APPLICATION OF BIOMIMETIC EXTRACTION TO MEASURE TOXICITY OF OIL SANDS PROCESS-AFFECTED WATER TO AQUATIC ORGANISMS

by

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Abstract

Accumulation of oil sands process-affected water (OSPW) in Alberta's Oil Sands Region poses a potential environmental problem if it encounters natural waters. As a result of Alberta's zero-discharge policy, OSPW is stored on site. One potential treatment solution being tested at the pilot scale is a constructed wetland treatment system (CWTS). This study aims to measure chronic toxicity of OSPW to aquatic organisms and assess the treatment efficacy of Imperial Oil's Kearl treatment wetland using passive sampling to measure changes in OSPW toxicity as it flows through the wetland. Biomimetic extraction (BE) using solid-phase micro-extraction (SPME) fibers measures freely dissolved concentrations of the acid-extractable organic (AEO) fraction of OSPW and gives insight into the toxicity of OSPW through calibration with in-vivo toxicity metrics from chronic toxicity tests. This passive sampling method has the potential to replace traditional animal toxicity testing for whole effluent screening. Chronic toxicity testing was performed with walleye (*Sander vitreus*) early life stages (ELS) and an aquatic invertebrate (*Ceriodaphnia dubia*) with untreated and wetland-treated OSPW to calibrate BE-SPME and determine OSPW treatment efficiency. Walleye and *C. dubia* exhibited similar sensitivity to OSPW for sublethal endpoints (IC_{25} = 64% and 64% OSPW, respectively). However, walleye survival was more sensitive to OSPW than *C. dubia* (LC_{50} = 71% and >100%, respectively). A reduction in toxicity to *C. dubia* survival and reproduction after wetland treatment and agreement between in-vivo toxicity testing and BE-SPME measurements was observed. These results support the application of constructed wetlands to treat OSPW and the feasibility of BE-SPME passive sampling as a monitoring tool.

Keywords: Oil sands process-affected water; Treatment wetlands; Biomimetic extraction; Solid-phase microextraction; Chronic toxicity

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List of Acronyms

µg/L	Microgram per litre
AEO	Acid extractable organic
AOSR	Athabasca Oil Sands Region
ASTM	American Society for Testing and Materials
BC	British Columbia
BE	Biomimetic extraction
CAPP	Canadian Association of Petroleum Producers
CCME	Canadian Council of Ministers of the Environment
CHWE	Clark hot water extraction process
CWTS	Constructed wetland treatment system
DMN	dimethylnaphthalene
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EC	Effect concentration
ECCC	Environment and Climate Change Canada
ELS	Early life stage
EPL	End pit lake
GC-FID	Gas chromatography flame-ionizable detector
GOA	Government of Alberta
H_3PO_4	Phosphoric acid
HRT	Hydraulic retention time
IC	Inhibitory concentration

KTW	Kearl treatment wetland
LC	Lethal concentration
mg/L	Milligram per litre
mmol	Millimolar
NA	Naphthenic acid
NAFC	Naphthenic acid fraction compound
NaOH	Sodium hydroxide
NR	Not reported
OSPW	Oil sands process-affected water
PAH	Polycyclic aromatic hydrocarbon
PDMS	Polydimethylsiloxane
SFU	Simon Fraser University
SPME	Solid phase microextraction
TLM	Target lipid model
TPH	Total petroleum hydrocarbon
TRAP	Toxicity relationship analysis program
US EPA	United States Environmental Protection Agency
WETA	West Effluent Tailings Area
WQG	Water quality guideline

Chapter 1. Introduction

1.1. Alberta Oil Sands

Canada holds the third largest oil reserve globally, with 97% of that reserve located in Alberta's oil sands region (AOSR) (Government of Alberta, 2017). Canada's oil sands are located within the Athabasca, Peace River, and Cold Lake areas of Alberta (Alberta Energy Regulator, 2021). The Athabasca River, along with many of its tributaries, runs adjacent to and often directly through the oil sands operations (Dube et al., 2021). Industrial development of the oil sands region began in 1967 and has since grown exponentially, with over 3 million barrels extracted per day by 2018 (Government of Alberta, 2020). Oil sands production is responsible for nearly 2/3 of total crude oil in Canada and extraction is expected to increase by 1.27 million barrels per day between 2019 and 2035 (Canadian Association of Petroleum Producers (CAPP), 2020). The oil sands industry is critical to the Canadian economy, providing $105 billion of the nation's gross domestic product (GDP) and almost 400,000 jobs (CAPP, 2019). While the entire country benefits economically from the oil sands' profitability, Alberta is most directly benefitted both through royalties ($4.5 billion in 2011-12) and employment (Pembina Institute, 2013).

1.2. Oil Sands Process-affected Water

Oil sands are loose or partially consolidated deposits of sand that are saturated with bitumen that can be refined to be used for gasoline, jet fuel, diesel fuel, and heating oil (Masliyah et al., 2004). The process of bitumen recovery varies but the most commercially-used technology is the Clark Hot Water Extraction (CHWE) process developed by Karl Clark in the 1920's (Pasternack and Clark, 1951). The CHWE process uses large volumes of hot water, steam, and a caustic agent (NaOH) for the separation of bitumen from the oil sands. A flotation separation process then occurs that creates bitumen froth, which is further processed and results in 88-95% bitumen recovery (OSRIN, 2010). The resulting tailings effluent is transported into tailings ponds as a slurry mixture made of sands, dispersed fines, water, and any remaining bitumen (Chalaturnyk et al., 2002). For every barrel of oil produced, approximately 2 barrels of

water are used (Energy Regulator, 2020). Most of the oil sands process-affected water (OSPW) from the recovery process is recycled to be used in future extraction (>75%). However, the reuse of settled OSPW leads to higher concentrations of organic and inorganic constituents left behind in the ponds. This has large environmental implications (Giesy et al., 2010). The volume of stored tailings was reported to be nearly 1 billion m^3 in 2017 and covers an area of 176 km^2 (Foght et al., 2017; Small et al., 2015).

The composition of OSPW is complex and can vary between sites. It is comprised of residual bitumen, sand, clay, water, and both organic and inorganic contaminants (Mahaffey and Dube, 2016). Although variable, OSPW water chemistry always includes major contaminants including naphthenic aids (NAs), polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethyl benzene, xylenes, phenols, heavy metals, and ions (Allen, 2008; Mahaffey and Dube, 2016). Currently, there is a zero-discharge policy implemented by the *Alberta Environmental Protection and Enhancement Act* (1993) requiring all tailings to be held on site. Oil companies are responsible for OSPW reclamation within ten years of a mine's end-of-life (Wilson et al., 2018). The current zero-discharge policy is in place due to concern over the toxicity of OSPW to the surrounding environment. The primary acute toxicity of OSPW has been attributed to the naphthenic acid fraction components (NAFCs), which account for <50% of all OSPW organic fraction components (Grewer et al., 2010; Mahaffey and Dube, 2016). Both whole effluent OSPW and extracted NAFCs have been shown to exhibit toxic effects including impaired reproduction and embryonic development, immunosuppression, and endocrine disruption (Anderson et al., 2012; He et al., 2012; Garcia-Garcia et al., 2011; White and Liber, 2020.). When NAFCs are removed from OSPW through various treatments, toxicity has been observed to decrease (Anderson et al., 2011; Wang et al., 2013).

The requirement for mining companies to have mine waste ready to reclaim within 10 years of a mine's end-of-life presents a challenge when large-scale tailings reclamation options are still needed. Seepage of OSPW from tailings storage ponds into natural waters, including the Athabasca River and its tributaries, in the Athabasca region is a concern shared among environmental regulators, scientists, and local communities including First Nations. Monitoring of the surrounding natural waters has revealed that groundwater seepage is occurring, but surface waters remain uncontaminated by OSPW (Fennel and Arciszewski, 2019). While this provides evidence for a low level of risk to

the surrounding biological systems, the volume of tailings waste is growing exponentially and reclamation solutions are necessary for safe release.

Reclamation solutions such as ozonation or the addition of petroleum coke to tailings are effective but labor intensive in comparison to passive or semi-passive wet-landscape solutions such as treatment wetlands (McQueen et al., 2017). Treatment wetlands have been used successfully in many other industries including wastewater reclamation (Sundaravadivel and Vigneswaran, 2001). The pilot-scale Kearl Treatment Wetland (KTW) operated by Imperial Oil Ltd provides an opportunity to measure the efficacy of this wet-landscape reclamation treatment for OSPW degradation and toxicity. If effective, treatment wetlands may be a viable large-scale solution that could be implemented by the oil sands industry. The goal of this research is to measure chronic toxicity of OSPW to aquatic organisms and changes in toxicity of OSPW after wetland treatment.

1.3. Toxicity and Degradation of OSPW

OSPW exerts acute and chronic toxicity on aquatic organisms. The acute toxicity of OSPW is attributed to NAs and more broadly, NAFCs (Meulen et al., 2021). The toxicity of NAFCs varies, with differences observed between OSPW-derived NAFCs and commercial mixtures. Commercial mixtures, which are extractions of petroleum distillates commonly used as emulsifiers and preservatives (Clemente et al., 2004), exert greater toxicity than bitumen-derived mixtures to aquatic invertebrates including *Hyalella azteca*, *Vibrio fischeri*, and *Lampsilis cardium* (Bartlett et al., 2017). For the purpose of this literature review, only bitumen-derived mixtures are included.

The structure of the specific NAFCs also has an impact on toxicity. NAFCs of a lower molecular weight contribute greater toxicity compared to those of higher molecular weight, although the mechanism driving this toxicity difference is still not well understood (Frank et al., 2008).

Whilst NAFCs drive most acute toxicity, the toxicity of raw OSPW is the focus of this research. Previous studies have found toxic effects from OSPW exposure on fish (He et al., 2012; Scarlett et al., 2013), invertebrates (Bartlett et al., 2017), and mammals (Garcia-Garcia et al., 2011). The acute impacts of whole OSPW toxicity have been well documented in many aquatic organisms. OSPW exposure impacts the respiratory and circulatory systems of *Daphnia manga* (Lari et al., 2017), as well as grazing behaviour

(Lari et al., 2016). It also can lead to growth impairment, oxidative stress, and endocrine disruption in *Chironomus dilutus* (Wiseman et al., 2013). Chronic impacts of OSPW exposure are much less documented (White and Liber, 2019). Reduced embryonic survival, premature hatching, and altered behaviour have been observed in fathead minnow during a 7-day OSPW exposure (*Pimephales promelas*) (He et al., 2012). Adult emergence of *C. dilutus* was significantly delayed (approximately 11 days) when exposed to fresh OSPW (Anderson et al., 2012). This emergence delay is attributed to oxidative stress caused by NAFCs, which has been observed in a similar study that observed reduced pupation and a 5-day emergence delay (White and Liber, 2020). There is minimal data available on the multi-generational impacts of OSPW exposure. An individual study on the persistent and transgenerational effects of raw and ozonated OSPW found that exposure had a trans-generational impact on larval activity, anxious behaviour, and swimming speed (Philibert et al., 2019). The authors emphasized the need for further study on long-term impacts of OSPW exposure.

Toxic constituents of OSPW including NAs are primarily degraded by sediment microbial communities under aerobic conditions (Del Rio et al., 2006). The rate of degradation for OSPW toxic constituents including NAs depends on factors such as molecular structure and the level of alkyl branching (Ajaero et al., 2020). Smaller NAs with less alkyl branching are “labile” and are quickly degraded by microbes. This is often associated with decreased toxicity as these labile NAs are responsible for the majority of acute toxic effects (Han et al., 2008). However, “refractory” NAs, which are larger and more persistent, are left behind after initial degradation occurs and can exert chronic toxic effects. Toor et al. (2013) measured degradation and associated changes to toxicity of OSPW in simulated wetlands after 52 weeks. They observed rapid degradation of “labile” NAs while the “refractory” NAs were persistent and likely responsible for chronic toxicity present in a Microtox assay (Toor et al., 2013). A study by Kavanagh et al. (2011) found similar results with aged (>15 years) OSPW impairing reproduction of fathead minnow (*Pimephales promelas).* These results highlight the need to find a viable treatment solution that addresses residual toxicity present after OSPW degradation.

1.4. Treatment of OSPW

The Government of Alberta's zero-discharge policy was initially put in place to encourage water re-use. However, the growing volumes of OSPW being stored as a result of this policy have created major concern due to potential for toxic effects if released into the environment (Martin, 2015). Oil companies in the AOSR are required to have all disturbed land ready to reclaim within 10 years of mine closure under Alberta's *Environmental Protection and Enhancement Act* (2021). This requirement includes the reclamation of new and existing OSPW with the ultimate goal of environmental release. Large-scale and cost-effective OSPW treatment solutions are necessary if environmental release is to be possible.

Treatment and reclamation strategies for OSPW are wide ranging, with many still in the pilot stages. Wet-landscape reclamation strategies are being considered due to their passive nature and the potential for them to be permanently incorporated into the natural environment. One example of wet-landscape strategies currently being implemented is end pit lakes (EPLs). EPLs store inactive fluid fine tailings (no fresh input) and are capped with freshwater (Dompierre et al., 2016). There is evidence supporting increased water quality of the surface waters over time (White and Liber, 2018). A more recent wet-landscape reclamation strategy that is yet to be implemented at a large scale is constructed wetland treatment system (CWTS). This literature review will focus on constructed wetlands as an OSPW reclamation strategy and provide evidence for their potential success as a solution to tailings management in the AOSR.

1.4.1. Constructed Wetland Treatment Systems

CWTSs are an effective treatment solution for refinery effluent (Huddleston et al., 2000), agricultural runoff (Beutel et al., 2009), and textile dye wastewater (Bulc and Ojstrsek, 2008). Recently, CWTSs have been proposed as a treatment solution for OSPW (Toor et al., 2013). Wetlands passively or semi-passively perform a number of natural processes that aid in chemical transformation, including photolysis, hydrolysis, oxidation, reduction, and biotransformation (Rogers and Castle, 2008). When constructing a CWTS, wetland characteristics must be considered such as the species of plants present, sediment type, water depth, flow rate, and indigenous microbial

community (Haakensen et al., 2015). These CWTS features affect biogeochemical conditions and can further impact degradation abilities.

Wetlands used for OSPW reclamation are still at the proof-of-concept stage and their efficacy, while promising, is under investigation. Laboratory wetland microcosms offer the ability to assess the efficacy of such a treatment solution in a controlled manner. Simulated wetlands exposed to two different sources of OSPW exhibited a reduction in NA concentrations and an associated decrease in toxicity to rainbow trout (*O. mykiss*) (Toor et al., 2013). However, residual NA toxicity was still observed in the simulated wetlands, indicating longer hydraulic retention times (HRT) than those used in the study. It may be necessary to couple wetland treatment with another treatment strategy such as oxidation to reach complete toxicity reduction. For example, combining a CWTS and a solar photocatalytic reactor decreased NA and metal concentrations as well as reduced toxicity to *C. dubia* (Simair et al., 2021). Constructed wetlands hold great potential for effectively reducing concentrations of toxic constituents of OSPW, although more research is necessary.

1.5. Passive Sampling as a Useful Tool

Quantifying dissolved organics is a crucial step in understanding the potential risks of exposure to OSPW. The complexity of mixtures such as OSPW makes it difficult to characterize the individual constituents and determine their toxicity (Parkerton et al., 2000). Total petroleum hydrocarbon (TPH) analyses have been used traditionally to determine toxicity of oil fractions to organisms (Redman et al., 2018). However, this technique is not an effective predictor of negative impacts on aquatic organisms. The use of TPH analysis includes undissolved, hydrophobic hydrocarbons that are not responsible for the substance's toxicity (Letinski et al., 2014). The target lipid model (TLM) allows for the quantification of dissolved hydrocarbon toxicity and assumes that aquatic toxicity is a result of the concentration of a chemical in an organism's lipids exceeding a critical threshold (McGrath and Di Toro, 2009).

Biomimetic extraction (BE) using solid-phase microextraction (SPME) fibers is a technique that can be used *in-situ* to determine the risk of contaminants that employ a narcotic mode of action (Leslie et al., 2002). SPME is a tool that is regularly used to quantify freely dissolved organic chemicals in water. It is often preferred over other techniques such as liquid-liquid extraction and solid-phase extraction due to its speed

and cost-effectiveness (Huang et al., 2021). Applying the SPME method is useful to measure the bioavailability of petroleum constituents in water, with polydimethylsiloxane (PDMS) fibers used as a surrogate lipid to predict toxicity to aquatic organisms (Redman et al., 2014). A major benefit to using BE is that unlike other exhaustive methods for extracting hydrophobic organic chemicals, it does not deplete the concentration of the toxicant in the experimental media. This is intended to mimic the level of uptake by biota in the natural environment (Leslie et al., 2002).

A growing number of studies have applied the BE-SPME method to predict the toxicity of petroleum substances to aquatic organisms including fish (Hedgpeth et al., 2019; Parkerton et al., 2000; Redman et al., 2014; Redman et al., 2018) and aquatic invertebrates (Letinski et al., 2014). These studies have found that thresholds determined by BE-SPME passive sampling were consistent with measured toxicity as well as target lipid model outputs. In combination with mass spectrometry, the SPME method using PDMS fibers could detect both NAs and hydrocarbons. This method can be used to test the efficacy of OSPW treatment solutions such as CWTSs and quantify the bioavailability of organic compounds in both aqueous media and sediment. Applications of the BE-SPME method are limited, with few studies focused on Alberta and minimal use of site-relevant species. Additionally, acute effects are most often considered.

1.6. Rationale for Research

The large volume of OSPW currently stored on-site in the AOSR because of the GOA's zero-discharge policy presents a challenge due to the potential for accidental release. Operators are currently required to store all OSPW on-site, with no option to discharge into the natural waters because of known toxic effects to aquatic organisms. If OSPW is to be discharged, successful treatment at a large scale is needed. Constructed treatment wetlands are one type of treatment technology currently being considered. In addition to treatment, methods for measuring and monitoring the constituents of concern, including NAFCs, is required.

The BE-SPME passive sampling method has been previously applied to measure bioavailability and predict toxicity of petroleum substances, including NAFCs (Redman et al., 2018; Piggott 2022). The goal of BE-SPME as a monitoring method is to accurately predict toxic thresholds for aquatic organisms and serve as an alternative to traditional animal toxicity testing. This study will contribute toward solving the challenge of OSPW treatment through collecting chronic toxicity data for two site-relevant species (*C. dubia* and walleye) and directly applying BE-SPME passive sampling to assess the efficacy of treatment wetlands at OSPW toxicity removal.

1.7. Research Objectives

The objectives of this research include:

1. Developing and application of a method for testing the chronic toxicity of OSPW to *C. dubia* and walleye using BE-SPME.

2. Applying BE-SPME to both bulk and wetland OSPW to quantify the bioavailable organic fraction and serve as a surrogate measure of toxicity for aquatic organisms.

Chapter 2. Application of Biomimetic Extraction to Measure Chronic Toxicity of OSPW to *Ceriodaphnia dubia* and Walleye

2.1. Summary

This study aims to measure chronic toxicity of OSPW to *Ceriodaphnia dubia* and walleye (*Sander vitreus*). Both *C. dubia* and walleye are site-relevant species that inhabit the natural waters within the AOSR. The invertebrate C. dubia is a ubiquitous organism that is commonly used in toxicity testing due to its common occurrence in natural waters, short life cycle, and important role in the aquatic food chain (ECCC, 2007). Walleye is a recreationally caught species found throughout the Athabasca River basin (Craig and Smiley, 1986). It is a predatory fish that sits high on the food web and consumes smaller fish species. Walleye is a culturally important species, consumed by local First Nations communities within the AOSR (O'Connor et al., 2022).

C. dubia were chronically exposed to OSPW during a 6-day survival and reproduction toxicity test. Additionally, a walleye early life-stage (ELS) toxicity test was done to measure chronic effects of OSPW throughout the embryonic and larval stages. Walleye is not a routinely used laboratory organism due to poor egg availability, weak larval stock, and cannibalism (Barrows and Lellis, 2006). However, they are a very site-relevant species, and are potentially at risk of exposure to OSPW-derived contaminants. The use of walleye gives this study greater relevance for the region and the Indigenous communities that depend on walleye as a resource.

BE-SPME is a passive sampling method that can predict toxicity of complex petroleum compounds to aquatic organisms through measuring the bioavailability of organic substances (Letinsky et al., 2022). In addition to traditional toxicity testing with *C. dubia* and walleye, BE-SPME was conducted on OSPW dilutions used during the exposures. This tool has the potential to be applied during routine monitoring to quickly assess the potential for toxic effects to aquatic organisms (Huang et al., 2020). In this study, BE-SPME was applied in conjunction with the toxicity testing, with the goal of developing a calibration curve between measured toxic effects and SPME concentration.

This method provides insight into the ability of BE-SPME to predict toxic effects of OSPW to aquatic organisms and could be used to interpret SPME-extracted ambient waters. If found to be successful at predicting toxic effects of OSPW to walleye, this passive sampling tool may provide a replacement for animal toxicity testing for routine monitoring purposes.

Overall, the objectives of this study were to:

- Measure chronic toxicity of OSPW to *C. dubia* and walleye.
- Apply the BE-SPME passive sampling method to the test treatment waters to determine critical thresholds to *C. dubia* and walleye, compare to measured chronic toxic effects, and predict OSPW toxicity.

2.2. Materials and Methods

2.2.1. OSPW Source

In the Summer of 2021, ~600L of fresh OSPW was collected from the West Effluent Tailings Area (WETA) at the Kearl Oil Sands site operated by Imperial Oil Resources Ltd. and shipped to SFU in a plastic tote. The water was stored in pails in SFU's Alcan Aquatic Research Center in Burnaby, BC until use. Prior to use, the water used for toxicity testing was filtered through a sieve (90 µm) to remove any large particulate matter.

2.2.2. Water Quality

Water quality parameters, including temperature, dissolved oxygen, conductivity, and pH, were measured each day of the 6-day exposure for *C. dubia* and the 28-day exposure for walleye when treatment waters were replenished. Water quality was only collected for the laboratory control[1], 1.56%, 12.5% and 100% vol/vol OSPW treatments

[1] Laboratory control for the *C. dubia* toxicity test was 20% Perrier® mineral water and 80% deionized water + 5 µg/L Se and 2 µg/L vitamin B12. Perrier® mineral water was added as a hardness correction. For the walleye toxicity test, control water was dechlorinated City of Calgary

for *C. dubia*, but was collected for all treatment waters for walleye. Additionally, the raw OSPW collected in Summer of 2021 was analyzed by ALS Environmental (Burnaby, BC) for physical parameters, anions and nutrients, and metals (total and dissolved). Measured concentrations in OSPW were compared against water quality guidelines to observe any exceedances that may contribute to toxicity to aquatic organisms. The GOA water quality guidelines (WQGs) for aquatic life were used. If a GOA guideline was not available, the Canadian Council of Ministers of the Environment (CCME) WQGs were consulted and applied where needed.

NAs in 100% OSPW were quantified using Orbitrap mass spectrometry (Orbitrap-MS), which has high resolution and accuracy for polar compounds (Ajaero et al. 2018). The analysis was performed by InnoTech Alberta (Calgary, Alberta). Total NAs were reported herein.

2.2.3. Toxicity Testing

Study Species

C. dubia

The cladoceran *C. dubia* was chosen as the representative aquatic invertebrate species for this study. Daphnids, commonly known as water fleas, are ubiquitous in freshwater environments and are a critical component of the food web. They are a commonly used organism for toxicity testing due to characteristics including their short life cycle, sensitivity to contaminants, and small size (ECCC, 2007). Nautilus (Burnaby, BC) used their maintained brood for toxicity testing.

Walleye

Walleye was the fish species chosen for this study due to its site relevance and identified cultural importance. Walleye is a freshwater piscivorous fish that is found throughout the Athabasca River basin. They are migratory fish that move through the river and tributaries at different times of the year. The migratory pattern of walleye includes overwintering in Lake Athabasca, moving into the mainstem Athabasca River in

tap water. The type of control water used in the tests likely differed due to the quantity of water needed for testing (e.g., large volume for walleye compared to *C. dubia*).

the Spring to spawn and feed, and then using adjacent tributary mouths for nursery functions and resting areas (Schwalb et al., 2015). Walleye spawn once per year during the Spring (Nelson and Paetz, 1992). They are a culturally significant species to Indigenous communities and are caught recreationally.

On May 5, 2022, newly fertilized walleye eggs were collected at a spawn camp at the Cold Lake Fish Hatchery in Lac Ste. Anne, Alberta. The eggs were transported to the Nautilus laboratory in Calgary, AB on the same day. Upon arrival, the fertilized eggs were transferred into a MacDonald hatch jar and the water temperature was maintained at 8 ± 2°C. The hatch jar was connected to a recirculation system using dechlorinated municipal tap water prior to testing.

Test Protocol

C. dubia Survival and Reproduction Exposure

C. dubia neonates (<24 hour old) were exposed to serial dilutions of OSPW at the Nautilus Laboratory in Burnaby, BC in October 2022. The testing protocol used was the Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *C. dubia*, EPS 1/RM/21 (ECCC, 2007). A summary of the protocol and test conditions is found in **Table 1**.

Table 1. Test conditions for *C. dubia* survival and reproduction toxicity test

Test Condition	Description
Test type	Static-renewal
Test duration	7 ± 1 day
Test vessel	20-mL glass test tube
Test volume	15 mL (depth = 10 cm)
Test concentrations	Seven concentrations (1.56%, 3.12%, 6.25%, 12.5%, 25%, 50%, and 100%), plus laboratory control
Test replicates	10 per treatment
Number of organisms	1 per replicate
Control/dilution water	20% Perrier® water and 80% deionized water ± 5 µg/L Se and 2 µg/L vitamin B12
Test solution renewal	100% renewal daily
Test temperature	25 ± 1°C
Feeding	Daily with *Pseudokirchneriella subcapitata* and Trout chow and Cerophyl (TCC) (3:1 ratio)
Light intensity	100 to 600 lux at water surface
Photoperiod	16 hours light / 8 hours dark
Aeration	None

Notes: mL = millilitre; cm = centimetre; µg/L = microgram per litre; % = percent; °C = degrees Celsius.

The measured endpoints included survival and reproduction. In order for the test to be considered acceptable and reliable, the following conditions must be met: ≧ 80% survival; ≧15 young per surviving control producing three broods; ≧60% of controls producing three or more broods; no ephippia present.

Walleye Early Life Stage (ELS) Exposure

Walleye embryos (24 to 48-h post-fertilization) were exposed to serial dilutions of OSPW at the Nautilus laboratory in June 2022 (**Table 2**). The testing protocol was adapted from United States Environmental Protection Agency (US EPA) 'Fish ELS Toxicity Test' (US EPA, 2016) and the American Society for Testing and Materials (ASTM) 'Standard Guide for Conducting ELS Toxicity Tests with Fishes' (ASTM, 2013). A summary of the protocol and test conditions is found in **Table 2**.

Table 2. Test conditions for walleye ELS toxicity test

Test Condition	Description
Test type	Static-renewal
Test duration	28 days
Test vessel	1.5-L glass jars
Test volume	1 L
Test concentrations	Five concentrations (6.25%, 12.5%, 25%, 50%, and 100%), plus laboratory control
Test replicates	4 per treatment
Number of organisms	30 per replicate
Control/dilution water	Dechlorinated City of Calgary tap water
Test solution renewal	50% renewals daily
Test temperature	Test was initiated at 8 ± 2°C and increased 1-2°C each week to mimic natural conditions up to a final temperature of 15 ± 1°C
Feeding	Fed three times daily, with newly hatched Artemia nauplii beginning ~8-days post-hatch
Light intensity	100 to 300 lux
Photoperiod	16 hours light / 8 hours dark
Aeration	Gentle, continuous

Notes: L = litre; % = percent; °C = degrees Celsius.

Measured endpoints included percent hatch, 21-day and 28-day post-hatch survival, 21-day and 28-day overall survival, 28-day dry weight, and normality of hatched larvae. Hatching normality was assessed based on presence or absence of deformities of hatched larvae, including spinal abnormalities, edema, craniofacial effects, and delayed

development. The test acceptability for the controls was >66% hatch and ≧70% post-hatch survival.

Figure 1. Photograph of the walleye toxicity testing set-up at Nautilus Environmental in Calgary, Alberta. Taken by Julia Brueggeman, June 2022.

2.2.4. Measuring Toxicity Reduction using BE

At the start of the *C. dubia* toxicity test, ~125 mL of water from each treatment, including the laboratory control, was subsampled in triplicate, and stored in amber glass bottles at 4°C prior to analysis at SFU. BE-SPME was conducted on all water samples at the Gobas Laboratory at SFU following the protocol outlined in Redman et al. (2018).

In summary, triplicate samples of the treatment water were added to 20 mL vials with no headspace and sealed with Teflon-faced septum caps. Samples were acidified to a pH of ~2.4 using 50 uL of phosphoric acid (H_3PO_4). BE-SPME was performed using the automatic method (Leap Technologies Combi-PAL Autosampler) and a fiber coated with 30 um PDMS. Samples were agitated at 250 rpm for 30 minutes and brought to a

consistent temperature (30˚C). The fiber was exposed to the sample for 100 minutes, or time to equilibrium, prior to being analyzed by a gas chromatography flame-ionizable detector (GC-FID). The chromatographs were analyzed using Agilent ChemStation software (Version A.02.12).

The FID response was normalized against a standard curve that was created using 0.5 µL of three known amounts of 2,3-dimethlylnaphthalene (2,3-DMN) (20, 100, and 200 µg/mL). The BE peaks were then normalized to the volume of the PDMS (0.132 µL) on the fiber (mmol/LPDMS). The chromatographs were analyzed using Agilent ChemStation software (Version A.02.12).

2.2.5. Statistical Analysis

Statistical analysis was performed on the laboratory toxicity data by Nautilus using CETIS Version 2.1.1 (Tidepool Scientific Software, 2021). The CETIS software uses the linear interpolation method to determine inhibition concentrations. Additionally, effect concentrations (LC_x/IC_x) were determined for survival and sub-lethal endpoints (reproduction, hatching) using the US EPA Toxicity Relationship Analysis Program (TRAP; Version 1.30a). TRAP applies a two-parameter nonlinear regression (logistic equation) with a log-transformed exposure variable to determine the point estimates. An ANOVA test in JMP (Version 16) was used to measure significant differences between treatment groups (% OSPW vol/vol). Post-hoc tests were used as required.

Toxic effects of OSPW to *C. dubia* and walleye were estimated using BE measurements of bulk OSPW. In this case, the BE measurement is used as the exposure metric as opposed to the concentration of OSPW. The relationship between the toxic effect and the concentration of OSPW derived from the BE measurements was determined by a two-parameter Weibull function that was fitted using the Solver tool in Microsoft Excel®, using the sum of least squares with the measured dose-response data obtained in this study for *C. dubia* (reproduction) and walleye (survival and hatch rate). The equation for a two-parameter Weibull distribution is as follows:

$$F(x) = 1 - e^{-\left(\frac{x}{\beta}\right)^{\alpha}}$$

$F(x)$ = toxic effect
x = SPME concentration measurement ($mmol_{2,3\text{-}DMN}/L_{PDMS}$)
β = determined using the Solver function in Microsoft Excel®
α = determined using the Solver function in Microsoft Excel®

The BE concentrations of total acid-extractable organics in OSPW (in units $mmol_{2,3\text{-}DMN}/L_{PDMS}$) were used as the exposure metric to explain the toxic effect to C. dubia reproduction (% effect), walleye hatch rate, and survival. The effects predicted by the model using BE measurements were then compared to the measured effects observed in toxicity testing. This comparison provides insight into the ability of BE measurements to accurately predict toxic effects to *C. dubia* and walleye. The Weibull distribution method was also used to determine modelled effect concentrations (EC_x) in BE terms using the equation above.

2.3. Results and Discussion

2.3.1. Water Quality Analysis

Laboratory Conditions

C. dubia

Table 3 and **Figure 2** show the water quality parameters of the water used to replenish treatment tanks during water changes throughout the 6-day *C. dubia* exposure. Temperature for the *C. dubia* test was maintained within 25 ± 1°C, as stipulated by the protocol (EPS 1/RM/21; ECCC 2007). Dissolved oxygen content of the bulk OSPW test was similar to that of the controls for all treatments and ranged from 7.6 to 8 mg/L. Conductivity increased with OSPW concentration with an average of 1,290 µS/cm in the 100% treatment, which is typical due to high salinity observed in fresh OSPW (Zubot et al., 2012). Similarly, pH was observed to increase with OSPW concentration but remained within the limits (6.5 - 8.5) outlined in the testing protocol.

Table 3. pH, temperature, conductivity, and dissolved oxygen for each OSPW dilution measured during the 6-day *C. dubia* toxicity test. Values are reported as mean ± standard deviation.

Parameter	Units	Laboratory Control	1.56% OSPW	12.5% OSPW	100% OSPW
pH	-	7.9 ± 0.25	8.1 ± 0.23	8.3 ± 0.10	8.5
Temperature	˚C	25 ± 0.41	24 ± 0.38	24 ± 0.42	24 ± 0.42
Conductivity	µS/cm	207 ± 3.8	221 ± 5.6	342 ± 5.6	1290 ± 19
Dissolved oxygen	mg/L	8.0 ± 0.05	7.5 ± 0.25	7.6 ± 0.23	7.8 ± 0.16

Notes: OSPW = oil sands process-affected water; % = percent; - = no units; ˚C = degrees Celsius; µS/cm = micro-Siemen per centimetre; DO = dissolved oxygen; mg/L = milligrams per litre.

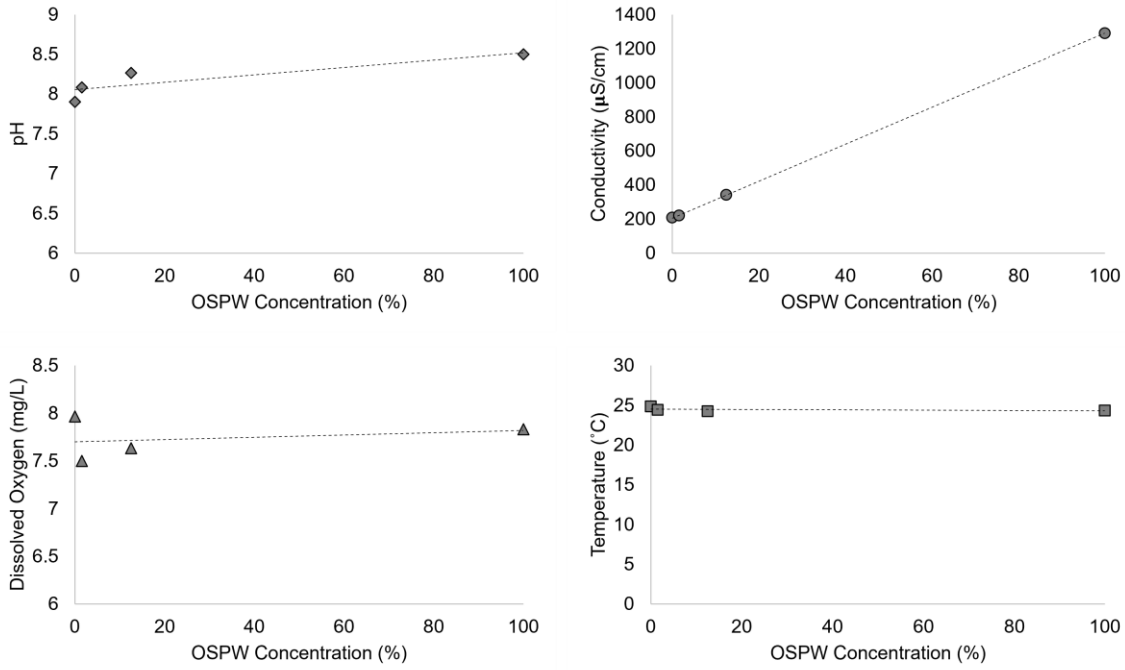

Figure 2. Average water quality (pH, conductivity, dissolved oxygen, and temperature) for each OSPW dilution measured throughout the 6-day *C. dubia* toxicity test.

Walleye

Water quality parameters including temperature, dissolved oxygen, conductivity, and pH were measured every day of the 28-day exposure during water changes. Measurements were taken on both the new and old water. Only water chemistry of the

new water was presented, except for the day of termination when a water change was not done. **Table *4*** and **Error! Reference source not found.** show the average water quality of the new water used to replenish during water changes throughout the exposure for each treatment group. The test protocol involved a temperature gradient that was initiated at a temperature of 8 ± 2°C and increased 1-2°C every week reaching a final temperature of 15 ± 1°C. This was intended to mimic the natural change in water temperature during the Spring season in the Athabasca River. Temperature was consistent across treatment groups and individual replicates and followed the intended temperature increase closely. Dissolved oxygen for the treatment waters was similar to the controls and ranged from 8.7 to 10.4 mg/L, meeting ECCC's protocol requirements of >90% saturation. Conductivity of the lower OSPW dilutions (e.g., 6.25% and 12.5%) were similar to the controls. Conductivity increased with increasing OSPW concentrations, consistent with previous observations because of high salinity in OSPW (Zubot et al., 2012). The pH of treatment waters was slightly higher than the control but within test protocol limits (6.5 – 8.5).

Table 4. pH, temperature, conductivity and dissolved oxygen of OSPW dilutions measured during the 28-day walleye toxicity test. Values are mean ± standard deviation.

Parameter	Units	Lab Control	6.25% OSPW	12.5% OSPW	25% OSPW	50% OSPW	100% OSPW
pH	-	7.96 ± 0.17	7.97 ± 0.25	8.03 ± 0.15	8.05 ± 0.12	8.11 ± 0.10	8.17 ± 0.93
Temperature	˚C	12 ± 2.3	12 ± 2.3	12 ± 2.3	12 ± 2.3	12 ± 2.3	12 ± 2.3
Conductivity	µS/cm	434 ± 37	472 ± 32	514 ± 47	620 ± 38	801 ± 58	1157 ± 69
DO	mg/L	9.4 ± 0.57	9.6 ± 0.53	9.6 ± 0.48	9.7 ± 0.48	9.7 ± 0.52	10 ± 0.33

Notes: OSPW = oil sands process-affected water; % = percent; - = no units; ˚C = degrees Celsius; µS/cm = microsiemens per centimetre; DO = dissolved oxygen; mg/L = milligrams per litre.

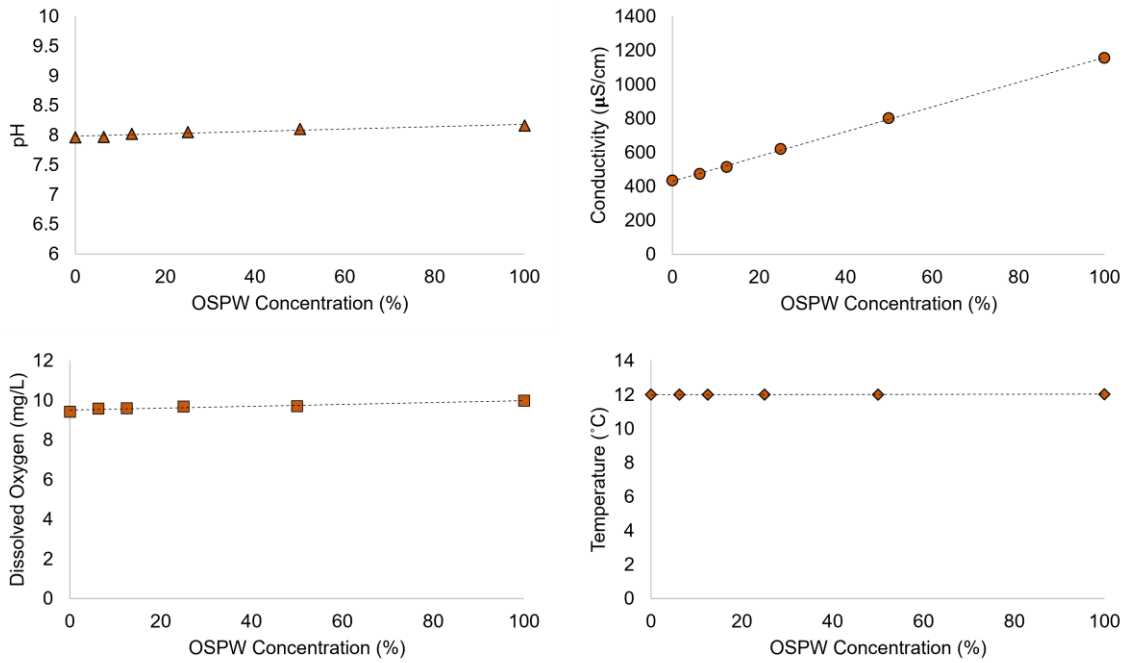

Figure 3. Average water quality (pH, conductivity, dissolved oxygen, and temperature) for each OSPW dilution measured throughout the 28-day walleye toxicity test.

Additional details of the analytical methods used for water quality analyses are found in Appendix C.

Water Quality Screening

Results of the OSPW water chemistry were compared against water quality guidelines (**Table 5**). All parameter concentrations were below GOA and CCME WQGs, with the exception of fluoride and aluminum (total and dissolved).

The measured fluoride concentration was 3.6 mg/L, which exceeds the CCME chronic WQG of 0.12 mg/L. However, this value is below observed fluoride IC_{20}/IC_{50} values for *C. dubia* (>7.8 mg/L) and therefore unlikely to contribute toward toxicity of OSPW (Pearcy et al. 2015). Acute toxicity of fluoride to rainbow trout was observed between 51 to 193 mg/L when a range of hardness values were tested (Pimentel and Bulkley, 1983). A study by Pearcy et al. (2015) measured chronic toxicity of fluoride to rainbow trout and determined an IC_{20} ranging from 6 to 21.6 mg/L with varying chloride concentrations. Both the acute and chronic effect concentrations measured in other studies confirm that the concentration measured in the bulk OSPW is unlikely to exert toxic effects to walleye.

Total and dissolved aluminum concentrations were both 528 µg/L, indicating that the aluminum in OSPW was fully dissolved. This concentration exceeds both the chronic (50 µg/L) and acute (100 µg/L; dissolved fraction only) GOA guidelines. Toxicity of aluminum to aquatic organisms is influenced by toxicity-modifying factors, including pH, dissolved organic carbon (DOC), and hardness. The chronic exceedance of aluminum has the potential to exert toxic effects on *C. dubia*, with similar species (*Ceriodaphnia silverstrii*) having an observed EC50 for aluminum of 520 µg/L (Castelhano Gebara et al., 2021). A study on fathead minnow by ENSR (1992; cited in ECCC 2022) observed a 7-day EC_{10} of 271 µg/L (normalized to pH = 7.5, DOC = 0.5 mg/L, and hardness = 50 mg/L). Although fathead minnow are not the ideal surrogate species for walleye, this observed effect supports the idea that the concentrations observed in this study have the potential to cause toxic effects to fish.

The concentration of NAs in the 100% OSPW treatment group was 11.5 mg/L (± 0.45 mg/L). Data on the chronic toxicity of NAFCs to *C. dubia* is limited. However, a study by Redman et al. (2018) measured toxicity of OSPW-derived NAFCs to *C. dubia* survival, immobilization, and reproduction. Effects to reproduction were observed after 7 days and had an IC_{25} value of 10 mg/L NAFC. Chronic toxicity testing (7-day) of NAFCs to other invertebrate species including *Hyalella azteca* was completed by Bartlett et al. (2017) and the IC_{25} values for survival ranged from 10.6 to 21.8 mg/L. The results of these studies indicate that the measured NA concentrations in the OSPW from this study are high enough to have a potential toxic effect to *C. dubia*. Most other toxicity testing with aquatic invertebrates available in the literature uses a commercial NA mixture, which has been shown to be an insufficient alternative to OSPW-derived NAFCs when measuring toxicity (West et al., 2011). For walleye, the measured concentration in bulk OSPW has been observed to cause effects (Marentette et al., 2015). Walleye hatch success had effect concentrations (EC_{50}) ranging between 9.5 and 11 mg/L NAFC. Sublethal effects were also observed, including deformities such as spinal curvature and cardiovascular and craniofacial defects.

It is important to note that water quality of this OSPW was analyzed in June of 2021 but not used for toxicity testing until June of 2022. Therefore, changes in parameters such as ammonia may have occurred that could have impacted the water quality used in testing. Although ammonia was not measured throughout the duration of the toxicity tests, water changes were completed daily (100% for *C. dubia* and 50% for

walleye, daily) to manage fluctuating ammonia levels. These changes would not be reflected in the presented water quality results. However, most parameters are assumed to be relatively stable and therefore should not have changed significantly. The concentration of NAs in the 100% OSPW treatment group was 11.5 mg/L (± 0.45 mg/L).

PAH concentrations in OSPW were not measured, although most were found to be below CCME and British Columbia WQGs in previous assessments of tailings water from Imperial Oil operations (Piggott, 2022). Those that exceeded guidelines included benzo(a)anthracene, benzo(a)pyrene, and pyrene. Whilst we did not measure individual PAHs, they do partition to the PDMS coating of SPME fibers and therefore should be accounted for in the acid-extractable organic fraction (King et al., 2004).

Table 5. Water Quality Screening of Bulk OSPW

Parameter	Unit	Guidelines for the Protection of Aquatic Life				Bulk OSPW
		Acute (GOA)	Chronic (GOA)	Acute (CCME)	Chronic (CCME)	
Conventional Parameters						
pH	-	-	6.5 - 9.0	-	6.5 - 9.0	8.3
Hardness, as $CaCO_3$	mg/L	-	-	-	-	235
Total alkalinity, as $CaCO_3$	mg/L	-	20	-	-	222
Total suspended solids	mg/L	-	-	-	-	6.7
Total organic carbon	mg/L	-	-	-	-	44
Dissolved organic carbon	mg/L	-	-	-	-	41
Turbidity	NTU	-	-	-	-	7.2
Conductivity	uS/cm	-	-	-	-	1,280
Hardness (as $CaCO_3$), from total Ca/Mg	mg/L	-	-	-	-	227
Major Ions						
Fluoride	mg/L	-	-	-	0.12	**3.6**$^{(C)}$
Total Metals						
Aluminum	µg/L	-	-	-	100	**528**$^{(C)}$
Dissolved Metals						
Aluminum	µg/L	100	50	-	-	**528**$^{(A, C)}$

Notes: GOA = Government of Alberta; CCME = Canadian Council of Ministers of the Environment; OSPW = oil sands process-affected water; CaCO3 = calcium carbonate; mg/L = milligrams per litre; NTU = nephelometric turbidity units; µS/cm = micro Siemens per centimetre; µg/L = micrograms per litre.
(C) = exceeds chronic water quality guideline.
(A) = exceeds acute water quality guideline.
Bolded and blue shaded value indicates a guideline exceedance.

2.3.2. Measuring Changes to OSPW Toxicity by BE

Figure 4 shows the relationship between OSPW concentration and acidified BE-SPME measurements for the *C. dubia* toxicity test. An overall linear relationship (y = 1.5812x + 54.105, R^2 = 0.8584) was observed between the OSPW dilutions of 1.56%, 3.12%, 6.25%, 12.5%, 25%, 50%, and 100% and corresponding BE concentration measurements. BE concentration measurements steadily increased with OSPW dilution concentration but experienced a reduction in concentration above OSPW concentration of 50% vol/vol. This pattern is consistent with results for walleye presented in **Figure 5**. The variability of BE measurements within each treatment was overall consistent across all treatments (i.e., low standard error).

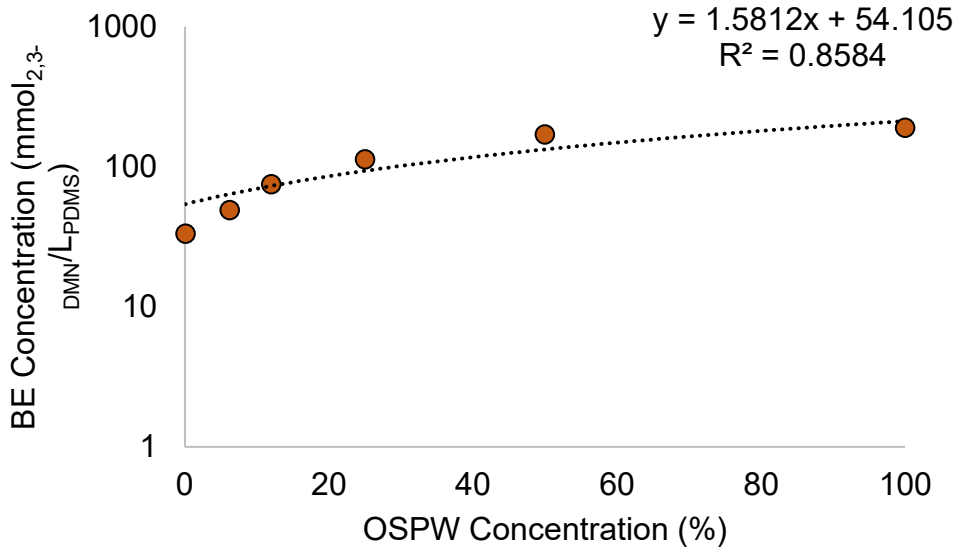

Figure 4. BE-SPME concentration ($mmol_{2,3\text{-}DMN}/L_{PDMS}$) by OSPW concentration (%) for *C. dubia* (n=3).

Figure 5 shows the relationship between OSPW concentration and acidified BE-SPME measurements with a log-transformed y-axis. An overall relationship was observed between the OSPW dilutions of 6.25%, 12.5%, 25%, and 100% (y = 1.9404x + 34.744, R^2 = 0.9054, **Figure 5**). BE measurements increased with OSPW dilution concentration. However, the BE concentrations begin to plateau between the 50 and 100% OSPW vol/vol treatment groups. This same pattern was also observed in the *C. dubia* toxicity test (**Figure 4**), which may be a due to the PDMS fiber reaching its

sorption capacity during the extraction. The variability of BE measurements within each treatment is overall consistent across all treatment, except for the 50% OSPW vol/vol treatment group. Higher variability was observed in this treatment group, which may be a result of inadequate mixing of treatment waters prior to taking the triplicate samples.

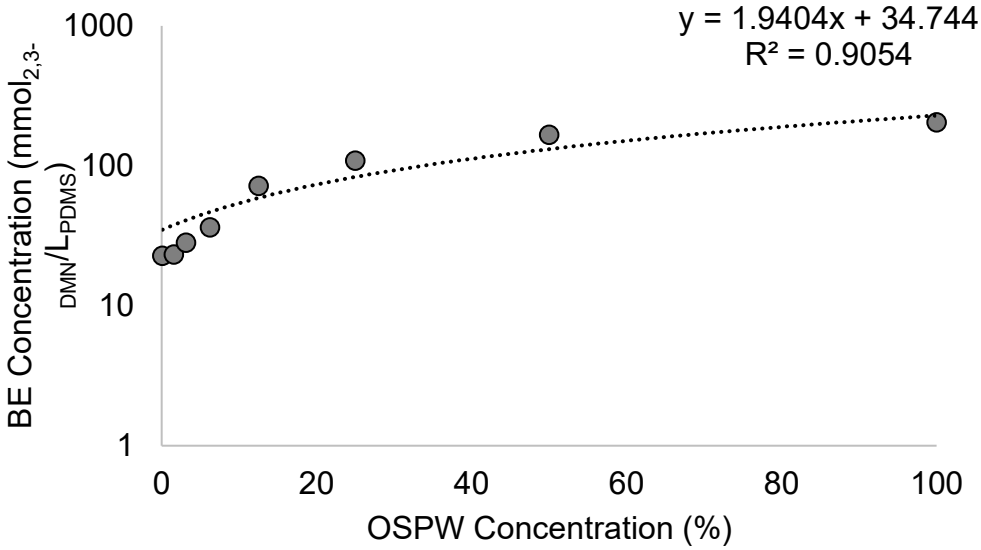

Figure 5. BE-SPME concentration ($mmol_{2,3\text{-}DMN}/L_{PDMS}$) by OSPW concentration (%) for walleye (n=3).

2.3.3. *C. dubia* Reproduction and Survival

Reproduction

Reproductive success of *C. dubia* was measured by the number of neonates produced by an individual organism after 3 broods over the 6-day exposure period. The IC_{25} for reproduction determined using TRAP was 80% vol/vol (63-102%). This is higher than the effect concentration (IC_{25}) provided by Nautilus (64% vol/vol; 53-79%), which is to be expected due to differences in statistical methodology (refer to Section 2.2.5). The response was similar between the laboratory control and the 1.56% to 50% vol/vol treatments. A statistically significant decrease in reproductive success was observed in the 100% treatment group ($P < 0.05$), with the mean number of neonates produced being 8.4 neonates compared to 18.6 neonates in the laboratory control (**Figure 6**, top).

Chronic toxicity of OSPW to *C. dubia* reproduction has been observed in previous studies. A study by Zubot et al. (2012) assessed the success of petroleum-coke

absorption at OSPW toxicity removal and observed chronic toxicity of OSPW (both treated and untreated) to *C. dubia*, which the researchers attributed to potential salt intolerance. Additionally, Hendrikse et al. (2018) observed a significant effect of untreated (wetland inflow) OSPW to *C. dubia* reproduction compared to the control ($P < 0.05$). However, the severity of this effect was determined to have unlikely ecological significance. The results of this study, in addition to previous work, suggest that *C. dubia* is a species tolerant to diluted OSPW concentrations, with the only significant effect occurring with untreated OSPW. This provides evidence in support of the dilution and/or treatment of OSPW to reduce toxicity to aquatic organisms.

Survival

The LC50 for *C. dubia* survival was >100% vol/vol, as no mortality was observed in any treatment groups (**Figure 6**, bottom). Previous studies that measured survival of *C. dubia* exposed to OSPW (Muskeg River Mine, Shell Canada Ltd.) found that when exposed to 100% OSPW, an effect to survival was observed (lowest observed effect concentration (LOEC) = 25% vol/vol OSPW) but the LC_{50} was 75% vol/vol, indicating an overall tolerance (McQueen et al., 2017). Additionally, *C. dubia* exposed to EPL water from Base Mine Lake had 100% survival after 8 days of exposure (White and Liber 2020). It is important to note that the composition of OSPW varies between sources, and toxicity testing results with different OSPW waters must be compared with caution.

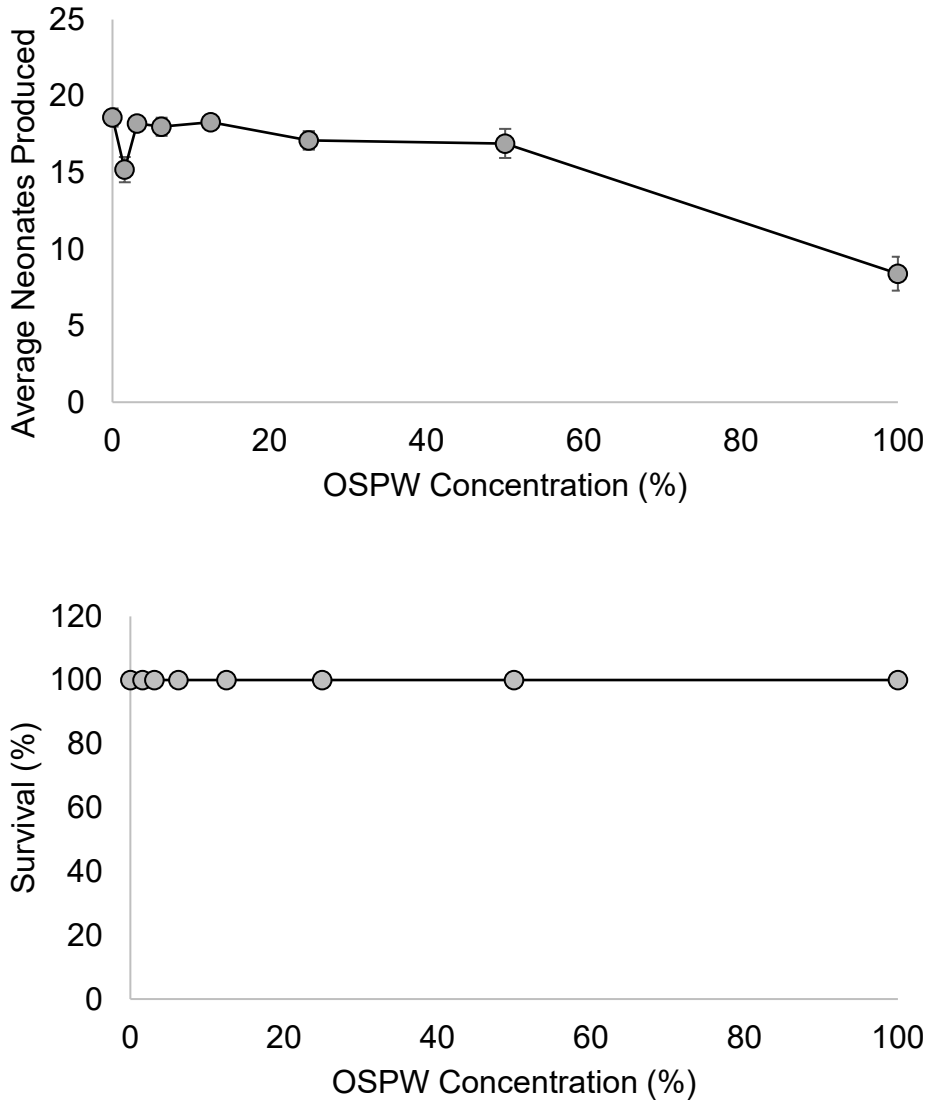

Figure 6. Measured endpoints for *C. dubia* 6-day reproduction and survival toxicity test by OSPW concentration. Top = average neonates produced; bottom = survival. Error bars in top figure represent standard error (n=10).

2.3.4. Walleye ELS Toxicity Test

Hatching

Hatching rate (%) and normality of hatched larvae (%) by treatment are shown in **Figure 7** (top left; top right). Hatching rate was determined as the proportion of hatched embryos to total embryos for each replicate, with the mean across the replicates shown above. Normality of hatched larvae was determined as the proportion of normally

hatched larvae to total embryos for each replicate, with the mean across the replicates shown above. Endpoint results are shown as raw results and as control-normalized results. Results were normalized to the control by dividing the treatment effect by the control effect and multiplying by 100.

Hatch rate was similar in the lab control and the 6.25% to 50% vol/vol OSPW dilutions. The hatch rate decreased in the 100% vol/vol OSPW dilution to an average of 26% across replicates (Figure 5, top right). CETIS determined an IC_{25} value of 64% (54-76%), which was lower than the TRAP-predicted IC_{25} of 91% vol/vol OSPW for hatch rate. There were identified errors associated with the TRAP IC_{25} derivation (e.g., large standard error, steepness at a maximum, large partial effects) that are likely due to the lack of spread of the data, with only the 100% vol/vol OSPW treatment showing a strong effect. This effect of OSPW to hatch rate is consistent with studies using other fish species, with a higher rate of premature hatching observed in fathead minnow exposed to untreated (i.e., 100%) OSPW (He et al., 2012). Additionally, this finding is consistent with what was observed in a study by Piggott (2022) in which rainbow trout (*O. mykiss*) were exposed to similar OSPW treatments and had little observed impact to hatch rate and total embryos hatched in all treatments except 100% OSPW. Hatching success is a strong predictor of ELS mortality in fish (Marentette et al., 2015).

Normality of hatched larvae was consistent between the laboratory control and the 6.25% vol/vol to 50% vol/vol OSPW dilutions. The percentage of normal neonates hatched significantly decreases to 26% in the 100% vol/vol OSPW dilution (Figure 5, top right). CETIS determined an IC_{25} value of 68% (56%-NR[2]) for post-hatch normality, which was lower than the TRAP-predicted IC_{25} value of 89% vol/vol OSPW. The same uncertainty associated with the hatch rate endpoint applies to normality of hatched larvae. A significant difference was observed between treatment groups ($P < 0.001$). A post-hoc Tukey's test determined that all treatment groups differed significantly from the 100% OSPW treatment group.

[2] Upper confidence limit was not reported.

Survival

Throughout the walleye exposure, percent survival was low in the control treatment groups, with an average of 48% survival in the controls by day 21 (**Figure 7**), bottom right). Control survival did not meet the test acceptability criteria (≥70%), therefore results should be taken with caution. A significant difference was observed in the 100% OSPW dilution compared to the control and all other treatment groups ($P < 0.001$), with no survival occurring by day 21. CETIS determined an EC_{50} value for 21-day survival of 71% (50-100%), which was similar to the TRAP-predicted EC_{50} of 78% vol/vol OSPW. A similar pattern was observed for post-hatch survival, with no survival observed for organisms in all replicates in the 100% treatment group (**Figure 7**, bottom left). CETIS and TRAP predicted the same IC_{50} values of 71 and 78% vol/vol OSPW, indicating no difference in effect between post-hatch survival and 21-day survival. Errors occurred during the TRAP runs (e.g., large standard error, steepness at a maximum, large partial effects) that add uncertainty to the resulting effect concentrations.

Many factors may contribute to the low survival in the laboratory control, which was similarly observed in an NAFC exposure with walleye by Marentette et al. (2015). Development of walleye embryos post-hatch is dependent upon the surrounding temperature. In this study, the maximum temperature reached was 15°C. It has been shown that warmer temperatures (i.e., 20°C) are necessary for continued embryonic development and overall survival (Koenst and Smith, 1976). Overall, walleye is considered to be a difficult laboratory organism, resulting in a wider range of accepted control survival compared to a more routine species such as fathead minnow (ASTM, 2012).

Growth

Growth (as mean dry weight) was not able to be assessed in the 100% vol/vol OSPW treatment because no survival was observed by day 21. No effect on growth was observed in all other treatment groups. The IC_{25} for growth (dry weight) was >50% vol/vol OSPW. Previous work by Lyons et al. (2018) similarly found that embryonic growth of zebrafish (*D. rerio*) was not negatively impacted by exposure to untreated OSPW. Due to low control survival, growth results should be taken with caution.

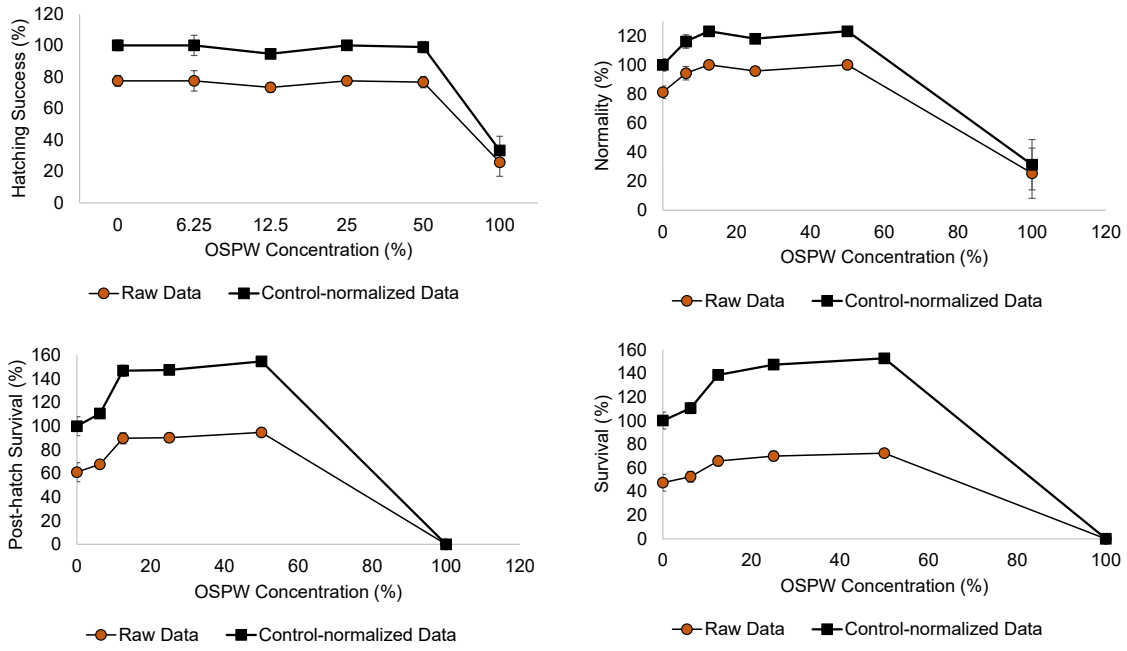

Figure 7. Measured endpoints (raw data and control-normalized) for walleye 28-day toxicity test by OSPW concentration (%). Top left = hatching success; top right = normality of hatched; bottom left = post-hatch survival; bottom right = overall test survival. Error bars represent standard error (n=4).

2.3.5. Predicting OSPW Toxicity using BE-SPME

C. dubia

The measured toxic response for *C. dubia* reproduction was compared to the modelled effect created using a Weibull function (**Figure 8**). BE concentration was used as the exposure metric. Overall, the measured values (toxic effect to reproduction; grey circles) from toxicity testing followed a similar pattern to the model-predicted effect (**Figure *8***), with some variability observed.

The IC_{25} effect for *C. dubia* reproduction derived from the Weibull function equation in BE terms was 185 $mmol_{2,3\text{-}DMN}/L_{PDMS}$, which corresponds to an OSPW concentration of 76% vol/vol (**Figure 4**). This value is higher than the IC_{25} determined by CETIS (64%) and lower than the IC_{25} produced by TRAP (80% vol/vol). Strong agreement between modelled toxic effects using BE-SPME and measured values for *C. dubia* reproduction was observed. These results align with the findings of Redman et al.

(2018) and provide further evidence for the reasonable use of BE as a surrogate measure of toxicity of organic mixtures to aquatic organisms.

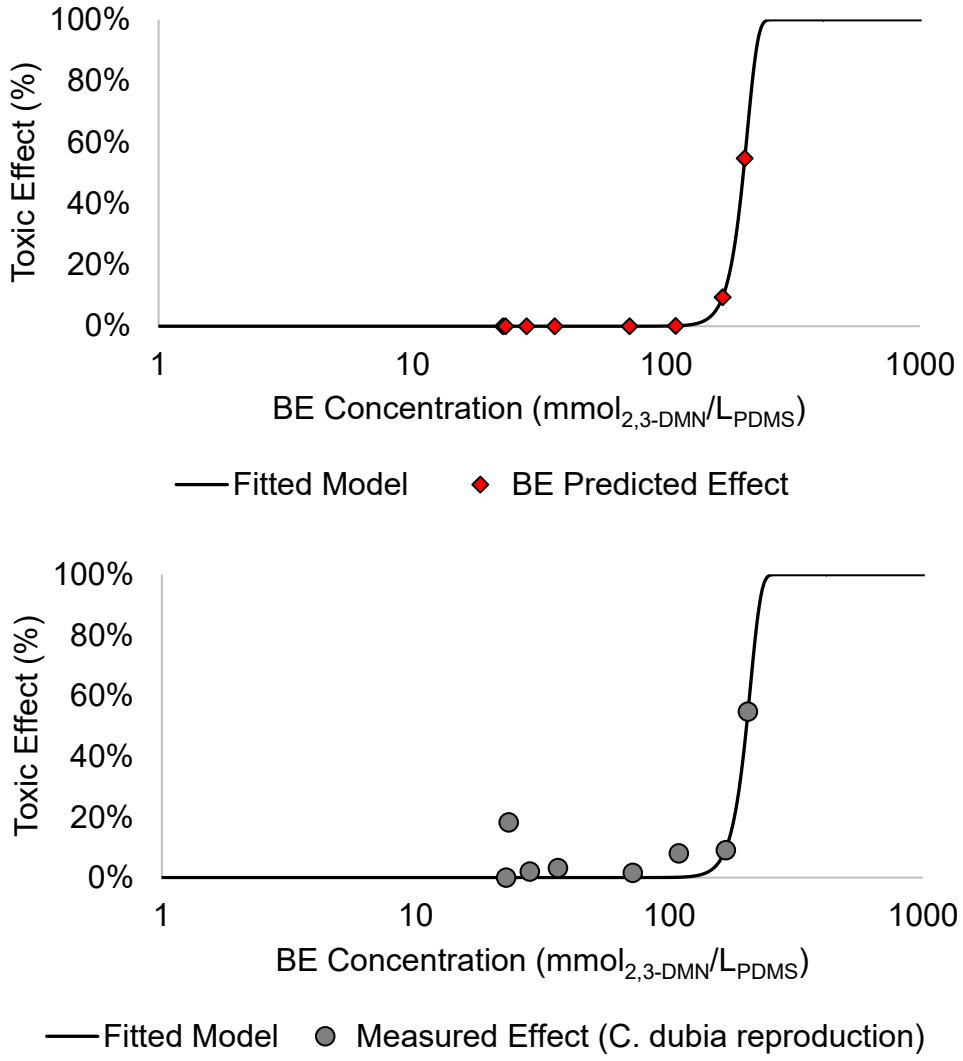

Figure 8. Top: Modelled toxic effect using the Weibull function for *C. dubia* reproduction. Red diamonds show BE-SPME concentrations (this study) of total acid extractable organics in OSPW and their corresponding toxic effect.
Bottom: Measured toxic effect to *C. dubia* reproduction after 6-day exposure as a function of BE concentration compared to the fitted model. Grey dots show measured toxicity data from this study.

Walleye

The measured toxic response for walleye hatch rate was compared to the modelled effect created using a Weibull function of the relationship between BE concentration and toxic effect (**Figure *9***). BE concentration ($mmol_{2,3\text{-}DMN}/L_{PDMS}$) was used

as the exposure metric. The measured values from the 28-day toxicity test followed a pattern that was overall similar to the modelled effect produced by the Weibull function using the BE-SPME concentrations measured in the treatment waters used in toxicity testing. This provides evidence for the efficacy of BE-SPME as a predictive tool to observe patterns of toxic effect to aquatic organisms for monitoring purposes.

The IC_{25} for hatch rate in BE terms was 95 $mmol_{2,3\text{-}DMN}/L_{PDMS}$, which corresponds to an OSPW concentration of 25% OSPW vol/vol (**Figure *5***). The modelled effect predicted a much lower IC_{25} compared to the value determined by TRAP (91% OSPW vol/vol) and by CETIS (84% OSPW vol/vol). The hatch rate IC_{50} in BE terms was 184 $mmol_{2,3\text{-}DMN}/L_{PDMS}$, which corresponds to an OSPW concentration of 82%. This value is very similar to the IC_{50} produced by CETIS of 84% (78-90%). The Weibull function was unable to predict the toxic effect for 21-day survival that was observed in the 100% OSPW treatment. This is likely due to the fact that lower OSPW concentrations (i.e., 50% OSPW) had similar BE concentrations to the 100% treatment but no toxic effect was observed.

The application of BE-SPME for predicting fish ELS effects from organic compounds is a relatively new use of this passive sampling method. Therefore, limited data are available to compare measures of toxicity using BE concentration as the exposure variable. In the case of survival, using BE measurements to predict toxicity was overall accurate when compared to measured data. However, the same was not the case for hatch rate, in which BE measurements overestimated the IC_{25}. The application of this method needs to be validated with additional testing with walleye and other species to get a more thorough understanding of its potential for predicting toxicity.

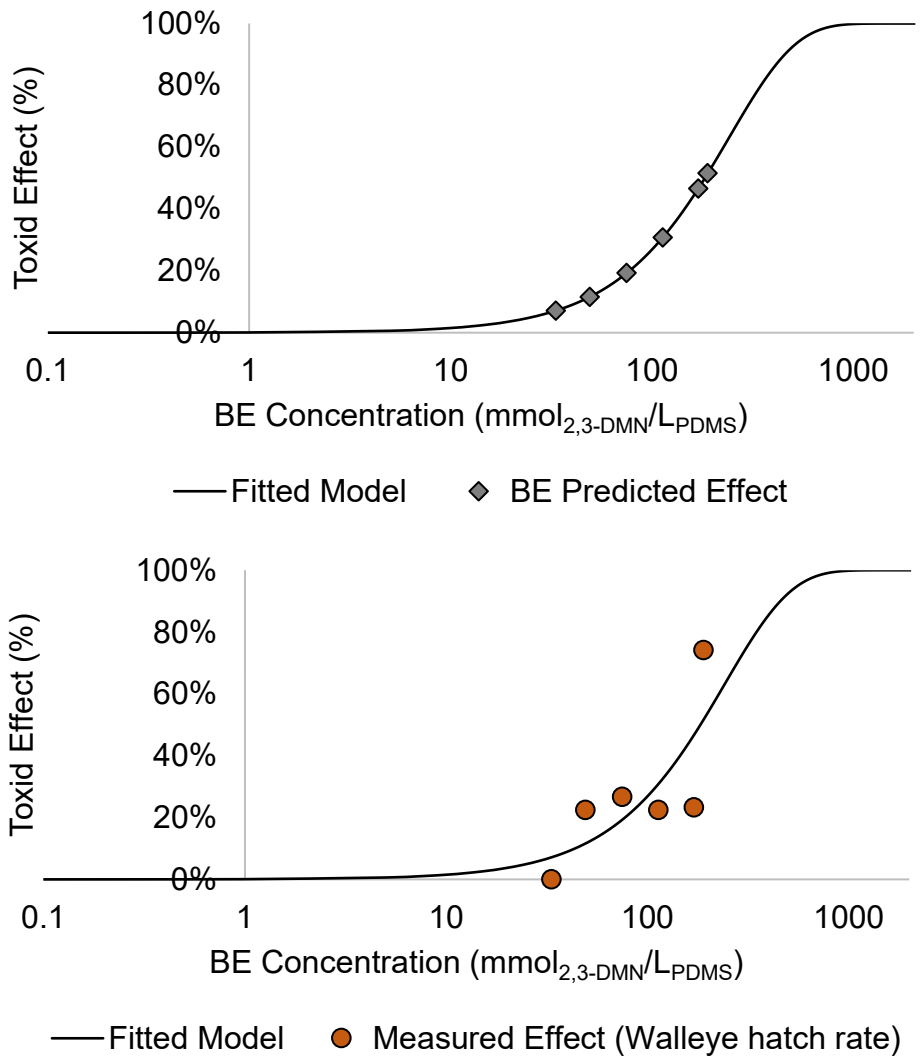

Figure 9. Top: Modelled toxic effect using the Weibull function for walleye hatch rate. Grey diamonds show BE-SPME concentrations (this study) of total acid extractable organics in OSPW and their corresponding toxic effect.
Bottom: Measured toxic effect to walleye hatch rate after 28-day exposure as a function of BE concentration compared to the fitted model. Red dots show measured toxicity data for walleye hatch rate from this study.

2.4. Key Findings

The key findings of this study are as follows:

- OSPW was chronically toxic to *C. dubia* reproduction. An IC_{25} was observed at an OSPW concentration of 64%

- Exposure to OSPW had no effect on *C. dubia* survival (LC_{50} > 100% vol/vol).
- A 74% effect to walleye hatch rate and 100% effect to post-hatch survival in the 100% OSPW treatment was observed.
- NAFCs in OSPW are the likely contributors to the observed toxicity but due to the complexity of the OSPW mixture, we are unable to definitively state that they are responsible for toxic effects in this study.
- Strong agreement was observed between modelled toxic effects produced with a Weibull function using the BE-SPME concentrations from treatment waters and the measured toxic effects for *C. dubia* reproduction. This provides evidence for the support of BE-SPME as a predictor of toxicity for sublethal effects to *C. dubia*.
- The BE-SPME extraction in the walleye 28-day test determined the toxic threshold of OSPW for walleye survival, which aligned with measured toxic effects. Greater variability was observed for the reproductive endpoints (e.g., hatch rate).
- The use of walleye in toxicity testing presents challenges (e.g., lower control survival) that require additional research and development if walleye is to be used routinely for toxicity testing.

Chapter 3. Application of Biomimetic Extraction to Measure Changes in Toxicity of OSPW to *Ceriodaphnia dubia* After Wetland Treatment

3.1. Summary

The application of CTWs has been proposed as a viable OSPW treatment solution (Ajaero et al., 2018; Cancelli and Gobas 2022; Hendrikse et al., 2018; McQueen et al., 2017; Simar et al., 2021). Treatment wetlands provide natural remediation processes, including sorption and biotransformation, that aid in the removal of contaminants (Rodgers and Castle, 2008). Wetland vegetation and microbial communities are responsible for the majority of contaminant degradation occurring within the system (Simair et al., 2021). The application of treatment wetlands for treatment of OSPW in the AOSR is a relatively new and pilot-scale concept but has been preliminarily shown to be effective.

This study assessed changes in chronic toxicity after wetland treatment in Imperial Oil's KTW located in northern Alberta. Toxicity reduction was assessed using traditional animal toxicity testing with the invertebrate *Ceriodaphnia dubia* in a chronic survival and reproductive toxicity test. This toxicity testing was paired with passive sampling using BE-SPME to assess the ability of the passive sampler at predicting the toxicity of the organism to OSPW.

In the Summer of 2022, OSPW from Imperial Oil's Kearl oil sands operation was pumped through the KTW for ~15 days. This water was collected and used for chronic toxicity testing to *C. dubia* to measure changes in toxicity throughout the course of the wetland flow-through process. BE measurements on the same wetland-treated water were collected to assess changes in the acid-extractable organic (AEO) fraction as a result of remediation processes occurring within the wetland. Toxicity removal to *C. dubia* survival was observed in addition to a partial removal of toxicity to reproduction. A similar toxicity reduction was predicted through BE measurements, which validates the tool as a predictor of toxic effects of OSPW to aquatic organisms. This study contributes to the growing body of research on the feasibility of the application of treatment wetlands to treat OSPW and more specifically the potential for toxicity reduction.

3.2. Materials and Methods

3.2.1. OSPW Source

Kearl Treament Wetland

The KTW is a free water surface-flow constructed wetland located at Imperial Oil's Kearl Lake oil sands operation, approximately 70 km from Fort McMurray, Alberta. The KTW is currently in the pilot stage of operation and was built to assess OSPW treatment. Due to weather constraints and extreme low temperatures during the Winter, the KTW operates only during the Summer (i.e., May to September). The wetland is a closed-circuit system comprised of six individual cells, including three deep pools (1.7m in depth) and three shallow areas (**Figure 10**). The deep pools have submerged vegetation while the shallow areas have dense vegetation dominated by species including the common cattail (*Typha latifolia*) and the water sedge (*Carex aquatilis*). OSPW is pumped through the KTW at a rate of 5 L/s with a hydraulic retention time of approximately 14 days. The total volume of the KTW is approximately 6,000 m^3 and the surface area of all wetland cells was estimated to be 7,894 m^2 (Cancelli and Gobas, 2020).

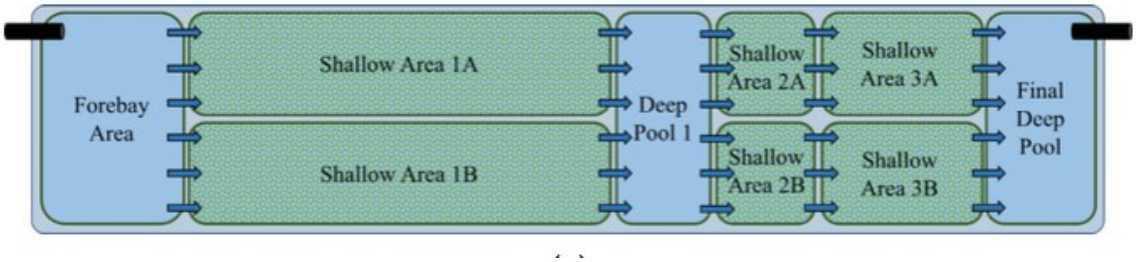

Figure 10. Schematic of the Kearl Treatment Wetland. Water flows through the wetland from left to right. Figure from Cancelli and Gobas, 2020.

In 2022, OSPW was pumped through the KTW between June and July for 20 days. OSPW from WETA was sourced and pumped to the wetland in a single-pump event. Compared to previous years, a less-diluted, higher concentration OSPW was pumped through the wetland in 2022. Aqueous samples were collected from the first

forebay by on-site contractors on days 0, 7, and 15. Water was stored in amber glass bottles, shipped to Simon Fraser University, and stored in the dark at 4˚C until use.

3.2.2. Toxicity Testing

Study Species

The cladoceran *C. dubia* was the representative invertebrate species used in this study. Refer to Section 2.2.3 for details on the species life history.

C. dubia Survival and Reproduction Exposure

C. dubia neonates (<24 hours old) were exposed to OSPW sampled on days 0, 7, and 15, at the Nautilus Laboratory in Burnaby, BC in October 2022. The testing protocol used was the Biological Test Method test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*, EPS 1/RM/21 (ECCC, 2007). The toxicity test was run as a pass/fail test with each day of sampling being its own treatment as opposed to having > 5 dilutions to determine an LC_{50} value. A summary of the protocol and test conditions is provided in Section 2.2.3 in **Table *1***. The measured endpoints included survival and reproduction, as measured through the total number of neonates produced by each individual female after it has reached 3 broods.

3.2.3. Water Quality

Water quality parameters were measured daily including pH, hardness, temperature, and conductivity. Wetland water from day 0 and day 15 of the flow-through process was subsampled and analyzed by ALS Environmental (Burnaby, BC) for physical parameters, anions and nutrients, and metals (total and dissolved). PAHs were not measured as they were not shown to exceed water quality guidelines in previous assessments (Piggott, 2022). Results of the water quality analysis were compared against federal and provincial water quality guidelines for aquatic life. This was done to assess whether any observed toxicity to *C. dubia* was a result of the acid-extractable organic fraction of OSPW or because of another contaminant present in the OSPW mixture. The GOA WQGs were prioritized and where a guideline was not available, CCME WQGs were used.

NAs in OSPW collected on days 0 and 15 of the wetland flow-through process were quantified using the Orbitrap-MS method, which has high resolution and accuracy for polar compounds (Ajaero et al. 2018). The analysis was performed by Innotech Alberta (Calgary, Alberta).

3.2.4. Measuring Toxicity Reduction using BE-SPME

At the beginning of the *C. dubia* toxicity tests, ~125 mL of water from each treatment (days 0, 7, and 15), including the laboratory control[3], was subsampled in triplicate and stored in an amber glass bottle at 4°C prior to analysis. BE-SPME was conducted on all water samples from both toxicity tests at the Gobas Laboratory at SFU following the protocol outlined in Redman et al. (2018). Refer to Section 2.2.4 for details of the BE-SPME protocol. Toxicity reduction (E_{tox}) of bulk OSPW was then calculated from the BE measurements taken on days 0 and 15 (complete hydraulic retention time). This metric is useful in determining the overall efficacy of the KTW.

3.2.5. Statistical Analysis

The concentration-response statistical analysis for the *C. dubia* test was performed using the software CETIS Version 2.1.1 (Tidepool Scientific Software, 2021). *The C. dubia* test was run as a pass-fail and therefore, effect concentrations were not determined. An ANOVA test was run in JMP (Version 16) to determine significant differences in toxic effect between treatment groups (days 0, 7, and 15). Post-hoc tests were used as required.

Toxic effects to *C. dubia* were estimated using measured BE concentrations from the sub-sampled OSPW treatment waters sampled during the wetland flow-through process and the measured dose-response data in this study. A Weibull function was used to estimate toxic effects from the measured BE concentrations (refer to Section 2.2.5).

[3] Laboratory control for the *C. dubia* toxicity test was 20% Perrier® mineral water and 80% deionized water + 5 µg/L Se and 2 µg/L vitamin B12.

3.3. Results and Discussion

3.3.1. Water Quality Analysis

Laboratory Conditions

. **Table 6** and **Figure 11** show the water quality of the water used to replenish treatments during daily water changes throughout the exposure. Overall, water quality parameters varied compared to the laboratory control, with the exception of temperature. Temperature was maintained within 25 ± 1°C, as stipulated by the protocol. Dissolved oxygen (DO) slightly increased between day 0 and 15 but was significantly lower in all treatment groups compared to the control ($P < 0.05$). The average dissolved oxygen concentration across treatment groups ranged from 6.6 to 6.9 mg/L. Both conductivity and pH slightly increased between days 0 and 15 ($P < 0.05$ for conductivity; $P > 0.05$ for pH). Conductivity in all treatment groups was significantly higher than that in the laboratory control ($P < 0.001$) due to the high salinity of OSPW (Zubot et al., 2012).

Table 6. Average water quality for each OSPW dilution measured during the 6-day *C. dubia* toxicity test. Values are mean ± standard deviation.

Parameter	Units	Laboratory Control	Day 0	Day 7	Day 15
pH	-	7.6 ± 0.24	8.1 ± 0.28	8.2 ± 0.28	8.3 ± 0.27
Temperature	˚C	25 ± 1	25 ± 1	25 ± 1	25 ± 1
Conductivity	µS/cm	209 ± 5.2	1,372 ± 28	1,462 ± 26	1,509 ± 22
Dissolved oxygen	mg/L	8.0 ± 0.05	6.6 ± 1.2	6.7 ± 0.97	6.9 ± 0.88

Notes: OSPW = oil sands process-affected water; % = percent; - = no units; ˚C = degrees Celsius; µS/cm = micro Siemen per centimetre; DO = dissolved oxygen; mg/L = milligrams per litre.

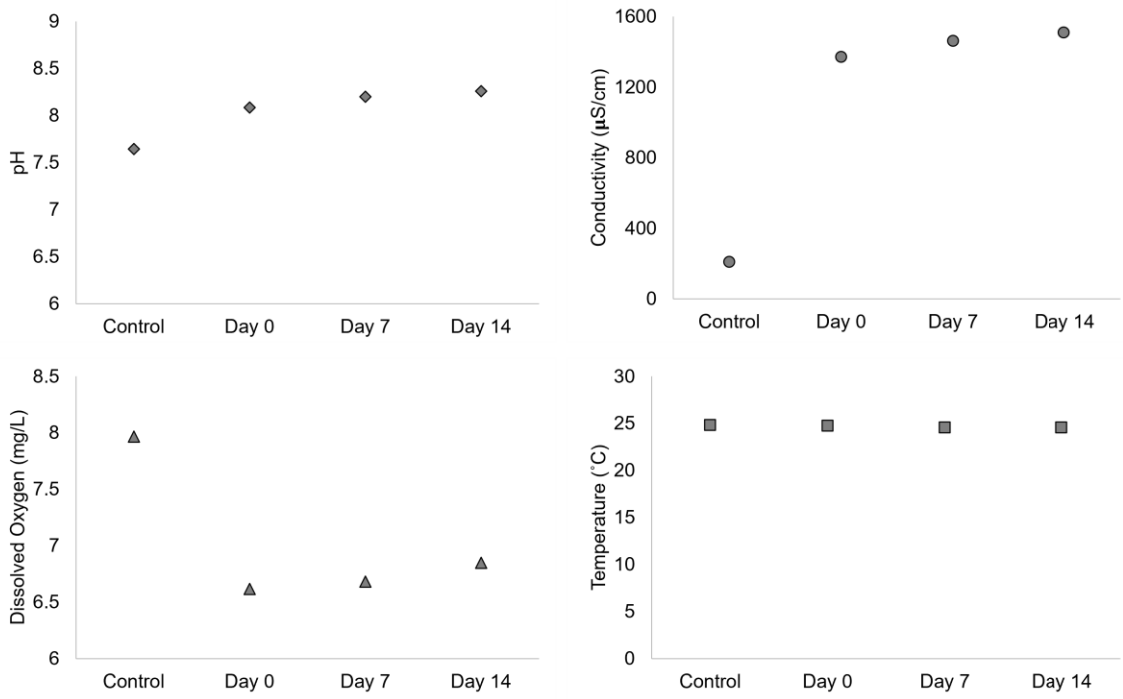

Figure 11. Average water quality (pH, conductivity, dissolved oxygen, and temperature) for each treatment group measured throughout the 6-day C*. dubia* toxicity test.

Water Quality Screening

The results of water quality screening against WQGs is shown in **Table *7***. The parameters that exceeded WQGs included alkalinity, fluoride, sulphate, aluminum, and molybdenum. Refer to Appendix C for the full water quality screening results.

Alkalinity ranged from 113 mg/L in the control water to 293 mg/L in the day 15 OSPW, exceeding the chronic WQG of 20 mg/L. Changes in alkalinity have been shown not to cause toxic effects, or influence toxicity of other parameters, to *C. dubia* reproduction (Lasier et al., 2006). Therefore, this exceedance is unlikely to contribute to toxic effects observed in this study. Fluoride was below the guideline for the control water but ranged from 2.95 mg/L to 3.29 mg/L in day 0 and 15 OSPW, exceeding the chronic WQG of 0.12 mg/L. Toxicity of *C. dubia* to fluoride is described in Section 2.3.1. The sulphate concentration of 432 mg/L marginally exceeded the chronic WQG (309 to 429 mg/L) in the day 15 OSPW treatment. Sulphate has not been shown to directly cause toxicity to *C. dubia*. However, it has been shown to mediate toxicity of other substances such as vanadium to *Daphnia pulex* (Meina et al., 2019). Additionally,

elevated sulphate concentrations can contribute towards eutrophication, which depletes oxygen in a water and sediment and can have negative impacts to surrounding biota (Harper 1992). However, the sulphate concentrations observed on the day 15 OSPW treatment are unlikely to contribute toward the overall toxicity of OSPW as DO concentrations in treatment waters were maintained throughout testing.

The total aluminum concentration of 130 µg/L exceeded the chronic guideline of 100 µg/L in the day 0 OSPW treatment. The toxic effects of aluminum to *C. dubia* are described in Section 2.3.1. There is the potential for this measured concentration of aluminum in OSPW to exert chronic effects, although the guideline exceedance is marginal and would likely not have a significant effect. The molybdenum concentration of 77 µg/L exceeded the chronic guideline of 73 µg/L in the day 0 OSPW treatment. The observed concentration was marginally above the chronic WQG. Chronic toxicity of molybdenum to *C. dubia* has a measured IC_{25} of 47,500 µg/L (Naddy et al., 1995). The concentration of molybdenum measured in this study is below the IC_{25} and is therefore unlikely to contribute to toxicity of OSPW to *C. dubia*.

Table 7. Water quality screening of OSPW collected from the KTW on days 0 and 15 for conventional parameters, major ions, and total metals.

Parameter	Unit	Guidelines for the protection of Aquatic Life				Laboratory Control	Day 0	Day 15
		Acute (GOA)	Chronic (GOA)	Acute (CCME)	Chronic (CCME)			
Conventional Parameters								
pH	-	-	6.5 - 9.0	-	6.5 - 9.0	8.3	8.4	8.4
Hardness, As Caco3	mg/L	-	-	-	-	170	232	336
Total Alkalinity, As Caco3	mg/L	-	20	-	-	113	235	293
Total Suspended Solids	mg/L	-	-	-	-	<3.0	9.1	<3.0
Total Organic Carbon	mg/L	-	-	-	-	0.67	41	40
Dissolved Organic Carbon	mg/L	-	-	-	-	1.0	39	42
Turbidity	NTU	-	-	-	-	0.27	5.4	0.96
Conductivity	uS/cm	-	-	-	-	539	1,360	1,480
Major Ions								
Fluoride	mg/L	-	-	-	0.12	0.022	**3.3**$^{(C)}$	**3.0**$^{(C)}$
Sulphate	mg/L	-	309 - 429	-	-	161	384	**432**$^{(C)}$
Total Metals								
Aluminum	mg/L	-	-	-	100	27	**130**$^{(C)}$	5.9
Molybdenum	mg/L	-	73	-	73	0.24	**77**$^{(C)}$	54

Notes: KTW = Kearl Treatment Wetland; GOA = Government of Alberta; CCME = Canadian Council of Ministers of the Environment; OSPW = oil sands process-affected water; CaCO3 = calcium carbonate; mg/L = milligrams per litre; NTU = nephelometric turbidity units; µS/cm = microsiemens per centimetre.
(C) = exceeds chronic water quality guideline.
No observed exceedances of acute water quality guidelines.
Bolded and blue shaded value indicates a guideline exceedance

Figure 12 shows the concentration of total NAs measured in OSPW on days 0 and 15 in 2021 and 2022. During both years, a significant reduction in total NA concentration was observed between days 0 and 15. In 2021, a 53% reduction was observed between day 0 (19.6 mg/L) and day 15 (9.1 mg/L). A less dramatic reduction was observed in 2022, which had a 22% reduction between day 0 (12.2 mg/L) and day 15 (9.5 mg/L). However, OSPW collected on day 15 during both years had very similar NA concentrations even though initial concentrations on day 0 differed. Previous work on treatment wetlands observed transformation of NAs with a greater relative abundance of oxygenated-NAs found in untreated OSPW compared to the untreated OSPW. This is due to the fact that the biodegradation of NAs is a result of oxidative processes (Ajaero et al., 2018). A pilot study by Hendrikse et al. (2018) saw a decrease in total NAFC concentrations from 43 mg/L in untreated OSPW to 10 mg/L after 16 days of wetland treatment, which is consistent with the results of this study. Overall, treatment wetlands have been shown to significantly decrease concentrations of NAs in OSPW.

The concentrations in OSPW after 15 days of wetland treatment reached levels between 9.1 and 9.5 mg/L during the two years that NAs were measured. Orbitrap mass spectrometry was used to quantify NAs during both years of testing. These concentrations are both under the reported IC_{25} for NAFCs of 10 mg/L reported by Redman et al. (2018). However, concentrations may still be high enough to have a toxic effect. A longer flow-through period of wetland treatment is likely needed to observe a more significant decrease in toxic effect. Refer to Section 2.3.1 for discussion on the toxicity of NAFCs to *C. dubia* from bulk OSPW.

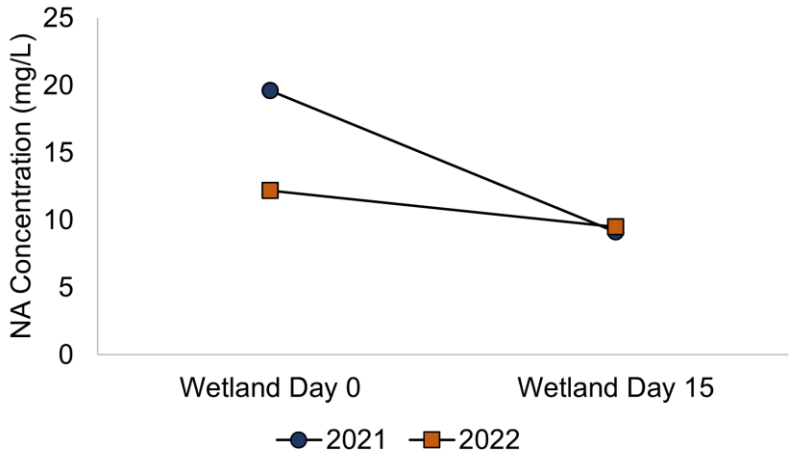

Figure 12. Concentrations of total naphthenic acids (mg/L) measured by Orbitrap-MS on days 0 and 15 of wetland flow-through process in 2021 (black dot) and 2022 (red dot).

3.3.2. Measuring Changes of OSPW Toxicity by BE

Figure 13 shows the relationship between wetland sampling day and acidified BE-SPME measurements. A linear relationship was observed (R^2 = 0.99). Incremental differences were observed between treatment days but overall, the BE concentrations remained consistent across sampling days. This result differs from work previously done on naphthenic acids in treatment wetlands. Research by Cancelli and Gobas (2022) measured a significant change in BE concentration after 15 days of wetland treatment, indicating a reduction in toxicity to aquatic organisms. This result was not observed in this study and therefore, it is important to identify the potential differences in methodology that may have contributed toward these differences in results. A major difference in methodology was the use of an autosampler for BE measurements in this study compared to the manual method applied in Cancelli and Gobas (2022). It has been shown during a round-robin study that the application of BE-SPME has high variability across laboratories, with the automated sampling method having greater reproducibility while the manual method had higher variability (Letinsky et al., 2022). This finding makes comparisons between studies that apply different methods difficult and introduces uncertainty to the method. Additional replication and a greater number of sampling days are needed to confirm the measured BE concentrations.

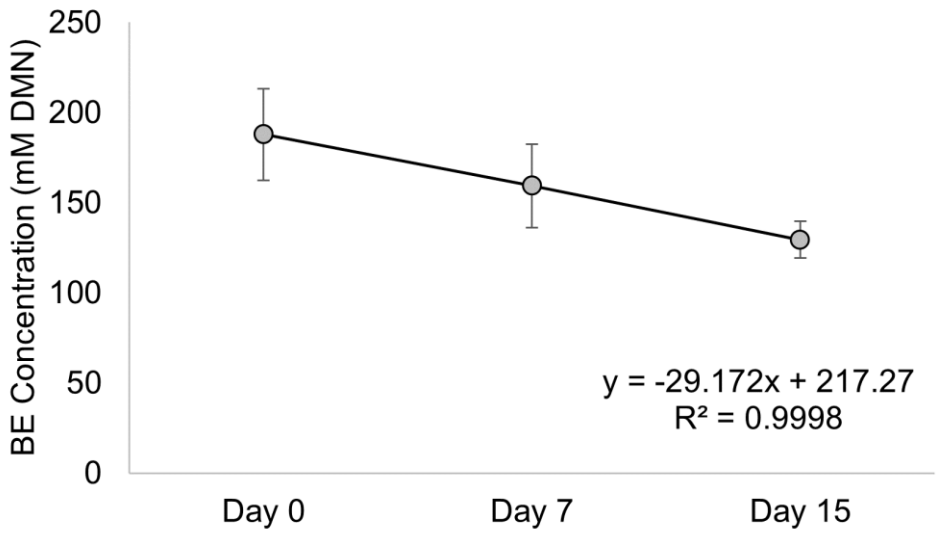

Figure 13. BE-SPME concentration ($mmol_{2,3\text{-}DMN}/L_{PDMS}$) by wetland sampling day with water collected in 2022. SE is shown for each sampling day (3 replicates per day). Error bars represent standard error (n=3).

3.3.3. *C. dubia* Survival and Reproduction

Reproductive success (i.e., number of neonates produced by an individual female) was significantly different between treatment groups ($p < 0.05$). **Figure 14** (left) shows a decrease in toxicity between days 0 and 15. All treatment groups (days 0, 7, and 15) had a mean neonate number that was significantly different from that in the laboratory control (17.9 ± 2.1 mean neonates produced; $P < 0.001$). The mean reproduction for day 15 (17.2 ± 1.9 mean neonates produced) was the closest to the laboratory control, indicating that toxicity of OSPW to *C. dubia* was substantially reduced after 15 days of wetland treatment. For the day 7 treatment, mean reproduction was greater than that on day 15 and the laboratory control (18.4 ± 1.8 mean neonates produced), showing that the toxic effect to reproduction was ameliorated by day 7 of wetland treatment. Day 0 had greatly reduced reproduction compared to the controls and the other two treatments (0.8 ± 1.0 mean neonates produced), indicating an effect prior to any wetland treatment.

Measured *C. dubia* survival for days 0, 7, and 15 of wetland treatment is shown in

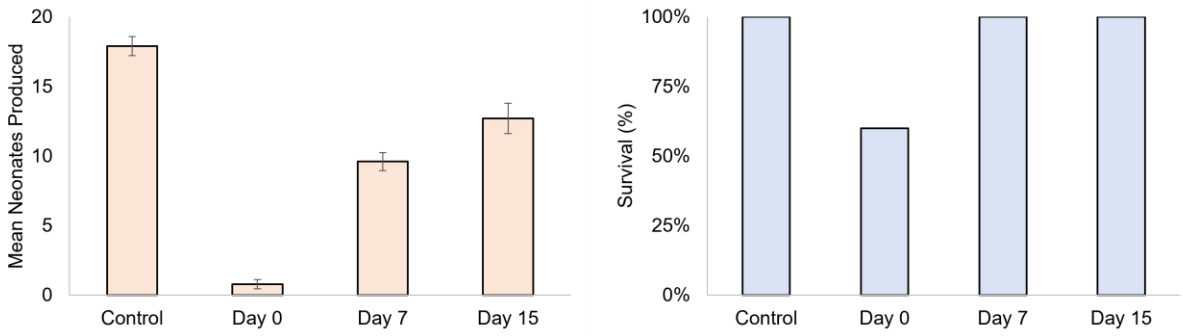

Figure *14* (right). During the 6-day exposure, mortality was observed for 3 individuals out of 9 in the day 0 treatment group, leading to overall survival of 60%. No other mortalities were observed in the other treatments. Both day 7 and day 15 treatment groups had 100% survival, indicating a complete reduction in toxic effect to *C. dubia* survival after 7 days of wetland treatment. This finding provides evidence for the efficacy of the treatment wetland at reducing OSPW toxicity to *C. dubia*.

The *C. dubia* survival and reproduction results observed in this study are consistent with the findings of Hendrikse et al. (2018), who measured changes in toxicity of OSPW to *C. dubia* after 16 days of wetland treatment and observed an elimination of toxicity after treatment. This study's results and the results of Hendrikse et al. (2018) study provide evidence for the efficacy of treatment wetlands at removing toxicity of OSPW to *C. dubia*.

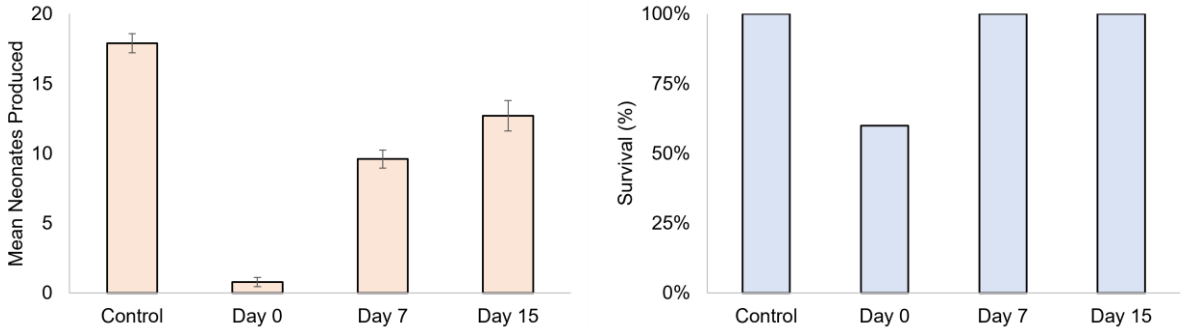

Figure 14. Measured endpoints for *C. dubia* 6-day reproduction and survival toxicity test by wetland sampling day. Left = average neonates produced; right = survival. Error bars on the left figure represent standard error (n=10).

3.3.4. Toxicity Reduction of OSPW after Wetland Treatment

The dose-response relationship of the reproductive effects to *C. dubia* after a 6-day exposure, as a function of the BE concentration of total acid-extractable organics in OSPW ($mmol_{2,3\text{-}DMN}/L_{PDMS}$), is shown in **Figure 15**. The modelled curve for *C. dubia* reproduction was derived from the dose-response data measured in Chapter 2 and the BE-SPME measurements from two years of wetland treatment were plotted along this curve. BE-SPME measurements of wetland-treated OSPW in 2019, 2021, and 2022 indicated that no toxicity is expected to *C. dubia* reproduction. However, toxicity was observed in the *C. dubia* toxicity test, with all treatment groups (days 0, 7, and 15) experiencing a significant effect to reproduction compared to the laboratory control. Additionally, survival in day 0 OSPW was 60%, which was not predicted by the BE measurements.

The ability of BE-SPME to predict toxic effects to *C. dubia* reproduction after wetland treatment was insufficient in this study when compared to measured toxicity data. This suggests that additional calibration of the BE-SPME method is needed prior to its application as a reliable monitoring tool. However, previous work by Cancelli and Gobas (2022) provided evidence in support of the application of BE-SPME to predict toxicity of wetland OSPW to *Danio rerio*. Additionally, data with rainbow trout have successfully applied BE-SPME as a predictive tool for toxicity (Piggott, 2022). These studies provide evidence for its usefulness and applicability with other species that was not otherwise observed with walleye. In future studies, greater replication (e.g., more sampling days) is needed. Continued research and validation of the BE-SPME tool would be beneficial to understand the inter-lab differences observed with the methodology, as described by Letinski et al. (2022), that may influence these results.

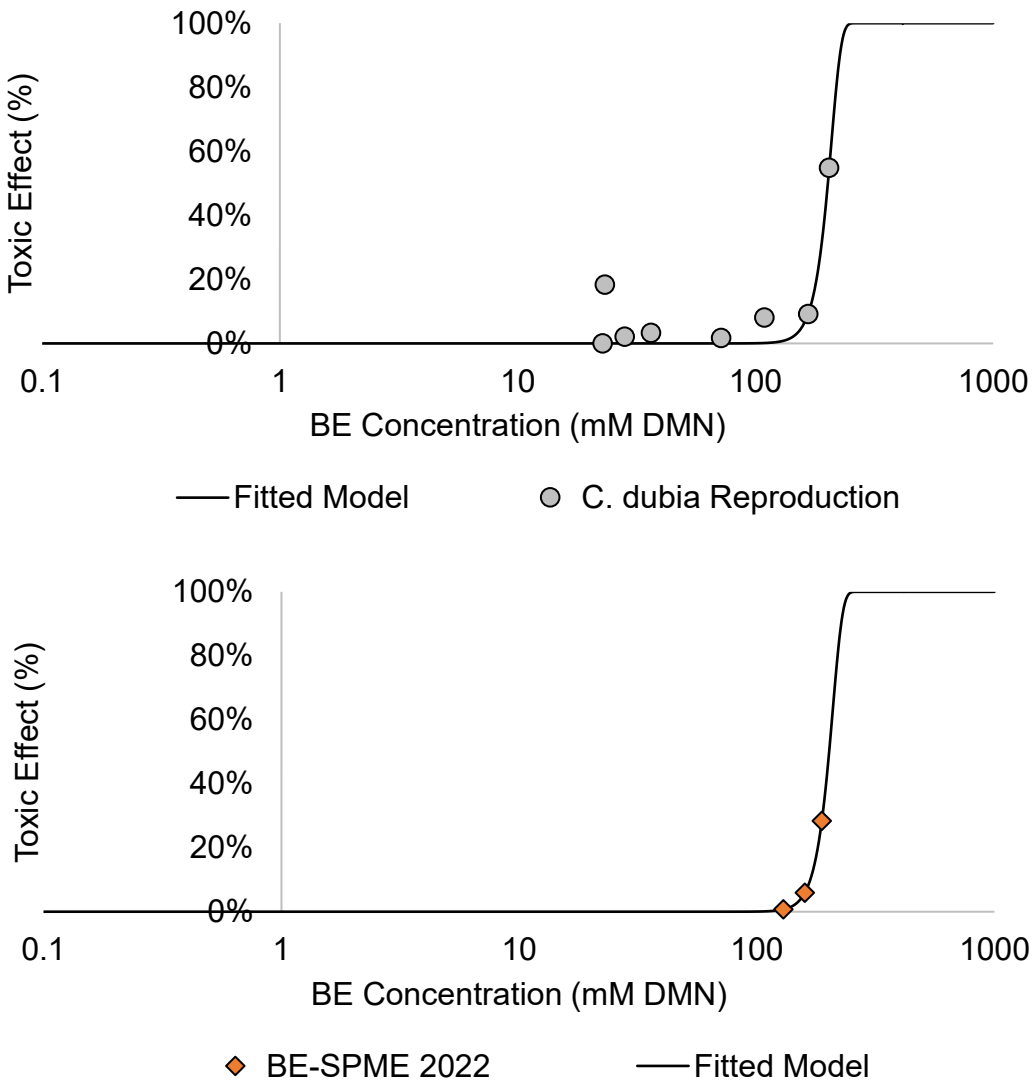

Figure 15. Top: Modelled toxic effect using the Weibull function for *C. dubia* reproduction (solid line) with measured toxicity data from this study shown in grey circles.
Bottom: Modelled toxic effect using the Weibull function for C. dubia reproduction (solid line) with BE-SPME concentrations from wetland treated waters (day 0, 7, and 15) collected in 2022 shown as red diamonds.

3.4. Key Findings

- Concentrations of NAs decreased by 22-53% after 15 days of wetland treatment to a total concentration that is unlikely to cause toxic effects to *C. dubia* survival and reproduction.

- Toxicity of OSPW to *C. dubia* survival and reproduction was ameliorated after 7 days of wetland treatment in the KTW.
- BE-SPME was unable to accurately predict the toxicity reduction to *C. dubia* reproduction as a result of wetland treatment.

Chapter 4. Conclusions and Future Direction

4.1. Overall Conclusions

The work described in this thesis contributed toward the goal of predicting toxicity of OSPW to aquatic organisms (*C. dubia* and walleye) through the application of BE-SPME to OSPW (refer to Research Objectives, Section 1.7). OSPW was chronically toxic to *C. dubia* reproduction, with an effect (55%) observed in the 100% OSPW dilution and a corresponding BE concentration of 204 $mmol_{2,3\text{-}DMN}/L_{PDMS}$. Chronic toxicity to walleye was observed for lethal and sublethal endpoints, with 100% mortality and an effect (74%) to hatch rate in the 100% OSPW dilution, with a corresponding BE concentration of 190 $mmol_{2,3\text{-}DMN}/L_{PDMS}$. BE-SPME was then further applied to a constructed wetland located in the AOSR to measure changes in toxicity after wetland treatment. A 31% change in BE concentration was observed between day 0 and 15 of wetland treatment, with respective corresponding BE concentrations of 130 and 188 $mmol_{2,3\text{-}DMN}/L_{PDMS}$. **Figure 16** shows the critical thresholds of endpoints for. *C. dubia* and walleye expressed as BE concentration ($mmol_{2,3\text{-}DMN}/L_{PDMS}$) and the BE concentration of OSPW after 15 days of wetland treatment (green arrow).

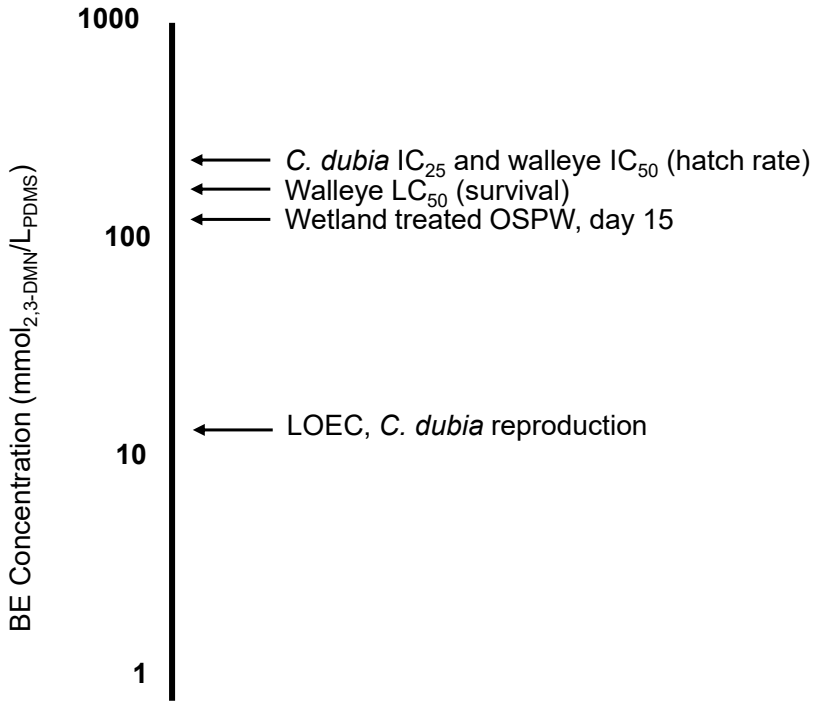

Figure 16. Critical thresholds of walleye survival (LC_{50}) and hatch rate (IC_{25}) and *C. dubia* reproduction (IC_{25}) and lowest observed effect concentration (LOEC) expressed in terms of BE concentration ($mmol_{2,3\text{-DMN}}/L_{PDMS}$). BE concentration of the wetland-treated OSPW collected on day 15 is also included.

Pairing BE-SPME measurements with traditional animal toxicity testing allows for the calibration of the passive sampling method and the potential for its application as a monitoring tool. This research provides evidence for its efficacy to predict chronic toxicity to *C. dubia* but additional calibration is needed for site-relevant fish species. Eventually, BE-SPME may provide alternatives to animal toxicity testing, which is a goal of the toxicological community (Norberg-King et al., 2018).

There is minimal available chronic toxicity data for OSPW, with a greater focus on acute toxicity. If the discharge of OSPW is considered, understanding the potential for chronic impacts to aquatic organisms is necessary when determining the overall risk of exposure. This study provides useful chronic toxicity data for both untreated and wetland- treated OSPW. Additionally, a culturally relevant species was used that is virtually absent from the literature, with the exception of work by Marentette et al (2015 and 2017). Collecting meaningful and useful chronic toxicity data with a culturally important species is a valuable contribution to oil sands toxicity research.

The application of BE-SPME as a surrogate measurement for toxicity to OSPW AEO fractions is a growing area of research that comes with limitations and uncertainty. A recent round-robin study by Letinsiki et al. (2022) showed significant inter-lab variability for BE-SPME, highlighting the sensitivity of the method and the potential for error as a result of differing methodologies. However, this thesis showed consistency between measured and modelled effects using BE-SPME in Chapter 2 and provides evidence for its usefulness as a monitoring tool.

4.2. Future Direction

The available research conducted within the oil sands region is increasing rapidly, with focus on toxicology, treatment technologies, and analytical methodologies to characterize the complexities of OSPW. There is a push from multiple stakeholders including government, industry, and First Nations to evaluate the impacts of oil sands mining on the natural environment in the AOSR and find solutions for OSPW treatment. This thesis contributes toward this growing field of research while also highlighting areas for future work.

Additional research into understanding the drivers behind the inter-laboratory variability in BE-SPME measurements of OSPW described in Letinski et al. (2022) is a key area of future work. While BE-SPME provides a potential alternative to animal toxicity testing, high variability has been observed depending on the method used (automated vs. manual). It was shown that the manual method was more variable as a result of differences in mixing speed and type during the equilibration stage of the BE-SPME extraction (Letinski et al., 2022). Standardizing this method is a necessary step if this tool is to be applied for monitoring purposes. Additionally, future work should rely on the automated method due to the lower variability and higher reproducibility compared to manual extraction.

Impacts to walleye and other regionally relevant species should continue to be considered when measuring effects of OSPW to aquatic organisms. This study has revealed the ability of walleye to withstand high concentrations of OSPW, indicating that with treatment, the effects to hatch rate and survival may be ameliorated. The inclusion of site-relevant species may present challenges in a laboratory setting, as observed in this study. However, it is still important to include such species to gain a more accurate

depiction of how the eventual treated OSPW discharge would affect the local ecosystems of the natural waters of the AOSR.

As policy around treatment and discharge of OSPW continues to evolve, several promising treatment technologies, including, but not limited to, treatment wetlands, ozonation, application of bioreactors, and petroleum-coke adsorption, are being explored. The potential success of these treatment solutions will influence the zero-discharge policy in place and may aid in reducing the overall volume of OSPW that has, and continues to be, produced. The future direction of this field will be to fill knowledge gaps on the complexity of OSPW mixtures and determine how to best proceed with treatment.

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Appendix A.

Toxicity testing and water quality laboratory reports

Description:

The accompanying PDF includes the laboratory reports for the *Ceriodaphnia dubia* and walleye toxicity testing from Nautilus Environmental and the water quality reports from ALS Environmental.

Filename:

Appendix A.pdf