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TOXICITY OF OIL SANDS TO EARLY LIFE STAGES OF FATHEAD MINNOWS (*PIMEPHALES PROMELAS*)

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Abstract—The present study examines the effects of exposure to oil sands on the early life stages (ELS) of fathead minnows (*Pimephales promelas*). Sediments within and outside natural oil sand deposits were collected from sites along the Athabasca River (AB, Canada). The ELS toxicity tests were conducted with control water, natural oil sands, reference sediments, and oil-refining wastewater pond sediments. Eggs and larvae were exposed to 0.05 to 25.0 g sediment/L and observed for mortality, hatching, malformations, growth, and cytochrome P4501A induction as measured by immunohistochemistry. Natural bitumen and wastewater pond sediments caused significant hatching alterations and exposure-related increases in ELS mortality, malformations, and reduced size. Larval deformities included edemas, hemorrhages, and spinal malformations. Exposure to reference sediments and controls showed negligible embryo mortality and malformations and excellent larval survival. Sediment analyses using gas chromatography–mass spectrometry revealed high concentrations of alkyl-substituted polyaromatic hydrocarbons (PAHs) compared to unsubstituted PAHs in natural oil sands (220–360 µg/g) and oil-mining wastewater pond sediments (1,300 µg/g). The ELS sediment toxicity tests are rapid and sensitive bioassays that are useful in the assessment of petroleum toxicity to aquatic organisms.

Keywords—Oil sands   Polyaromatic hydrocarbons   Early life stages   Fathead minnow

INTRODUCTION

The Athabasca River basin of northeastern Alberta (Canada) is an area rich in petroleum reserves (Fig. 1). The Athabasca Oil Sands region contains deposits of bitumen, fine sands, clays, and water [1]. Bitumen is a naturally weathered, heavy crude oil composed of a complex mixture of hydrocarbons, hetero-organics, and metals. Early life stages (ELS) of fish exposed to weathered crude oils [2,3] and oil sand extracts [4] have shown significant toxicological responses that have been linked to chronic polyaromatic hydrocarbon (PAH) exposure. As industrial development in the Athabasca region continues, concerns regarding the potential negative impacts on aquatic ecosystems are increasing.

Several industries commercially extract crude oil from this location, producing large volumes of process-affected (tailings) waters. These tailings waters contain residual bitumen, organic acids, and PAHs, which have been shown to be acutely toxic to aquatic organisms [1]. A recent study of adult yellow perch (*Perca flavescens*) showed that exposure to tailings waters was associated with altered mixed-function oxygenase activity and bile PAH equivalent concentrations [5]. In field mesocosms, native fathead minnows (*Pimephales promelas*) reared in oil sands wastewater exhibited reduced growth compared to fish growing in nonprocessed water [6]. Tributaries that flow through the Athabasca Oil Sands region have eroded bitumen deposits, thereby distributing naturally derived hydrocarbons throughout the watershed. Thus, a need exists to determine the possible ecological effects arising from oil sands–related compounds (OSRCs) leaching from sediments both naturally and because of anthropogenic disturbances.

Few studies have investigated the effect of natural oil sands on aquatic biota in the tributaries of the Athabasca River. Recent studies have demonstrated altered reproductive function, biochemical responses, and differences in health parameters of native fish populations exposed to naturally occurring OSRCs [7,8]. These studies did not assess the direct effects of PAH contamination, specifically the chemical constituents in waters and sediments. Headley et al. [9] recently characterized the degree of natural PAH contamination in this region and showed that tributary sediments contain alkyl-substituted PAHs at much higher concentrations than their corresponding, unsubstituted PAHs. Because little is known regarding the toxicity of alkyl-substituted PAHs, it is important to assess whether PAHs and other OSRCs are bioavailable and have the potential to cause effects in fish. If so, it would be important in further studies to identify the causative compounds to understand the effects of growing industry against the background of any potential effects from natural oil sands in this river basin.

To our knowledge, studies concerning the effects of natural oil sands on early fish development have not been undertaken. The objective of the present study is to examine the effects of natural and anthropogenic oil sands exposure on the ELS of fathead minnows. This is an ideal test species because it is a standard toxicological bioassay organism [10] and indigenous to the Athabasca River watershed [11]. In addition, this research compares sediment PAH concentrations from natural and anthropogenic sites to assess what types of compounds are potentially bioavailable to the ELS of laboratory fish.

MATERIALS AND METHODS

Study area

The Athabasca River flows northward and discharges into Lake Athabasca (Fig. 1). Bitumen-rich oil deposits are exposed along the banks of the river for approximately 100 km, and

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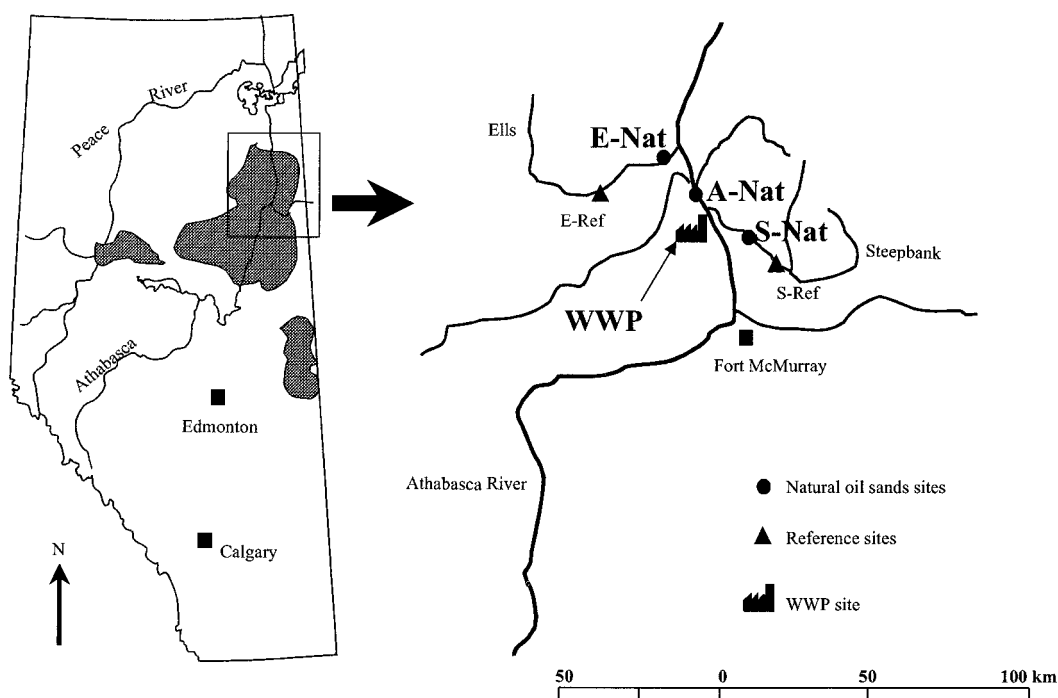* To whom correspondence may be addressed (maria.colavecchia@cciw.ca).

Fig. 1. Map of rivers and sediment collection sites in the Athabasca oil sands area of northern Alberta (Canada). Oil sands deposits are shaded. See text for locations and descriptions of sites. WWP = wastewater pond.

tributaries draining into this area cut into these deposits [9]. Two tributaries that were accessible for sampling and identified as typical natural source inputs of oil sands material were the Ells River and the Steepbank River (AB, Canada). In each of these tributaries, river sediments and bitumen were collected from two locations, namely downstream (lower) and upstream (upper) sites. River sediments represented natural PAH burdens encountered along the banks of these tributaries. The downstream samples were collected within the oil sands area and would provide natural PAH source inputs (E-Nat, 57°16.01′N, 111°42.51′W; S-Nat, 57°1′23″N, 111°28′30″W), whereas reference sediments were taken upstream of the oil sands deposits (E-Ref, 57°13.52′N, 111°53.15′W; S-Ref, 56°55′40″N, 111°13′56″W) (Fig. 1). No oil sands deposits were visible at either upstream site. In contrast, downstream portions of these rivers had streambeds that resembled asphalt-like pavement, with patches of sand, rubble, and bedrock lying on and/or in the surface. Oil sheens were visible in many pools and banks along downstream sites of both these tributaries. In addition, sediments were collected from the Athabasca River (A-Nat, 57°08′03″N, 111°35.47′W) and from a wastewater pond (WWP) located at an oil sands mining facility (Suncor Energy, Fort McMurray, AB, Canada). Visible oil sheens were observed on the pond's surface, and a characteristic tar odor was detected as sediments were disturbed during sampling. This WWP contains cooling water used in the refinery as well as runoff from the site, and it discharges to the Athabasca River. These sediments are settled solids collected from the pond, so they differ in composition from the other bitumens and natural river sediments tested (Table 1). This latter site is directly impacted by oil sands mining operations (anthropogenic).

Sediment collection

In September 2000, several surface sediment samples (depth, <10 cm) were collected from each site, composited, and stored at 4°C in sealed, 5-L, food-grade plastic containers. Wastewater pond sediments (depth, <30 cm) were obtained using an Ekman grab (Wildco, Buffalo, NY, USA). Composite, 10-g samples from each site were split, labeled, and shipped to National Water Research Institute (NWRI; Burlington, ON, Canada) for PAH and particle grain size (PGS) analyses. Results for the PGS analysis, determined gravimetrically using sieves, are provided in Table 1. Particle grain size classifications account for 100% of particles and include gravel (particle size range, 2–4 mm), fine sands (250–62.5 μm), silt (62.5–3.9 μm), and clay (3.9–0.06 μm). On arrival at NWRI, samples were freeze-dried and stored at −20°C until PAH analysis. Sediment samples for PGS and ELS fish testing were stored in the dark at 4°C, and tests were started eight weeks after field collection.

Extraction and fractionation of parent and alkyl PAHs

Sediment samples (5 g) were freeze-dried and homogenized before extraction. Also before extraction, all samples and method blanks were spiked with 100 μl of a surrogate standard mixture containing four perdeuterated PAHs: [^{2}H$_8$]naphthalene, [^{2}H$_{10}$]fluorene, [^{2}H$_{12}$]pyrene, and [^{2}H$_{12}$]benzo[a]pyrene. Samples were extracted using accelerated solvent extraction (ASE) with 100% dichloromethane (DCM) at 100°C at a pressure of 2,000 psi using a Dionex ASE (model 200; Sunnyvale, CA, USA). The extraction procedure consisted of an oven heat-up time of 10 min, a static time of 10 min, a flush volume of 70% of the extraction cell volume, and a nitrogen purge at 150 psi for 60 s. The samples were extracted in 11-ml extraction cells topped up with prewashed (using DCM) Ottawa Sand Standard (20–30 mesh; Fisher Scientific, Nepean, ON, Canada). With every set of 12 samples, a method blank and two reference standards were run. The method blank was Ottawa Sand Standard prewashed with DCM. The DCM extracts were passed through sodium sulfate columns to remove any residual water and then transferred to 250-ml, round-bottom flasks and solvent exchanged into 4 ml of hexane. The hexane extracts were quantitatively transferred to 15-ml disposable centrifuge tubes and

Table 1. Total polycyclic aromatic hydrocarbon (PAH) concentrations (ng/g) for the Athabasca River (AB, Canada) and tributary sediments[a]

	Anthropogenic WWP	Downstream (natural oil sands)			Upstream (reference)	
		E-Nat	S-Nat	A-Nat	E-Ref	S-Ref
Particle grain size category	Silt and clay	Sand/gravel	Sand/gravel	Sand/gravel	Sand	Sand
Naphthalene (N)	56	1	0	0	1	0
C_1-naphthalene (N1)	300	6	34	0	7	ND
C_2-naphthalene (N2)	300	50	270	260	57	ND
C_3-naphthalene (N3)	430	340	620	440	370	ND
C_4-naphthalene (N4)	1,600	1,900	2,900	1,900	540	ND
Acenaphthylene (AC)	0	0	0	0	0	0
Acenaphthene (AE)	12	1	33	0	5	2
Fluorene (F)	110	4	24	0	10	3
C_1-fluorene (F1)	19	20	0	0	16	ND
C_2-fluorene (F2)	330	75	210	0	0	ND
C_3-fluorene (F3)	750	1,600	940	540	0	ND
Phenanthrene (P)	510	1,200	370	0	92	12
Anthracene (A)	770	0	0	0	0	0
C_1-phenanthrene/anthracene (P1)	7,300	22,000	4,900	1,400	330	ND
C_2-phenanthrene/anthracene (P2)	24,000	58,000	14,000	9,000	370	ND
C_3-phenanthrene/anthracene (P3)	34,000	53,000	44,000	22,000	240	ND
C_4-phenanthrene/anthracene (P4)	28,000	25,000	36,000	22,000	33	ND
Fluoranthene (FL)	350	760	560	410	11	3
Pyrene (PY)	510	1,200	2,300	1,300	21	5
C_1-fluoranthene/pyrene (FLPY1)	21,000	5,500	19,000	7,800	57	ND
C_2-fluoranthene/pyrene (FLPY2)	31,000	6,100	30,000	16,000	48	ND
C_3-fluoranthene/pyrene (FLPY3)	29,000	14,000	36,000	19,000	18	ND
C_4-fluoranthene/pyrene (FLPY4)	27,000	21,000	43,000	27,000	14	ND
Benzo[*a*]anthracene (BA)	620	130	130	0	2	0
Chrysene (CY)	11,000	2,500	2,500	1,800	3	1
C_1-benzo[*a*]anthracene/chrysene (BAC1)	11,000	8,600	16,000	11,000	18	ND
C_2-benzo[*a*]anthracene/chrysene (BAC2)	220,000	11,000	35,000	24,000	24	ND
C_3-benzo[*a*]anthracene/chrysene (BAC3)	290,000	6,200	31,000	23,000	8	ND
C_4-benzo[*a*]anthracene/chrysene (BAC4)	200,000	2,100	18,000	11,000	3	ND
Benzo[*b*]fluoranthene (BB)	80,000	560	890	780	3	1
Benzo[*jk*]fluoranthene (BJK)	11,000	110	160	170	2	0
Benzo[*e*]pyrene (BE)	530	11	1,200	960	2	0
Benzo[*a*]pyrene (BAP)	22,000	22	500	270	2	0
C_1-benzofluoranthene/pyrene (BFLPY1)	150,000	2,800	9,400	6,600	14	ND
C_2-benzofluoranthene/pyrene (BFLPY2)	110,000	2,300	7,800	5,400	4	ND
Perylene (PYL)	0	69	1,100	750	22	2
Indeno[1,2,3-*cd*]pyrene (IP)	0	100	220	170	2	0
Dibenzo[*a,h*]anthracene (DA)	0	81	210	150	1	0
Benzo[*ghi*]perylene (BP)	190	200	440	350	3	1
Total PAH (ng/g)	1,300,000	250,000	360,000	220,000	2,400	30

[a] ND denotes compounds that were less than detection limits. Particle grain size ranges account for 100% of particles. Site abbreviations are noted in text and include; WWP = wastewater pond; E-Nat, S-Nat, and A-Nat = natural oil sands sites on the Ells, Steepbank, and Athabasca Rivers, respectively.

concentrated to approximately 1 ml using a Turbo Vap LV Evaporator (Zymark, Hopkinton, MA, USA). The extracts were then quantitatively transferred to an 8-g, fully activated silica gel clean-up column (500 × 20 mm) and eluted with 40 ml of hexane followed by 70 ml of 50% DCM in hexane. The first fraction was discarded. The second fraction, containing the aromatic hydrocarbons, was solvent exchanged into iso-octane and concentrated to a final volume of 1 ml. After the addition of an internal standard containing three perdeuterated PAHs ([$^2H_{10}$]acenaphthylene, [$^2H_{10}$]fluoranthene, and [$^2H_{12}$]chrysene), the samples were analyzed by gas chromatography–mass spectrometry (GC-MS).

GC-MS analysis of parent and alkyl PAHs

An Agilent 6890 gas chromatograph equipped with a 5973 mass-selective detection (Folsom, CA, USA) was used for the analysis of parent and alkyl PAHs. The mass spectrometer was operated in electron-impact mode. The GC capillary fused-silica column used was an Agilent HP-5ms (length, 30 m; inner diameter, 0.25 mm; film thickness, 0.25 μm). Samples were injected (2 μl) in pulsed-splitless mode with an initial pressure

of 25 psi, held for 1.25 min, and then maintained at a constant flow rate of 1.2 ml/min. The column temperature was programmed as follows: 80°C for 2 min, 50°C/min to 100°C, 5°C/min to 300°C, and then held for 5 min. The injector temperature was set at 260°C, the transfer line at 300°C, and the ion source and quadrupole at 230°C and 106°C, respectively. Helium was used as the carrier gas. Two ions were monitored for each analyte and the perdeuterated PAH standards. Concentrations of parent PAHs were based on calibrations using a five-point curve that was checked after every six injections for continuing performance. Authentic standards were used to generate the relative response factors (RRFs) for the parent PAH. Refer to Table 1 for abbreviations of PAH analytes. The N1 concentrations were calculated using the RRF for 1-methyl naphthalene, N2 through N4 using the RRF of 2,6-dimethylnaphthalene, P1 using the RRF of 1-methylphenanthrene, P2 through P4 using the RRF of 3,6-dimethylphenanthrene, FLPY1 through FLPY4 using the RRF of 2-methyl fluoranthene, BAC1 through BAC4 using the RRF of 4-methyl chrysene, and BFLPY1 and BFLPY2 using the RRF of 7-methyl benzo[*a*]pyrene. Extraction recoveries were in the 34 to 119%

range. Detection limits varied with the compound but generally were less than 1 ng/g.

ELS maintenance

We modified standard protocols for the 7-d fathead minnow growth bioassay [10] by starting sediment exposures with newly fertilized embryos less than 24 h postfertilization (HPF). Eggs and larvae were exposed for approximately 12 d, which included a 4- to 5-d embryonic exposure followed by a 7-d posthatch (DPH) larval exposure. Eggs and larvae were maintained in a controlled incubator ($25 \pm 1°C$) in dechlorinated, charcoal-filtered, Burlington (ON, Canada) municipal water adjusted to a hardness of 130 ppm with $CaCO_3$ on a 16:8-h light:dark photoperiod.

Fathead minnow eggs were shipped from Aquatic Research Organisms (Hampton, NH, USA) to NWRI. Eggs were collected from three to four nest tiles and shipped the day they were laid. On arrival, eggs were pooled and cleaned of debris, and opaque, unfertilized, or abnormal embryos were discarded.

ELS sediment assay and sampling

Eggs (<24 HPF, $n = 20$ per container) were placed in glass Nitex (Aquatic Eco-Systems, Apopka, FL, USA) mesh-bottomed cups (size, 500 μm) suspended 2 cm above the bottom of 1-L covered glass beakers. We developed a high- to low-exposure regime in similar sediment to water ratios as those described by Munkittrick et al. [12]. Beakers contained varying amounts of sediments (25.0, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.098, and 0.049 g), 1 L of overlaying laboratory water, and moderate aeration. Sediments were obtained from tributaries of the Athabasca River and included reference, natural oil sands, and anthropogenic sites (Table 1). Both sediments and water were renewed daily and allowed to equilibrate approximately 24-h before placement of embryos and larvae. Feeding of larvae started at 1 DPH, and each beaker received a slurry of concentrated brine shrimp (*Artemia salina*) twice daily, providing approximately 10 μl of brine shrimp/larva, which was adjusted daily to account for remaining live larvae. Dilution water pH, dissolved oxygen, temperature, conductivity, and ammonia levels were measured daily in each beaker before water changeover. Ammonia concentrations were estimated using a commercial Freshwater Ammonia (NH_3/NH_4) Test Kit (33A Aquarium Pharmaceuticals, Mississauga, ON, Canada). The ranges for temperatures, dissolved oxygen, pH, conductivity, and ammonia in exposure waters were as follows: 24 to 26°C, 8.8 to 10.9 mg/L, 7.45 to 8.33, 0.16 to 0.34 μS/cm, and 0.05 to 1.0 ppm, respectively. No significant deviations were observed in water quality of the dilution waters among treatments during the exposures.

All beakers were checked daily for hatching and mortality, and dead embryos and larvae were noted and removed. At hatching, larvae were scored as positive or negative for the prevalence of malformations. The presence or absence of predominant larval deformities was recorded; these deformities included severe subcutaneous pericardial (heart) and peritoneal (yolk sac) edemas, hemorrhages (includes hemostasis), and skeletal malformations. Swelling of the pericardium and/or anterior margins of yolk membranes with clear, fluid-filled areas indicated heart and yolk sac edemas. Vertebral abnormalities included larvae with coiled spinal columns and curved larvae. On day 12, remaining larvae were killed and preserved in 70% ethanol. Within three weeks of preservation, larvae were removed from ethanol, rinsed in distilled water, blotted dry, placed into preweighed weigh boats, dried at 60°C for 5 to 8 h, and then weighed. At hatch, subsamples ($n = 5$ per group) of larvae were sampled for later histology and cytochrome P4501A (CYP1A) protein induction.

Statistical analysis

Data are expressed as the mean ± standard error of the mean, with four replicates per exposure group. Egg mortality refers to the cumulative number of embryos that died before hatch and includes incomplete hatches. Larval mortality refers to the cumulative number of larvae that died from hatch to the termination of the exposure. Mortality data of embryos and larvae in each beaker were converted to percentage mortality before statistical analysis. Although abnormal larval morphology was characterized by numerous malformations (see *Results*), only the cumulative prevalence of pericardial edema, hemorrhages (includes hemostasis), spinal deformities, and mortality was determined by site for each exposure group. Threshold exposures were defined by the no-observable-effect concentration to lowest-observable-effect concentration (LOEC) range. When parametric assumptions of normality and homogeneity of variance could not be met by logarithmic transformation, nonparametric statistics were used. Mortality and deformity responses across treatment groups were compared post hoc (Kolmogorov-Smirnov test) against controls, with differences analyzed using Kruskal-Wallis tests. For S-Nat- and E-Nat-exposed groups, endpoints were compared to both water controls and corresponding reference-exposed groups (S-Ref and E-Ref, respectively). Normal distributions and homogenous variances were observed for larval dry weights and hatching times (time to 50% hatch). Hence, raw data were treated by one-way analysis of variance, and exposed groups (by each site) were compared to controls. When analysis of variance showed significant treatment differences, means were compared post hoc (Tukey's test for honestly significant differences) to controls using Dunnett's *t* test. Data analyses were conducted with Systat 10.0 (Systat, Evanston, IL, USA), and significant differences were identified at $p < 0.05$ for all tests.

RESULTS

Sediment PAH concentrations

Sediments from different river sites differed in their content of gravel, sand, silt, and clay (Table 1). River sediments from both upstream sites were sandy. Downstream bitumen deposits were sandy gravel mixtures, and WWP sediments were mainly composed of silt and clay fractions.

Among sediments from various sites, differences were found in the concentrations of total PAH (TPAH) (Table 1). The TPAH concentrations were highest in anthropogenic sediments (WWP, 1,300 μg/g), followed by high TPAH concentrations in natural bitumen deposits (220–360 μg/g), then intermediate TPAH concentrations in natural river sediments (22–54 μg/g). Sediment TPAH concentrations from both reference sites were comparatively very low (0.03–2.4 μg/g). The WWP sediments had TPAH concentrations that were approximately fivefold higher than those in natural bitumen deposits. As well, WWP sediments had TPAH concentrations that were more than three orders of magnitude higher than TPAH in reference sediments. In WWP sediments, the highest concentrations of PAH were the alkylated PAH and included benzo[*a*]anthracene/chrysene compounds (BAC1 through BAC4, 80–290 μg/g), C_1- and C_2-benzofluoranthene/pyrene (BFLPY1, 150 μg/g; BFLPY2, 110); C_1- through C_4-fluoranthene/pyrene (FLPY1 through FLPY4, 21–31 μg/g), and C_1- through C_4-phenanthrene/anthracene (P1 through P4, 7.3–34 μg/g) (Table 1).

Sediment PAH composition

The concentrations of alkyl-substituted PAH dominated the chemical profiles of all natural oil sands and anthropogenic sediments (Table 1). Substituted PAHs included C_1- through C_4-naphthalene (N1 through N4), C_1- through C_3-fluorene (F1 through F3), C_1- through C_4-phenanthrene/anthracene (P1 through P4), C_1- through C_4-fluoranthene/pyrene (FLPY1 through FLPY4), C_1- through C_4-benzo[*a*]anthracene/chrysene (BAC1 through BAC4), and C_1- and C_2-benzofluoranthene/pyrene (BFLPY1 and BFLPY2). Alkyl-substituted PAH comprised 93 to 99% of the TPAH of these sediments, whereas concentrations of unsubstituted PAH ranged from 1 to 7% (Table 1). However, substituted PAH compounds were not detected in sediments from the S-Ref site, which was outside the oil sands formation (Table 1).

Similar chemical profiles and compounds were found in natural oil sands, with three predominant alkylated groups comprising approximately 95% of the total measured PAH (Table 1). These classes were BAC1 through BAC4 (~60%), FLPY1 through FLPY4 (~20%), and P1 through P4 (~15%). Benzofluoranthene/pyrene (~3%) and alkylated naphthalenes (<1%) and fluorenes (<1%) were found in trace amounts. Both the S-Nat and A-Nat sites were nearly identical in PAH composition (Table 1). The E-Nat site showed some similarity in profile to the S-Nat and A-Nat sites; however, the abundances of predominant alkylated PAH groups varied, with P1 through P4 comprising a larger portion (50%), followed by BAC1 through BAC4 (31%) and then FLPY1 through FLPY4 (15%) (Table 1). The WWP sediments contained similar types of compounds; however, BAC1 through BAC4 made up the greatest proportion of the TPAH concentration (85%), followed by BFLPY1 and BFLPY2 (9%), FLPY1 through FLPY4 (3%), and P1 through P4 (3%) (Table 1).

Hatching success and ELS toxicity

Control and reference sediment–exposed eggs demonstrated greater than 95% hatch across replicates within 4 d. Hatching success was greater than 99% among control eggs (exposed to dechlorinated laboratory water), and total percentage larval mortality did not exceed 10% (7.9% ± 1.8%). Hatching success was greater than 98% among reference sediment–exposed embryos, and total percentage larval mortality did not exceed 15% (E-Ref, 7.5% ± 2.0%; S-Ref, 12.3% ± 2.1%). No statistical differences were found between control and reference sediment–exposed groups in hatching success, time to hatch (TTH), and larval survival.

Abnormal embryo development and reduced movements were observed in some bitumen- and WWP-exposed eggs (data not shown). These abnormalities included edemas and hemorrhages. In contrast, control and reference sediment–exposed embryos displayed vigorous movements within the chorion and no visible malformations during embryo development. Eggs exposed to oil sands from all three sites demonstrated exposure-related increases in mortality (Fig. 2A). All bitumen-exposed groups at and above the threshold of 1.5 g/L (i.e., LOEC) had significantly increased mortality compared to controls (Table 2). Eggs exposed to WWP sediments showed exposure-related increases in mortality at and above a threshold of 3.1 g/L (Fig. 2A and Table 2).

The TTHs of control and reference sediment–exposed groups were similar (~4 d). Embryos exposed to WWP sediments showed significant alterations in TTH compared to control eggs ($p < 0.001$) (Fig. 2B). Low-exposure WWP sediments (0.05–0.4 g/L) resulted in significant premature hatching (3.0–3.5 d), whereas in high-exposure groups (1.5–6.2 g/L),
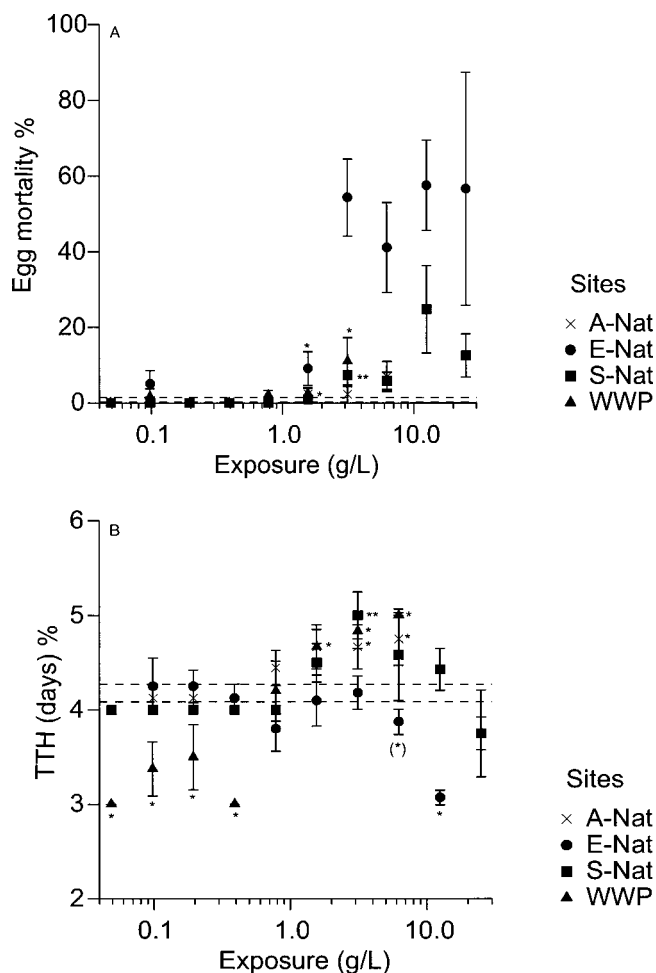

Fig. 2. Egg mortality (**A**) and time to 50% hatch (TTH) (**B**) across exposure groups for each site. Symbols represent the mean ± standard error, with $n = 4$ replicates/group. Egg mortality and TTH for control and reference sediment–exposed groups were pooled and are defined by the upper and lower 95% confidence limits (dashed bars). Exposures showing statistical significance from pooled controls are noted with asterisks (**B**) and represent estimated lowest-observed-effect concentration values (**A**). An asterisk in parentheses represents a marginally significant difference from pooled controls. *$p < 0.05$, **$p < 0.001$. WWP = wastewater pond.

embryos hatched significantly later (4.7–5.0 d) than controls. A-Nat-exposed eggs showed a significant exposure-responsive increase in hatching time compared to control embryos (Fig. 2B and Table 2). Embryos exposed to S-Nat hatched later (4.5–5 d) than controls; however, this finding was only significant at 3.1 g/L ($p < 0.001$) (Fig. 2B). In high-exposure groups of E-Nat (6.2 and 12.5 g/L), embryos showed significant premature hatching compared to laboratory water controls (3.8 d, $p = 0.058$, marginally significant; 3.1 d, $p < 0.001$, respectively) and reference sediment–exposed eggs ($p < 0.05$, Fig. 2B).

During hatching, some embryos died partially emerged from the chorion or half-hatched. Most sediment exposure–related mortality occurred at hatching or 1 to 3 DPH, and only in those larvae showing malformations. Larval mortality showed exposure-related increases with natural oil sands and anthropogenic sediments (Fig. 3). Both A-Nat- and S-Nat-exposed groups had significantly greater larval mortality than controls above the threshold of 0.8 g/L (i.e., LOEC), whereas E-Nat-exposed groups had significantly greater mortality

Table 2. Results for Kruskal-Wallis analyses of variance and post-hoc analyses of embryo and larval responses[a]

Endpoints	WWP (g/L)	E-Nat (g/L)	S-Nat (g/L)	A-Nat (g/L)
Egg mortality (LOEC)	3.1*	1.5* (*)	3.1** (*)	1.5*
Larval mortality (LOEC)	0.05**	0.1* (*)	0.8** (*)	0.8*
% Pericardial edema (LOEC)	0.1*	0.4** (*)	0.8* (*)	3.1**
% Spinal deformity (LOEC)	0.4*	0.8** (*)	1.5* (*)	3.1*
% Hemorrhages (LOEC)	0.4**	0.8** (*)	3.1** (*)	1.5*
Larval weight (LOEC)	0.2*	0.4* (*)	1.5* (*)	1.5*
Time to 50% hatch	Premature at ≤ 0.04* Delayed at ≥ 1.5*	Premature at ≥ 3.1** (*)	Delayed at 3.1** (NS)	Delayed at ≥ 1.5*

[a] Parameters were significant (* $p < 0.05$, ** $p < 0.001$) unless indicated by NS (not significant). For S-Nat and E-Nat, endpoints were compared to both water controls and corresponding upstream (reference) sediments (E-Ref and S-Ref, respectively). Values in parentheses represent these p values. For other sediments, endpoints were compared to water controls alone. WWP = wastewater pond; LOEC = lowest-observed-effect concentration.

above the threshold of 0.1 g/L (Table 2). Exposure to all doses of WWP sediment significantly increased larval mortality compared to controls ($p < 0.001$) (Fig. 3). Thus, the threshold for WWP causing larval mortality was less than 0.05 g/L, the lowest exposure tested in the present study (Table 2).

Few malformations were present in reference sediment–exposed groups (~1%) and laboratory water controls (~2%) (Fig. 4). Water controls and reference sediment–exposed groups showed normal larval morphology, with normal pericardial development and yolk sacs (edema absent), straight trunks (~1% had spinal deformities), and minimal hemorrhages (~1% had hemorrhages). Newly hatched WWP- and bitumen-exposed larvae showed obvious pathologies that included pericardial edema, hemorrhages, and spinal deformities (Figs. 4 and 5). Multifocal hemorrhages and hemostasis were visible in several areas, including the caudal region, yolk sac, heart, ocular, and cranial tissues. Skeletal abnormalities included larvae with coiled spinal columns and curved larvae with varying degrees of lordosis and scoliosis. Bitumen- and WWP-exposed larvae showed additional malformations in approximately 10% of individuals. These malformations included craniofacial deformities (underdeveloped jaws and domed skulls), eye alterations, and reduced pigmentation. These individuals exhibited severe pericardial edema and pauses (10–45 s) in the normal sequence of heart movements, followed by a resumption of reduced heart movements. Many of these severely edematous individuals emerged viable but showed necrotic tissues (trunk, head, heart, and/or yolk sac fractions), despite continued heart activity. Many exposed larvae appeared to be lethargic, stayed near the bottom of the glass cups, and occasionally displayed erratic patterns of twitches. Exposed larvae demonstrated these impaired swimming behaviors at hatching to 1 to 3 DPH. Generally, the prevalence of malformations (pericardial edema, spinal malformations, and hemorrhages) showed significant exposure-responsive increases with bitumen- and WWP-exposed groups ($p < 0.05$ and $p < 0.001$, respectively) (Figs. 4 and 5), and LOECs for these responses are noted in Table 2.

Larval dry weight was reduced, as shown by an overall decrease in biomass, with increasing exposure in WWP (LOEC, 0.2 g/L) and bitumen groups (LOEC, 0.4–1.5 g/L) (Table 2). Dry weights of larvae in higher-exposure treatments were significantly reduced from those of control and reference sediment–exposed larvae (Fig. 6).

DISCUSSION

ELS toxicity and mechanisms

The results of the present study indicate that several mechanisms may play a role in the toxicity of oil sands to the ELS

of fathead minnows. Reduced hatching success with exposure to oil sands suggests that PAHs and/or OSRCs may have a cytotoxic effect on the developing embryo. Cytotoxic effects on rainbow trout have been correlated with certain two- and three-ring PAHs [13]. Pink salmon embryos [14] and Pacific
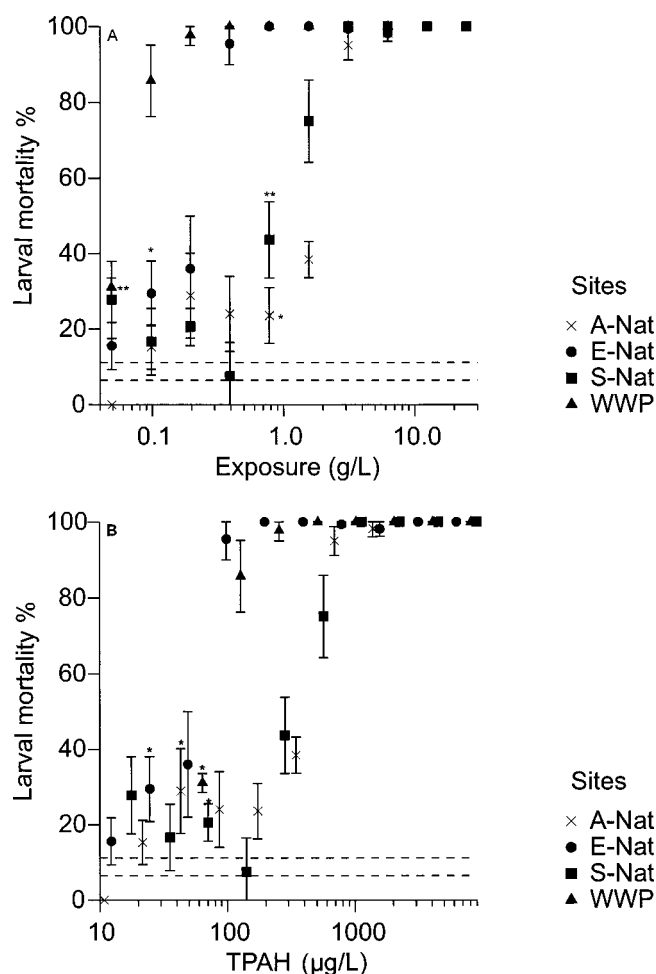

Fig. 3. Cumulative larval mortality (12 d posthatch) across exposure groups for each site expressed as (**A**) grams of sediment per liter and (**B**) total polycyclic aromatic hydrocarbon (TPAH; μg/L), where symbols represent the mean ± standard error with $n = 4$ replicates/group. Larval mortality for control and reference sediment–exposed groups (pooled) was 10 to 15% and are represented by the dashed bars (95% confidence intervals). Exposures showing statistical significance from pooled controls are noted with asterisks and represent estimated lowest-observed-effect concentration values. *$p < 0.05$, **$p < 0.001$. WWP = wastewater pond.
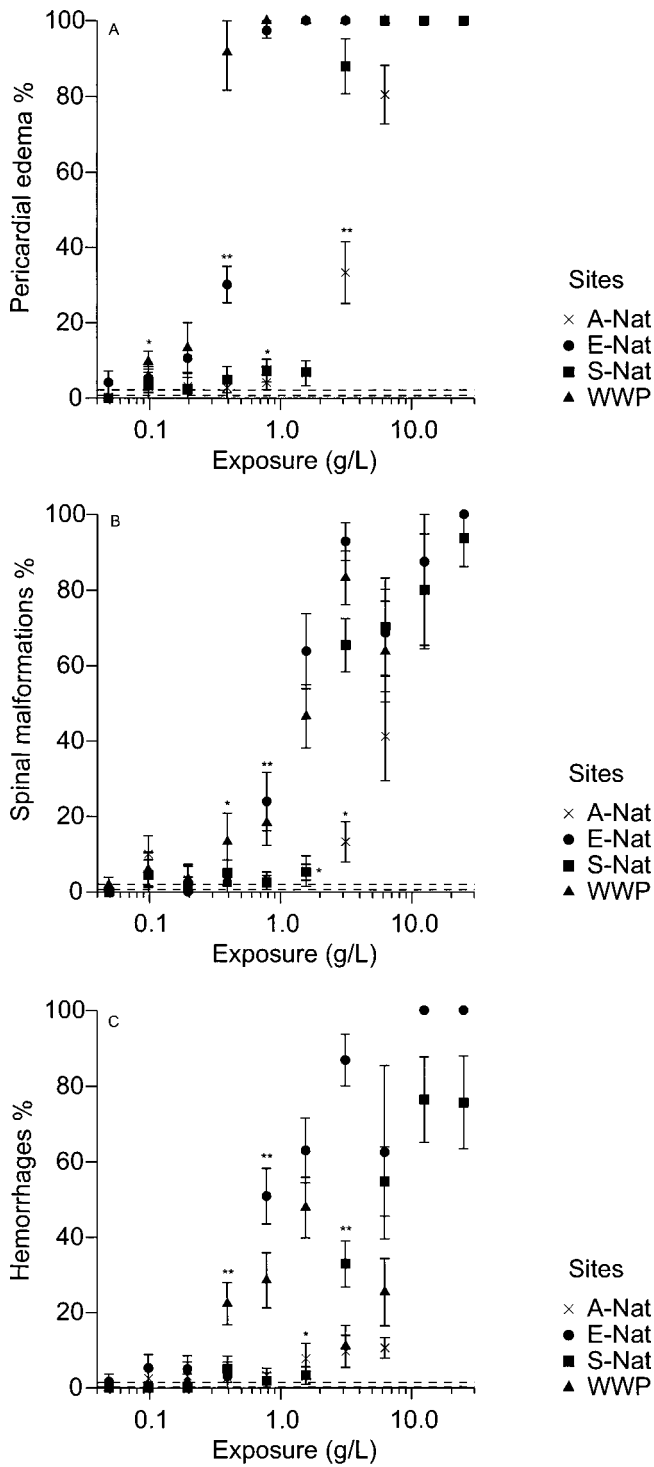
Fig. 4. Prevalence of (**A**) pericardial edema, (**B**) spinal malformations, and (**C**) hemorrhages of larvae at hatch among sites and exposures (g sediment/L), where symbols represent the mean ± standard error, with $n = 4$ replicates/group. Control and reference sediment–exposed responses for all variables were (≤2% and are represented by the dashed bars (95% confidence intervals). Exposures showing statistical significance from pooled controls are noted with asterisks and represent estimated lowest-observed-effect concentration values. *$p <$ 0.05, **$p <$ 0.001. WWP = wastewater pond.

Fig. 5. Prevalence of (**A**) pericardial edema, (**B**) spinal malformations, and (**C**) hemorrhages of larvae at hatch expressed as total polycyclic aromatic hydrocarbon (TPAH; μg/L), where symbols represent the mean ± standard error, with $n = 4$ replicates/group. Control and reference sediment–exposed responses (pooled) are represented by the dashed bars (95% confidence intervals). Exposures showing statistical significance from pooled controls are noted with asterisks and represent estimated lowest-observed-effect concentration values. $p <$ 0.05. WWP = wastewater pond.

herring larvae exposed to *Exxon Valdez* oil [2,3] also exhibited genetic damage that was related to TPAH concentrations. Researchers have demonstrated that fish exposure to PAHs during early developmental stages results in high bioaccumulation by
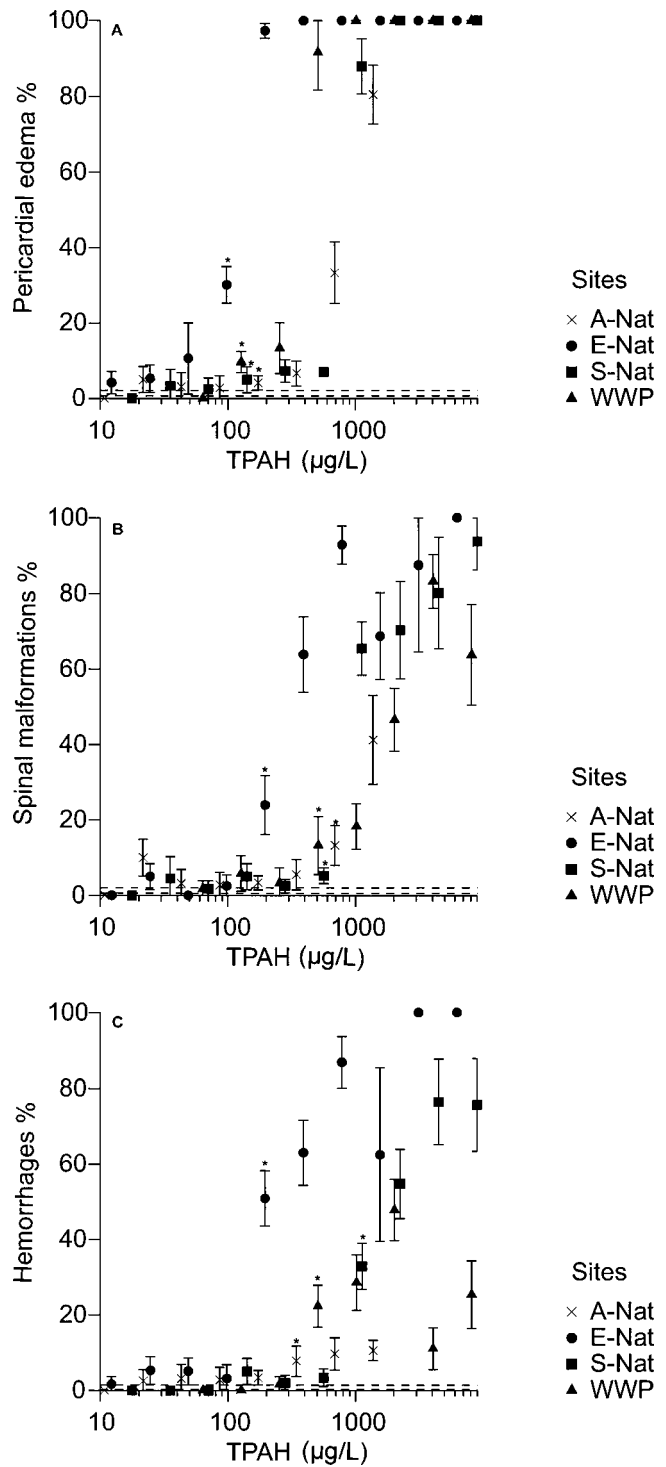
eggs, presumably because of low metabolic capability and the relatively high lipid contents of fish embryos [15]. The majority of egg mortality in the present study occurred during organogenesis, when a rudimentary liver may have formed
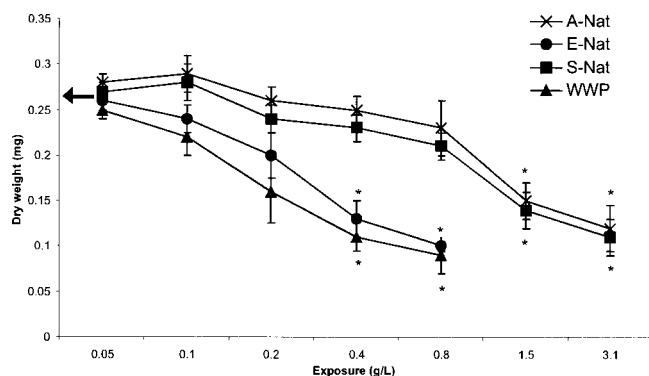
Fig. 6. Dry weights of larvae at 5-d posthatch (end of exposure) exposed to natural oil sands and wastewater pond (WWP) sediments, where symbols represent the mean ± standard error weighed in batches of 10 individuals for all sites ($n = 4$). Arrow indicates control response, and asterisks denote statistically significant differences from control larvae.

[11]. The liver produces CYP1A enzymes involved in the detoxification and activation of several lipophilic contaminants [16]. Metabolism of PAHs can lead to the formation of highly reactive radical cations, some of which are cytotoxic and mutagenic. Therefore, enzymatic oxidation and DNA damage during early development may inhibit the differentiation and/or growth of certain structures and result in the prehatch mortality observed in the present study. Furthermore, several researchers have reported precocious hatching with embryonic exposure to benzo[*a*]pyrene [17] and crude oil fractions [18]. Other studies, as well as the present study, have shown both premature and delayed hatching, depending on the source of the crude oil and exposure concentration [19,20].

For exposed larvae, pericardial edema was the most specific morphological marker of damage from exposure to oil sands. The prevalence of pericardial edema in the present study was directly related to exposure in the bitumen and WWP groups (Fig. 4A), and 100% of the larvae that died had edema. This suggests that edema was associated with cardiac insufficiency and may be one of the causes of ELS mortality.

Subcutaneous hemorrhage is a common response in embryonic exposure to PAHs [17] and is considered to be one of the earliest signs of cardiovascular dysfunction [21,22]. In the present study, hemorrhage was dependent on exposure and was often associated with several abnormalities (Fig. 4C). Numerous studies have reported pericardial edema, bradycardia, and arrhythmia in fish embryos exposed to oil fractions [19,23]. Recently, Vines et al. [24] reported decreased heart rates and arrhythmia in creosote-exposed herring eggs. These exposed embryos exhibited a pattern of sustained tremors, which those authors speculated could be caused by reduced blood flow to developing skeletal muscles. Edema pathogenesis in this present study was not evaluated, but increased fluid pressure around the heart may lead to decreased blood flow to tissues, resulting in degenerative tissues, reduced growth, and development [25]. Although blood flow was not assessed in this study, edemas may be restricting blood flow, which may have led to necrosis and death in exposed larvae.

Many deformations in fathead minnows were seen in the skeletal system, primarily exposure-dependent increases in spinal malformations (Fig. 4B). Pacific herring exposed to weathered crude oil showed significant dose-related increases in spinal curvatures and reduced swimming ability [2]. In addition, fathead minnow growth decreased with increasing oil sands exposure. Fish embryos exposed to alkyl PAHs [26] and oil

fractions showed retarded embryonic growth [2,25,27]. The growth of larval fish, although an indicator of contaminant exposure, also has great implications for recruitment and survival. Retarded development and spinal deformities may be a consequence of reduced blood flow [26]. For a better understanding of the possible relationship between these malformations, it would be useful to look at the temporal succession and the severity of the observed deformities. The similarity of exposure–response curves for the various malformations indicates that the mechanism of toxic action among oil sands sites was similar.

The observed symptoms in fathead minnows are consistent with those seen in several fish species exposed to a variety of toxic substances that bind with the aryl hydrocarbon receptor, including dibenzo-*p*-dioxins [21,28], alkyl phenanthrenes [22,26], and oil-contaminated sands and gravel [25,28,29]. Dioxin-induced blue sac disease (BSD) is a syndrome characterized by increased ELS mortality and malformations and has been associated with CYP1A induction in endothelial tissues of exposed fish. Thus, it has been postulated that vascular dysfunction may lead to edema [16]. The toxic mechanism of aryl hydrocarbon receptor–binding chemicals is still under investigation and may involve CYP1A induction, oxidative stress, and endothelial cell damage [30]. Immunohistochemical analyses have detected increased CYP1A protein expression in several tissues of bitumen- and WWP-exposed larvae compared to control and reference groups (M.V. Colavecchia, unpublished data). Further research is needed to investigate the possibility of similar mechanisms for the cardiovascular effects observed.

Sediment PAH concentrations and composition

Site differences in TPAH concentrations are related to proximity to natural and anthropogenic PAH inputs and follows an expected pattern. The TPAH concentrations were highest at anthropogenic sites (WWP, 1,300 μg/g), intermediate at natural oil sands sites (220–360 μg/g), and very low to negligible at upstream sites (2.4–0.03 μg/g) (Table 1). The WWP site had very high TPAH concentrations because these are settled solids collected from a WWP that contains cooling waters and runoff from the refinery site. Therefore, these sediments are directly influenced by anthropogenic sources (oil sands transport, extraction, and refining activities). Natural bituminous materials were collected from oil sands areas that were removed from industrial developments. Therefore, only natural erosion of oil sands have contributed PAHs to these sediments, resulting in intermediate TPAH values for natural oil sands sites. Differences in PAH concentration and composition among natural oil sands reflects the patchiness of PAHs in sediments, which likely is related to natural variability, as well as heterogeneity in the degree of weathering and localized soil erosion [9]. Because reference sediments were sampled from sites outside natural oil sands deposits, PAH levels were, as expected, very low, and some PAH compounds were not detected at the E-Ref and S-Ref sites. These sediments did not have natural or anthropogenic PAH inputs. Low PAH values from upstream sites and downstream river sediments were similar to previously reported concentrations [9]. Thus, the pattern of sediment TPAH concentrations across sites largely reflects proximity to anthropogenic versus natural PAH sources.

The PAH compositions of these sediments were typical of other petroleum-source PAHs, in which the alkyl-substituted PAHs predominate over unsubstituted PAHs [4,9,31]. Alkylated derivatives have received less study; thus, little information exists regarding the toxicity of these chemicals to ELS

of teleosts [32]. Increased BSD and CYP1A induction have been shown in several fish species exposed to alkyl phenanthrenes [22,26]. Increased malformations and mortality and decreased growth of fish eggs exposed to crude oil have been related to PAH concentrations [27], specifically alkyl derivatives of naphthalene, phenanthrene, fluorene, dibenzothiophenes (DBTs), and chrysene [3,25,33]. Japanese medaka exposed to oil sands tailings extracts show increased BSD and mortality, which were attributed to alkylated PAHs [4]. Because the symptoms of toxicity in fathead minnows exposed to oil sands are similar to those observed in other fish species exposed to alkylated PAH, these compounds likely may have contributed to the ELS toxicity observed in the present study. Because of the complexity of oil sands materials, toxicity identification evaluation studies are necessary to definitively correlate toxic effects with specific chemicals. Several toxic chemicals present in oil sands were not evaluated in the present study, and these include hydrocarbon degradation products, naphthenic acids (NAs), DBTs, metals, and sulfides. The toxicity of weathered crude oils to marine organisms has also been correlated with polar, low-molecular-weight hydrocarbon degradation products [34]. Naphthenic acids are a family of carboxylic acids that are abundant oil sands wastes [6] and are acutely toxic to aquatic organisms [1]. In addition, C_1- through C_4-DBTs are sulfur-containing compounds that are typically found at high levels in natural oil sands and tailings extracts [4,31]. Rhodes [4] suggested that the high proportion of C_2-substituted DBTs in oil sands extracts may be contributing to the increased BSD observed in exposed medaka. Although these compounds were not analyzed in the present study, further research is needed to assess the environmental risks of low-molecular-weight hydrocarbon degradation products, NAs, and/or DBT contamination from these sediments. The myriad of chemical constituents makes it difficult to correlate toxic effects with specific compounds; hence, elucidation of the toxic fractions of oil sands warrants further investigation.

The toxicity ranking of sediments followed a pattern similar to sediment TPAH concentrations. The toxicity ranking (from highest to lowest) based on estimated LOEC values is as follows: WWP >>> natural oil sands (E-Nat > S-Nat = A-Nat) >>> reference sediments. The ELS mortality and malformations were negligible in reference sediment–exposed groups and were similar to water controls. The lack of toxicity with reference sediment–exposed groups was expected, because these sediments contained very low TPAH concentrations, along with little (E-Ref) or no (S-Ref) alkyl-substituted PAHs.

The WWP-exposed groups demonstrated the greatest toxicity, as evidenced by LOEC values for larval mortality, malformations, and weight (as low as 50 mg/L). Along with having the highest sediment TPAH absolute concentration, WWP sediments were primarily composed of high-molecular-weight (HMW) compounds (four- to six-ring aromatics), namely benzo[*a*]anthracene/chrysene (85%) and benzofluoranthene/pyrene (9%). In laboratory studies exposing herring eggs to less-weathered oil and more-weathered oil (MWO), exposed larvae showed increased malformations and mortality as well as decreased size and swimming [2]. The MWO was more harmful to herring than equivalent amounts of less-weathered oil, which had relatively lower proportions of HMW PAHs. Researchers concluded that the increased toxicity of MWO was attributed to larger (HMW) and more alkyl-substituted PAHs, thereby demonstrating the importance of PAH composition [2]. Natural oil sands (E-Nat, S-Nat, and A-Nat sites) had relatively lower proportions of HMW PAHs (48–85%) compared to WWP sediments. Fathead minnow embryos exposed to these

natural bitumens showed dose–responses that generally paralleled those with WWP, but toxicity was lower (LOEC values were higher) for the natural bitumens, thereby demonstrating the importance of PAH composition. Similar toxicity was observed with the S-Nat and A-Nat exposures and may be related to the compositional similarity of these materials, as evidenced by nearly identical PAH ratios (i.e., BAC:FLPY:P:BFLPY, 60%:20%:15%:3%). Although the E-Nat site contained relatively similar TPAH concentrations (250 μg/g), its exposure elicited stronger toxicological responses than the S-Nat and A-Nat bitumens. The PAH composition of E-Nat differed considerably (31%:15%:50%:2%) and contained relatively larger quantities of phenanthrene/anthracene and its alkylated derivatives, which may have resulted in higher toxicity relative to other natural oil sands. Hence, the differential toxicity of anthropogenic and natural oil sands may have resulted from alterations in chemical composition and concentration among these sediments. Because of the chemical complexity of these sediments, a variety of compounds (alkyl PAHs, hydrocarbon degradation products, NAs, DBTs, and metals) may be contributing to the toxicity observed in fathead minnows.

The extrapolation of data from the laboratory to the field always involves uncertainty, and the possibility exists that indigenous fish species could be more or less sensitive to oil sands compared to fathead minnows. In nature, decreased hatching success would have a negative impact on the reproductive potential of fish populations. The potential environmental significance of smaller and malformed larvae would be impaired swimming and feeding ability. Therefore, these larvae may experience increased vulnerability to predation, starvation, infection, and mortality compared to their larger counterparts. Season, diet, general health, and interspecies differences in metabolism, excretion, and behavior are just a few factors that could influence the response of native fish species to oil sands exposure. The presence of other contaminants and their potential interactions, along with natural variability of physical and chemical factors, would be expected to modify the toxicity of these sediments. The bioavailability and effects of PAH in oil sands might be altered by factors such as natural water flows, volatilization, evaporation, and ultraviolet radiation [35]. Because laboratory exposures were static, covered, and involved controlled lighting conditions, these factors were not assessed in the present study. In addition, varying sediment to water ratios and physiochemical characteristics of oil sands materials may affect PAH bioavailability and exposure, so sediment PAH concentrations may not accurately predict toxicity. Therefore, the best method for characterizing exposure would be the measurement of tissue PAH, CYP1A induction, and/or PAH metabolites, which in turn could be used to construct dose–response curves. Ongoing laboratory studies are currently addressing this important research need.

CONCLUSION

Fathead minnows exposed to WWP sediments and to oil sands from fertilization to several days posthatch showed decreased hatching success, altered TTH, increased malformations, reduced size, and increased larval mortality. Effects were dependent on the exposure, and prevalence increased at sediment exposures of greater than 50 mg/L. Symptoms were not observed or were negligible in controls or larvae exposed to reference sediments collected on the same rivers outside the oil sands formation. Effects similar to those observed in the present study have been observed in fish embryos exposed to

weathered oils and their components [2,3,25–27,33]. Differences in PAH concentration and composition among oil sands sites likely contributed to the toxicological effects observed. The present study demonstrates that exposure to OSRCs from natural oil sands and refinery WWP sediments can have significant negative impacts on the survival and embryolarval development of the fathead minnow.

REFERENCES

1. Alberta Department of Energy. 1995. Fine tails and process water reclamation. In Hamaza H, Lord, T, Nelson R, Sheeran D, Sury K, Stone J, eds, *Advances in Oil Sands Tailings Research,* 1st ed. Oil Sands and Research Division, Edmonton, AB, Canada, pp 3–17.

2. Carls MG, Rice SD, Hose JE. 1999. Sensitivity of fish embryos to weathered crude oil: Part I. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environ Toxicol Chem* 18:481–493.

3. Marty GD, Hose JE, McGurk MD, Brown ED, Hinton DE. 1997. Histopathology and cytogenetic evaluation of Pacific herring larvae exposed to petroleum hydrocarbons in the laboratory or in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill. *Can J Fish Aquat Sci* 54:1846–1857.

4. Rhodes SM. 2002. Investigating the effects of dimethylated PAH on the embryo/larval development of the Japanese medaka (*Oryzias latipes*). MSc thesis. University of Waterloo, Waterloo, ON, Canada.

5. Van den Heuvel MR, Power M, MacKinnon MD, Dixon DG. 1999. Effects of oil sands related aquatic reclamation on yellow perch (*Perca flavescens*). II. Chemical and biochemical indicators of exposure to oil sands related waters. *Can J Fish Aquat Sci* 56: 1226–1233.

6. Siwik PL, Van Meer T, MacKinnon MD, Paszkowski CA. 2000. Growth of fathead minnows in oil sand–processed wastewater in laboratory and field. *Environ Toxicol Chem* 19:1837–1845.

7. Parrott J, Portt C, Baker M, Colavecchia M. 1999. The use of SPMDs and small sentinel fish species to assess naturally-occurring oil sands compounds in tributaries of the Athabasca River. *Proceedings,* 26th Annual Aquatic Toxicity Workshop, Edmonton, AB, Canada, October 4–6. *Can Tech Report Fish Aquat Sci* 2293:88.

8. Tetreault GR, McMaster ME, Dixon GD, Parrott JL. 2003. Using reproductive endpoints in small forage fish species to evaluate the effects of Athabasca oil sands activities. *Environ Toxicol Chem* 22:2775–2782.

9. Headley JV, Akre C, Conly MF, Peru KM, Dickson LC. 2001. Preliminary characterization and source assessment of PAHs in tributary sediments of the Athabasca River, Canada. *Journal of Environmental Forensics* 2:335–345.

10. Environment Canada. 1992. Biological test method: Test of larval growth and survival using fathead minnows. EPS 1/RM/22 Technical Report. Ottawa, ON.

11. Robinson CLK, Tonn WM. 1986. Influence of environmental factors and piscivory in structuring fish assemblages of small Alberta lakes. *Can J Fish Aquat Sci* 46:81–89.

12. Munkittrick KR, Blunt BR, Leggett M, Huestis S, McCarthy LH. 1995. Development of a sediment bioassay to determine bioavailability of PAHs to fish. *J Aquat Ecosys Health* 4:169–181.

13. Schirmer K, Dixon DG, Greenberg BM, Bols NC. 1998. Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. *Toxicology* 127:129–141.

14. Roy NK, Stabile J, Seeb JE, Habicht C, Wirgin I. 1999. High frequency of K-*ras* mutations in pink salmon embryos experimentally exposed to *Exxon Valdez* oil. *Environ Toxicol Chem* 18:1521–1528.

15. Petersen GI, Kristensen P. 1998. Bioaccumulation of lipophilic substances in fish early life stages. *Environ Toxicol Chem* 17: 1385–1395.

16. Guiney PD, Smolowitz RM, Peterson RE, Stegeman JJ. 1997. Correlation of 2,3,7,8-tetracholorodibenzo-*p*-dioxin induction of cytochrome P4501A in vascular endothelium with toxicity in early life stages of lake trout. *Toxicol Appl Pharmacol* 143:256–273.

17. Winkler DL, Duncan KL, Hose JE, Puffer HW. 1983. Effects of benzo[*a*]pyrene on the early development of California grunion, *Leuresthes tenuis* (Pisces, Atherinidae). *Fish Bull* 81:473–481.

18. Leung TSY, Bulkley RV. 1979. Effects of petroleum hydrocarbons on length of incubation and hatching success in the Japanese medaka. *Bull Environ Contam Toxicol* 23:236–243.

19. Linden O. 1978. Biological effects of oil on early development of the Baltic herring, *Clupea harengus membras*. *J Mar Biol* 45: 273–283.

20. Paine MD, Leggett WC, McRuer JK, Frank KT. 1992. Effects of Hibernia crude oil on capelin (*Mallotus villosus*) embryos and larvae. *Mar Environ Res* 33:159–187.

21. Henry TR, Spitsbergen JM, Hornung MW, Abnet CC, Peterson RE. 1997. Early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in zebrafish (*Danio rerio*). *Toxicol Appl Pharmacol* 142: 56–68.

22. Brinkworth LC, Hodson PV, Tabash S, Lee P. 2003. CYP1A induction and blue sac disease in early developmental stages of rainbow trout (*Oncorhynchus mykiss*) exposed to retene. *J Toxicol Environ Health* 66:627–646.

23. Middaugh DP, Chapman PJ, Shelton ME. 1996. Responses of embryonic and larval inland silversides, *Menidia beryllina*, to a water-soluble fraction formed during biodegradation of artificially weathered Alaska North Slope crude oil. *Arch Environ Contam Toxicol* 31:410–419.

24. Vines CA, Robbins T, Griffin FJ, Cherr GN. 2000. The effects of diffusible creosote-derived compounds on development of Pacific herring (*Clupea pallasi*). *Aquat Toxicol* 51:225–239.

25. Marty GD, Short JW, Damback DM, Willits NH, Heintz RA, Rice SD, Stegeman JJ, Hinton DE. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Can J Zool* 75:989–1007.

26. Billiard SM, Querbach K, Hodson PV. 1999. Toxicity of retene to early life stages of two freshwater fish species. *Environ Toxicol Chem* 18:2070–2077.

27. Couillard CM. 2002. A microscale test to measure petroleum oil toxicity to mummichog embryos. *Environ Toxicol* 17:195–202.

28. Helder T. 1981. Effects of 2,3,7,8-tetracholorodibenzo-*p*-dioxin (TCCD) on early life stages of rainbow trout (*Salmo gairdneri* Richardson). *Toxicology* 19:101–112.

29. Kiparissis Y, Lee K, Ho C, Reynolds J, Henry N, Hodson PV. 2001. Chronic toxicity of oiled sediments to Japanese medaka. *Abstracts,* 22nd Annual Meeting, Society of Environmental Toxicology and Chemistry, Baltimore, MD, USA, November 11–15, p 265.

30. Cantrell SM, Joy-Sclezinger J, Stegeman JJ, Tillitt DE, Hannink M. 1998. Correlation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced apoptotic cell death in the embryonic vasculature with embryotoxicity. *Toxicol Appl Pharmacol* 148:24–34.

31. Madill REA, Brownlee BG, Josephy PD, Bunce NJ. 1999. Comparison of the Ames *Salmonella* and Mutatox genotoxicity assay for assessing the mutagenicity of polycyclic aromatic compounds and of porewater from Athabasca Oil Sands mature fine tailings. *Environ Sci Technol* 33:2510–2516.

32. National Research Council. 1985. *Oil in the Sea: Inputs, Fates, and Effects.* National Academy Press, Washington, DC.

33. Heintz RA, Short JW, Rice SD. 1999. Sensitivity of fish embryos to weathered crude oil: Part II. Increased mortality of pink salmon embryos (*Oncorhynchus gorbuscha*) incubating downstream from weathered *Exxon Valdez* crude oil. *Environ Toxicol Chem* 18:494–503.

34. Middaugh DP, Chapman PJ, Shelton ME, McKenney CL, Courtney LA. 2002. Effects of fractions from biodegraded Alaska north slope crude oil on embryonic inland silversides, *Menidia beryllina*. *Arch Environ Contam Toxicol* 42:236–243.

35. Oris JT, Giesy JP. 1986. The photo enhanced toxicity of anthracene to juvenile sunfish (*Lepomis* spp.): Photoperiod effects and predictive hazard evaluation. *Environ Toxicol Chem* 5:761–768.

PAPER

Chemical speciation and partitioning of trace metals (Cd, Co, Cu, Ni, Pb) in the lower Athabasca river and its tributaries (Alberta, Canada)†

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Concentrations of Cd, Co, Cu, Ni and Pb were measured in particulate and dissolved phases at 11 sites located upstream and near Athabasca oil sands development. The *in situ* discrimination between non-labile and labile dissolved metals was done using diffusive gradients in thin-films (DGT) devices. The DGT-labile fraction of Co and Ni was 30% lower near development sites whereas Cu, Cd and Pb showed minor changes spatially. It was found that an 8-fold increase in dissolved organic matter (DOM) near development induced a rapid decrease in DGT-labile metals. Dissolved metal concentrations were used along with DOM, major ions, nutrients, pH and conductivity to calculate the distribution of dissolved metal species using the speciation model WHAM. Labile-DGT metal concentrations agreed well with WHAM-predicted concentrations. It was also found that a significant amount of metals were associated with the non-DGT labile fraction (*i.e.* colloidal DOM) and colloid abundance was more important than suspended particulate matter abundance in influencing metal mobility near Athabasca oil soils development. Since changes in colloidal DOM levels are likely to be the result of surface mining activities, this confirms the serious effects of oil sands activities on metal biogeochemical cycles in the lower Athabasca River.

Introduction

The speciation of trace metals plays a critical role in their distribution and fate in natural aquatic ecosystems. It is an important issue to consider when assessing potential environmental impacts in terms of metal mobility and bioavailability. Whereas the total concentration of metal is routinely measured, it is not a reliable indicator of environmental fate.[1] It has been shown that physical speciation (*i.e.* the distribution of the analyte according to its molecular size) influences the mobility and bio-

logical effects of trace metals. When metals are introduced into an aqueous system by riverine and/or atmospheric input, they interact with inorganic and organic molecules and particles. The affinity of the metals for particles, which settle through the water column, determines the residence time, their concentrations and ultimate fate in the aquatic system. When bound with dissolved ligands such as dissolved organic matter (DOM), metals have a greater residence time. They are generally less bioavailable, because their size prevents them from crossing cell membranes. Partition distribution between particulate and dissolved phases (*i.e.* Kd) plays a key role in regulating the concentrations of metals in natural water systems. Kd is often used in monitoring metal mobility in dynamic systems.[2] The free/labile metals are generally considered to be more bioavailable than metals in strong complexes or associated with DOM, colloids and/or suspended particles.[1] Several techniques including diffusive gradient in thin film (DGT) have been shown to be useful tools for the

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Environmental impact

The environmental performance of Athabasca oil sands development is under intense public scrutiny. This present study will contribute to assessing the water quality of a watershed as strongly recommended by the oil sand advisory panel on water monitoring for the Lower Athabasca River Basin and connected waterways. Canada's and Alberta's water guidelines for the protection of aquatic life were not exceeded for study metals (Co, Cu, Ni and Pb) at most sites except for Cd. On the other hand, size and chemical speciation of the six study metals were significantly altered near the oil sand development relative to upstream sites. The changes in DOM concentrations and composition were found to affect strongly metal speciation near oil sand development.

measurements of free ions and/or labile species.[3–5] Simultaneous use of different techniques such as size fractionation (particulate *vs.* dissolved) and DGT (*i.e.* labile fraction) would thus provide complementary information that results in a more comprehensive picture of metal fate in aquatic systems.

Over the past years, oil sands development in Alberta has been intensified and presents new challenges for environmental management in Canada.[6–9] Since the predominant technologies for extracting bitumen from the oil sands rely on large amounts of freshwater, concerns about the water quality of natural water bodies and its impacts on aquatic life and human health are increasing. The aim of the present study is to determine metal partitioning between particulate, dissolved and DGT-labile fractions in the lower Athabasca River. Change in mobility and bioavailability of metals (Cd, Co, Cu, Ni, Pb) will be discussed considering the potential influence on oil sand development to the biogeochemical cycles of metals in the aquatic ecosystem.

Materials and methods

Sample collection

Surface river water sampling was carried out in June/July 2008, August 2008, June 2009 and July 2009 (Fig. 1). Six sites were chosen on the main stem Athabasca River (AR01-03, AR05, AR09-10) and 4 tributaries (Hangingstone - AR02b, Clearwater - AR04, Muskeg – AR06-07a and Mackay Rivers – AR08) were sampled. Sites upstream of Fort McMurray (AR01-04) were designated as upstream whereas the downstream sites (AR05-AR10) were designated as near development. The July 2008 and June 2009 samplings took place at the end of the spring freshet with a river flow of 879 and 833 m^3/s (Athabasca River below Fort McMurray; Water Survey of Canada). The mid-summer sampling (August 2008) was considered to be at lower flow regime (745 m^3/s) whereas the July 2009 sampling was done at

Fig. 1 Map showing the sampling location in the Athabasca River watershed. AR2b and AR04 are located on Hangingstone and Clearwater Rivers, respectively.Upstream sites (AR01-04) were designated as upstream whereas the downstream sites (AR05-AR10) were designated as near development.

high flow regime (905 m^3/s) as a result of heavy rains in the watershed.

Unfiltered and filtered waters were collected for trace metals (Cd, Co, Cu, Ni and Pb) and major elements (Ca, Na, K, Mg) at each site. Surface water filtration was performed immediately in the field through a 1 μm acid-washed Nuclepore filter into a 60 mL acid-washed polypropylene bottle. Similar manipulations were performed with milli-Q water under field conditions to control the cleanness of the sampling and these field blanks showed metal concentration under the detection limit of the instrument (0.007, 0.007, 0.098, 0.068, 0.040 ppb for Cd, Co, Cu, Ni and Pb, respectively). Ultra clean conditions were maintained during all stages of sample collection, transport, handling, processing and analysis. Samples were acidified with 2% triple distilled HNO$_3$ and kept at 4 °C until analysis.

Triplicates of pre-loaded open and restricted pore diffusive gradients in thin film (DGT) units from DGT Research Ltd. (www.dgtresearch.com) were deployed directly in the river and fixed at 10–20 cm from the surface at five sites for 65 to 94 h in July 2009. All DGT deployment sites were located near development except AR02b. The open pore hydrogel (OP) allows the diffusion of simple metal ions and small organic metal species whereas the restricted pore hydrogel (RP) is thought to allow the free diffusion of simple metal ions and significantly retards the diffusion of humic substances and metal-humic complexes.[3,10] The simultaneous deployment of OP- and RP-DGT helps us revealing the importance of metal–organic complexes based on their capacity to dissociate in the diffusive layer. The resins were retrieved with acid-washed Teflon tweezers, placed in acid-washed centrifuged tubes and stored at 4 °C until analysis. The elution was performed with 1M triple distilled HNO$_3$ in clean room. These solutions were left overnight to allow extraction of metals from the resin before analysis. Non-exposed DGT units were used to assess possible contamination during assembling, deployment, retrieval and extraction. Contaminations of DGT blanks were negligible in comparison with masses of metal accumulated in exposed DGTs.

Trace metal analysis

Metal concentrations (Cd, Co, Cu, Ni, Pb) were measured by high resolution ICP/MS (Element2, Thermo) with indium and rhodium used as internal standards. The accuracy of the ICP/MS measurements was assessed using 1643e (National Institute of Standards and Technology, USA) and SLRS-4 reference water (National Research Council, Canada). Particulate metal concentrations were obtained by difference between unfiltered and filtered samples.

Analysis of other parameters

Calcium and magnesium in unfiltered and filtered waters were analyzed by ICP-OES (Varian Vista MPX). The accuracy of the major element analysis was checked using SLRS-4 reference water (National Research Council, Canada).

Total dissolved organic carbon (DOC) and nutrients (NO$_3^-$, PO$_4^{3-}$, SO$_4^{2-}$) were determined on GFF filtrate (pre-combusted at 450 °C, Whatman) at each station. DOC concentration was measured by the high temperature combustion method on

a Shimadzu TOC-V$_{CPH}$ analyzer.[11] Three to five replicate injections (100 μL each) were performed for each sample, resulting in a typical coefficient of variation <2%. Nutrients were measured by ion chromatography (Dionex). Conductivity, pH and temperature were measured using an AP85 Accumet (Fisher, Canada). Chlorophyll-a (Chl-a), collected on a GF/F filter and extracted with 90% acetone, was measured by the method described in Strickland and Parsons.[12] Total suspended particulate matter (SPM) concentrations were determined by filtering a known volume of river water sample through a pre-weighted glass fiber filter membrane (GF/F, Whatman) dried at 60 °C. The filters were dried and weighed 2–3 times until a constant weight was obtained.

Calculations

Diffusive gradients in thin films (DGT) rely on establishing a linear gradient through the gel while they are deployed.[13] The accumulated mass of metal, M, is measured after elution from the Chelex-100 with nitric acid and used to calculate the concentration C of DGT-labile metal. According to Fick's laws of diffusion, the time averaged concentration of DGT-labile metal is then calculated as:

$$C = M.\Delta g/(D.t.A)$$

where Δg is the thickness of the diffusion layer (0.091–0.095cm), D is the diffusion coefficient at a given temperature, t is the deployment time (from 66 to 94 h) and A is the area of the exposed gel (3.14 cm^2).

Theoretically, the concentrations of metal complexes [Me]$_{1-5nm}$ with a diameter larger than 1 nm but smaller than 5 nm could be estimated based on the following relationship

$$[Me]_{1-5nm} = D_{OP-DGT}/D_{Me-DOM} ([Me]_{OP-DGT} - [Me]_{RP-DGT})$$

where D_{OP-DGT}, D_{RP-DGT} and D_{Me-DOM} are the diffusion coefficients for free metals in OP-DGT, RP-DGT and metal bound to dissolved organic matter, respectively. The range of D_{Me-DOM} values (0.8 to 2.8 × 10^{-6} cm^2/s[14]) reflects the variability in DOM composition and metal binding properties.

Chemical speciation using WHAM

The DGT-labile metal concentrations were compared with those of inorganic species predicted by the Windermere Humic Aqueous Model version 6.0 (WHAM 6). The input variables were the measured dissolved concentrations of metals, major ions, nutrients and physical chemical parameters. The concentration of humic substances was estimated from the DOC concentration as 60% of the DOC with a fulvic to humic acid ratio of 9 : 1.[15] The default binding constant values provided with the model were used.

Results and discussion

Field measurements

The pH values averaged 7.9 ± 0.4 with the lowest values found during rain events in July 2009 (Fig. S1†). As the sampling was performed in summer, the water temperatures were relatively

high (13.8–23.5 °C) with the lowest temperatures recorded during rainy events (July 2009) (not shown). Varying within a narrow range, these two parameters did not account for the change in metal speciation. In contrast, conductivity varied by a factor of 2 between stations with a mean value of 224 ± 32 μS/cm. Average values of DOC ranged from 5.6 ppm-C at the most upstream station (AR01) to 47.7 ppm-C in the development area (i.e. AR05). In open-pit mining, the peatlands covering the Athabasca oil sands region are destroyed to access the underlying bitumen. The high DOC levels in the development area reflect the degradation of peatland and release of DOC into aquatic environment. Strong differences were observed in SPM and concentrations of nutrients between stations and sampling time. For example, AR07-08 located in near development had the lowest SPM and nutrient contents.

Since hardness was not measured directly, it was computed from the results of the calcium and the magnesium determinations. The calculated average hardness ranged from 60 to 100 mg/L as CaCO$_3$ in August 2008 and July 2009, respectively with an average of 77 ± 30 mg/L for both sampling. According to the regional aquatic monitoring program (www.ramp-alberta.org) the hardness as CaCO$_3$ ranged from 88 to 110 mg/L in the Mackay River mouth (close to AR08) and from 87 to 109 mg/L in the Athabasca River downstream of development in summer 2008 and 2009. Similar results were found in the long-term monitoring study of the Athabasca River.[16]

Particulate vs. dissolved metals

The average concentrations of Cd, Co, Cu, Ni and Pb in bulk phases were 0.06 ± 0.05, 0.42 ± 0.53, 1.29 ± 1.51, 1.94 ± 1.36 and 0.53 ± 0.87 ppb, respectively (Fig. S2†). Co and Pb were mainly found in the particulate phase (55–62%) whereas Cu and Ni were mostly found in the dissolved phase (63–72%) in early and mid-summer, and during summer flood events (Fig. 2). On the other hand, Cd partitioning was dominated by particulate phase in early summer (83%). The summer flood sampling showed a 2.3 fold reduction in particulate Cd with 36% of bulk concentration found associated with SPM. With the exception of Cd, spatial distribution of metals indicated that the dissolved phase was more dominant near development (p < 0.05). For example, the dissolved copper fraction averaged 51 and 76% at upstream and near development sites, respectively.

Average bulk and dissolved concentrations of Cd, Cu, Ni and Pb were compared with water quality standards of the Canadian Drinking Water Quality Guidelines,[17] the Alberta Environment Surface Water Quality Guidelines[17] and the US EPA.[18] Currently, there is no national or provincial standard for cobalt limits in Canadian freshwater. Freshwater guidelines for Cd, Cu, Ni and Pb were applied at water hardness ranging from 60 to 100 ppm CaCO$_3$ (based on Ca and Mg measurements). Bulk concentrations of Ni were well below the recommended water quality guidelines (65 ppb[17]) (Fig. S2†). Total concentration of Cu and Pb were below the CCME metal guidelines (0.018–0.033, 2 and 1–2 ppb, respectively) except at AR01 (Cu and Pb; mid-summer flood), AR03 (Cu; mid-summer) and AR09 (Cu; mid-summer flood). On the other hand, bulk concentrations of Cd were above the maximum acceptable concentrations (0.018–0.033 ppb[17]). For example, the bulk Cd guidelines were exceeded
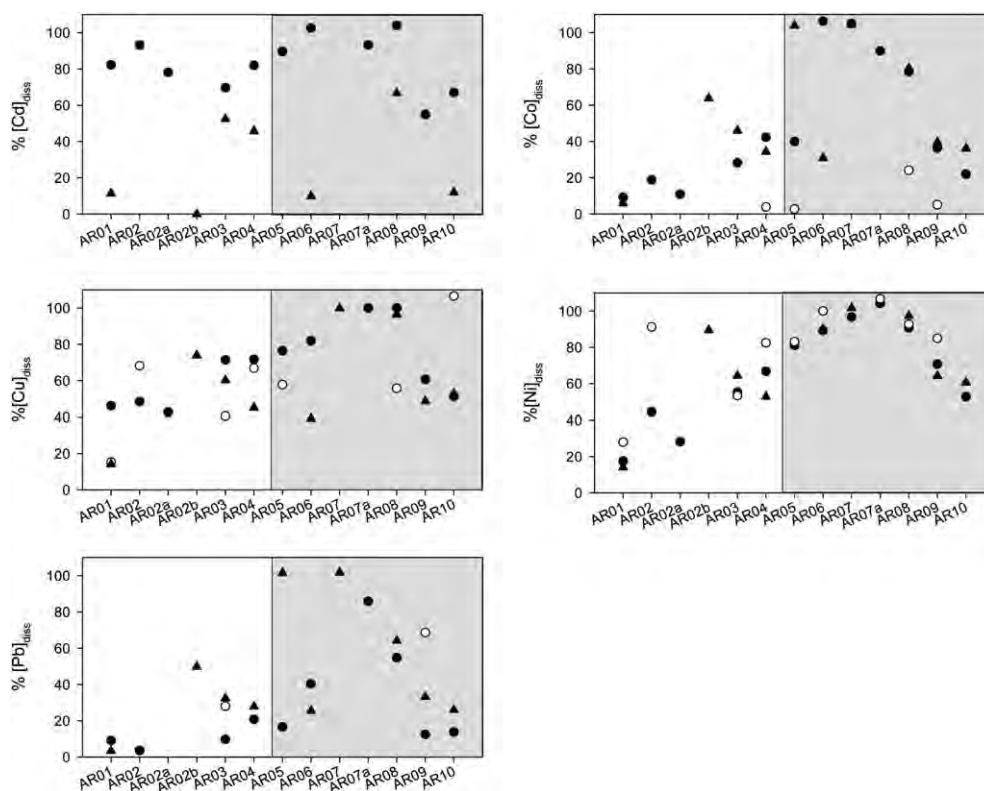
Fig. 2 Percent dissolved metal concentrations in bulk waters in (●) early summer, (○) mid-summer and (▲) mid-summer flood. The shaded area indicates near development sites.

by 1.2 and 1.9 at AR08 in early summer 2009 and mid summer flood, respectively. Alternatively, dissolved Cd concentrations were above the recommended guidelines at AR07 in early summer 2009, at AR04-05 and AR09 in mid summer flood, and at AR08 in both 2009 sampling. The maximum Cd concentrations were measured at AR08 in both bulk and dissolved fractions.

Access restriction to sites near the oil sand development limited our independent assessment of the RAMP data to only one site (i.e. AR08). Moreover, the majority of the RAMP sampling was performed in September-October. Collectively
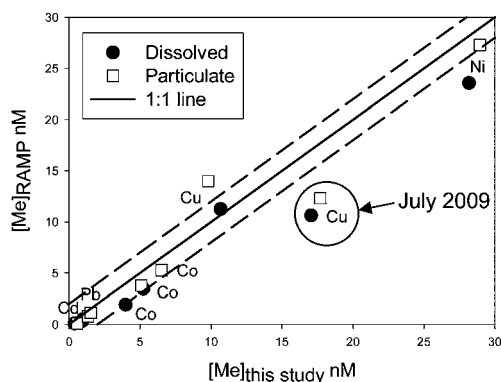

Fig. 3 Intercomparison of trace metal concentrations (Cd, Co, Cu, Ni and Pb) in the dissolved (<1μm filtered) and particle-bound phases between RAMP and our study at AR08.
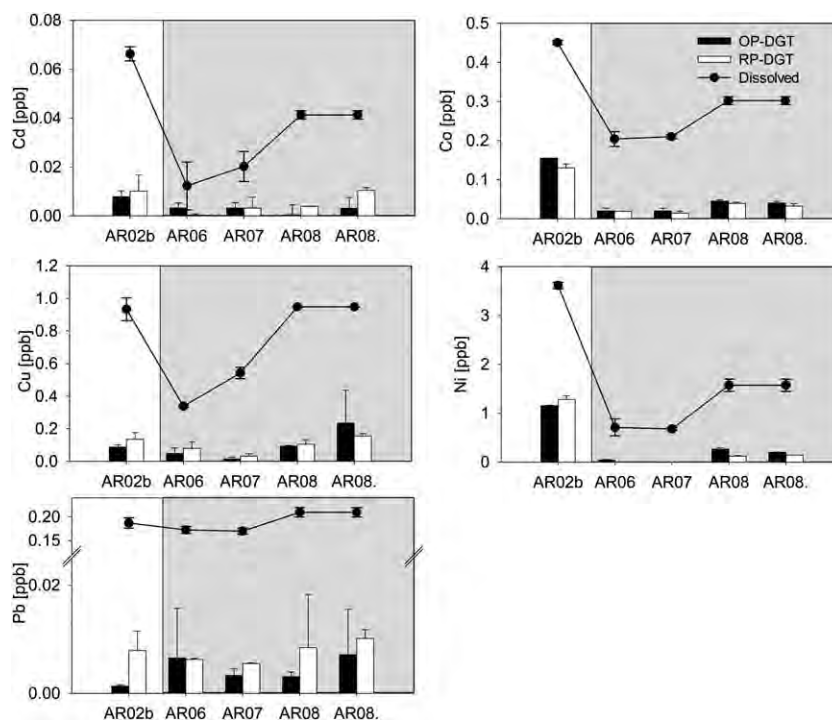
a good agreement was found for all metals at AR08 between the two datasets except for Cu in July 2009 (Fig. 3). Although based on limited samples (i.e. July 2008 and July 2009), water quality monitoring yielded similar metal concentrations in two independent assessments (industry/government funded RAMP program and this work). Such a comparison was strongly recommended by the scientific community.[9]

DGT-labile metals

DGT-labile metal concentrations (Fig. 4) ranged from below detection limit to 34% of dissolved concentrations. An average of 12–16% of the dissolved fraction of Cd, Co, Cu and Ni was DGT-labile whereas only 2% of dissolved Pb was available to the DGT devices. Similar DGT-labile metal fractions were found in previous freshwater studies.[4,19] The upstream site, AR02b, was characterized with the highest DGT-labile fractions of Co and Ni (34 and 32% of the dissolved concentrations, respectively). A 3-fold difference was observed between the upstream and near development sites for both Co and Ni. On the other hand, DGT-labile Pb content was the lowest at all sites with values ranging from 1 to 4% of the dissolved concentration. Average DGT-labile metal concentrations did not exceed the maximum acceptable dissolved concentrations at any sites (2.2 ppb Cd, 9.0 ppb Cu, 52 ppb Ni and 2.5 ppb Pb[18]). Although DGT-labile concentrations were low and may not induce toxic effects based on individual concentrations, metal mixtures can have additive, synergistic and antagonistic effects to aquatic biota.[20,21]

Fig. 4 Trace metal concentrations (Cd, Co, Cu, Ni and Pb) in the dissolved (<1μm filtered) and, OP- and RP-DGT-labile phases. The shaded area indicates near development sites.

Metals unable to diffuse through the DGT gel layer represented a significant fraction (> 84%) in the Athabasca River watershed. The non DGT-labile fraction of Co and Ni was 1.3 fold greater near development whereas Cu, Cd and Pb showed minor changes spatially. It is most likely that the fraction of metal not detected by DGT was complexed by strong large ligands such as DOM and was thus unable to either diffuse through the DGT gel layer or dissociate fast enough in order for the metal to be accumulated by the resin.[13,5] Indeed, fluorescence spectroscopy showed a significant red-shift in DOM fingerprint at the near development sites,[22] suggesting an aromaticity increase. The presence of highly aromatic DOM such as humic substances typically found in peatland at the near development sites favored the formation of highly stable complexes, hence reducing the concentration of free/labile metal ions.

Theoretically, the combined use of OP- and RP-DGT discriminates metal complexes with different size and diffusion coefficients. Previous studies[10,23] suggested that RP-DGT allows diffusion of small inorganic species and excludes labile organic species. In contrast OP-DGT allows the labile fraction of metal associated with larger ligands. Significantly lower DGT-labile concentrations were found for Co and Ni at most sites when using RP-DGT (Fig. 4). The 1–5 nm DGT-labile fraction comprised 1–30%, and 6–55% of the dissolved concentration of Co and Ni, respectively. This can be attributed to a fraction of metals complexed to large ligands unable to diffuse through the RP hydrogel.[13] Cu, Cd and Pb on the other hand did not show any significant difference between the two hydrogels at most sites (Fig. 4). This suggests that small inorganic species (< 1nm) were the predominant DGT-labile forms of Cu, Cd and Pb in the Athabasca River. Also since no significant fractions of Cd, Cu

and Pb were being discriminated by OP-DGT the larger, rapidly dissociating organic complexes were not dominant for these three metals.

The heterogeneity parameter, Γ, derived from the slope of the plot of DGT-labile metal concentration *versus* the ratio of dissolved concentration and DOC,[24,25] is shown in Table 1. For a true kinetically limited binding, $\Gamma > 0.50$ indicates homogeneous ligand while lower Γ values reflect increasing heterogeneity. The Γ values for Cd, Co and Ni of 0.72, 0.67–0.68 and 0.62–0.71, respectively, were consistent with homogeneous binding. These values were comparable to those obtained previously using DGT of 0.8–1.0 for Cd and 0.6 for Ni.[24] They however were slightly lower than those reported in a headwater stream study[25] (1.23 and 1.10 for Cd and Ni, respectively). With correlation coefficients < 0.3, Cu and Pb showed some kinetic limitations and heterogeneous binding. The significant scattering observed in the plot of log [Me]$_{DGT}$ *vs.* log ([Me]$_{dissolved}$/DOC) likely arose from a change in concentration and composition of ligands. This is consistent with significant change in structural composition and molecular weight of DOM observed in the Athabasca River.[22] Cu and Pb are known to complex strongly

Table 1 Values of the heterogeneity parameter, Γ, derived from the slopes of the plots [Me]$_{DGT-labile}$ *vs.* [Me]$_{dissolved}$/DOC.[a]

	Cd	Co	Cu	Ni	Pb
OP-DGT	n/a	0.68	n/a	0.71	n/a
RP-DGT	0.72	0.67	n/a	0.62	n/a

[a] n/a indicates an unreliable linear regression.

with DOM.[26,27] The high stability constant for Cu and Pb binding to DOM might result in slow dissociation kinetics and thus enhance the kinetic limitation.[28]

Comparison of the experimental DGT results with the WHAM VI predictions

Predictions of trace metal speciation in the Athabasca River tributaries was made using WHAM VI. There was good agreement between the WHAM VI predicted metal species and the experimentally determined metal species by DGT in the Athabasca River tributaries ($pMe_{WHAM}/pMe_{DGT} = 0.98 \pm 0.07$; n = 25). The six metals considered varied in the extent of their interactions with the other constituents of the surface waters. This led to substantial differences in their species distributions, and in the concentrations of the labile and organically complexed species. The simulation of metal speciation by WHAM showed that DOM-complexed metal comprised between 4 and 99% of dissolved metal concentration (Table 2). The association with DOM was significant for Cu and Pb (85–99%) whereas Co-DOM and Ni-DOM were always minor species (4–24%). Cd-DOM exhibited intermediate behavior (35–71%). The relative importance of organically-complexed metals agrees with previous studies on river waters [Tipping *et al.*, 1998]. For the studied metals the following order of complexation with DOM was observed: Cu, Pb > Cd > Co, Ni. In general, this order of decreasing binding between metals and DOM was in accordance with the Irving-Williams series of transition element affinity (Cu > Ni > Co) for organic ligands such humic substances. The lower organically complexed fractions of Co and Ni relative to Pb corresponded well with the weaker binding parameters previously reported.[29]

Simulations were performed to infer the impacts of DOM on metal speciation at AR-08 located near a First Nations settlement (Table 2). The hypothetical decrease in DOC contents at AR-08 to that of AR-02b (upstream site) showed that the association with DOM would decrease down to about 46% for Co, 45% for Ni, and 28% for Cd while the amount of Cu and Pb associated with DOM would only decrease marginally by 2 and 3% respectively. The hypothetical changes in organically-complexed metals are a direct consequence of the ability of metal ions to form stable complexes with DOM. Since Co and Ni were significantly below the water guidelines, the increase in labile fractions as a result of hypothetical decrease in DOM would not increase significantly the ecological risks. However for Cd with natural concentrations greater than the Canada's and Alberta's water guidelines, the decrease in DOC concentration will enhance the labile fraction by 40%, which could further enhance potential ecological threat for the ecosystem.

Effects on metal partitioning

The partition coefficients between the particulate and dissolved fractions (Kd) are reported in Fig. 5. A strong affinity between the metal and the particulate phase is revealed by a high Kd value. The average Kd values decreased from 4.47 to 3.56 in the series: Pb = Cd > Co = Ni = Cu. The highest distribution coefficients were found for Cd and Pb, indicating that these elements were preferentially scavenged by particles. The log values of Kd ranged from 4.23 and 5.15 and, from 4.15 to 5.90 for Cd and Pb, respectively. Similar values were found in the freshwater studies.[30–32] As for Co, Cu and Ni, values of log Kd varied from 3.42 to 5.02. These values were in the lower range of Kd values in surface waters based on a literature survey.[33] These slightly lower values of Kd might be due to the effect of organic matter on the solubility of these elements.[34] High DOC concentrations increased the metal binding in the dissolved phase with a consequent decrease in the partition coefficients. In this study, an 8-fold increase in DOC (Fig. S1†) led to a significant decrease
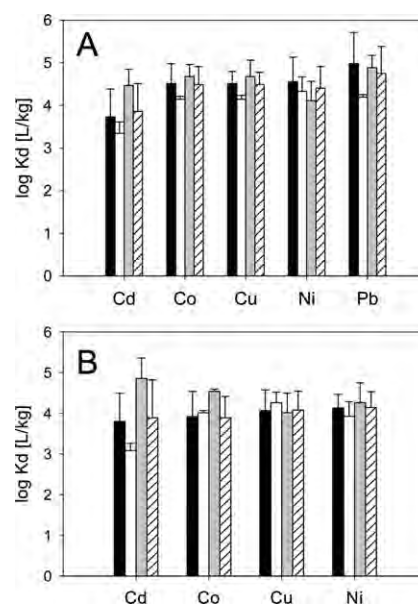

Fig. 5 Temporal variation in metal distribution coefficient between particulate and dissolved phases at (A) upstream sites and (B) near development. Early summer (black), mid-summer (white), flooding event (gray), average (shaded).

Table 2 Percentage of metal bound to DOM calculated by WHAM at DGT sites *, ** indicates the impact of decline in DOC levels from moderate to low DOC levels, respectively, on AR08 metal speciation (see text for more details)

	DOC [ppm-C]	Cd-DOM	Co-DOM	Cu-DOM	Ni-DOM	Pb-DOM
AR02b	18	34.8	4.4	85.4	5.5	86.4
AR06	34	55.5	7.0	97.1	10.6	98.1
AR07	32	70.8	18.9	96.7	24.4	98.8
AR08	33	58.9	5.4	97.8	7.5	96.8
AR08*	25	50.9	4.1	96.9	5.6	95.5
AR08**	18	42.5	3.0	95.5	4.1	93.6

in Kd for Co, Cu and Pb near development sites ($p < 0.05$). The result implies that the release of high amount of DOM during surface mining activities increased the mobility and solubility of metals in the aquatic systems. This agrees well with WHAM model showing the dominance of DOM complexation in the dissolved phase (Table 2). Similar results have been found in freshwater studies.[34] Change in ionic strength, and in particular the competition with major ions including Ca, Na and K, may affect the metal fraction associated with the dissolved fraction.[2,27] No significant correlation with major ions and conductivity were observed in this study.

The metal fraction associated with the dissolved phase was significantly higher near development ($p < 0.05$; Fig. 2). This results in significantly lower Kd values near development relative to upstream sites for all metals except Cd ($p < 0.05$; Fig. 5). The absence of significant spatial difference in SPM and percentage of dissolved Cd support the lack of significant difference in Kd values between upstream and near development sites. The other metals (*i.e.* Co, Cu, Ni and Pb) showed significant higher Kd values at upstream sites. For example, the mean distribution coefficients for Cu decreased from 4.37 at upstream sites to 4.07 near development. Higher Kd values were found during the rainy events for Co, Cd and Pb as a result of lower dissolved metal concentration.

Based on a limited dataset, partition between particulate and DGT-labile (Kp) ranged from 4.73 to 6.38 with Cd and Pb having the highest Kp values (5.46–5.95 and 5.87–6.84, respectively). At all sites, Kp was greater than Kd. This result suggests that a significant amount of metals were associated with the non DGT-labile phase (*i.e.* metal associated with DOM) and this phase was more important than SPM in influencing metal cycling and transport in the Athabasca River system. The average molecular weight of DOM in the Athabasca River measured by field flow fractionation was 1030 ± 60 Da,[22] within size range for colloids (>1nm or ~1000 Da). The 8-fold increase in DOC observed near development sites would enhance the significance of colloids in metal binding. This may explain the reduction in DGT-labile metal fraction and the high Kp's near development relative to upstream sites.

Conclusion

Size and chemical speciation of trace metals in the lower Athabasca River affected by the oil sands development are reported at different flow conditions. Concerns exist over the concentrations of Cd and, Cu and Pb at some sites, as they were above the Canadian and Albertan guidelines for the protection of aquatic life. A noticeable decrease in DGT-labile metal concentration was observed near development as a result of a 8-fold DOC increase. The reasonable agreement between the levels of DGT-labile metals and those of inorganic species, as calculated by the WHAM speciation model, indicate that DGT have measured inorganic labile species. The partitioning particulate/dissolved and particulate/labile showed notable differences between upstream and near development sites. These results suggest that a significant amount of metals were associated with colloidal size DOM instead of particles or kinetically DGT labile species. The colloid abundance was also more important than SPM abundance in influencing metal cycling and transport near Athabasca

oil sands development. This study represents a benchmark for future metal monitoring assessment in the region following expansion of the industry and represents valuable information for future studies. Indeed, the assessment of metal mobility and bioavailability is necessary to identify potential changes associated with the rapid oil sands expansion.
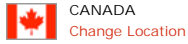
References

1 A. Tessier, D. R. Turner, 1995. *Metal speciation and bioavailability in aquatic systems*. J. Buffle, H. P. VanLeeuwen (Eds), John Wiley and Sons, Chichester.
2 C. Guéguen and J. Dominik, *Appl. Geochem.*, 2003, **18**, 457–470.
3 H. Zhang and W. Davison, *Pure Appl. Chem.*, 2001, **73**, 9–15.
4 P. S. Tonello, A. H. Rosa, C. H. Abreu Jr. and A. A. Menegario, *Anal. Chim. Acta*, 2007, **598**, 162–168.
5 M. H. Tusseau-Vuillemin, C. Gourlay, C. Lorgeoux, J. M. Mouchel, R. Buzier, R. Gilbin, J. L. Seidel and F. Elbaz-Poulichet, *Sci. Total Environ.*, 2007, **375**(1–3), 244–256.
6 Golder Associates. April 2003, report 992-2229, 150p.
7 E. N. Kelly, D. W. Schindler, P. V. Hodson, J. W. Short, Radmanovich and C. C. Nielsen, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 16178–16183, DOI: 1073/pnas.1008754107.
8 E. N. Kelly, J. W. Short, D. W. Schindler, P. V. Hodson, M. Ma, A. K. Kwan and B. L. Fortin, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 22346–22351, DOI: 1073/pnas.0912050106.
9 D. Schindler, *Nature*, 2010, **468**, 499–501, DOI: 10.1038/468499a.
10 H. Zhang and W. Davison, *Anal. Chem.*, 2000, **72**, 4447–4457.
11 C. Guéguen, L. Guo, D. Wang, N. Tanaka and C. C. Hung, *Biogeochemistry*, 2006, **77**, 139–155.
12 J. D. H. Strickland, T. R. Parsons, 1972. *A practical handbook of seawater analysis*, Vol. 167. Bulletin of the Fisheries Research Board Canada.
13 H. Zhang and W. Davison, *Anal. Chem.*, 1995, **67**, 3391–3400.
14 I. J. Allan, J. Knutsson, N. Guiges, G. A. Mills, A. M. Fouillac and R. Greenwood, *J. Environ. Monit.*, 2007, **9**, 672–681, DOI: 10.1039/b701616f.
15 G. C. Dwane and E. Tipping, *Environ. Int.*, 1998, **24**, 609–616.
16 T. Hebben, 2009. Analysis of water quality conditions and trends for the long-term river network: Athabasca River, 1960–2007. Alberta Environment, March 2009.
17 CCME, 1999. *Canadian Environmental Quality Guidelines*. Canadian Council of Ministers of the Environment. Environment Canada. Hull, Quebec; 8 Chapters.
18 USEPA, 1999. *National recommended water quality criteria – correction. Office of Water 4304*, United States Environmental Protection Agency. EPA 822-Z-99–001, 25pp.
19 N. Odzak, D. Kistler, H. Xue and L. Sigg, *Aquat. Sci.*, 2002, **64**, 292–299.
20 S. Preston, N. Coad, J. Townend, K. Killham and G. I. Paton, *Environ. Toxicol. Chem.*, 2000, **19**, 775–780.
21 N. M. Franklin, J. L. Stauber, R. P. Lim and P. Petocz, *Environ. Toxicol. Chem.*, 2004, **21**, 2412–2422.
22 C. Guéguen and C. W. Cuss, *J. Chromatogr., A*, 2011, **1218**, 4188–4198.
23 M.-H. Tusseau-Vuillemin, R. Gilbin and M. A. Taillefert, *Environ. Sci. Technol.*, 2003, **37**, 1645–1652.
24 R. M. Town, P. Chakraborty and H. P. van Leeuwen, *Environ. Chem.*, 2009, **6**, 170–177.
25 K. W. Warnken, A. J. Lawlor, S. Lofts, E. Tipping, W. Davison and H. Zhang, *Environ. Sci. Technol.*, 2009, **43**, 7230–7236.

26 J. Buffle, 1988. *Complexation Reactions in Aquatic Systems: An Analytical Approach*. Ellis Horwood, John Wiley and Sons, New York.

27 E. Tipping, 2002. *Cation binding by humic substances*. Cambridge University Press.

28 K. W. Warnken, W. Davison, H. Zhang, J. Galceran and J. Puy, *Environ. Sci. Technol.*, 2007, **41**, 3179–3185.

29 R. M. Town and M. Filella, *Anal. Chim. Acta*, 2002, **466**, 285–293.

30 A. M. Kraepiel, J. F. Chiffoleau, J. M. Martin and F. M. M. Morel, *Geochim. Cosmochim. Acta*, 1997, **61**, 1421–1436.

31 J. M. Garnier and C. Guieu, *Mar. Environ. Res.*, 2003, **55**(1), 5–25.

32 P. Monbet, *Estuaries*, 2004, **27**, 448–459.

33 J. D. Allison, T. L. Allison, 2005. U.S. Environmental Protection Agency, EPA/600/R-05/074.

34 J. Vesely, V. Majer, J. Kucera and V. Havranek, *Appl. Geochem.*, 2001, **16**, 437–450.

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The Shell True North Forest

CONSERVING LAND FOR GENERATIONS TO COME

The Shell True North Forest is a 1,820 acre (740 ha) tract of land in northern Alberta's boreal zone. The privately owned land was previously used for cattle grazing and hay production. Its purchase on behalf of Shell's oil sands business will conserve area more than twice the size of Vancouver, BC's Stanley Park.

Shell has a land and reclamation strategy in place to guide environmental performance in our oil sands business. As oil sands reclamation - opens in new window takes decades to complete, conserving land allows us to take action in the short term. Over the next decade Shell plans to accelerate the pace of land reclamation and develop technologies to reduce future land disturbance.

The Athabasca Oil Sands Project (AOSP) has been conserving habitat in the boreal wilderness since 2007 as part of a commitment with the Oil Sands Environment Coalition (OSEC). The AOSP committed to spend $2 million over ten years to help mitigate, and partially offset, land and habitat disturbances resulting from existing mining operations. With the addition of the True North Forest, we have now conserved over 3000 acres of habitat offset land.

Location

The Shell True North Forest is located 70 km north of Grand Prairie, Alberta and lies less than one km south of Moonshine Provincial Park. The park offers a range of recreational activities like camping, fishing and hiking. Nearby Jack Bird Pond provides outstanding nature walking and birdwatching opportunities.The Blueberry Mountain Conservation Site is less than nine kilometres northwest of the property.

Managing Biodiversity

Land conservation plays a key role in managing biodiversity. The Shell True North Forest contains mixed woodlands, grasslands, wetlands and habitat along the Ksituan River which runs through the property.

The mixed habitat of established forest and new re-growth creates an ideal environment for high densities of elk, deer and moose, which in turn support cougars and other predators known to inhabit the area. Among the abundance of birds present in the area, is the barred owl, a Species of Special Concern in Alberta.

Future Land Use

The land was secured through an arrangement with the Alberta Conservation Association - opens in new window. Together both parties will develop a conservation management plan to guide future activity on the property. The plan will identify potential opportunities to enhance wetlands or plant additional trees. As portions of the land were previously used for cattle grazing and hay production, these areas will be allowed to reforest over time, naturally returning to their original boreal forest state.

Low impact recreational activities will also be considered as part of the planning process. With excellent road access, the property offers potential for a diverse selection of activities such as hiking and bird watching.

Responsible management will ensure the Shell True North Forest remains a protected natural haven well into the future.

Learn more about the Shell True North Forest (PDF, 2571 KB) - opens in new window

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