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## Effects of oil sands process-affected waters and naphthenic acids on yellow perch (*Perca flavescens*) and Japanese medaka (*Orizias latipes*) embryonic development

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#### Abstract

Syncrude Canada Ltd. is currently developing environmentally acceptable oil sands process-affected water management methods as part of their land reclamation strategy. Surface waters of the "wet landscape" reclamation option characteristically have elevated concentrations of sodium sulphate and naphthenic acids (NAs), with low levels of PAHs. The following experiment compared early-life stage responses of yellow perch (*Perca flavescens*) to those of Japanese medaka (*Oryzias latipes*) when exposed to Mildred Lake settling basin (MLSB) surface water and a commercial sodium naphthenate (Na-NA) standard. Perch eggs were fertilized and incubated in: 100%, 50%, 20%, 4%, 0.8%, and 0.16% dilutions of MLSB water, as well as 20, 10, 5, 2.5, and 1.25 mg/l solutions of the commercial standard. Medaka embryos were exposed to the same treatments, post-fertilization. Both species demonstrated an increase in the incidence of deformity, and a decrease in length at hatch as NA concentrations increased. MLSB surface water contained higher levels of NAs than the commercial standard, however, showed consistently higher NA threshold effect concentrations for both species. Significant differences between the MLSB water and the Na-NA standard suggest that they contain NA congeners with different toxicity, or other compounds such as PAHs. Species differences in thresholds could be explained by the difference in developmental stage in which the exposures were initiated.

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### 1. Introduction

The Athabasca oil sands deposit, located north of Fort McMurray, Alberta, is the world's largest single oil reserve. Separation of bitumen from the oil sand utilizes large amounts of water; approximately 4 m<sup>3</sup> of fluid tailings are produced for each m<sup>3</sup> of oil sand processed (Holowenko et al., 2002; MacKinnon et al., 2005). Of this water, greater than 70% is recycled back into the process, but the net effect of the oil sands processing is an ever-growing inventory of process-affected waters and tailings materials. By the end 2004, approximately 500 million m<sup>3</sup> of oil sands processaffected waters (OSPW) in various sinks (free water, fine tails, engineered tailings, sand deposits) had accumulated at Syncrude Canada Ltd.'s processing facility since operations began in 1978 (Madill et al., 2001).

After oil sands processing, extraction tailings are pumped to settling basins. As the fine tailings consolidate, the pore water is released (Boerger et al., 1992; Mikula

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et al., 1996). This pore water contains ultra-fine particles of clay that do not settle out, as well as high concentrations of both naphthenic acids (NAs) and sodium sulphate (FTFC, 1995). Fresh OSPW is acutely toxic to aquatic organisms, and most of this toxicity has been attributed to the high concentrations of naphthenic acids (NAs) (Clemente and Fedorak, 2005). When aged under aerobic conditions the toxicity is reduced (Verbeek et al., 1993; FTFC, 1995; Schramm et al., 2000).

Naphthenic acids are a family of low molecular weight, naturally-occurring carboxylic acid surfactants. The NAs are released from the bitumen into water under elevated pH conditions used in the oil-sand extraction process. In addition to NAs, OSPW will contain elevated levels of ions derived from the oil sand and process aids, so that conductivity ranges from about 3000 to 5000  $\mu$ S cm<sup>-1</sup>, with the primary ions being Na, Cl, HCO3 and SO4. The contribution of sodium sulfate to the toxicity of fine-tailings has not been thoroughly investigated. Understanding the potential toxicity of OSPW to fishes is crucial for the evaluation of water management (potential discharge) and reclamation of the oil sands leases by "wet landscape" options (wetlands, pit lakes, water capped lakes) for land reclamation and tailings management on oil sands land leases (Boerger et al., 1992; FTFC, 1995). The water bodies resulting from this "wet landscape" option will involve lining of mined out depressions with fine tailings, followed by capping with non-process affected water, to establish water bodies of quality sufficient to support viable aquatic communities. Several experimental water-capped tailings ponds have been constructed on the Syncrude lease in northern Alberta to investigate this reclamation option and factors that could affect their success.

This study was part of a larger program to examine the impacts of the wet landscape option on adult fish responses (van den Heuvel et al., 1999a,b, 2000) and on the early life stage toxicity to fishes. Preliminary studies in the latter, have shown that surface waters from the experimental ponds containing NAs and salts had no effect on fertilization or pre-gastrulation mortality rates in yellow perch (Perca flavenscens). There was, however, an inverse relationship of NA concentration and conductivity to egg size and larval length (Peters, 1999). To further examine the potential environmental impacts of the wet landscape strategy, basic questions regarding the toxicity of water released during the consolidation of fine tailings to early-life-stages of fish were investigated. Although Japanese medaka (Oryzias latipes) are often used in such laboratory-based investigations, it can be difficult to extrapolate results to resident wild fish populations (Kimball and Levin, 1985). Therefore, eggs collected from wild yellow perch, a popular sport fish in the study area and a species for which development and growth have been well documented, (Post and McQueen, 1994; Sandström et al., 1995) were included in these studies. Running tests with the two species in parallel also allowed us to establish their relative tolerance, and determine if their responses were comparable to the stressors examined. Since yellow perch eggs are only available seasonally, while medaka eggs are available year round, the results of this comparison might allow us to undertake more intensive exposure-response studies with medaka with greater assurance that the results could be used for environmental risk assessment applicable to perch. Briefly, eggs from both medaka and yellow perch were exposed to varying levels of NAs from both OSPW from Mildred Lake settling basin (MLSB, an active settling basin on Syncrude's site), and a commercially-available sodium naphthenate (Na-NA). Toxicity was assessed as changes in the incidence of larval deformities and larval length at hatch.

#### 2. Materials and methods

#### 2.1. Exposure solutions

Commercial Na-NA was supplied as a 50% (w/v) aqueous solution by Pfaltz-Bauer Inc. (Waterbury, CT; N00910). Treatment dilutions of MLSB water and the Na-NA standard for the perch exposures were prepared with water from Gregoire Lake (south of Fort McMurray, AB; 56°27'N, 111°12'E), that also served as the reference water. Gregoire Lake water had an average conductivity of  $106 \ \mu S \ cm^{-1}$  and pH of 7.1, and contained a low but measurable background level of NAs. For the medaka tests, embryo rearing medium (ERM, Kirchen and West, 1976) was used to dilute the MLSB and Na-NA standard solution, and as the control. NAs analyses were conducted at Syncrude's Edmonton Research Centre using Fourier transform infrared (FTIR) spectroscopy (Jivraj et al., 1995). Conductivity, pH, and NA concentrations for the test waters and their dilutions are listed in Table 1. The nominal concentrations of the Na-NA solutions were higher than the actual concentrations measured in the treatments. This reflects the 50% solution of the Na-NA

Table 1

Chemistry of the reference, treatment dilutions of Mildred Lake settling basin (MLSB) surface water, and the commercial sodium naphthenate (Na-NA) standard used in the yellow perch and medaka embryo–larval exposures

Treatment	pН	Conductivity (µS/cm)	Naphthenic acids (mg/l)	
			Perch <sup>a</sup>	Medaka <sup>b</sup>
Gregoire Lake (ref)	7.06	107	0.25	< 0.1
100% MLSB water	8.34	2880	84.66	71.03
20% MLSB water	7.87	694	16.25	13.76
4% MLSB water	7.49	226	3.48	2.89
0.8% MLSB water	7.17	133	1.06	0.65
0.16% MLSB water	6.68	114	0.54	0.16
20 mg/l Na-NA	7.18	112	4.89	4.49
10 mg/l Na-NA	7.16	109	2.5	2.08
5 mg/l Na-NA	7.1	110	1.12	1.1
2.5 mg/l Na-NA	7.17	107	0.69	0.48
1.25 mg/l Na-NA	7.19	111	0.41	0.27

<sup>a</sup> Dilutions prepared using Gregoire Lake water.

<sup>b</sup> Embryo rearing media used for reference and treatment dilution in place of Gregoire Lake water.

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