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Fathead minnow (*Pimephales promelas*) reproduction is impaired in aged oil sands process-affected waters

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ABSTRACT

Large volumes of fluid tailings are generated during the extraction of bitumen from oil sands. As part of their reclamation plan, oil sands operators in Alberta propose to transfer these fluid tailings to end pit lakes and, over time, these are expected to develop lake habitats with productive capabilities comparable to natural lakes in the region. This study evaluates the potential impact of various oil sands process-affected waters (OSPW) on the reproduction of adult fathead minnow (*Pimephales promelas*) under laboratory conditions. Two separate assays with aged OPSW (>15 years) from the experimental ponds at Syncrude Canada Ltd. showed that water containing high concentrations of naphthenic acids (NAs; >25 mg/l) and elevated conductivity (>2000 μS/cm) completely inhibited spawning of fathead minnows and reduced male secondary sexual characteristics. Measurement of plasma sex steroid levels showed that male fathead minnows had lower concentrations of testosterone and 11-ketotestosterone whereas females had lower concentrations of 17β-estradiol. In a third assay, fathead minnows were first acclimated to the higher salinity conditions typical of OSPW for several weeks and then exposed to aged OSPW from Suncor Energy Inc. (NAs ∼40 mg/l and conductivity ∼2000 μS/cm). Spawning was significantly reduced in fathead minnows held in this effluent and male fathead minnows had lower concentrations of testosterone and 11-ketotestosterone. Collectively, these studies demonstrate that aged OSPW has the potential to negatively affect the reproductive physiology of fathead minnows and suggest that aquatic habitats with high NAs concentrations (>25 mg/l) and conductivities (>2000 μS/cm) would not be conducive for successful fish reproduction.

1. Introduction

Alberta's oil sands developments account for more than 40% of Canada's oil production (CAPP, 2009). Oil sands ore consists of a mixture of bitumen (heavy, biodegraded crude oil), sand, clay, and formation water. In surface mining operations, modifications of the Clark Water Extraction process are used to separate the bitumen from the oil sands. In this process, an aqueous digestion with hot water, usually with NaOH as a process aid, will yield recoveries of >90% (FTFC, 1995). This results in large volumes of fluid fine tailings that slowly de-water, producing a soft fines-rich suspension, known as mature fine tails (MFT). The resulting released water is reused in the oil sands extraction process (process water) and accounts for >80% of the water used in a surface oil sands operation. Large settling basins are required during the operational phase of the surface mine development as an essential part of current water management strategies, but eventually they must be reclaimed when no longer actively being used (ERCB, 2009). Current inventories of fluid tailings (process affected water and MFT) within the various settling basins exceed 10^9 m^3 of tailings and are a focus of concern (Del Rio et al., 2006).

As a result of the extraction and water recycling activities in the surface mine operations, the resulting oil sands process-affected waters (OSPW) will have elevated concentrations of naphthenic acids (NAs) and inorganic ions (e.g. Na^+, Cl^-, SO_4^{2-}, and HCO_3^-) relative to surface waters in the region (MacKinnon and Boerger, 1986; Schramm et al., 2000). The un-recovered bitumen in the oil sands process-affected material comprises primarily saturated and polar

Abbreviations: OSPW, oil sands process-affected waters; MFT, mature fine tails; PAHs, polycyclic aromatic hydrocarbons; MFO, mixed-function oxygenase; NAs, naphthenic acids; PVC, polyvinyl chloride; GSI, gonadosomatic indices; LSI, liver somatic indices; SSI, spleenosomatic indices; CF, condition factor.

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organic compounds, with a minor fraction being polycyclic aromatic hydrocarbons (PAHs), present predominately as the alkylated series of the PAHs. These petrogenic PAHs are natural constituents of bitumen that have very low solubility in OSPW (Madill et al., 2001).

Freshly produced OSPW can be acutely toxic to aquatic organisms; NAs in their dissociated ionic form are thought to be primarily responsible (Frank et al., 2008; MacKinnon and Boerger, 1986; Madill et al., 2001). These NAs are a mixture of saturated acyclic, monocyclic, and polycyclic carboxylic acids that are highly soluble natural surfactants associated with the oil sands bitumen fraction (Clemente and Fedorak, 2005). The acute toxicity of "fresh" OSPW declines over time, likely due to a decrease in the proportion of lower molecular weight NAs (Holowenko et al., 2002; MacKinnon and Boerger, 1986). The natural biodegradation of NAs in OSPW is slow and yields an increasing proportion of mono-, di- and trioxidized NAs (Han et al., 2009).

The wet landscape reclamation option to deal with the large amounts of MFT and residual OSPW involves their transfer to geotechnically-secure mined-out pits. It is expected that the toxicity of the waters in these end pit lakes will diminish over time leading to the development of stable and viable lake habitats with a biological capability similar to natural lakes in the region. In establishing the effectiveness of this remediation approach, chronic effects of aged OSPW on semi-aquatic and aquatic organisms in these systems must be understood.

Fish studies have been conducted to assess the effects of OSPW exposure. Peters et al. (2007) demonstrated that fresh OSPW can cause deformities in yellow perch (*Perca flavescens*) and Japanese medaka (*Oryzias latipes*). Histopathological changes in gill and liver tissue have been reported in several fish species exposed to aged OSPW (Nero et al., 2006b; van den Heuvel et al., 1999). Increases in liver size and hepatic mixed-function oxygenase (MFO) activity have also been observed in fish exposed to oil sands constituents (Tetreault et al., 2003; van den Heuvel et al., 1999). These constituents may also have an adverse effect on the reproductive physiology of fish. *In vitro* studies on gonadal tissues demonstrated reduced production of sex steroids (basal and forskolin-stimulated) in slimy sculpin (*Cottus cognatus*) collected within the oil sands area relative to fish from a reference site (Tetreault et al., 2003). Goldfish (*Carassius auratus*) held in ponds containing aged OSPW had lower plasma steroid levels than reference fish (Lister et al., 2008).

This study was designed to determine the effects of aged OSPW on the reproductive physiology of fathead minnow (*Pimephales promelas*). Sexually mature fathead minnows were exposed to aged OSPW in the laboratory for 21 days and the numbers of eggs spawned, secondary sexual characteristics, and plasma sex steroid levels were monitored. Fathead minnows are native to the oil sands region and these forage fish are expected to be present in aquatic reclamation options such as end pit lakes. The OSPW was collected from experimental ponds at Syncrude Canada Ltd. and Suncor Energy Inc., within the Alberta oil sands region. In the study using Suncor OSPW, fathead minnows were acclimated to the higher salinities typically associated with OSPW prior to the start of the experiment; in the Syncrude OSPW exposures there was no acclimation. This research will aid in predicting potential challenges regarding the success of fish in the wet landscape reclamation option and will help to identify the risks that must be addressed as oil sands operators implement end pit lakes.

2. Materials and methods

2.1. Collection of OSPW

OSPW with various concentrations of NAs for this study were sourced from ponds on the leases of Syncrude Canada Ltd. and Suncor Energy Inc. in Northern Alberta. The waters from these sources were naturally aged OSPW and this water, through a battery of standard bioassays (fish, invertebrates, bacteria), has been demonstrated to be not acutely toxic. To evaluate the wet landscape reclamation option, Syncrude Canada Ltd. constructed a series of test ponds on part of their lease in Northern Alberta (57°05.050′N, 111°41.505′W) in 1989 and 1993. These ponds contained MFT and OSPW collected from Syncrude's Mildred Lake Settling Basin. Pond 5 was constructed in 1989 and filled with 1000 m^3 of MFT and capped with 1000 m^3 of OSPW (depth 2.5 m). Pond 9 was constructed in 1993 and filled with approximately 50,000 m^3 of OSPW with no MFT (maximum depth of 5 m). Demonstration pond was constructed in 1993 and filled with approximately 70,000 m^3 of MFT (maximum depth of 12 m) and capped with approximately 70,000 m^3 of non-OSPW (muskeg surface water from the area). Aged OSPW was also collected from Suncor Energy Inc.'s North and South MFT ponds (56°59.478′N, 111°32.138′W). These ponds were constructed in 1991 and filled with MFT (~14,500 m^3) and capped with OSPW (approximately 12,000 m^3, 2.5 m depth). Gregoire Lake (56°27′06″N, 111°07′38″W), located approximately 15 km south of Fort McMurray, Alberta, was used to source reference water for each year of study.

Water from all of the locations listed above was collected using a submersible pump positioned approximately 1.0 m below the surface and then stored in 1000 L polypropylene containers before they were used. Test waters used for fish exposures were characterized (Table 1) at Syncrude's Research Facility in Edmonton, AB using standard aquatic chemical methods. The concentrations of total NAs were measured using Fourier transform infrared (FTIR) spectroscopy (Holowenko et al., 2001). Kodak NAs (Eastman Kodak Company, Rochester, NY) were used as standards in the FTIR analysis.

2.2. Fathead minnow reproduction assay

Fathead minnow reproduction assays were conducted in a trailer located on Syncrude's site. Each of the tanks ($n = 4$ per treatment) contained four female and two male fathead minnows and three halved polyvinyl chloride (PVC) pipes which served as the spawning substrate. The tanks were filled with 15 L of Gregoire Lake water and placed inside a reservoir maintained at 25 ± 1 °C, under a photoperiod of 16 h light:8 h dark. Dissolved oxygen remained above 8 mg/l throughout the exposures. Fathead minnows were fed frozen adult brine shrimp (*Artemia* sp.) *ad libitum* twice daily. After a 14–21 day pre-exposure period, the fish were transferred to tanks containing either Gregoire Lake water (reference) or one of the treatment waters (OSPW) described below for an additional 21 days. Water renewals occurred every two days.

The endpoints monitored included the number of spawns, fecundity (number of eggs spawned), fertility, hatching success, and survival to swim-up. Spawning and fecundity were determined by counting the numbers of eggs laid on the PVC pipe and their fertility was checked under a dissecting microscope. A subsample of these eggs was kept to measure hatching success and survival to swim-up (96 h post hatch). This involved transferring 10 eggs into a Petri dish containing water from the corresponding treatment and placing them in an incubator (25 ± 2 °C, 16 h light:8 h dark). There were four Petri dishes for each tank and each tank was evaluated twice. The eggs were checked daily for mortality and hatching and any dead eggs or larvae were recorded and removed. Approximately 70% of the test waters in the Petri dishes were changed daily.

2.3. Assay 1: Syncrude aged OSPW exposure

Fathead minnows (~1–2 years old) were purchased from Silhanek Baitfish Farms (Bobcaygeon, ON, Canada) and transported to