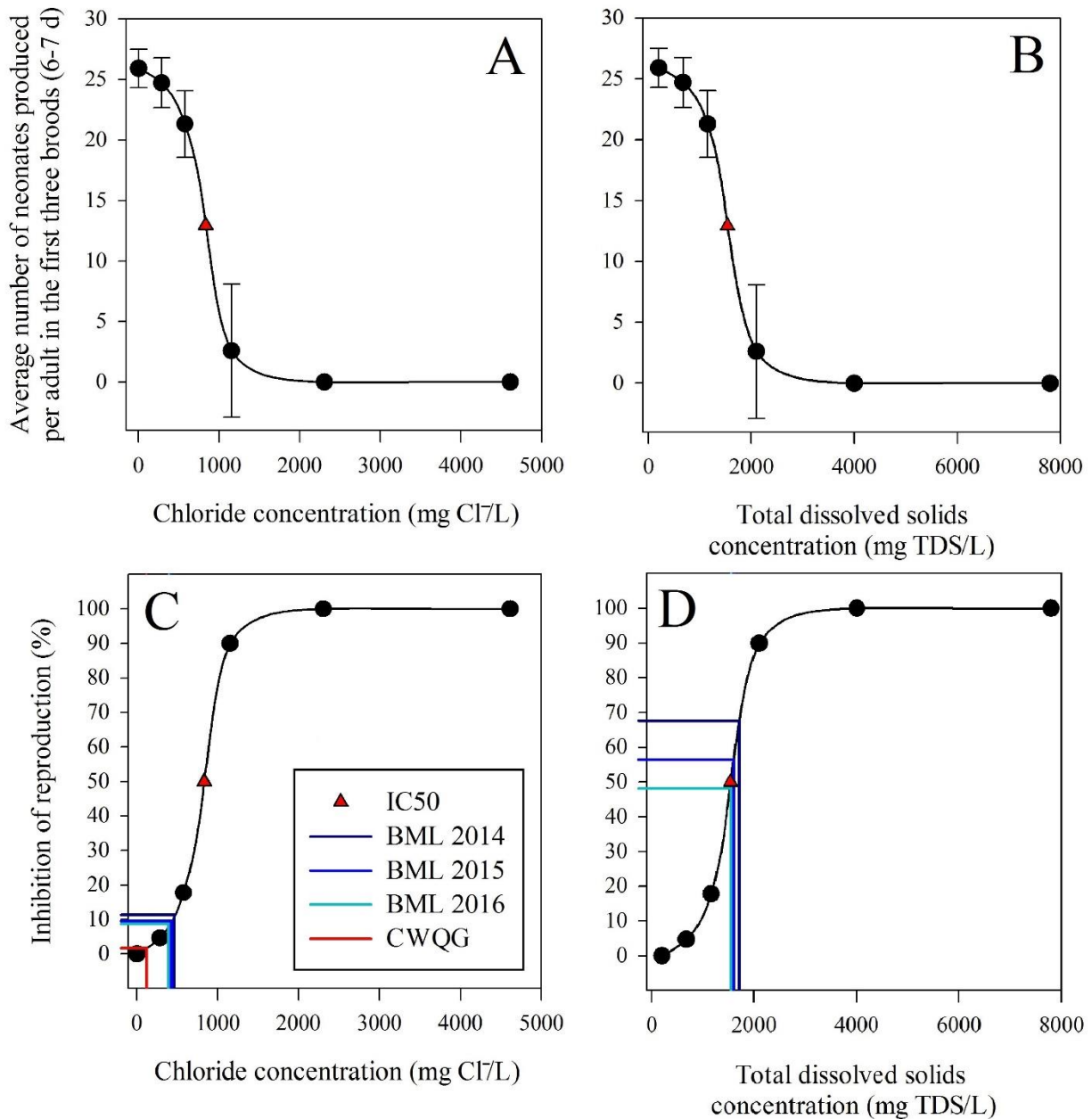
Figure 4.3 – Average number of neonates produced per Ceriodaphnia dubia adult in the first three broods (A, B) and converted values for inhibition of reproduction (C, D) after exposure to sodium chloride (NaCl) in moderately hard reconstituted water. Concentrations are expressed as both chloride (A, C) and total dissolved solids (B, D). Error bars represent one standard deviation of the mean. Dose response curves for reproduction inhibition were plotted against the concentration of chloride and total dissolved solids detected in 2014, 2015, and 2016 BML surface water (C, D), as well as the Canadian Water Quality Guideline (CWQG) for chloride (C), in order to predict the influence of salinity in subsequent tests with reconstituted BML salt water and BML surface water.

4.3.2　3-brood survival and reproduction (salt water control – 60 mg/L hardness)

Results of the first full *C. dubia* test with reconstituted BML salt water and BML surface water were unexpected. At concentrations of 25% and 50% BML salt water and surface water, no effects on survival after 8 d were observed in either the 2014 or 2015 treatments (all ≥90%). In the 100% treatments, survival in the 2014 salt water decreased to 30%, while the 2015 salt water had a survival of 70%. Interestingly, the same effects were not observed in the 100% BML surface water treatments – both 2014 and 2015 survival were ≥90%. These results suggested that the salinity of BML surface water alone was more toxic to *C. dubia* than the whole BML mixture (Figure 4.4).
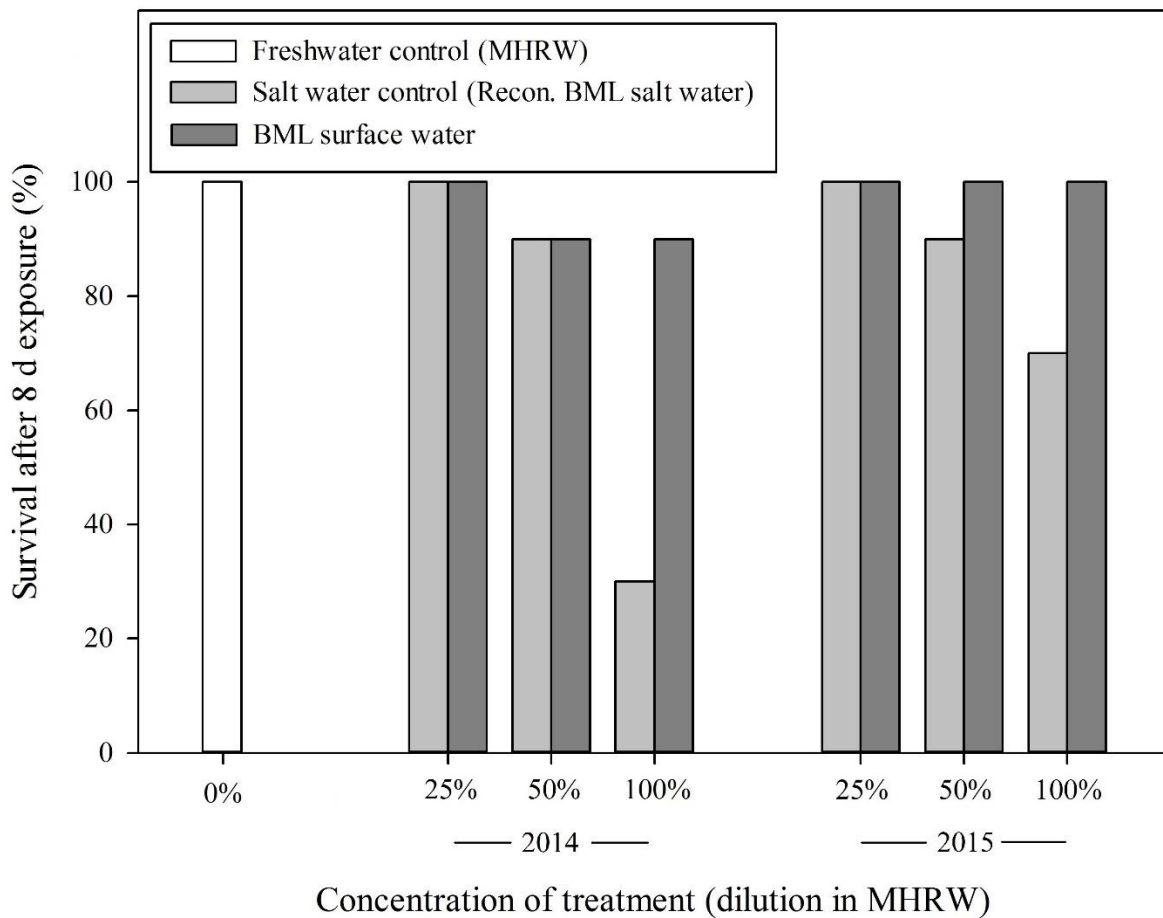
Figure 4.4 – Survival of *Ceriodaphnia dubia* neonates after 8 d exposure to freshwater control (moderately hard reconstituted water), 60 mg/L hardness salt water control (reconstituted BML 2014 and 2015 salt water), and BML 2014 and 2015 surface water.

Similarly, no statistically significant differences were observed in reproduction between any of the treatments or between the two years at the 25% and 50% dilution levels. However, statistically significant decreases in reproduction were observed in both the 100% BML salt water as well as the 100% BML surface water treatments (Figure 4.5A). For clarity, the neonate production values were compared to the controls to derive percentage-based reproduction inhibition values for these treatments (Figure 4.5B). Although no statistically significant differences were detected between 2014 and 2015, the 100% BML salt controls caused significantly greater average reproduction inhibition (95.7% and 80.7%, respectively) than the BML surface waters (40.4% and 37.3%, respectively). Again, these results suggested that the salinity alone was more toxic than the whole mixture of chemicals within BML surface water.
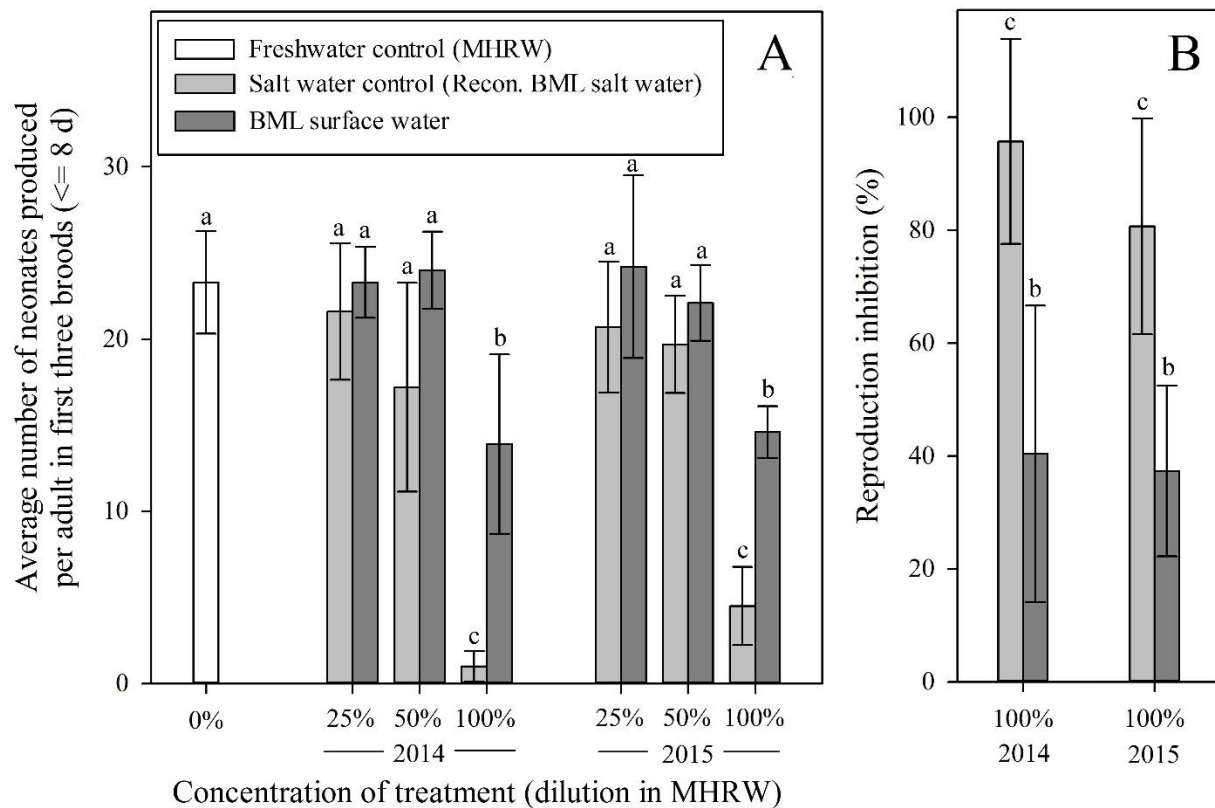
Figure 4.5 – Average number of neonates produced per Ceriodaphnia dubia adult in the first three broods (A) and converted values for reproduction inhibition (B) after exposure to freshwater control (moderately hard reconstituted water), 60 mg/L hardness salt water control (reconstituted BML 2014 and 2015 salt water), and BML 2014 and 2015 surface water. Error bars represent one standard deviation of the mean. Results did not pass Shapiro-Wilk normality test therefore significant differences among treatments were determined using Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's post-hoc test (n=6-10, α=0.05) and are denoted by letters.

4.3.3   3-brood survival and reproduction (salt water control – 80 mg/L hardness)

As previously discussed, based on the results of the first *C. dubia* test, the hardness of the reconstituted BML salt water was increased from 60 to 80 mg CaCO$_3$/L and the test was run again. This small change appeared to completely remove all mortality observed in the salt water control treatments of the previous test. Aside from the freshwater control with a survival of 90%, 100% survival was observed in all of the treatments after 8 d of exposure (Figure 4.6).

Figure 4.6 – Survival of *Ceriodaphnia dubia* neonates after 8 d exposure to freshwater control (moderately hard reconstituted water), 80 mg/L hardness salt water control (reconstituted BML 2014 and 2015 salt water), and BML 2014 and 2015 surface water.

As in the previous test, no statistically significant differences were observed in reproduction among any of the treatments or between the two years at the 25% and 50% dilution levels, except for 2015 BML surface water which caused a small, but statistically significant increase in reproduction. Statistically significant decreases in average reproduction were again observed in both the 2014 and 2015 100% BML salt water (53.3% and 52.0% respectively), as

well as the 2014 100% BML surface water (19.2%) treatments, however, the 100% BML 2015 surface water no longer caused any inhibition of reproduction (Figure 4.7). Although the 100% BML salt water treatments still caused significant reproduction inhibition, the effects were less severe than those observed in the test at 60 mg $CaCO_3$/L. This ameliorative effect of water hardness on the toxicity of salinity to *C. dubia* reproduction was consistent with the effects observed on survival and provided further evidence for the role of salinity in the toxicity of BML. It is important to note that the water hardness of BML surface water is even higher (100 mg $CaCO_3$/L) thus the effects of salinity within BML may be further reduced.
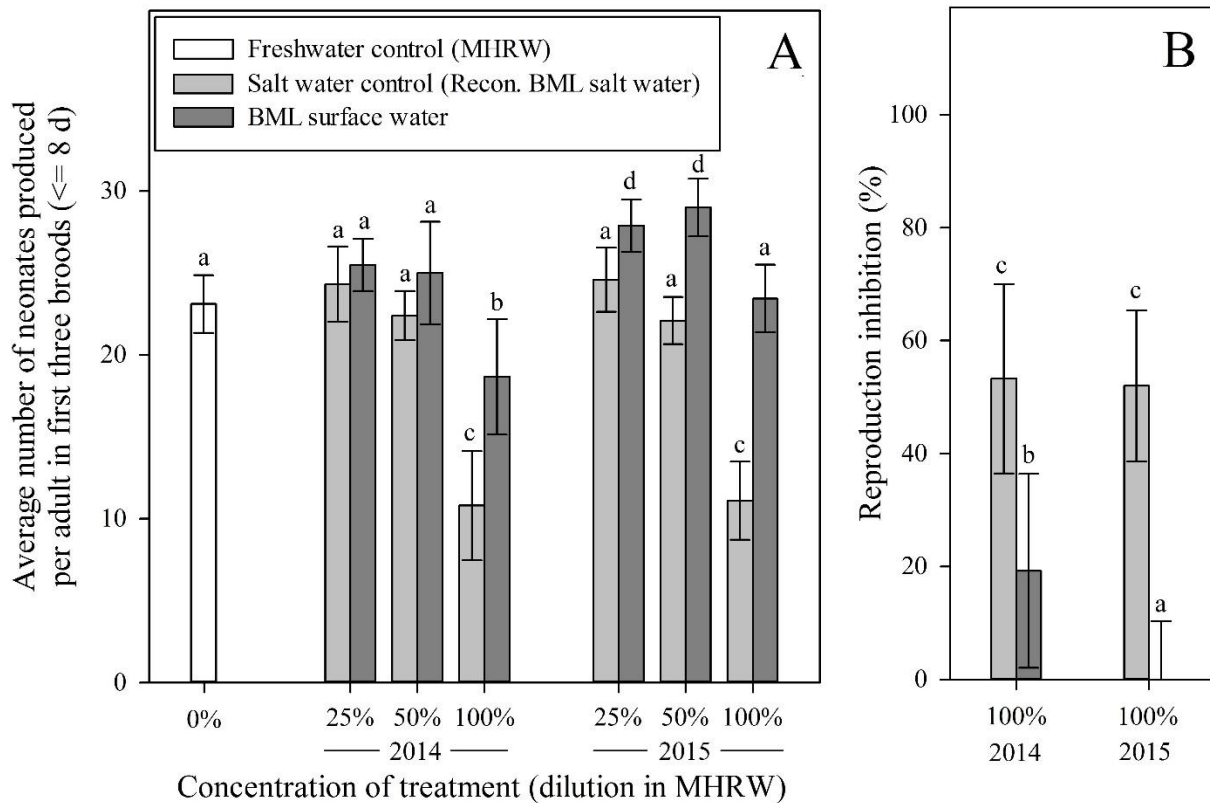
Figure 4.7 – Average number of neonates produced per Ceriodaphnia dubia adult in the first three broods (A) and converted values for reproduction inhibition (B) after exposure to freshwater control (moderately hard reconstituted water), 80 mg/L salt water control (reconstituted BML 2014 and 2015 salt water), a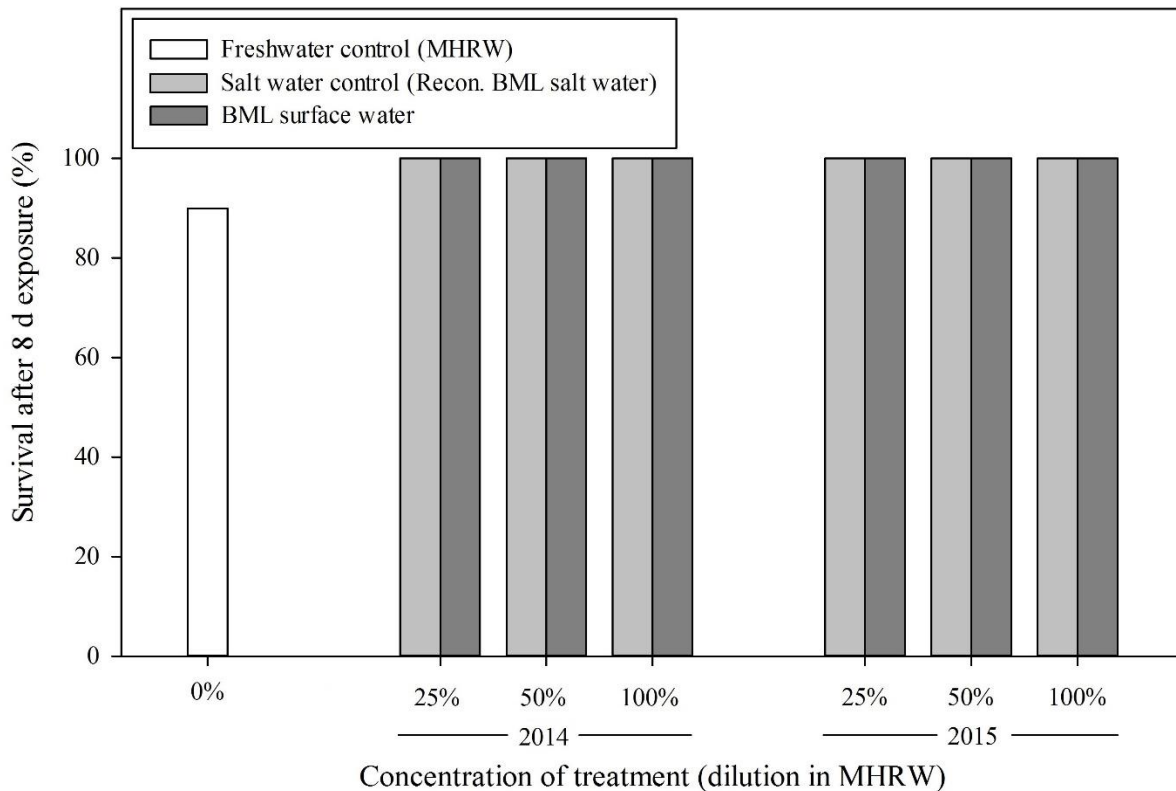nd BML 2014 and 2015 surface water. Error bars represent one standard deviation of the mean. Significant differences among treatments were determined using one-way ANOVA followed by Tukey's post-hoc test (n=6-10, α=0.05) and are denoted by letters.

4.4   Discussion

Although the results of the NaCl reference toxicant test and reconstituted BML salt water exposures were consistent with predictions of the toxicity of BML salinity, the whole BML surface water mixture was significantly less toxic to *C. dubia* than expected.

The steep dose-response of chronic salt toxicity to *C. dubia* in this study was consistent with previous studies (Lasier and Hardin, 2010; Mount *et al*., 2016), with reproduction only a slightly more sensitive endpoint than survival (7-d LC_{50}=937 mg Cl^-/L vs. 7-d IC_{50}=834 mg Cl^-/L). In moderately hard water, measured IC_{50} values were similar to those reported by Lasier and Hardin (2010) for Cl^- (834 mg/L vs. 653 mg/L) and nearly identical when expressed instead as TDS (1534 mg/L vs. 1569 mg/L). The measured IC_{25} for Cl^- was also similar to the value reported by Lasier and Hardin (2010), as well as that of Elphick *et al*. (2011) used in the derivation of the CWQG for Cl^- (635, 456, and 454 mg/L, respectively).

As previously discussed, NaCl only represents a portion of the salinity within BML surface water, thus all predictions of toxicity were based instead on TDS as a surrogate for total salinity. Although the toxicity of salts to *C. dubia* can vary widely depending on the ions tested (Lasier and Hardin, 2010; Mount *et al*., 2016; Erickson *et al*., 2017), TDS has been show to explain approximately 70% of observed chronic reproduction toxicity in *C. dubia* (Lasier and Hardin, 2010). Despite these inconsistencies, TDS can therefore still provide a rough estimate of the toxicity of total salinity within BML surface water to *C. dubia*. Based on the modeled dose-response trend (Figure 4.3), TDS alone was expected to cause a 50 to 70% inhibition of reproduction in *C. dubia* exposed to BML surface water. This effect was indeed observed in the 100% reconstituted BML salt water treatments, but not in exposures to whole BML surface water.

4.4.1   Hardness 60 test

Results of the first full *C. dubia* test with reconstituted BML salt water and BML surface water were somewhat unexpected. The most surprising result was the lack of mortality and lower than expected inhibition of reproduction in the BML surface water treatments, even at 100% strength. Reproduction inhibition rates were only 40%, on average, which was lower than what was previously predicted based on salinity (TDS) alone. In addition to salinity, whole BML surface water contains many dissolved organic acids and metals, as previously discussed, which are expected to add to the toxicity of the whole mixture (see Section 1.3). However, whole BML

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surface water was less toxic than the reconstituted BML salt water treatments, which suggested that either some component of the reconstituted water increased the toxicity of the major ions, or that some component of BML surface water decreased toxicity. It is also worth noting that although the 2015 treatment waters were slightly less toxic than the 2014 treatment waters (likely due to dilution, see Chapter 2), this difference was not statistically significant.

Although the lack of effects on survival and reproduction in the 25% and 50% reconstituted treatments was consistent with the steep slope of the salinity dose-response curve, mortality and reproduction inhibition in the 100% salt treatments were both greater than predicted by TDS. While some of this discrepancy may simply be due to inaccuracy in using TDS to predict complex salt mixtures, the increased toxicity of reconstituted BML salinity was most likely due to the lower than desired water hardness (especially when results of the Hardness 80 test are considered, see next section). At low to moderate water hardness, relatively small decreases in water hardness can significantly increase the toxicity of major ions to *C. dubia* (Lasier and Hardin, 2010; Mount *et al*., 2016; Erickson *et al*., 2017). Knowing this, the reconstituted BML salt water recipes were made to have a water hardness as close to BML surface water as possible (100 mg/L as $CaCO_3$). However, the final test solutions were much softer than desired (approximately 60 mg/L as $CaCO_3$) due to the formation of a visible salt precipitate – likely $CaCO_{3(s)}$ – during aging and aeration prior to initiating the test. A similar phenomenon was observed by Mount *et al*. (2016) in reconstituted waters with very high alkalinity due to the addition of large amounts of $NaHCO_3$ salt (as in this study). In order to control for the potential effects of water hardness, the study was repeated at a water hardness of 80 mg/L as $CaCO_3$.

4.4.2 Hardness 80 test

Even though the water hardness of 80 mg/L as $CaCO_3$ was still lower than that found in BML surface water, this increase of only 20 mg/L corresponded to a significant amelioration in the toxicity of the reconstituted BML salt water to *C. dubia* – reproduction improved by approximately 30 to 40%, and mortality was eliminated (a 70% increase in survival in the 2014 treatment). The resulting reproduction inhibition rates of approximately 50% were also consistent with those previously predicted by TDS. Despite these improvements in the apparent success of the second test, the toxicity of both 2014 and 2015 BML surface water in this test was also reduced (by approximately 20 to 40%), even though these treatment waters were identical to those of the

original test (only the reconstituted BML salt water treatments were amended with increased water hardness). Furthermore, while there was no difference between the toxicity of the 2014 and 2015 BML surface water samples in the original test, the 2015 BML treatments were significantly less toxic than those of 2014 in the second test – the 100% treatment no longer caused a decrease in reproduction, and the 25% and 50% treatments had significantly increased reproductive success compared to the control. The exact reason for the inconsistency between the two tests is unclear, but may have simply been due to a combination of differences in water hardness and perhaps subsampling and sample preparation. The hormesis-like effects observed in the 2015 BML surface water exposure were likely due to the very dilute concentrations of the trace metals, many of which are essential micronutrients for *C. dubia* (Naddy *et al*., 1995), which further highlights the fact that the reconstituted recipes used in these studies could be improved upon. A recent investigation of major ion toxicity to *C. dubia* by Mount *et al*. (2016) used amended salt waters instead of reconstituted salt waters – additional salts were added to a pre-existing natural water as a base, instead of synthetic laboratory water. Natural field-collected water contains background alkalinity without the need for the excessive addition of chemical $NaHCO_3$ salt, while also containing trace nutrients which provide beneficial effects and a more realistic exposure scenario (Mount *et al*., 2016). To try to control for these additional factors, a third and final test was conducted with reconstituted BML salt water at 100 mg/L water hardness, as well as an additional exposure to granular activated charcoal (GAC)-treated BML surface water. Granular activated charcoal treatment of OSPW from the same source as BML has been previously shown to remove a significant portion of the dissolved organic and metal constituents, while leaving the salinity relatively unaffected (Anderson, 2012b). The resulting GAC-treated water can therefore be used as a more natural approximation of BML surface water salinity, and provide a more environmentally relevant exposure comparison. Unfortunately, while the dissolved organic fraction was almost entirely removed, the GAC treatment leached a significant amount of Al and some other metals into the treated water which confounded the study and rendered the results inconclusive (see Appendix D for more details).

4.4.3 Influence of dissolved organic carbon on bicarbonate ion toxicity

Whatever the cause(s) of the inconsistency between the two tests may be, the one conserved observation was that whole BML surface water was significantly less toxic than the reconstituted BML salt water. Since the major ion concentrations in both test waters were nearly identical, and since dissolved metals within BML surface water are unlikely to pose much risk to *C. dubia* (see Chapter 2), the only major difference between the reconstituted and natural BML waters was the absence of dissolved organic carbon (DOC) in the salt water controls. It was therefore hypothesized that the high concentration of DOC within BML surface water may have an attenuating effect on the toxicity of the major ions. This apparent antagonism between major ion toxicity and dissolved organic compounds (specifically naphthenic acids, NAs) has previously been observed in preliminary studies of oil sands process-affected water (OSPW) exposure to both *C. dubia* and chironomids (Turcotte *et al*., 2009; Kennedy, 2012), however, the results were never published. As a result, testing of this hypothesis was an additional aim of the failed GAC exposure. Despite a lack of published studies, Lasier and Hardin (2010) found increasing DOC to potentially decrease the toxicity of HCO_3^- to *C. dubia* reproduction, although the effect was not observed on the toxicity Cl^- or SO_4^{2-}. Interestingly, a very similar interaction was observed in fathead minnow (*Pimephales promelas*) reproduction by Kavanagh *et al*. (2012) with the addition of $NaHCO_3$ causing a significant decrease in NA toxicity, while $NaCl$ and Na_2SO_4 had no effect. The conservation of this bicarbonate-organic acid interaction between different species and research groups (and the apparent lack of similar interactions with Cl^- and SO_4^{2-}) suggests a significant and specific mechanism of action, and supports the conclusions of both groups that the effect may be due to altered uptake (Lasier and Hardin, 2010; Kavanagh *et al*., 2012). Conversely, a study by Nero *et al*. (2006) found the addition of Na_2SO_4 to significantly reduce NA-associated mortality in yellow perch (*Perca flavescens*). Improved survival was similarly attributed to reduced uptake, however, this effect was instead due to inflammation reducing gill surface area.

Studies of chronic toxicity of major ions and salinity on the growth and reproduction of cladocerans such as *C. dubia* generally attribute effects to altered energy allocation due to impairment of osmoregulation (Soucek, 2007; Lasier and Hardin, 2010; Freitas and Rocha, 2011; Simmons, 2012; Mount *et al*., 2016; Erickson *et al*., 2017). The hemolymph of freshwater cladocerans such as *Ceriodaphnia* is hyperosmotic relative to the external environment, thus osmoregulation is largely dependent on the active uptake and concentration of major ions against

a concentration gradient, such as Cl^- exchange for HCO_3^- via active Na^+/K^+ ATPase (adenosine triphosphatase) transport pumps (Aladin, 1991; Hoke *et al*., 1992; Aladin and Potts, 1995). Since the body wall of cladocerans has very low permeability to water and ions, these transport processes occur via specialized, highly permeable membranes containing mitochondria-rich ion-transporting cells (also known as chloride cells or ionocytes) localized at the epipodites – gill-like structures attached to the base of the legs (Aladin, 1995). In environments with increased salinity such as BML, concentration gradients across these membranes are altered, resulting in greater energy required to maintain homeostasis and leading to stress. Several other related mechanisms of effect have also been discussed including direct damage to embryos (Freitas and Rocha, 2011), altered membrane permeability (Baker *et al*., 2017), competition at uptake sites (Simmons *et al*., 2012; Soucek and Dickinson, 2016), and decreased filter-feeding rates (Soucek, 2007). Recent studies of HCO_3^--specific toxicity (Harper *et al*., 2014; Vera *et al*., 2014) have attributed effects to the original mechanism proposed by Hoke *et al*. (1992) who suggested that HCO_3^- toxicity is due to the disruption of active Cl^-/HCO_3^- exchange and regulation resulting in the inhibition of Cl^- uptake by cladocerans. High concentrations of HCO_3^- have also recently been observed to cause significant reductions in Na^+/K^+-ATPase activity in fathead minnows (Farag and Harper, 2014), which provides further evidence for the role of HCO_3^- in altering osmoregulation in aquatic organisms. Unfortunately, even after decades of testing, the exact mechanisms of toxicity of many major ions, including HCO_3^-, to *C. dubia* and other aquatic organisms are still unclear (Lasier and Hardin, 2010; Simmons, 2012; Baker *et al*., 2017). This makes it exceedingly difficult to determine whether this HCO_3^--DOC interaction is in fact occurring, and if so, the mechanism through which it acts. It seems most likely that the high concentration of DOC within BML surface water is somehow reducing the availability of major ions such as HCO_3^- to interfere with these osmoregulatory functions, however, the exact mechanism for this interaction is unknown.

4.4.4 Conclusion

Base Mine Lake surface water collected in 2014 and 2015 was found to have no effect on the chronic survival of *Ceriodaphnia dubia*, and only caused minimal inhibition of reproduction. These effects were significantly lower than those predicted and observed in treatments of BML salinity alone. This suggests that some component of BML surface water, possibly the dissolved organic fraction, has an attenuating effect on the toxicity of the major ions present. Bicarbonate likely accounts for the majority of this major ion-associated toxicity and risk as it is the single most toxic anion to *C. dubia* and also the most concentrated within BML surface water. A few studies have previously observed a similar antagonistic interaction between HCO_3^- and dissolved organic compounds, such as naphthenic acids, which may explain this attenuating effect, however, the data are extremely limited and should be the focus of future characterization of BML surface water toxicity. Furthermore, the concentrations of these dissolved organic acids within BML surface water are expected to gradually decrease over time due to natural aging and biodegradation processes while the concentrations of the inorganic constituents such as major ions will persist indefinitely. It is therefore possible that as BML ages, surface water quality may begin to decrease again due to increased salt toxicity as this antagonistic and ameliorative effect becomes depleted.

CHAPTER 5 – GENERAL DISCUSSION

5.1   Project rationale and research objectives

As conventional oil and gas sources are depleted, global demand for non-conventional sources such as bitumen within the Alberta oil sands is increasing, leading to plans for increased expansion and production (CAPP, 2015). In the Athabasca region of the Alberta oil sands, the extraction of bitumen via surface mining and chemical separation processes has led to significant environmental impacts including removal of overburden, creation of mined-out end pits, and significant production of fluid fine tailings (FFT) and oil sands process-affected water (OSPW). Due to a lack of sufficient treatment methods, after 40 years of mining activity and development, Alberta oil sands mine operators have accumulated nearly 1 trillion L of 'legacy' fluid tailings (AEP, 2015a) which are currently stored in tailings ponds covering over 88 km^2 of the Athabasca region (AEP, 2015b). In recent years, several provincial groups have been established in order to manage and assess these environmental impacts, including the Alberta Environmental Monitoring, Evaluation and Reporting Agency (AEMERA) and the Alberta Energy Regulator (AER). While these groups have had some success in reducing the rate of production of new tailings, the accumulation and storage of legacy tailings remains a pressing concern. As of 2014, less than 10% of the oil sands mining industry's 933 km^2 footprint is in some stage of reclamation, and only 1 km^2 of land has been certified as reclaimed (AEP, 2015d). Over the years many reclamation strategies and techniques have been proposed and tested by oil sands mine operators, however, water-capped tailings (WCT) in end pit lakes (EPLs) – the storage of accumulated fluid tailings in old mined-out bitumen ore pits capped with a surface layer of water – is perhaps the most popular and has many distinct advantages over other methods. By incorporating waste products (FFT and OSPW) directly into the reclamation landscape through the establishment of artificial lakes (EPLs), oil sands mine operators can solve both issues of tailings accumulation and storage. The goal of this reclamation method is for EPLs to ultimately resemble natural aquatic ecosystems which can then be fully connected and incorporated into the surrounding landscape and watershed.

Unfortunately, due to the physical and chemical processes used in the extraction and separation of bitumen from oil sands ore, the FFT (pore water) and OSPW within these EPLs contain high concentrations of various constituents of concern – dissolved organic compounds, metals, and salts. These constituents cause adverse effects in aquatic organisms and therefore

reduce surface water quality and impede the development of EPLs into functional aquatic ecosystems. To address these concerns, Syncrude initiated the Base Mine Lake (BML) experiment in December of 2012 – the first large-scale demonstration of WCT EPLs within the Alberta oil sands. Base Mine Lake is an 8 km^2 WCT EPL which contains approximately 186 billion L of FFT capped with 65 billion L of OSPW and fresh water (Dompierre and Barbour, 2016). The purpose of the BML experiment is to demonstrate the WCT method on a much larger scale to improve industry understanding of EPL design and operation, specifically the time required to reach acceptable surface water quality necessary for the development of a functional aquatic ecosystem.

The purpose of this thesis was to evaluate Syncrude's BML experiment in order to more adequately assess the sustainability and environmental risks of WCT and EPLs for the reclamation of Alberta oil sands surface mining activity. The studies herein were specifically designed to complement the goals of Syncrude's chemical performance monitoring plan outlined in the BML Start-Up Phase (2013 to 2016) and to address the following research objectives:

1) a. Identify inorganic constituents of concern within BML surface water (specifically metals and major ions) and track annual changes in their concentrations over the course of three years (2014 to 2016) to establish trends and predict future concentrations [Chapter 2]

   b. Evaluate how annual changes in surface water chemistry (specifically metals and major ions) affect toxicity and potential risk to aquatic organisms to estimate the time required to reach acceptable surface water quality [Chapter 2]

2) Characterize the toxicity of BML surface water to apical endpoints of freshwater species representative of those involved in the colonization and succession of aquatic ecosystems
   a. Assess the survival, growth, and development of the benthic invertebrate *Chironomus dilutus* exposed to 2014 BML surface water for most of its lifecycle (30 to 40 days) [Chapter 3]
   b. Assess the survival and reproduction of the sensitive zooplankton *Ceriodaphnia dubia* exposed to both 2014 and 2015 BML surface water samples for 8 days [Chapter 4]

5.2   Summary of results

5.2.1   Base Mine Lake surface water chemistry and risk

Despite a general decreasing trend, BML surface water chemistry remained relatively consistent with respect to concentrations of the inorganic constituents over the three-year course of this study (2014 to 2016). Due to the lack of significant changes, concentrations of major ions and dissolved metals within BML surface water remained significantly elevated compared to background Athabasca River water (ARW) concentrations. As of 2016, BML surface water was still approximately 10x more saline (as total dissolved solids, TDS) than ARW, primarily due to approximately 50x higher concentrations of Na^+ and Cl^-, though significantly elevated concentrations of HCO_3^- and SO_4^{2-} also contributed. Salinity decreased by approximately 10% from 2014 to 2016 and was due to similar decreases in the concentrations of Na^+ and Cl^- (14 and 12%, respectively). Previous hydrological studies have shown that surface water concentrations within BML are primarily controlled by two competing processes: 1) influx: increases due to movement of constituents from FFT pore water into the overlying surface water via advective-diffusive transport; and 2) efflux: decreases due to the continuous pumping of water within BML by Syncrude (freshwater in, process water out) resulting in removal of constituents and dilution of the system (Dompierre and Barbour, 2016; Dompierre, 2017). Since Cl^- generally acts independently of biogeochemical reactions within aquatic systems, the decreases observed in BML surface water were nearly perfectly explained by the net result of these two processes (based on data reported by Dompierre, 2017). Similarly, even though Na^+ is involved in ion exchange at clay surfaces within BML surface water, these reactions have a negligible effect on the total dissolved concentration thus annual changes in Na^+ follow a similar trend to that of Cl^-. Conversely, the concentrations of HCO_3^- and SO_4^{2-} within BML surface water remained relatively unchanged over the course of this study as their influx is controlled by an additional biogeochemical process – anaerobic respiration (methanogenesis) occurring within the FFT pore water (Siddique *et al.*, 2014a; 2014b). Similar to the major ions, certain dissolved metals such as B and Mo were significantly elevated above background ARW concentrations (73x and 52x higher, respectively) though the majority were ≤10x higher. However, the annual trends were much less clear as the concentrations of some metals in BML surface water increased over time, some decreased, but most stayed relatively constant. While the primary efflux process controlling the dissolved metals will be identical to that of the major ions, the influx processes from FFT pore water to the overlying

water will vary greatly for each metal depending on the rate of diffusion, as well as both physical (adsorption) and chemical (reduction, precipitation) processes. Without a more thorough understanding of the concentrations of dissolved metals within FFT pore water and the geochemical processes controlling them, it is difficult to predict how the concentrations within BML surface water will change over time.

The toxicological risk of BML surface water was initially assessed using the most recent (August 2016) water chemistry data and was based on Canadian Water Quality Guideline (CWQG) values for the long-term protection of freshwater aquatic life, as well as chronic toxicity (reproduction inhibition) to the sensitive freshwater zooplankton, *Ceriodaphnia dubia*. As of 2016, B and Cl$^-$ were the only inorganic constituents which exceeded their respective CWQG values with hazard quotients of 1.3 and 3.4, respectively, and were therefore deemed to be of 'very high' concern. However, it should be noted that at the time of writing no CWQGs existed for many of the major ions previously identified as being of potential concern (Na$^+$, HCO$_3^-$, and SO$_4^{2-}$). In addition to B and Cl$^-$, concentrations of As, Mo, and Se within BML surface water corresponded to hazard quotients of approximately 0.4 to 0.5, and were therefore deemed to be of 'moderate' concern. Concentrations of the remaining metals and ions fell well below CWQGs, where available. Since CWQGs are designed to be conservative, comparisons to *C. dubia* toxicity thresholds generally resulted in much lower risk outcomes. Most major ions and metals within BML surface water were predicted to be of 'low' to 'very low' risk to *C. dubia*, although Cl$^-$, HCO$_3^-$, and Ni were all flagged as posing 'high' or greater risk. It should be noted that the risk of Ni may be overestimated as speciation and complexation modeling (Visual MINTEQ) indicated that only 16% of the Ni within BML surface water should be present as the free Ni^{2+} ion; due to the high concentrations of dissolved organic carbon (DOC) and alkalinity, Ni-(DOC) and Ni-(HCO$_3^-$/CO$_3^{2-}$) complexes were predicted to be predominant. It is also important to note that significant interactions will likely occur within solution between many of the major ions and dissolved metals (with respect to both chemistry and toxicity), thus individual risk outcomes should be interpreted with caution. Although the risk assessment approach used in this study was relatively basic and limited, the results were consistent with the consensus that salinity (as Na$^+$, Cl$^-$, and HCO$_3^-$) is the primary driver of inorganic toxicity of OSPW to sensitive aquatic organisms, while dissolved metals are of little concern.

5.2.2 Base Mine Lake surface water toxicity

In order to verify the predicted toxicological risk, a series of toxicity tests were performed with BML surface water collected in October 2014 and August 2015. Since the risk assessment had identified salinity within BML surface water as being of 'very high' risk to potential aquatic organisms, reconstituted BML salt waters were designed to mimic the concentrations of all major ions within BML surface water (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, SO_4^{2-}, and NO_3^-) and used in each toxicity test to control for the potential effects of salinity on the test organisms.

The first series of toxicity tests were designed to complement previous studies of OSPW on *Chironomus dilutus* performed by Anderson *et al*. (2012a; 2012b). No significant effects were observed on larval survival or growth in a preliminary 10-d toxicity test using 100% 2014 BML surface water, therefore a partial lifecycle test (approximately 30 to 40 d of exposure) was initiated to examine more sensitive endpoints. Test organisms were exposed directly as egg masses to mimic oviposition in a natural scenario, and exposed until emergence of adults occurred. Across all endpoints examined in the test, no significant effects were observed in the reconstituted BML salt water control as *C. dilutus* is relatively tolerant to salinity. After 23 d of exposure to the 100% 2014 BML surface water treatment, significant reductions in larval growth, activity, and case size and integrity were all observed, although there was no effect on larval survival. At test termination, there was no effect on the final mass or sex ratio of successfully emerged adults. However, a significant delay in emergence timing and reduction in emergence success were observed in test organisms exposed to the 100% 2014 BML surface water treatment. These effects were consistent with those observed by Anderson *et al*. (2012b) in *C. dilutus* exposed to aged OSPW samples (with similar water chemistry to BML surface water) collected from small-scale WCT reclamation ponds. Based on a mechanistic investigation by Wiseman *et al*. (2013a), the developmental effects observed in this study were attributed to the dissolved organic fraction (naphthenic acids, NAs) within BML surface water causing oxidative stress and endocrine dysfunction. Conversely, the reduced emergence success appeared to be due to a significant proportion of adults which fully emerged from their exuviae but drowned, and was therefore attributed to a potential surfactant effect of these organic acids in reducing surface water tension.

Due to the complete lack of salt toxicity, and only limited effects of long-term exposure to 100% BML surface water to *C. dilutus*, the second series of toxicity tests were conducted with one

of the most sensitive standard test species, *Ceriodaphnia dubia*, to complement the earlier toxicological risk assessment. A preliminary reference toxicant test with NaCl was used to determine the sensitivity of the in-house culture to salinity and indicated that the concentration of TDS within BML surface water alone would likely cause a 50 to 70% inhibition of reproduction in exposed *C. dubia*. As a result, all tests were conducted with paired BML surface water and reconstituted BML salt control treatments at 25, 50, and 100% strength, however, no effects on survival or reproduction were observed in any of the tests or treatment waters at 25 or 50%. This lack of effects at more dilute concentrations was consistent with the steep dose-response curve of salt toxicity observed in the reference toxicant test. Each test was also conducted with both 2014 and 2015 surface water and salt control treatments in order to examine whether annual changes in BML water chemistry affected toxicity. Results of the first toxicity test were unexpected as the 100% salt water controls were found to be significantly more toxic than whole 100% BML surface water. The 100% 2014 salt water control in particular caused 70% mortality and nearly 100% inhibition of reproduction in *C. dubia* after only 8 d of exposure. However, these effects were not attributed directly to the concentrations of major ions, but rather a lower than desired water hardness in the reconstituted salt water controls (only 60 mg/L compared to 100 mg/L as $CaCO_3$ in BML surface water). A similar study has previously shown that the addition of large amounts of $NaHCO_3$ salt to achieve high alkalinity in reconstituted waters (as in this study) can lead to significant precipitation of Ca^{2+} ions as $CaCO_{3(s)}$ (Mount *et al*., 2016). A reduction in Ca^{2+} can then cause an increase in the toxicity of major ions to *C. dubia* (Lasier and Hardin, 2010; Mount *et al*., 2016) and was therefore the most likely cause of the greater than expected toxicity in the salt water control. To account for this effect, the test was repeated at a water hardness of 80 mg/L as $CaCO_3$ which significantly reduced the toxicity of the salt control treatment – no effect on *C. dubia* survival and only 50% inhibition of reproduction. While the magnitude of these effects was more in line with the salinity dose-response curve from the reference toxicant test, both the 2014 and 2015 100% BML surface water samples were still much less toxic – 20% and 0% inhibition of reproduction, respectively, and no effect on survival. Since the previous toxicological risk assessment suggested limited influence of dissolved metals on the toxicity of BML surface water to *C. dubia*, the only significant difference between the reconstituted salt water treatment and whole BML surface water was the absence of the dissolved organic fraction. The results of these tests therefore suggested that the dissolved organic acids within BML surface water may attenuate

the toxicity of the major ions and salinity to *C. dubia*. Similar antagonistic effects have previously been reported by several different authors (Turcotte, 2009; Lasier and Hardin, 2010; Kavanagh *et al*., 2012; Kennedy, 2012), however, the exact mechanism remains unknown.

5.3   Risk assessment and significance to Alberta oil sands reclamation

Major ions – specifically Na^+, Cl^-, and HCO_3^- – were found to be the inorganic constituents of highest concern within BML surface water, although their contribution to the overall toxicity of the mixture was negligible in the species tested; *Chironomus dilutus* was tolerant of the level of salinity within BML surface water, while the presence of dissolved organic acids appeared to reduce the toxicity of these major ions to the more sensitive *Ceriodaphnia dubia*. Furthermore, despite their elevation above background levels, the concentrations of dissolved metals within BML surface water were of negligible concern, consistent with the consensus that dissolved metals generally contribute little to the overall toxicity of OSPW relative to dissolved salts and organic acids. The results of this thesis suggest that while the water chemistry of BML surface water will continue to pose some risk to colonizing aquatic organisms, current surface water quality conditions are now sufficient to support the survival, growth, development, and reproduction of limited populations of certain freshwater species – especially those able to tolerate elevated levels of salinity. This conclusion was confirmed by the discovery of wild *Daphnia pulex* adults and neonates successfully growing and reproducing in the August 2016 BML surface water sample (*D. pulex* 21-d reproduction $IC_{10} = 368$ mg Cl^-/L; CCME, 2011). This also indicates that BML surface water quality has reached a sufficient level to sustain populations of some primary producer(s) (e.g. bacteria, phytoplankton) necessary to support higher trophic level organisms such as cladocerans, chironomids, and ultimately fish. However, the time required for such colonization, ecological succession, and development of BML into a robust aquatic ecosystem is unknown and will depend on the extent of Syncrude's intervention in natural processes.

As previously discussed, it is crucial to remember that the maintenance of current surface water quality and concentrations of inorganic constituents of concern within BML surface water is entirely due to continuous pumping activity (freshwater in, process water out) by Syncrude. As long as all current processes remain constant, the efflux of chemical mass within BML from pumping activity will continue to exceed and offset the influx of chemical mass from FFT pore water, leading to the gradual dilution of surface water over time. However, based on current

concentrations and chemical flux rates reported by Dompierre (2017), this net dilution effect is very slow and will take approximately 21 years to reduce the concentration of Cl^- within BML surface water below the CCME guideline value of 120 mg/L. Furthermore, this dilution effect only removes approximately 0.07% of the total Cl^- within BML annually (as pump-out water). Thus, despite potentially low concentrations within BML surface water, a significant and persistent chemical mass of major ions and dissolved metals will continue to exist within FFT pore water for decades. As a result of this artificially-maintained concentration gradient between BML surface water and FFT pore water, if Syncrude were to ever stop, or even reduce pumping rates, the advective-diffusive flux of chemicals from FFT pore water into overlying water could drastically reduce surface water quality back towards pre-dilution (2013) levels and could adversely affect any established ecosystem. This is concerning, as it means the sustainability of WCT and EPLs as a method for long-term oil sands mining reclamation will be dependent on continuous manual dilution of surface water by oil sands operators for decades. Syncrude has proposed that BML will eventually be incorporated into the surrounding 430 km^2 watershed, however, it is unlikely that natural water flow through this system would be sufficient to continue offsetting advective-diffusive chemical flux rates in order to maintain acceptable surface water quality.

Despite these concerns, the BML experiment has been a success in many aspects. Results of the toxicological risk assessment and toxicity tests, as well as the discovery of *D. pulex* colonization, all show that acceptable surface water quality for the establishment of an aquatic ecosystem has been reached in BML in under four years. Instead of leaving reclamation to natural biodegradation processes, Syncrude's intervention and pumping activity has drastically reduced the time required to improve surface water quality. The water chemistry of BML surface water, as well as the magnitude of effects observed in the tests with *C. dilutus*, are now similar to that of surface water from smaller-scale WCT reclamation ponds which have been aging for over 15 years (Table 5.1). Base Mine Lake (FFT capped with freshwater and OSPW in 2012) most closely resembles Big Pit (FFT capped with freshwater in 1993) and FE5 (FFT capped with OSPW in 1989). While some of the inorganic constituents of concern in BML surface water remain elevated above the concentrations found within Big Pit and FE5, many others are in fact lower, even though BML was more than 10 years less-aged at the time of sampling. In addition to the pumping activity, Syncrude also added a significant amount of alum $(KAl(SO_4)_2 \cdot 12H_2O)$ – a chemical flocculant – to BML surface water in September 2016 in order to accelerate the settling of suspended solids

(fine clays). While this may affect certain water chemistry parameters within BML, it will further ameliorate surface water quality and may also accelerate the development of a proper sediment layer at the FFT-water interface within the lake which could help reduce the transport of chemical mass from FFT into the overlying water.

Table 5.1 – Water chemistry of fresh and aged Syncrude oil sands process-affected waters.

		Oil Sands Process-Affected Waters				
		WIP†	TPW†	Big Pit†	FE5†	BML
Sampling Date		Summer 2009	Summer 2009	Summer 2009	Summer 2009	August 2016
Age at Sampling		Fresh (0 years)	16 years	16 years	20 years	<4 years
Conductivity	(µS/cm)	3702	-	-	2790	2700
pH		8.85	-	-	8.93	8.7
Hardness	(mg/L)	67	-	-	182	115
Alkalinity	(mg/L)	467	-	-	454	610
Naphthenic acids	(mg/L)	72	35	23	13	25*
Major Ions	**(mg/L)**					
Chloride	Cl^-	616	271	116	147	405
Sulfate	SO_4^{2-}	583	135	211	793	200
Nitrate	NO_3^-	94	1.8	4.6	3.9	3.1
Sodium	Na^+	1024	645	447	672	540
Potassium	K^+	20	5.9	4.6	9.4	9.4
Magnesium	Mg^{2+}	15	16	20	44	27
Calcium	Ca^{2+}	14	7.8	12	21	12
Dissolved Metals	**(µg/L)**					
Aluminum	Al	70	61	43	37	11
Arsenic	As	7.7	6.9	8.7	4.9	2.7
Cadmium	Cd	0.5	0.3	0.4	0.3	0.02
Cobalt	Co	2.6	0.5	0.4	0.4	0.8
Molybdenum	Mo	274	6	4	3	32
Nickel	Ni	8.5	2.2	2.6	2.0	6.6
Lead	Pb	0.06	0.05	0.05	0.04	0.06
Selenium	Se	3.8	1.4	1.3	1.2	0.5
Strontium	Sr	2710	283	627	1132	631
Uranium	U	28.7	9.3	6.2	3.1	4.8
Vanadium	V	12	4	6	2	4

† Water chemistry data for WIP, TPW, Big Pit, and FE5 taken from Anderson *et al.* (2012b)
* Reported naphthenic acids concentration from October 2014 sample, not August 2016
WIP: Fresh, untreated OSPW
TPW: OSPW only; aging since 1993
Big Pit: FFT capped with freshwater; aging since 1993
FE5: FFT capped with OSPW; aging since 1989
BML: FFT capped with freshwater and OSPW; aging since December 2012

Under the Alberta provincial *Environmental Protection and Enhancement Act*, oil sands operators have historically been legally required to return the land to a 'functionally equivalent state' after surface mining has ceased. However, the recently introduced Tailings Management Framework for the Mineable Athabasca Oil Sands (TMF) has amended these requirements to allow for reclaimed land to have a different use in order to account for the creation of artificial lakes (EPLs) where previously there were none. Unfortunately, the TMF mainly focuses on reducing the production of new tailings, rather than managing existing legacy tailings. It does, however, acknowledge that new criteria and guidelines such as enhanced wastewater (OSPW) characterization must be established in order to properly regulate oil sands reclamation, especially in the case of WCT and EPLs. Specifically, there is currently no definition or binding criteria for what constitutes 'acceptable' surface water quality with respect to oil sands EPLs, which makes it difficult to assess the current progress and success of the BML experiment. Based on the results of this thesis, it is clear that salinity (as Cl$^-$) within BML surface water will continue to exceed the freshwater CWQG for several decades, however, this has not prevented the initial colonization of salt-tolerant aquatic species. Even though BML and other oil sands EPLs may never fully resemble other natural freshwater aquatic ecosystems within the region, it could be argued that the establishment of a less diverse brackish or estuarine ecosystem is still an acceptable endpoint for permanent reclamation, as long as the ecosystem is functional and appears healthy.

Widespread industry adoption of WCT and EPLs will be largely site-specific and dependent upon the success and viability of the BML experiment (COSIA, 2012). The Government of Alberta is currently developing specific WCT policies and performance criteria for the oil sands which will be used by the AER in developing a regulatory approach for managing future WCT and EPL operations (AER, 2015b). The AER is also responsible for developing water quality criteria and conditions for the potential release of EPL surface water to the environment (Government of Alberta, 2015). The AER has already conditionally approved several future oil sands WCT and EPL operations, however, these proposals must be accompanied by an alternate treatment method in case the BML experiment fails or is deemed insufficient for treating accumulated FFT and OSPW (AER, 2015b). Despite the advances made by Syncrude with the BML experiment, surface water quality remains the largest industry and regulatory concern currently facing WCT and EPL implementation and will therefore continue to be the focus of environmental monitoring and research for many years.

5.4 Future research and considerations

Conclusions drawn from annual changes in water chemistry and the toxicological risk assessment of BML surface water within this thesis should be interpreted with caution as all studies and analyses were performed with only three samples total – one for each year spanning 2014 to 2016 – all sampled at the same location during the same time of year (August to October). These samples therefore represent estimations of BML surface water chemistry and risk only, as a previous study has shown that the geochemistry of BML may vary both seasonally and spatially within the lake (Dompierre *et al*., 2016). The most important extension of the work presented in this thesis would be for continuous, in-situ monitoring of water chemistry within BML in order to confirm whether surface water quality remains consistent over time. Since surface water quality within BML appears to have now reached an acceptable level for certain salt-tolerant species, this could be further investigated by field surveys of aquatic organisms (such as *D. pulex*) already present within the lake, as well as caged *in situ* toxicity studies with other potential aquatic ecosystem colonizers. This would be of particular interest for studies of *C. dilutus* to allow for a full lifecycle of exposure, as the lack of reproduction data was a key limitation highlighted both within this thesis and that of Anderson (2011). Furthermore, while not a direct extension of the work presented in this thesis, more detailed characterization of FFT pore water concentrations and chemical mass flux rates from this compartment into the overlying surface water within BML are necessary to more accurately predict and model future concentrations of the inorganic constituents of concern within surface water. In addition to the broad investigations of surface water chemistry and quality, two relatively unexplored and specific effects of BML surface water were identified in this thesis. First, the surfactant-like nature of dissolved organic acids appears to reduce the surface tension of BML surface water leading to impaired emergence success of *C. dilutus* and likely other aquatic invertebrates which transition from an aquatic larval stage to a terrestrial adult stage. Second, the high concentrations of major ions and dissolved organic acids appear to interact antagonistically and mitigate the predicted toxicity of BML surface water to salt-sensitive aquatic species such as *C. dubia*. Both phenomena are likely to affect the success of colonization and ecological succession within BML and merit further investigation.

5.5 Conclusion

The studies herein were designed to complement Syncrude's chemical performance monitoring plan for Base Mine Lake, as well as previous studies on the effects of fresh and aged oil sands process-affected water on aquatic ecosystems. This thesis clearly demonstrates that, despite some persistent toxicological risks and effects of inorganic constituents of concern – specifically Na^+, Cl^-, and HCO_3^- – the surface water quality of BML has reached an acceptable level for the colonization of certain salt-tolerant aquatic organisms in under four years of aging. This rapid achievement in surface water quality was attained by continuous water cycling and efflux by Syncrude (freshwater pumped in, process water pumped out) which currently offsets the influx of chemical mass (major ions, dissolved metals) from the underlying fluid fine tailings pore water into the overlying surface water. The net effect of these two processes is the gradual dilution of BML surface water and improvement in surface water quality over time. However, if Syncrude were to reduce or stop this pumping activity, it is very likely that concentrations of these inorganic constituents of concern would begin to increase back to pre-dilution levels resulting in a significant increase in toxicity and reduction in surface water quality. The results of this thesis will therefore assist Syncrude in evaluating the sustainability and efficacy of water-capped tailings and end pit lakes as methods for the long-term reclamation of oil sands surface mining activity.

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APPENDIX

APPENDIX A – Chemical characterization of acid-extractable organic compounds

Detailed characterization of the dissolved organic fraction within Base Mine Lake (BML) surface water was primarily performed by another group within the University of Saskatchewan Toxicology Centre as well as the University of Alberta (see Morandi *et al*., 2015). For the purposes of this study, a simple chemical analysis was performed on the 2014 BML surface water sample only, based on liquid-liquid extraction methods described by Ross *et al*. (2012) and Pereira *et al*. (2013). Briefly, 100 mL of vacuum-filtered (0.7 µm) 2014 BML surface water was acidified to pH <2 with 18 M sulfuric acid, spiked with an internal standard (^{13}C-tetradecanoic acid), and extracted twice into 200 mL of dichloromethane (DCM). The combined 400 mL of DCM extract was evaporated to 5 mL via rotary evaporation, reduced to dryness with nitrogen gas, then the dry extract was dissolved in 1 mL methanol for chemical analysis via Orbitrap mass spectrometry paired with high-performance liquid chromatography. See Morandi *et al*. (2015) for more detailed instrumental techniques used at the University of Saskatchewan Toxicology Centre. All acid-extractable organic compounds detected in BML surface water are depicted in Figure A.1.
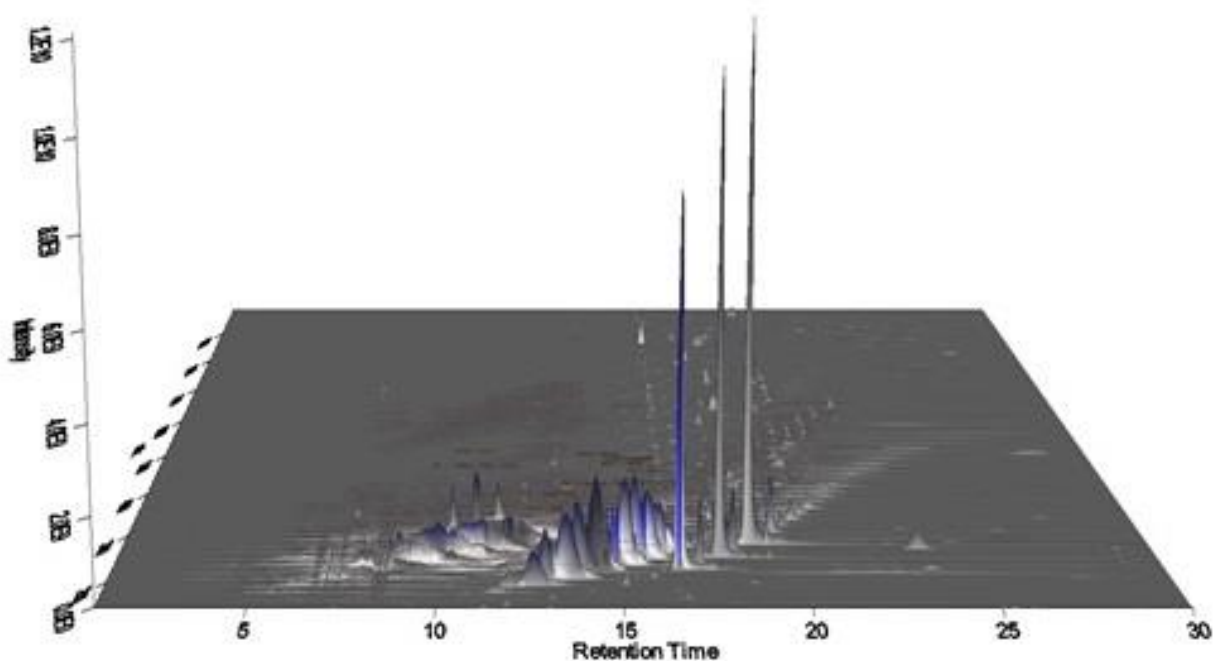

Figure A.1 – Intensity (y-axis), retention time (x-axis), and molar mass (z-axis) of all acid-extractable organic compounds detected via high resolution Orbitrap mass-spectrometry and high-performance liquid chromatography of Base Mine Lake surface water collected October 2014.

Base Mine Lake surface water contains a diverse and complex mixture of dissolved organic acids, of which naphthenic acids (NAs) comprise a large proportion. Naphthenic acids (also referred to as O2-compounds) are a group of cyclic carboxylic acids described by the general formula $C_nH_{2n+z}O_2$, where n is the number of carbon atoms and z is the degree of unsaturation associated with ring structures or double bonds. In Figure A.1 these compounds are represented by the group of peaks at a molecular weight of 200 g/mol to 300 g/mol which elute between approximately 12 and 17 minutes and correspond to the peaks depicted in Figure A.2A. Some of the other peaks shown in Figure A.1 likely represent acid-extractable organics (including compounds containing additional O-, as well as S- and N-atoms) previously identified in BML surface water by Morandi *et al*. (2015). Aside from the identified NA groups, over 30,000 other unidentified acid-extractable organic compounds were detected in 2014 BML surface water.

It is important to note that there is currently no analytical method available to determine the exact concentrations of specific NAs in water samples. All peaks and bars depicted in Figures therefore only represent general groups of compounds which share similar properties (number of carbons and z value), and may contain hundreds of different structural isomers. In order to estimate the concentration of NAs within BML surface water, as well as the relative profile of these groups, a calibration curve was created based on a standard NA technical mixture (SigmaAldrich, CAS#1338-24-5) containing petroleum-based alkylated cyclopentane carboxylic acids. The peak intensity of each NA group detected in the sample can thus be converted to concentration (C) based on the following equation:

$$C = \frac{I \times I_A \times C_B}{m \times I_B \times C_A}$$

Where I is the intensity of NAs in the sample; I_A is the intensity of the internal standard in the NA standard; C_A is the concentration of the internal standard in the NA standard; I_B is the intensity of the internal standard in the sample; and C_B is the concentration of the internal standard in the sample. The slope of the NA standard calibration curve (m) is calculated as I_C/C_C where I_C is the intensity of the NA standard and C_C is the concentration of the NA standard (y-axis and x-axis, respectively, on the calibration curve). Peak intensity, retention time, and molar mass of each identified NA class are shown in Figure A.2A. Using these values, NA groups can be further classified based on carbon number and z value, and concentrations can be estimated (Figure A.2B).
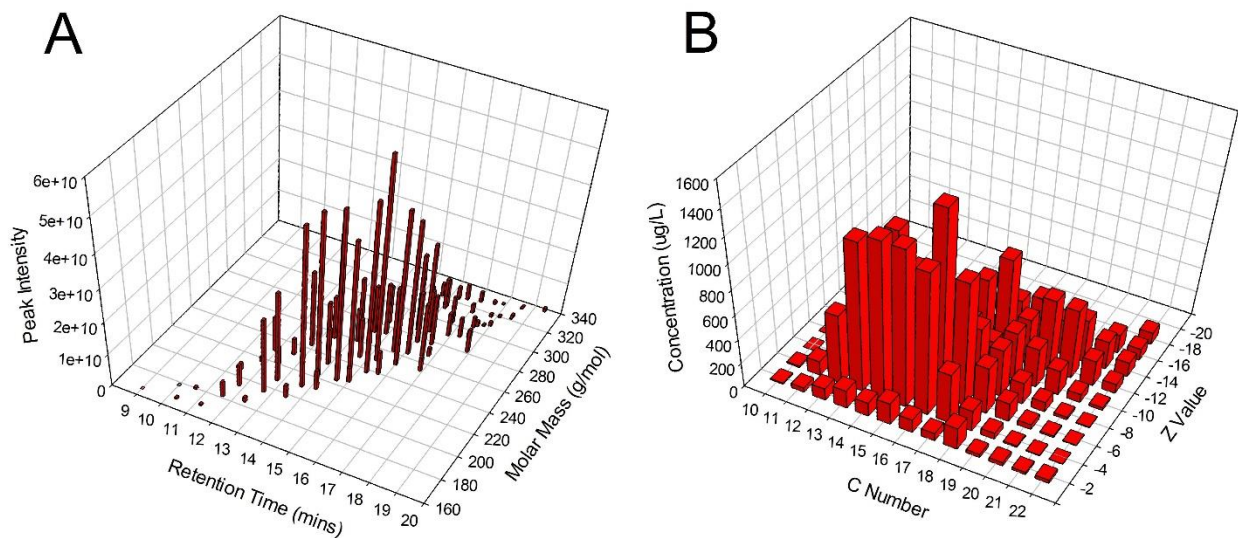
Figure A.2 – (A): Naphthenic acid (NA) groups (containing multiple isomers) detected within 2014 Base Mine Lake surface via Orbitrap mass spectrometry and high-performance liquid chromatography. Separation of NA groups is based on retention time within the column and molar mass (g/mol). (B) Due to the high mass sensitivity of Orbitrap mass spectrometry, identified NA groups can also be separated based on their carbon number and z-value (degree of unsaturation), from the general NA formula $C_nH_{2n+z}O_2$. Using an internal standard and NA standard calibration curve, peak intensities can be converted into estimates of concentration.

In total, 76 different NA groups were identified within 2014 BML surface water. Of these 76 groups, approximately 50% of the total concentration was attributed to the C(13 to 17)Z(-4 to -6) groups alone. C(16)Z(-6) was the single most abundant group (NA formula of $C_{16}H_{26}O_2$) with a concentration of approximately 1.5 mg/L. The average total concentration of NAs within BML surface water (sum of all groups) was estimated to be 25.26 ± 3.59 mg/L. This concentration is similar in magnitude to those previously reported for aged oil sands process-affected water collected from smaller-scale Syncrude reclamation ponds (13, 23, and 35 mg/L) which were based on an older Fourier transform infrared spectroscopy (FTIR) detection method (Anderson *et al.*, 2012). The relative intensity and profile of these groups was also consistent with those previously reported for other Syncrude oil sands process-affected waters (Han *et al.*, 2009; Toor *et al.*, 2013).

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APPENDIX B – Visual MINTEQ modeling of pH-dependent BML metal complexation

Table B.1 – Predominance of metal complexes at pH 8.0, 8.5, and 9.0 in BML (all values in %)

	Metal Cations	pH 8.0	pH 8.5	pH 9.0		Metal Cations [cont'd]	pH 8.0	pH 8.5	pH 9.0
Cd	Cd^{2+}	29.6	17.5	7.8		$Pb(CO_3)_2^{2-}$	11.4	31.4	59.3
	Cd-DOM	23.9	28.4	23.3		$PbCO_{3(aq)}$	48.3	44.4	29.9
	$CdOH^+$	0.1	0.3	0.4		$PbHCO_3^+$	2.9	0.8	0.2
	$CdCl^+$	17.0	10.0	4.5	Ag	Ag^+	2.9	2.9	2.9
	$CdCl_{2(aq)}$	0.6	0.3	0.2		$AgCl_{(aq)}$	48.4	48.4	48.4
	$CdSO_{4(aq)}$	3.7	2.2	1.0		$AgCl_2^-$	48.0	48.0	48.0
	$CdHCO_3^+$	6.0	3.4	1.3		$AgCl_3^{2-}$	0.7	0.7	0.7
	$CdCO_{3(aq)}$	17.7	31.4	39.4	Tl	Tl^+	94.8	94.8	94.8
	$Cd(CO_3)_2^{2-}$	1.2	6.3	22.0		$TlCl_{(aq)}$	2.9	2.9	2.9
Cr	$Cr(OH)_2^+$	2.6	0.8	0.3		$TlSO_4^-$	2.3	2.3	2.3
	$Cr(OH)_{3(aq)}$	97.3	99.0	99.3	U	$Ca_2UO_2(CO_3)_{3(aq)}$	7.6	1.7	0.3
	$Cr(OH)_4^-$	0.0	0.1	0.4		$CaUO_2(CO_3)_3^{2-}$	63.4	41.9	20.8
Co	Co^{2+}	47.4	32.3	17.2		$UO_2(CO_3)_2^{2-}$	0.5	0.3	0.2
	$CoOH^+$	0.6	1.3	2.1		$UO_2(CO_3)_3^{4-}$	28.5	56.1	78.7
	$Co(OH)_{2(aq)}$	0.0	0.3	1.5	Zn	Zn^{2+}	23.5	10.4	3.5
	$CoCl^+$	0.1	0.1	0.0		Zn-DOM	29.9	26.8	16.2
	$CoSO_{4(aq)}$	5.1	3.5	1.9		$ZnOH^+$	1.5	2.1	2.2
	$CoCO_{3(aq)}$	23.1	47.2	70.1		$Zn(OH)_{2(aq)}$	1.6	7.0	23.1
	$CoHCO_3^+$	23.7	15.3	7.2		$ZnCl^+$	0.4	0.2	0.1
Cu	Cu^{2+}	0.4	0.1	0.0		$ZnSO_{4(aq)}$	2.8	1.2	0.4
	Cu-DOM	14.3	7.5	2.9		$ZnCO_{3(aq)}$	34.5	45.9	42.5
	$CuOH^+$	0.9	0.7	0.5		$ZnHCO_3^+$	4.8	2.0	0.6
	$Cu(OH)_{2(aq)}$	0.1	0.4	0.8		$Zn(CO_3)_2^{2-}$	1.1	4.4	11.4
	$CuCO_{3(aq)}$	67.3	52.4	31.1		**Metal Anions**	**pH 8.0**	**pH 8.5**	**pH 9.0**
	$Cu(CO_3)_2^{2-}$	16.7	38.8	64.7	As	$HAsO_4^{2-}$	94.2	98.0	99.1
Ni	Ni^{2+}	27.3	15.7	7.8		$H_2AsO_4^-$	5.7	1.9	0.6
	Ni-DOM	22.0	25.5	23.2	B	H_3BO_3	93.4	81.8	58.7
	$NiOH^+$	0.2	0.4	0.6		$H_2BO_3^-$	6.4	17.7	40.2
	$Ni(OH)_{2(aq)}$	0.0	0.1	0.4		$NaH_2BO_{3(aq)}$	0.2	0.5	1.1
	$NiCl^+$	0.1	0.0	0.0	Mo	MoO_4^{2-}	96.0	98.6	99.5
	$NiSO_{4(aq)}$	2.9	1.7	0.8		$MgMoO_{4(aq)}$	2.7	0.9	0.3
	$NiCO_{3(aq)}$	25.9	44.7	62.0		$CaMoO_{4(aq)}$	1.3	0.4	0.2
	$NiHCO_3^+$	21.6	11.8	5.2	Se	$HSeO_3^-$	60.9	33.1	13.5
Pb	Pb^{2+}	0.6	0.2	0.0		SeO_3^{2-}	39.1	66.9	86.5
	Pb-DOM	35.7	22.1	9.7	V	HVO_4^{2-}	22.3	47.5	74.1
	$PbOH^+$	0.9	0.9	0.6		$H_2VO_4^-$	77.7	52.5	25.9

APPENDIX C – Values used in toxicological risk assessment calculations

Canadian Water Quality Guideline (CWQG) values for the long-term protection of freshwater aquatic life used in the toxicological risk assessment of Base Mine Lake (BML) surface water are summarized in Table B.1. Modern CWQGs (since 2009) are based on a species sensitivity distribution (SSD) approach that sets the guideline value equal to the concentration that protects 95% of aquatic organisms (HC$_5$). However, the CWQGs for most dissolved metals are based on toxicity values, regulatory conclusions, or previous guidelines that are now over 30 years old. These were generally based solely on toxicity to the most sensitive species and endpoint tested, and often had a safety factor applied (0.1x) in order to be more conservative.

Table C.1 – Canadian Water Quality Guideline values used in toxicological risk assessment

		Canadian Water Quality Guidelines (CWQG) for the (Long-term) Protection of (Freshwater) Aquatic Life			
Major Ions		**Value**	**Reference**	**Type**	**Details**
Chloride Cl$^-$		120 mg/L	CCME 2011a	SSD	-
Nitrate NO$_3^-$		13 mg/L	CCME 2012	SSD	-
Dissolved Metals					
Aluminum	Al	100	CCRM 1987	pH-dependent (>6.5)	Based on 1973 US EPA guideline
Arsenic	As	5.0	CCRM 1987	Safety Factor (0.1x)	14-d EC$_{50}$(growth) *S. obliquus* = 50 ug/L
Boron	B	1500	CCME 2009	SSD	-
Cadmium	Cd	0.16‡	CCME 2014	SSD	-
Chromium	Cr(VI)	1	CCME 1999a	Safety Factor (0.1x)	14-d LOEC *C. dubia* = 10 ug/L
	Cr(III)	8.9		Safety Factor (0.1x)	102-d LOEC(mortality) *O. mykiss* = 89 ug/L
Cobalt	Co	2.5	EC 2013	SSD	-
Copper	Cu	2.36‡	CCRM 1987	Equation (Hardness)	Based on 1985 US EPA guideline
Iron	Fe	300	CCRM 1987	Unknown	Based on 1978 ON IJC guideline
Lead	Pb	3.2‡	CCRM 1987	Equation (Hardness)	Based on 1985 US EPA guideline
Molybdenum	Mo	73	CCME 1999b	Safety Factor (0.1x)	28-d LC$_{50}$ *O. mykiss* = 730 ug/L
Nickel	Ni	96‡	CCRM 1987	Equation (Hardness)	Based on 1980 US EPA guideline
Selenium	Se	1	CCRM 1987	Unknown	Based on 1981 ON IJC guideline
Silver	Ag	0.25	CCME 2015	SSD	-
Thallium	Tl	0.8	CCME 1999c	Safety Factor (0.1x)	14-d EC$_{50}$ *L. minor* = 8 ug/L
Uranium	U	15	CCME 2011b	SSD	-
Vanadium	V	120	EC 2016	SSD	-
Zinc	Zn	30	CCRM 1987	Unknown	Based on 1976 ON IJC guideline

All guideline values in μg/L (ppb) unless otherwise marked
‡ Guideline value dependent on water hardness; calculated at BML surface water hardness of 100 mg/L as CaCO$_3$
CCME: Canadian Council of Ministers of the Environment Guideline [current]
CCRM: Canadian Council of Resource and Environment Ministers Guideline [archived]
EC: Environment Canada Federal Environmental Quality Guideline (FEQG)
ON IJC: Ontario Great Lakes Water Quality International Joint Commission Guideline
US EPA: United States Environmental Protection Agency Guideline

Chronic toxicity values for *Ceriodaphnia dubia* used in the toxicological risk assessment of BML surface water are show in Table B.2. All values with the exception of As are based on reproduction inhibition. Where available, toxicity values were taken from their respective CWGQ. For CWQGs which do not include *C. dubia* toxicity in their derivation, toxicity values were instead taken from the US EPA ECOTOX database (US EPA, 2016). In order to be as conservative and relevant as possible, preference was given to toxicity tests which were performed at similar water hardness to BML surface water (moderately hard, 100 mg/L as $CaCO_3$), concentrations which were measured (not nominal), and the most sensitive reported endpoint (generally IC_{10}).

Table C.2 – *Ceriodaphnia dubia* chronic toxicity values used in toxicological risk assessment

Major Ions		*Ceriodaphnia dubia* Chronic Toxicity (Reproduction Inhibition)			
		Value	Endpoint	Reference	CWQG
Chloride	Cl^-	454 mg/L	7-d IC_{25}	Elphick *et al.*, 2011	CCME, 2011a
Sulfate	SO_4^{2-}	1060 mg/L	7-d IC_{25}	Lasier & Hardin, 2010	*N/A*
Bicarbonate	HCO_3^-	379 mg/L	7-d IC_{25}	Lasier & Hardin, 2010	*N/A*
Nitrate	NO_3^-	50 mg/L	7-d IC_{25}	In: CCME, 2012	CCME, 2012
Dissolved Metals					
Aluminum	Al	448	≤8-d IC_{10}	Zuiderveen & Birge, 1997	*N/R*
Arsenic	As	1000	7-d LOEC†	Spehar & Fiant, 1986	CCME, 2001
Boron	B	13400	14-d MATC	In: CCME, 2009	CCME, 2009
Cadmium	Cd	4.9	14-d MATC	In: CCME, 2014	CCME, 2014
Chromium	Cr(VI)	10	14-d LOEC	In: CCME, 1999	CCME, 1999a
	Cr(III)	1680	≤8-d IC_{10}	Zuiderveen & Birge, 1997	*N/R*
Cobalt	Co	7.9	21-d EC_{10}	In: EC, 2013	EC, 2013
Copper	Cu	33.1	≤8-d IC_{10}	Zuiderveen & Birge, 1997	*N/R*
Iron	Fe	399	≤8-d IC_{10}	Zuiderveen & Birge, 1997	*N/R*
Lead	Pb	29.2	≤8-d IC_{10}	Zuiderveen & Birge, 1997	*N/R*
Molybdenum	Mo	34000	≤8-d IC_{12}	Naddy, 1995	CCME, 1999b
Nickel	Ni	6.22	≤8-d IC_{10}	Zuiderveen & Birge, 1997	*N/R*
Selenium	Se	510	≤8-d IC_{12}	Naddy, 1995	*N/R*
Silver	Ag	0.78	MATC	In: CCME, 2015	CCME, 2015
Thallium	Tl	76.6	7-d LOEC	Spehar, 1989	*N/R*
Uranium	U	73	7-d IC_{10}	In: CCME, 2011b	CCME, 2011b
Vanadium	V	250	≤8-d LOEC	Puttaswamy & Liber, 2012	*N/R*
Zinc	Zn	82.9	≤8-d IC_{10}	Zuiderveen & Birge, 1997	*N/R*

All guideline values in μg/L (ppb) unless otherwise marked
† Denotes toxicity endpoint other than reproduction inhibition (immobilization)
N/A: No Canadian Water Quality Guideline (CWQG) available
*N/R*: CWQGs which do not use *C. dubia* toxicity in their derivation

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APPENDIX D – Granular activated charcoal treatment and toxicity test

An additional test was performed with *Ceriodaphnia dubia* to elucidate the potential effects of water hardness and dissolved organic carbon (DOC) on major ion toxicity as observed in Chapter 4, although two modifications were made to the test procedure. First, the reconstituted salt water treatments were prepared at even higher initial water hardness to compensate for the precipitation of $CaCO_{3(s)}$. Despite the increased addition of calcium, the final water hardness of the reconstituted Base Mine Lake salt waters was effectively the same as in the previous Hardness 80 Test, although a water hardness of 90 mg/L as $CaCO_3$ was achieved in the 2015 test water. This suggests that a complete change in the recipe (e.g. significantly reduced addition of $NaHCO_3$) may be required to reach higher levels of water hardness although this would result in a lower than desired level of alkalinity. Secondly, BML surface water treated with granular activated charcoal (GAC) to remove DOC was added as an additional exposure. This was based on the method used by Anderson *et al*. (2012) who found a 4-h treatment with GAC to remove approximately 90% of the dissolved organic fraction within oil sands process-affected water. In the present study, a double treatment was performed (extracted twice, 8 h total) in an attempt to remove an even greater amount. Overall DOC removal rates in the 2014 and 2015 BML surface water samples were 87% and ≥92%, respectively (Table C.1). It should be noted that the GAC treatment was the second treatment method used to remove DOC from BML surface water. Since GAC treatment also affects the metals and ions within BML surface water, samples were initially liquid-liquid extracted using dichloromethane (refer to method in Appendix A), however, this method significantly altered the conductivity of the samples due to the required pH manipulations (addition of $H_2SO_{4(aq)}$ to reduce pH to 2 pre-extraction, then addition of $NaOH_{(aq)}$ to raise pH back to 9 post-extraction).

Even though the GAC treatment was very successful in removing DOC from BML surface water, several factors confounded the toxicity tests making the results inconclusive. Results of the chronic survival and reproduction *C. dubia* tests (for the 2014 and 2015 water samples) are shown in Figure C.1. Based on the hypothesis that salinity and DOC have an antagonistic effect on toxicity, the GAC treatments were expected to exhibit similar toxicity to the reconstituted BML salt water treatments, however, significantly greater than expected toxicity was observed. Furthermore, reproductive success in the freshwater controls was much lower than previously observed with the same *C. dubia* cultures (see Figures 4.5 and 4.7). These effects were attributed to significantly elevated concentrations of certain dissolved metals in the test waters (Table C.1).
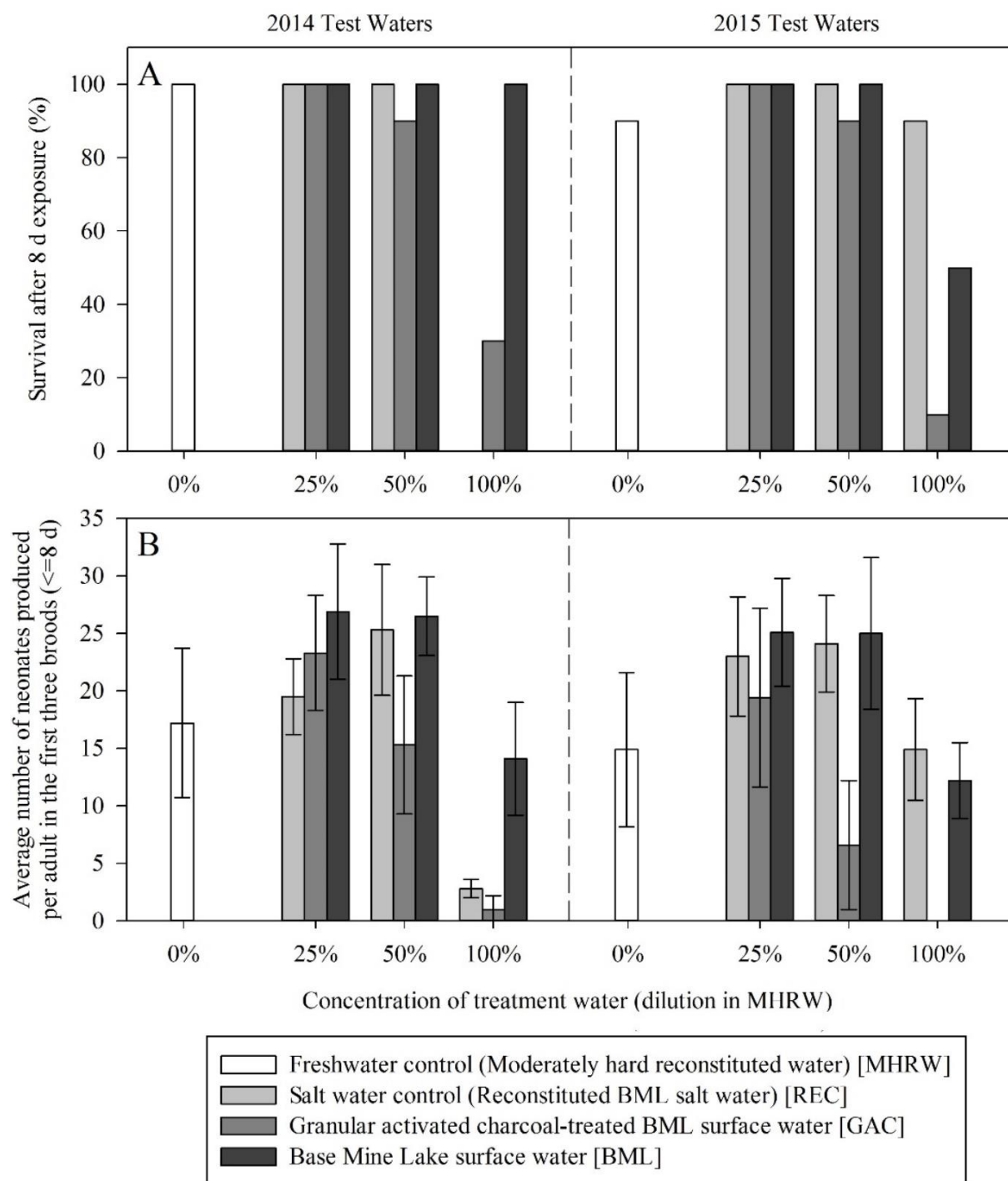
Figure D.1 – Exposure of *Ceriodaphnia dubia* to freshwater control (moderately hard reconstituted water), salt water control (reconstituted BML 2014 and 2015 salt water), granular activated charcoal-treated BML 2014 and 2015 surface water, and whole BML 2014 and 2015 surface water. A) Survival of C. dubia after 8 d of exposure; B) Average number of neonates produced per adult in the first three broods. Error bars represent one standard deviation of the mean.

Table D.1 – Chemical characterization of test waters used in activated charcoal toxicity test

Water Quality		MHRW	2014 Test Waters			2015 Test Waters		
			REC	GAC	BML	REC	GAC	BML
Conductivity	(µS/cm)	340	2800	2700	2900	2600	2800	2800
TDS	(mg/L)	210	1600	1500	1700	1500	1700	1600
pH		8.0	8.9	9.0	9.0	8.8	9.0	8.9
Hardness	(mg/L)	100	80	90	100	90	110	90
Alkalinity	(mg/L)	60	500	460	610	500	680	590
DOC	(mg/L)	-	1.8	3.2	24	<0.50	<0.50	6.3
Major Ions	**(mg/L)**							
Sodium	Na^+	27	600	500	630	580	620	630
Potassium	K^+	1.8	7.6	23	8.9	8.0	36	9.2
Calcium	Ca^{2+}	17	4.6	19	22	9.3	11	18
Magnesium	Mg^{2+}	15	16	11	11	16	21	11
Chloride	Cl^-	2.3	440	400	440	410	340	410
Sulfate	SO_4^{2-}	110	210	290	210	180	260	190
Bicarbonate	HCO_3^-	72	540	470	620	570	690	620
Carbonate	CO_3^{2-}	<0.50	39	43	64	23	67	48
Nitrate	NO_3^-	0.3	11	1.5	11	6.7	0.5	6.6
Dissolved Metals (ug/L)								
Aluminum	Al	1.03	3.53	3596*	5.58	2.38	4135*	3.80
Antimony	Sb	0.01	0.03	2.02	0.62	0.01	2.64	0.69
Arsenic	As	0.02	0.06	4.38	3.18	0.06	3.57	2.18
Barium	Ba	2.44	2.40	123	261	2.93	62.9	208
Boron	B	36.4	39.2	335	2126	41.6	29.9	2190
Cadmium	Cd	0.001	0.012	0.042	0.032	0.002	0.032	0.019
Chromium	Cr	0.17	0.38	2.56	0.78	0.42	0.67	0.63
Cobalt	Co	0.007	0.006	0.046	0.617	<0.005	0.014	0.142
Copper	Cu	5.71	0.42	1.45	2.39	0.31	1.33	8.70
Iron	Fe	1.59	3.96	3.54	6.16	1.68	4.27	16.1
Lead	Pb	0.20	0.11	0.58	0.10	0.05	0.67	0.05
Manganese	Mn	1.48	1.34	2.01	1.86	0.23	1.55	0.11
Molybdenum	Mo	0.43	0.45	72.9	40.5	0.58	63.9	33.0
Nickel	Ni	0.74	0.76	0.97	6.51	0.71	0.92	5.46
Selenium	Se	0.06	0.14	4.97	0.50	0.12	4.84	0.36
Silver	Ag	<0.005	0.007	<0.005	0.006	<0.005	<0.005	<0.005
Strontium	Sr	12.0	2.75	189	613	7.88	170	599
Thallium	Tl	0.011	0.031	0.009	0.008	0.005	0.006	0.008
Titanium	Ti	<0.12	0.16	0.24	0.28	0.12	0.75	<0.12
Uranium	U	0.02	0.01	4.48	4.35	0.01	6.68	4.26
Vanadium	V	0.01	0.10	21.0	3.59	0.10	15.5	2.31
Zinc	Zn	5.52	6.26	4.55	3.85	6.01	5.74	2.79

MHRW: Moderately hard reconstituted water (Freshwater control)
REC: Reconstituted Base Mine Lake salt water (Salt water control)
GAC: Granular activated charcoal-treated Base Mine Lake surface water
BML: Base Mine Lake surface water
* GAC treatment leached a significant amount of Al into test waters

Chemical characterization of the test waters revealed a massive concentration of dissolved Al in the 2014 and 2015 GAC-treated BML surface waters (3.6 and 4.1 mg/L, respectively). Not only were these concentrations 1000x higher than those found in untreated BML surface water, they also significantly exceeded both the CWQG and *C. dubia* toxicity value for Al (see Appendix C) and were therefore identified as the cause of the higher than anticipated toxicity in the GAC-treated waters. Since the concentrations were higher than those naturally observed in BML surface water, it was proposed that the GAC treatment itself was the source of the Al. Similarly, a significant concentration of Cu was detected in the freshwater control which was likely the cause of the reduced reproduction, and was also attributed to some method error. A series of method blanks were performed in which the carboys, metal sieves used to remove GAC post-treatment, and the GAC itself, were all rinsed with reverse osmosis (RO) water and analyzed (Table C.2).

Table D.2 – Dissolved metals leached from test materials following RO rinse (method blanks)

Dissolved Metals (ug/L)		RO Blank	Method Blanks (RO Rinse)		
			MHRW Carboy	Metal Sieves	Activated Charcoal
Aluminum	Al	0.79	1.02	8.46	2325‡
Antimony	Sb	0.01	0.02	0.03	0.92
Arsenic	As	<0.03	<0.03	0.04	0.27
Barium	Ba	0.06	0.63	1.84	32.0
Boron	B	<0.02	0.03	0.03	0.03
Cadmium	Cd	<0.01	<0.01	0.06	<0.01
Chromium	Cr	0.26	0.34	1.32	0.57
Cobalt	Co	<0.01	0.01	0.09	0.01
Copper	Cu	0.27	7.68*	167†	2.57‡
Iron	Fe	1.03	1.81	4.44	2.00
Lead	Pb	0.04	0.12	203†	0.03
Manganese	Mn	<0.03	0.27	1.10	1.60
Molybdenum	Mo	0.19	0.53	0.52	12.0
Nickel	Ni	<0.20	0.22	3.51	0.28
Selenium	Se	<0.07	<0.07	<0.07	1.08‡
Silver	Ag	<0.003	0.004	0.006	<0.003
Strontium	Sr	0.07	3.96	5.17	169
Thallium	Tl	0.004	0.008	0.015	0.019
Titanium	Ti	<0.02	0.04	0.10	0.17
Uranium	U	0.02	0.02	0.03	0.21
Vanadium	V	0.02	0.02	0.31	2.29
Zinc	Zn	0.51	5.19	854†	2.78

RO: Reverse osmosis water (deionized)
MHRW: Moderately hard reconstituted water (uses RO as base water)
* Metals (Cu) leached at concentrations above CWQG values from the carboy used to store MHRW during testing
† Metals (Cu, Pb, Zn) leached at concentrations above CWQG values from sieves used in activated charcoal treatment
‡ Metals (Al, Cu, Se) leached at concentrations above CWQG values directly from granular activated charcoal

As predicted, the carboy used to store the MHRW used in the test leached a significant amount of Cu (7.68 µg/L; 28x higher than the background concentration detected in the RO water used as the base to prepare MHRW). The source of this Cu contamination is unknown and surprising as the carboys were acid-washed and cleaned thoroughly prior to test initiation. After BML surface water was treated with GAC, the test water was passed through metal sieves to remove the large pieces of charcoal prior to subsequent vacuum filtration. These sieves were found to leach a significant amount of Cu, Pb, and Zn as they are mostly made of brass (an alloy of Cu and Zn). Interestingly, elevated concentrations of these same metals were not detected in the final GAC test waters, which suggests that the smaller GAC particles which remained in solution after sieving removed any metals leached from the sieves prior to vacuum filtration. However, future studies should still use plastic filtration mesh instead of metal if possible to avoid potential leaching. Finally, the GAC itself was found to be the source of the elevated Al detected in the GAC-treated waters, with the GAC-rinse containing 2.3 mg/L of Al (compared to 0.0008 mg/L in the RO), as well as elevated concentrations of several other metals including Sb, As, Ba, Cu, Mo, Se, Sr, V, and Zn. After this discovery, it was noted that Anderson *et al*. (2012) also reported elevated concentrations of Al in GAC-treated OSPW in their raw chemistry data but never discussed this increase. However, the concentration was somewhat lower than that observed in this study (0.9 mg/L) likely due to the shorter treatment duration, but perhaps also due to the source and pre-treatment of the activated charcoal. Furthermore, while the GAC-treated water in this study was significantly more toxic than the pre-treatment water due to the leached Al, the GAC-treatment nearly eliminated toxicity of the source OSPW tested by Anderson *et al*. (2012). This discrepancy is likely due to the difference in species sensitivity to Al – *Chironomus dilutus* used by Anderson *et al.* (2012) is generally far less sensitive to metals than *Ceriodaphnia dubia*. Therefore, although more expensive, future GAC studies with *C. dubia* and other metal-sensitive aquatic organisms such as *Daphnia* and *Hyalella* should use GAC which has undergone a more extensive pre-treatment regime (e.g. acid-washing) to remove adsorbed cationic metals which may leach into treated waters.

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