Meltwater from snow contaminated by oil sands emissions is toxic to larval fish, but not spring river water


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Fathead minnow
Oil sands
Snow
Spring melt water
PAHs, toxicity

HIGHLIGHTS

• Snow sampled near oil sands surface mining sites decreased larval fish survival.
• Snow sampled far from oil sands surface mining sites caused no chronic toxicity.
• Spring melt water sampled at industry sites was not toxic to exposed larval fish.
• Snow dilution and mixing with river water during spring melt was protective.

GRAPHICAL ABSTRACT

ABSTRACT

To assess the toxicity of winter-time atmospheric deposition in the oil sands mining area of Northern Alberta, embryo-larval fathead minnow (Pimephales promelas) were exposed to snowmelt samples. Snow was collected in 2011–2014 near (<7 km) oil sands open pit mining operations in the Athabasca River watershed and at sites far from (≥25 km) oil sands mining. Snow was shipped frozen back to the laboratory, melted, and amended with essential ions prior to testing. Fertilized fathead minnow eggs were exposed (<24 h post-fertilization to 7–16 days post-hatch) to a range of 25%–100% snowmelt. Snow samples far from (25–277 km away) surface mining operations and upgrading facilities did not affect larval fathead minnow survival at 100%. Snow samples from sites near surface mining and refining activities (<7 km) showed reduced larval minnow survival. There was some variability in the potencies of snow year-to-year from 2011 to 2014, and there were increases in deformities in minnows exposed to snow from 1 site on the Steepbank River. Although exposure to snowmelt from sites near oil sands surface mining operations caused effects in larval fish, spring melt water from these same sites in late March–May of 2010, 2013 and 2014 showed no effects on larval survival when tested at 100%. Snow was analyzed for metals, total naphthenic acid concentrations, parent PAHs and alkylated PAHs. Naphthenic acid concentrations in snow were below those known to affect fish larvae. Concentrations of metals in ion-amended snow were below published water quality guideline concentrations. Compared to other sites, the snowmelt samples collected close to mining and upgrading activities had higher concentrations of PAHs and alkylated PAHs associated with airborne deposition of fugitive dusts from mining and coke piles, and in aerosols and particles from stack emissions.

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1. Introduction

Oil sands surface mining operations in northern Alberta release oil sands-related compounds (OSRCs) into the air from refinery process stacks (National Pollutant Release Inventory, Environment and Climate Change Canada, 2015). In addition, compounds may be released from large tailings ponds (Galarneau et al., 2014), coke piles (Zhang et al., 2016), and mining dusts (Jautzy et al., 2013; Kurek et al., 2013). Numerous studies have shown deposition of OSRCs near oil sands operations (Evans et al., 2016; Harner et al., 2013; Hsu et al., 2015; Jariyasopit et al., 2016; Kelly et al., 2010; Kelly et al., 2009; Kirk et al., 2014; Kurek et al., 2013; S.M. Li et al., 2017; Manzano et al., 2017; Manzano et al., 2016a; Schuster et al., 2015; Zhang et al., 2015).

Recent studies of atmospheric deposition to snow in the oil sands mining area of northern Alberta have shown significant deposition of polycyclic aromatic hydrocarbons (PAHs) and metals. Kelly et al. (2009) found high depositions of PAHs and alkyl-substituted PAHs (APAHS) in areas closest to oil sands stacks. Deposition of total PAHs + APAHS in snow near oil sands surface mining facilities was 2–8 mg/m², with about 95% in the particulate matter and 5% in the dissolved phase (Kelly et al., 2009). In a related study, these authors also found that deposition of certain metals was also highest close to oil sands stacks (Kelly et al., 2010). High concentrations of Be, Pb, Hg, Sb, As, Cd, Cr, Cu, Ni, Ag, Ti, and Zn were deposited in snow particulates close to (<50 km) oil sands upgrading facilities, and concentrations decreased exponentially with distance from the source (Kelly et al., 2010). These studies have assessed the concentrations of OSRCs in melted snow, but have not thus far assessed if the mixtures of chemicals deposited with snow are associated with effects on aquatic organisms. The authors of these studies speculated that the concentrations of PAHs + APAHS in the snow melt pulse could cause toxicity in exposed biota (Kelly et al., 2009). Whether oil sands surface mines’ atmospheric deposition of PAHs is sufficient to harm aquatic species is currently unknown (Hodson, 2013).

Fish embryos and larval stages are particularly sensitive to the effects of PAHs and APAHS (reviewed in Hodson, 2017). Many of the fish species present in the Athabasca River and its tributaries spawn in the spring (late April through May). These species include walleye (Sander vitreus), northern pike (Esox lucius), longnose sucker (Catostomus catostomus), white sucker (Catostomus commersoni), and slimy sculpin (Cottus cognatus) (Bond, 1980; Bond and Machniak, 1979; RAMP, 2015, 2016; Tripp and McCart, 1979). Thus there is potential for exposure of sensitive fish embryos and larvae to PAHs and APAHS when OSRCs-impacted snow melts into the river.

To assess the potential impacts of atmospheric OSRC deposition on aquatic organisms, we investigated the response of exposure to melted snow. We chose sites based on those sampled in Kelly et al., 2009, and were coordinated with a field campaign assessing temporal trends in atmospheric contaminant deposition in 2011, 2012, and 2013 (Kirk et al., 2014). In 2013 and 2014, snow sampling sites were chosen to focus on the Ells and Steepbank Rivers to align with fish and invertebrate sampling sites carried out under the Joint Oil Sands Monitoring (JOSM) program. All snow samples were collected over ice on the river, and spring melt water samples (200 L collected by pump into 20 L stainless steel canisters) were taken directly from the river as the snow was melting, so that the results would represent snow that entered the river when snowmelt occurred, or actual spring melt water (see Supplemental data Table S1 of snow and spring melt water sampling sites and dates, and Fig. S1 of spring melt water sampling times on plots of daily discharge of Athabasca River).

The embryo-larval fathead minnow tests were designed to determine if snow and spring melt water had the potential to affect fish survival and growth. The relevance of any observed effects from the snowmelt exposures was further addressed in the testing of spring melt water, representing what fish would be exposed to in the real environment during spring snowmelt. Spring melt water was always tested at 100% concentration (i.e., undiluted) as this was the concentration to which wild fish would be exposed.

2. Methods

2.1. Site selection

For snow and spring melt water sampling, study sites were chosen to be near (<7 km) industry and upgrading activities, or far from (>25 km) these locations (Fig. 1). Snow and spring melt water samples were collected from sites on the Athabasca River, and two major tributaries, the Ells and Steepbank Rivers. A list of sites with latitude and longitude and dates of sampling is provided in Table S1. Sites were chosen based on those sampled in Kelly et al., 2009, and were coordinated with a field campaign assessing spatial trends in atmospheric contaminant deposition in 2011, 2012, and 2013 (Kirk et al., 2014). In 2013 and 2014, snow sampling sites were chosen to focus on the Ells and Steepbank rivers to align with fish and invertebrate sampling sites carried out under the Joint Oil Sands Monitoring (JOSM) program. All snow samples were collected over ice on the river, and spring melt water samples (200 L collected by pump into 20 L stainless steel canisters) were taken directly from the river as the snow was melting, so that the results would represent snow that entered the river when snowmelt occurred, or actual spring melt water (see Supplemental data Table S1 of snow and spring melt water sampling sites and dates, and Fig. S1 of spring melt water sampling times on plots of daily discharge of Athabasca River).

Fathead minnow embryos were exposed to the melted (ion-adjusted amended) snow for 5 days, and hatched minnows larvae were exposed for a further 7 to 16 days. Embryos and larvae were assessed for % hatch, % deformed fry, growth (length, weight, condition factor) and survival, after exposure to 25%, 50%, and 100% amended melt snow.

2.2. Snow and spring melt water sampling

Since all snow samples were collected on thick river ice, we ensured that the snow collected (and the OSRCs contained in it) would have entered the river during melt. Bulk snow samples (0.37–0.74 M³ from each site) were collected via helicopter and transported immediately to freezer trucks and kept frozen during shipment and in storage until fish exposures began in Burlington, ON, 2–5 months later. Spring melt water was collected through holes bored in ice that covered the river, and 200 L from each site was transported via helicopter, and then transported to Burlington via freezer truck at −20 °C until fish exposures began several weeks after collection.

Snow was sampled in March of each year so that we sampled during maximum snowpack depth but before any significant melting. Historical Fort McMurray snowpack accumulation data from Environment Canada’s National Climate Data and Information Archive was reviewed to target sample collections for maximum snowpack depth. A 100 m² site far from the landing-circle disturbance of the helicopter’s rotors (i.e., 50–100 m distance) was delimited, and snow was first sampled from five 1 m × 1 m areas, focusing initially on the corners and centre of the large square, followed by collection from additional 1 m × 1 m
areas, if necessary. GPS coordinates were recorded from the centre of the sampling area. Snow samples were collected from the surface snow to the depth of the frozen ice surface over the river, to ensure that snow samples represented the full extent of seasonal aerial deposition. Totes were transported to large freezer trucks by helicopter and maintained at −20 °C until toxicity tests were performed. One week prior to the fathead minnow embryo-larval exposures, snow was melted (in food grade polyethylene bags within the covered totes) at room temperature (over 3 days), and snowmelt from totes was pooled and mixed in a large 200 L covered rain barrel container (see Fig. S2 in Supplemental data). Melted snow was transferred to 20 L stainless steel canisters where salts were added (see below), and then snowmelt was stored at 4 °C prior to and during the bioassays. Analyses of contaminants in snow (PAHs, APAHs, metals, and naphthenic acids (NAs)) was done prior to and after salt additions. In 2011 there was enough snow to expose larval fish only until 7 days post-hatch (dph). In 2012, three snow samples were taken at each site, 50 m apart, to assess the spatial consistency of the response observed in 2011. The three snows sampled from each site in 2012 were referred to as snows A, B, and C. Because all of the snowmelt waters were low in essential ions, prior to testing they were augmented with several dry salts to bring the major ion levels up to those observed in the Athabasca River (sampled in the fall of 2009, see Supplemental data Table S2a). The salt additions (see Table 1) enabled fish to survive in the ion-poor snowmelt.

Spring melt water was collected in March–May depending on the year (see Table S1), commencing prior to ice break-up, during the period of snowmelt. See Supplemental data Fig. S1 for spring melt water sampling times and Athabasca River water daily discharge volumes. Spring melt water was pumped into stainless steel canisters and transported at 4 °C to Burlington, ON. Spring melt water was tested undiluted (i.e., at 100% concentration), and was not amended with ions prior to embryo-larval fish exposures.

2.3. Laboratory exposures of fathead minnow

Animal handling and experimental procedures were approved by the Animal Care Committee, which oversees the Aquatic Life Research Facility in Burlington, ON (Animal Use Protocols # 1013-14, 1111-13, 1211, 1310, 1410) operated under the approval of the Canadian Council of Animal Care. Fathead minnow embryos were exposed to oil sands environmental samples (snow or spring melt water) for 19–21 days (except for 2011 when exposure was for 12 days). Detailed methods for the fathead minnow tests have been published previously (Marentette et al., 2015b; Parrott et al., 2016). In tests conducted after December 2013, we improved the method by feeding 2 x per day so that food was always present in the beaker, and decreasing the number of eggs/larvae per beaker to 20 (from 30). This change resulted in better survival rates.

Table 1

<table>
<thead>
<tr>
<th>Salts to add</th>
<th>mM required</th>
<th>mg salt/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂·2H₂O</td>
<td>0.4455</td>
<td>65.5</td>
</tr>
<tr>
<td>CaSO₄·2H₂O</td>
<td>0.4455</td>
<td>76.7</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.5176</td>
<td>43.5</td>
</tr>
<tr>
<td>NaBr</td>
<td>0.0100</td>
<td>1.03</td>
</tr>
<tr>
<td>KCl</td>
<td>0.0294</td>
<td>2.19</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.4059</td>
<td>100.1</td>
</tr>
</tbody>
</table>

Final mg/L for each ion

<table>
<thead>
<tr>
<th>Ion</th>
<th>mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO₄²⁻</td>
<td>81.79</td>
</tr>
<tr>
<td>Ca</td>
<td>35.71</td>
</tr>
<tr>
<td>Mg</td>
<td>9.87</td>
</tr>
<tr>
<td>Cl</td>
<td>32.63</td>
</tr>
<tr>
<td>Na</td>
<td>12.13</td>
</tr>
<tr>
<td>K</td>
<td>1.15</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>25.37</td>
</tr>
<tr>
<td>Br</td>
<td>0.799</td>
</tr>
</tbody>
</table>
larval fish growth over the 16 days post-hatch. Dilution water for snowmelt exposures was obtained from Environment Canada’s Aquatic Life Research Facility in Burlington. The dilution water was obtained from the municipal system (City of Burlington, ON) and was dechlorinated, charcoal-filtered, and UV-sterilized and used as control water exposures for each series of embryo-larval fathead minnow snow exposures. Water quality for the control waters and for the snow dilution waters is shown in Supplemental information, Table S2a.

Replicate exposure beakers (1 L glass beakers, 3 per concentration, 6 per control water) contained 20–30 newly-fertilized fathead minnow eggs (20 eggs for tests from January 2014 forward). Eggs were purchased from Aquatox Laboratories (Guelph, ON) or obtained from an in-house breeding colony. The eggs used in testing had been fertilized 2–18 h before the start of the exposure. Eggs from ≥4 breeding groups were used to provide 20–30 eggs per replicate. Beakers and eggs were randomly assigned treatments and randomly assigned locations in incubators that held temperature at 25 °C and kept lighting at 16 h light: 8 h dark. All snow and spring melt water exposure solutions were changed daily. Fathead minnow eggs and larval fish were held in a nylon mesh-bottomed glass egg cup. The cup facilitated the daily transfer of the embryos or larvae to a new exposure beaker. Beakers were covered and solutions were aerated gently, and changed daily.

Embryos and larvae were inspected daily for deformities or mortality. Immobilized or deformed larvae were removed and euthanized. For larval tests done in 2010–2013, larvae were fed 20 μL/fish of a newly-hatched brine shrimp slurry daily (mean density of 6 nauplii/μL) 2 h prior to the solution changeover. For tests done from 2014 onward, the feeding volumes were 10 μL (0–8 dph) with mean density of 15 nauplii/μL, and 20 μL (9–16 dph) with half of the food allotment fed 1 h after solution changes and the remaining half following solution change. The number of larvae in the beaker was randomly culled at 2 h before solution changes and the remaining half following solution changes were assessed for total length to 0.01 mm (at 6.3× magnification), and mass (to 0.01 mg). At day 19–21 of the test (14–16 dph), surviving larvae were euthanized and measured.

The exposure period covered the sensitive windows of embryo-differentiation, hatching and early development of the larval fathead minnow. The exposure period included time during the embryonic stage (4–5 days), as well as the hatching stage. Exposures continued as the larval fish began to feed and grow for up to 16 days post-hatch (or up to 7 dph for snow collected in 2011). Exposure solutions were renewed daily so that there was a relatively-constant concentration of any OSRCs throughout the 21-day exposure. The endpoints assessed in the eggs and larval fish were survival, growth, and deformities. Specific endpoints assessed in the larval fathead minnows were % survival (egg to hatch, egg to 7–9 days post-hatch (dph), egg to 14–16 dph), growth (length, weight, and condition factor) at 7–9 dph and 14–16 dph, and % deformities at hatch. Deformities were assessed at hatch as in Marentette et al. (2015b), and included pericardial and/or yolk-sac edemas, spinal curvatures, hemorrhages, abnormal (tube-shaped) hearts, and craniofacial abnormalities (such as microphthalmia, edema around the ocular sockets, and/or abnormally small or large jaws).

2.4. Analysis of PAHs, alkylated PAHs, metals, and naphthenic acids in snow and spring melt water

Samples of snowmelt and spring melt water (1 L) were analyzed for 20 unsubstituted PAHs and 17 groups of APAHs (see Table S6a for list of individual PAHs and alkylated PAHs measured) by gas chromatography–mass spectrometry (GC–MS). In 2012, chemical analyses were conducted both prior to and after amending the snowmelt with salts. In 2013, salt-amended snowmelt samples were analyzed for PAHs and alkylated PAHs prior to and after fish exposure to determine if concentrations changed over the 24 h period between solution changes in the fathead minnow embryo-larval bioassay. Separation and characterization of PAHs and alkylated PAHs was conducted using an Agilent 6890 GC equipped with an Agilent 5973 mass selective detector using the methods described in (Wang et al., 2014). Total PAHs and alkylated PAHs are reported in Supplemental data (Table S6b), and detailed results are found in a companion paper (Parrott et al., in prep).

Concentrations of 45 dissolved and total metals (and Hg and methyl-Hg) were measured in snow and spring melt water. Melted snow and spring melt water samples (10 mL) were collected and preserved with nitric acid (Omni-pure) to a final concentration of 2%. Total and dissolved metals were analyzed at Environment and Climate Change Canada’s National Laboratory for Environmental Testing (NLET) in Burlington, ON. The method employed Hotblock digestion followed by inductively coupled plasmaduplomer mass spectrometry with collision/reaction cell capability (SOP#2005, Nelac Method ID: B0401 W, Environment and Climate Change Canada). Dissolved metals refer to those species that are soluble in water under natural environmental conditions. To determine the dissolved metals in water the sample was filtered through a 0.45 μm cellulose acetate filter paper immediately after sampling and before acidification. The 45 elements analyzed included numerous crustal and rare earth elements as well as 13 elements (Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Se, Sb, Ti, Zn) considered priority pollutant elements under the US Environmental Protection Agency’s Clean Water Act. NLET is a certified member of the Canadian Association for Environmental Analytical Laboratories and undergoes regular external reviews to maintain this accreditation. Detailed results for all of the metals are found in a companion paper (Parrott et al., in prep).

Concentrations of total NAs in 2011 snow were determined using high resolution (120,000 resolving power) negative ion electrospray ionization mass spectrometry as in (Headley et al., 2011). Samples were concentrated 100× by passing 100 mL of water through Isolute ENV+ solid-phase extraction cartridges (Biotage, Charlottesville, VA, USA) preconditioned with water and methanol. The cartridges were washed with 10 mL water, and the oil sands organics were eluted with 6 mL methanol. The eluent was evaporated to dryness under N2 and the residue re-dissolved in 1 mL 50:50 acetonitrile/water with 0.1% ammonium hydroxide. A subsample of 5.0 μL of concentrate was introduced into the eluent stream (200 μL/min) using an Accella liquid chromatograph system (ThermoFisher Scientific). Mass spectrometry analysis was conducted using a LTQ Orbitrap Velos (ThermoFisher Scientific) mass spectrometer as detailed in (Frank et al., 2016). In brief, the mass spectrometer was operated in full scan at a m/z scan range of 100 to 600 and the following conditions: sheath gas flow rate, 25 (arbitrary units); spray voltage, 2.90 kV; auxiliary gas flow rate, 5 (arbitrary units); S lens radiofrequency level, 67%; heater temperature, 50 °C; and capillary temperature, 275 °C. The mass accuracy was <2 ppm error for all mass assignments. Software used for molecular analysis was Xcalibur Ver 2.1 (ThermoFisher Scientific) and Composer Ver 1.0.2 (Sierra Analytics). The detection limit for total NAs was 0.007 mg/L, and the limit of quantitation (calculated as 3 x the detection limit) was 0.02 mg/L. Detailed results of NA profiles and total NAs for all snow and spring melt water samples are found in a companion paper (Parrott et al., in prep).

2.5. Statistical analyses

All data were analyzed using Systat 11.0 (Systat Software Inc., San Jose, CA). Statistics was performed on mean values per beaker, with n = the number of replicate beakers per exposure concentration. Fathead minnow survival, growth, and deformity data were assessed for normality (using the Shapiro–Wilks normality test) and statistical analyses were performed on untransformed data as over 87% of the data were normally distributed.

Statistical comparisons of these endpoints to those of controls (lab water exposed embryos and larvae) were carried out using ANOVA to assess whether there was an overall treatment effect. After that, post-hoc two sample t-tests (using Bonferroni’s adjusted p value, with
separate variances) compared mean values from control fish to mean 
values in fish exposed to specific snow or exposure concentrations 
(for example, comparing mean % survival of control larvae to mean % 
survival in larvae exposed to 50% ST2 melted snow).

3. Results

3.1. Water quality parameters for snow and spring melt water

Within each year of experiments there were no differences in expo-
sure conditions of temperature, dissolved oxygen, or ammonia mea-
sured in the fish exposure beakers over the 5 years of testing spring 
melt water and snowmelt, compared to lab water controls. Exposure 
temperatures ranged from 22.8 (± 0.1, standard deviation, s.d.) to 
23.9 (± 0.4 °C) in the various years. Dissolved oxygen ranged from 7.9 
(± 0.2) to 8.6 (± 0.6) mg/L. Free ammonia ranged from 0.000 (non-det-
tectable) to 0.002 and total NH3 + NH4+ ranged from 0.015 to 
0.034 mg/L (Table S2b). The ammonia concentrations in the snowmelt 
exposures of fathead minnow were at least 10 fold below the no ob-
served effect concentration (NOEC) for free ammonia on fathead min-
nov reproduction (NOEC = 0.025 mg/L free ammonia), and at least 
60 fold below the NOEC (of 2.19 mg/L) for total NH3 + NH4+ 
(Armstrong et al., 2012).

Melted snow samples lacked the essential ions necessary to support 
aquatic life. Conductivities measured in melted snow collected in 2011 
ranged from 9 μS/cm for snowmelt from the furthest reference site 
CA1 (277 km away from oil sands surface mines) to 67 μS/cm for snow-
melt from sites close to oil sands surface mining facilities (data in Supple-
mental data Table S2b). Because conductivities in 100% melted 
snow were so low (similar to conductivity of deionized water, approx. 
7 μS/cm), we added several dry salts (CaCl2·2H2O, CaSO4·2H2O, 
NaHCO3, NaBr, KCl, MgSO4·7H2O) to bring essential ions up to the levels 
in Athabasca River water at Fort McMurray (target major ion concentra-
tions in exposure water, in mg/L: SO4 346, Ca 35.7, Mg 9.87, Cl 3.2, Na 
11.9, and K 1.2) and to bring conductivities up to 340–380 μS/cm. With 
essential ion additions the conductivities of 100% snowmelt col-
lected in 2011 ranged from 346 to 392 μS/cm (see Supplemental data, 
Table S2b).

Some snowmelt samples had lower pH than laboratory water prior 
to the addition of salts. Snowmelt collected in 2011 from site CA1 fur-
thest from oil sands surface mines had a pH of 6.8 (± 0.2, s.d.). Other 
snow samples collected from sites far from surface mines, AR1 and 
AR15, both had pH of 7.6, and snowmelt from sites AR6 and ST2 (close 
to oil sands facilities) both had pH of 8.1. The addition of salts to the 
snowmelt raised the pH, and resulted in melted snow with pH of 8.0 
to 8.3 during the fathead minnow exposures, while control lab water 
pH was 8.4 (± 0.0) (Supplemental data Table S2b). The addition of dry 
 salts and resultant increase in the pH of snowmelt waters could affect 
the availability and thus the toxicity of metals in the snowmelt 
(Paquin et al., 2000). However, it should be noted that the snowmelt 
samples with the lower pHs were the ones furthest from oil sands sur-
face mines that generally contained lower concentrations of metals.

Adding essential ions to the snowmelt was effective in supporting 
aquatic life. Survival of embryos through to larval fish in deionized 
(DI) water with essential ions added (78.9 ± 12.6%, mean ± standard 
development, Fig. 2, DI + salts) was identical to control water survival 
(80.5 ± 10.2%).

3.2. Vulnerable developmental stages

Exposure to snow collected near surface mines and emission stacks 
rarely affected fathead minnow embryonic stages but effects were 
seen in larval fish from hatching onwards. From the time-course of 
exposure in Fig. 2 (exposure to 100% snow collected in 2012), it is evident 
that fathead minnow eggs were unaffected by melted snow during the 
5 d exposure of the eggs. Hatch occurred on day 4.5 to day 5 of the 
exposure, and there were no differences in hatch success with any 
snowmelt tested over the 4 years (2011–2014). One exception was snow from ST2 collected in 2014 where hatch was decreased to 38% in 
100% snow, compared to 95% hatch in control water (p = 0.019, 
Bonferroni’s adjusted p value with separate variance comparing treat-
ment mean to control mean) (see Table S3 for survival data for all 

Most of the larval fish mortality occurred after 4 dph (9 total days of 
exposure, Fig. 2). During the snowmelt exposures, larval fish survival 
decreased during the period between 4 dph and 8 dph (days 9–13 of 
the exposure), with some further decreases in survival observed be-
 tween 8 and 15 dph (days 13 to 20 of the exposure, Fig. 2).

3.3. Snowmelt toxicity - survival

Melted snow sampled at three sites close to (<7 km) from surface 
mixing operations and upgraders (AR6, AR7, and ST2) in 2012 was 
toxic to larval fish at exposure concentrations of 50 and 100% (Fig. 2). 
In comparison, larval fish exposed to 100% snowmelt collected in 2012 
from sites far from (site CA1, 277 km, site AR1 at Fort McMurray 
33 km, and site AR15 44 km from oil sands mines) oil sands surface 
mines had good survival, statistically similar to controls over the 20 d 
exposure (control survival 80.5 ± 10.2%, mean ± standard deviation).

Melted snow sampled from sites close to upgraders and surface 
mines reduced larval fathead minnow survival (to 35% and 13% of con-
trol survival) even at diluted concentrations of 25 and 50%, respectively 
(Fig. 3). Melted snow collected on the Athabasca River (AR6, AR7) or 
onto the Steepbank River (ST2) close to mining and upgrading sites caused 
reduced survival in exposed fathead minnow larvae in the lab (Fig. 3, 
all survival data shown in Supplemental data Table S3). Larval survival 
was also decreased by exposure to melted snow collected in 2013 and 
2014 at site ST3, located ~1 km from mining operations on the lower 
Steepbank River.

Snow collected from sites close to (<7 km) industrial activities con-
sistently reduced larval fish survival (Fig. 3). Significant reductions in 
survival were seen in larval fish exposed to snowmelt from sites AR6 
and ST2 from 2011 to 2014 (Fig. 3, p ≤ 0.05, all survival data in 
Table S3). In 2013 and 2014 Steepbank Upper (ST1) and Steepbank 
Lower (ST3) sites were added as they corresponded to wild fish, inver-
brate community, and sediment sampling sites of other components of 
the Joint Oil Sands Monitoring Program (JOSMP) (McMaster et al., 
2017). In 2013 significantly reduced larval fish survival was seen in 
exposures to snowmelt collected from ST2 and Steepbank Lower (ST3)
sites with 29% (p = 0.038) and 59% (p = 0.025) survival, respectively, until 16 dph for the 100% exposure group, compared to 88% survival in the controls (Table S3). In 2014, significantly decreased survival was seen in larval fish exposed to snow collected from ST2 and ST3 sites, with 2% and 47% survival, respectively, until 16 dph for the 100% exposure group (p ≤ 0.001) for both sites compared to 94% survival for the controls (Table S3).

There was some variability in the potencies of snow over the collection years (Fig. 3). Snow from AR6 and ST2 collected in 2011 and 2012 was toxic at 25% concentration, but snow collected from these same sites in 2013 and 2014 did not significantly affect larval fish survival at concentrations of 25% snowmelt, although higher exposure concentrations did reduce larval fish survival (Fig. 3).

3.4. Snowmelt toxicity – deformities and growth

Exposure to snow collected in 2014 near oil sands sites increased fathead minnow deformities at hatch. The percentages of deformities at hatch in control fish (exposed to lab water only) varied with each test and over the 4 year period, but were always under 8%. Similarly, the percentages of deformities in fish exposed to snows collected far from oil sands surface mining (CA1, AR1, AR15, EL2, EL3) sites was always under 7% over the 4 years of snow collections. Trends of increased deformities at hatch were observed in embryos/larvae exposed to snow collected from ST2 in 2011 (44%) and 2012 (16% for snow A, 29% for snow B), but these were not significantly different (p = 0.065–0.165) from the control fish that had 3–8% deformities (all deformity data are in Supplementary data, Table S4). In 2014, exposure of fathead minnow eggs to snow collected from ST2 caused a significant increase in the % deformities in hatched larvae (p = 0.004–0.007, Fig. 4). The most common deformities seen in ST2 exposed hatched larvae were edemas (pericardial and yolk-sac edema or peritoneal edemas), followed by craniofacial malformations, spinal curvature, bubbles (sub-epidermal edemas), and hemorrhages.

Length and weight of larval fish was affected by some of the near-industry snow samples, but changes in these length and weight endpoints did not show increased sensitivity compared to overall fish survival (Fig. 5). Length and weight of larval fish was affected at similar concentrations where decreased survival was observed (all growth data are shown in Supplemental data Table S5).

3.5. Toxicity of spring melt water

Spring melt water collected under ice in March 2010, April and May 2013, and April 2014 was always non-toxic to larval fathead minnow at concentrations of 100%. Most survival to 7–9 dph in spring melt water ranged from 91 to 104% of control fish survival (Fig. 6). Spring melt water collected in 2014 from site ST3 (lower Steepbank River) between the layers of ice, had the lowest survival of all the spring melt waters tested (70 ± 15% survival compared to 93 ± 4% survival for controls, mean ± s.d.), but were not significantly different (p = 0.110) compared to controls (Fig. 6).
Concentrations of PAHs (and especially APAHs) were elevated in unfiltered samples of melted snow from the four sites closest to industry, AR6, AR7, and ST2 and ST3 (Table S6b). At these sites concentrations of PAHs ranged from 550 to 74,500 ng/L and APAHs ranged from 3150 to 199,000 ng/L. Alkylated PAHs made up 71 to 93% of the PAHs measured in snow sites close to industry. Concentrations of PAHs measured in snow sites far from (＞25 km) industry ranged from 30 to 455 ng/L and APAHs ranged from 20 to 1890 ng/L. Alkylated PAHs made up 20 to 84% of the PAHs at sites far from industry (CA1 AR1, AR15, EL2, EL3).

Concentrations of total PAHs and total APAHs were low in unfiltered samples of snow collected at sites far from (＞25 km) surface mining operations. In snow collected far from oil sands surface mining operations (site CA1 277 km away), 25% of the PAHs were alkylated, and concentrations of PAHs and APAHs were low (68 and 23 ng/L, respectively). Snow collected on the Athabasca River close to the town of Fort McMurray (site AR1 which was 33 km south of, or upstream from, the oil sands surface mines) had 50% APAHs in 2011 (150 ng/L PAHs and 145 ng/L APAHs), and 66–67% APAHs in 2012 (201–290 ng/L PAHs and 410–580 ng/L APAHs). Snow collected on the Athabasca River north of oils sands facilities (site AR15) had 39% APAHs in 2011 (60 ng/L PAH and 39 ng/L APAH), and 71% APAHs in 2012 (210–350 ng/L PAH and 520–850 ng/L APAH). All data for PAHs and APAHs are in Table S6b.

Concentrations of total PAHs and total APAHs quantified in filtered snow samples in 2013 and 2014 did not show differences among sampling sites regardless of proximity to mining operations (complete data set in Table S6b). Filtered melted snow samples from AR6 sampled in 2013 had 120 ng/L PAHs and 40 ng/L APAHs, while filtered ST2 snow had 60 ng/L PAHs and 120 ng/L APAHs. Sites with no larval fish toxicity farther from industry (EL2 and EL3 sampled in 2014) had respective filtered concentrations of 20, 74, and 78 ng/L PAHs and 70, 21, and 28 ng/L APAHs. Filtered melted snow from ST2 sampled in 2014 had 87 ng/L PAHs and 29 ng/L alkylated PAHs. These PAH and APAH concentrations in filtered snow samples were similar to concentrations in filtered spring melt water samples collected in 2014 (54–100 ng/L PAHs and 14–65 ng/L APAHs). In 2013 and 2014, there were very few differences seen in PAHs and alkylated PAHs among the filtered snow and spring melt water samples, and most concentrations of individual PAHs and alkylated PAHs in filtered melted snow and filtered spring melt water were close to detection limits.

Concentrations of PAHs and APAHs were relatively stable over the 24-hour fish exposure period (solutions renewed every 24 h). For selected snows collected in 2013, pre-exposure and post-exposure PAHs and APAHs were measured to assess whether the daily renewal of the exposure solutions was sufficient to maintain a constant exposure. The results showed that there was little degradation or loss of these compounds during a 24-hour exposure period. For snowmelt samples collected on the Steepbank River in 2013 (sites ST2 Mid Upper and ST3), post-exposure PAHs and APAHs were 95–105% of pre-exposure concentrations (Supplemental data Table S6c).

Total NA concentrations in melted snow were elevated from sites close to industry (0.21 to 0.25 mg/L) compared to sites far from oil sands surface mines (0.08 to 0.11 mg/L), but concentrations at all sites were ＜0.3 mg/L (Supplemental data Table S7). Full NA data profiles are in a companion manuscript (Parrott et al., in prep).

Thirteen of the 45 metals measured have water quality guidelines (WQGs) for the protection of aquatic life set by the Canadian Council of Ministers of the Environment (CCME, 1999). Mercury was not included in this list. Only Al, Cu, Fe, Pb, and Zn exceeded the WQG in some unfiltered (total), non-amended water samples, though only Cd, Cu, and Fe exceeded the WQ in some of the filtered (dissolved) non-amended water samples (Parrott et al., in prep). However, once the water samples were amended with essential ions to allow for toxicity testing, none of the dissolved metals exceeded their WQG (Table S8). Full metal data profiles are in a companion manuscript (Parrott et al., in prep).

4. Discussion

In this study (over the study period 2011–2014) snow samples collected from four sites close to (<7 km) oil sands surface mining operations caused significantly decreased fathead minnow larval survival. There were also elevated rates of deformities in embryos exposed to snowmelt from one of these sites. In contrast, fathead minnow embryos exposed to melted amended snow samples collected from sites far from oil sands mining and upgrading activities showed no elevated toxicities or rates of deformities relative to controls.

In rivers in the oil sands region, fish embryos and larvae are not exposed to pure melted snow, as snowmelt mixes with river waters prior to exposure. Thus, the significance of the snow toxicity needed to be assessed after snow mixed with river water. To do this, under ice melt waters (spring melt water) were collected from the Athabasca River in 2010, and from the Steepbank and Ells Rivers in 2013 and 2014. The spring melt water samples collected under ice represent the combination of melted snow with Athabasca, Steepbank, and Ells River waters. Exposures of fathead minnows in the lab showed these spring melt waters were not toxic and did not cause deformities in exposed minnows. This multi-year study suggests that although the mixtures of chemicals in snow collected close to (<7 km) oil sands facilities affect larval fish survival in the lab, the mixing and dilution of the snow as it melts and combines with river waters ameliorated any effects. This theory is supported by comparing the sum of PAHs + APAHs in snow and spring melt water from AR6 and ST2. The total concentration of PAHs + APAHs in AR6 spring melt water was about 50 ng/L while the concentrations in AR6 snow ranged from 23,815 to 273,140.
studies are required to assess the impact of meltwaters on the Athabasca River could be exposed to spring melt waters for several weeks, future studies are required to assess the impact of meltwaters on fish species that have longer embryonic development periods than the fathead minnow, which hatches in 5 days. Species such as walleye and white sucker have embryonic developmental periods of 18–20 days, and so they may be more sensitive than fathead minnow to the OSRCs in snow and spring melt waters (Colavecchia et al., 2006; Raine et al., 2017).

The potencies of snow collected at the sites near oil sands facilities varied over the four years of sampling. The least potent snow samples were collected in 2013 and the most potent in 2012. Variation may have been caused by differences in releases, or changes in wind patterns that affected fugitive dust deposition, and variations in snow deposition/snow melt periods. Our observations of 2012 snow being the most potent and having the highest PAHs, and 2013 being the least potent snow agree with polycyclic aromatic compound (PAC) loading estimates in snow samples collected over the same period. Manzano et al. (2016a) estimated that within a 50 km radius of oil sands industries 1800 kg PAC was deposited in 2012, compared to 1236 kg in 2011, 814 kg in 2013, and 1367 kg in 2014.

The sources of the OSRCs in snow and the toxicity (or availability) of the OSRCs to larval fish appeared to differ between the two near-industry sites AR6 and ST2. When survival was plotted against the total concentrations of 17 measured alkylated PAHs in the unfiltered snow, the ST2 sites were similar (close together) and the AR6 sites were similar and in another grouping (Supplemental data, Fig. S3). Although there was variability year-to-year, it appeared that effects in fish occurred at lower total alkylated PAH concentrations for ST2 snow (2000–120,000 ng/L) and higher total alkylated PAH concentrations for AR6 snow (10,000–200,000 ng/L). The observations of the colour of the melted snow from the AR6 and ST2 sites were consistent with the differences observed in snow potencies and PAC profiles. AR6 snow was black, while ST2 snow was generally brown with finer particulate (see Supplemental data, Fig. S4 for picture of filtered samples of snow particulates). By visual inspection, it appeared that the AR6 snow contained particulate dusts from the piles of petcoke stored nearby on the oil sands industrial site. This is supported by findings of other recent studies. At sites within 30 km of oil sands facilities, snow samples had similar profiles of heterocyclic aromatics as delayed petcoke (Manzano et al., 2017). Analyses of living mosses and historical cores from peat bogs near oil sands facilities also suggested that petcoke contributed 45–95% of the PAHs, and scanning electron microscopy showed that large petcoke particles were present in snow samples close to industry sites (Zhang et al., 2016).

The types of particles in the snow may affect (A)PAH bioavailability and thus larval fish toxicity. Particles in AR6 snow were black, and comprised largely of petcoke (discussed above). The high concentrations of (A)PAHs on the AR6 petcoke particles may have been largely unavailable to larval fish. By contrast, ST2 particles were brown and finer grained, and although they had lower (A)PAH concentrations, the (A)PAHs on the particles may have been more available to the fish (see Fig. S5 for profiles of PAHs and APAHs in AR6 and ST2 snow particulates). This may explain the discrepancy between toxicity at the two sites: ST2 snow had lower total (A)PAHs but was more toxic than AR6 snow.

Snow collected far from (<25 km) upgraders and active surface mining sites did not affect larval fathead minnow survival (see green rectangle in Fig. 6). The sites CA1, AR1, AR15, Ells downstream, and Ells Upstream caused no significant negative effects on survival of larval minnows exposed for up to 21 days to 100% melted snow (Fig. 6 and Table S3).

The survival data from exposure to pure snow collected at the four sites close to industrial operations should be interpreted with caution. It shows that the OSRCs accumulated in some snows over the 4–5 month period were able to affect fish survival. However, it does not indicate the environmental relevance of the deposition, as the exposure of wild fish would be to the melting snow diluted in spring with river waters. This is why we tested in parallel the effects of the spring melt water sampled over several years.

Spring melt water samples were not toxic to exposed larval fish. In all cases, river water collected during spring melt in 2010, 2013, and 2014 did not significantly affect fathead minnow embryo-larval survival even when exposure was to 100% spring melt water (see blue ellipse in Fig. 6). In 2010, survival after exposure to 100% spring melt water ranged from 82 to 85%, in 2013 it ranged from 80 to 89%, and in 2014 survival ranged from 70 to 97% during the spring freshet (snow melting and ice melting period), the dilution of the snowmelt with river water was able to ameliorate the negative effects of the snow on the survival of larval fish. When plotted against the concentrations of alkylated PAHs in the snow exposure solutions (Fig. 7), the spring melt water samples (open symbols) have >75% survival and low concentrations of total alkylated PAHs (10 to 100 ng/L).

The timing of the spring melt water collection is important, as spring melt is characterized by an uneven concentration of contaminants in melt water. Concentrations of contaminants in snowmelt can be affected by many parameters including water solubility of the contaminants, snow grain size (fine or coarse), and the particles present in snow (Meyer et al., 2011; Meyer and Wania, 2008, 2011; Parajulee et al., 2016).
In general, water-soluble organic substances are released in high concentrations at the beginning of the melt period, while hydrophobic organic pollutants (such as larger PAHs) associated with particles are released at the end of melting (Meyer et al., 2009a, 2009b). Particles in snow can affect contaminant release: The release of phenanthrenes from clean snow occurs mostly at the start of the melt, while release from dirty snow occurs almost entirely as a pulse at the end of the snowmelt (Meyer and Wania, 2011). We collected most of our spring melt water samples after the snow had melted on the river (but snow remained in the surrounding catchment), and a thick layer of ice remained on the river. In 2013, we collected early (April) and a late (May) spring meltwater samples, and the late samples contained higher concentrations of total PAHs + APAHs (110 and 125 ng/L) compared to early spring melt waters (0–25 ng/L). We did not collect multiple spring melt water samples over time (within a year) to assess the potential differences in contaminant concentrations. Collection of spring melt waters (over time as the snow melts in the entire river catchment) at the sites closest to oil sands surface mines would provide important data on the peak concentrations of OSRCs in spring melt waters and freshet.

It is not likely that naphthenic acids in snow close to industrial operations caused the decreased larval fish survival observed. Although total naphthenic acids in melted snow were elevated from sites close to industry (~7 km), concentrations were <0.3 mg/L, which were well below the concentrations of naphthenic acid mixtures from multiple sources that caused mortality in fathead minnow eggs and larvae (about 5–10 mg/L) (Marentette et al., 2015b). Although measurement of total NAs does not take into account the possibility of highly toxic individual NAs, the majority of literature indicates that toxicity does not vary widely across NAs (Arens et al., 2015; Bauer et al., 2017; Kinley et al., 2016; C. Li et al., 2017; Marentette et al., 2015a; Marentette et al., 2015b; Morandi et al., 2015) although to date, very few individual NAs have been tested. Similarly, based on the fact that the concentrations of dissolved metals in the 2012 amended snows were below existing water quality guidelines, it is unlikely that metals were responsible for the observed effects. However, only 13 of the 45 metals measured have a water quality guideline (CCME, 1999).

Our results suggest that the cause of decreased survival of larval fish was likely the parent and alkylated PAHs in the snow. Aqueous total PAH concentrations from 300 to 60,000 ng/L have been found to result in lethal and sublethal effects in various species of fish (reviewed in Lee et al., 2015). Although there is a lot of scatter in the data in Fig. 7, the relationship between fathead minnow survival and APAHs (for all samples of snow and spring melt waters collected over the five years of this study) fits a sigmoidal curve with a chronic LC50 of 13,000 ng/L (Supplemental data Fig. S6). This chronic LC50 for snow collected close to oil sands surface mines is within the range of total PAH LC50s in fish. It is also possible that other PACs containing N and S heteroatoms recently characterized by Manzano et al. (2016b), could be associated with these effects. Concentrations of parent and alkylated PAHs were high in snow collected close to (~7 km) industry sites. Alkylated PAHs made up 71–90% of the PAHS in the snow samples collected close to industry. There are several studies showing that alkylated PAHs increase deformities and reduce survival of larval fish (Hodson, 2017; Lin et al., 2015; Rhodes et al., 2005; Scott et al., 2011). Studies of PAHs and APAHs in crude oil have shown that most of the cardiac toxicity in larval fish is caused by the phenanthrenes and dibenzothiophenes (Incardona et al., 2009; Incardona et al., 2004; Incardona et al., 2006) and that fractions containing 3–4 ring (A)PAHs cause most of the embryo toxicity (Adams et al., 2014). However, more fish toxicity data are needed on individual APAHs, N- and S-containing (A)PAHs, as well as effects-directed analyses of extracts containing (A)PAHs mixtures, to determine which compounds (or groups of compounds) are responsible for the observed toxicity.

It is not clear whether the concentrations of dissolved PAH in our study were high enough to decrease survival in fathead minnows.

Most studies have confirmed that PAHs need to be in the dissolved form to enter an aquatic organism (Carls et al., 2008; McIntyre et al., 2016). Total dissolved concentrations of PAHs and alkylated PAHs were low in this study (about 20–100 ng/L), with similar concentrations in the most potent snows and in the non-toxic snows or in non-toxic spring melt waters. Most studies of dissolved PAHs show that higher concentrations than those in this snow study are required to elicit effects in larval fish. Dissolved PAHs from crude oil are toxic to fish embryos and larvae (mahi mahi, Coryphaena hippurus) at concentrations of ΣPAHs0 (sum of 50 PAHs + alkylated PAHs) in the range of 3000 to 20,000 ng/L (LC50s) (Esbaugh et al., 2016) and 7000 to 15,000 ng/L (Edmunds et al., 2015). Dissolved concentrations of 3-ring PAHs that affect mahi mahi embryos are lower, ranging from 3000 to 4000 ng/L (Edmunds et al., 2015; Esbaugh et al., 2016). Embryonic exposure to total dissolved PAHs (sum of 35 PAHs and alkylated PAHs) of 230 ng/L reduced juvenile Pacific herring (Clupea pallasi) swimming speed after 7 months in clean water (Incardona et al., 2015).

As expected, most of the PAHs in the snows we collected close to industry were in the particle fraction, with very low concentrations in the dissolved fraction. This is similar to Zhang et al. (2016) who reported that 88% of the total PAHs in snows from the oil sands area were associated with the particle fraction, and Manzano et al. (2016a) who found highest percentages of particle-bound PAHs and APAHs close to oil sands surface mines. However, the presence of the particles with very high concentrations of parent and alkylated PAHs may have continuously replenished the dissolved fraction of PAHs and alkylated PAHs in the AR6, ST2, and ST3 snow samples. Total PAHs (particulate + dissolved) in snow samples close to industry were up to 273,000 ng/L. The dissolved PAHs in the spring melt water or in far-from-industry snow samples could not have been replenished as the total PAHs were much lower (up to 130 ng/L for spring melt water, and ~2000 ng/L in snow far from (~25 km) surface mining operations). The dissolved PAHs in snow samples (collected close to industry that contained particles high in PAHs and APAHs) could have been accumulated by larvae and reached toxic concentrations about 9–13 days into the exposure. We have not measured tissue concentrations of the PAHs and alkylated PAHs in fish bodies, so we cannot confirm this ‘replenished dissolved PAH exposure’ theory.

Alternatively, the particles could be the source of the toxicity in the snows collected close to oil sands mines. Particles were the source of PAH-related toxicity in zebrafish embryos exposed to sediment pore waters from a contaminated site (Fang et al., 2014). Larval fish ingested some of the snow particles during the exposures to AR6 snow, as black particulate was observed in their stomachs after exposure to 2012 snow from site AR6 (see Supplemental data, Fig. S7). The concentrations of alkylated PAHs and PAHs in the unfiltered snow ranged from about 24,000–273,000 ng/L at sites AR6 and 4000–21,000 ng/L at site ST2. The concentrations in unfiltered snow are within the range of PAH and alkylated PAH concentrations that affected larval fish in studies of crude oil (LC50s of 9000–46,000 ng/L, total PAHs; (Esbaugh et al., 2016)).

This is the first study to show that aerial deposition of OSRCs at sites close to industries can affect larval fish survival. However, the impact of the melted snow on fish early-life stages may be mitigated, as river water collected during spring melt water at the same locations did not affect fathead minnow embryo-larval survival. The snow and spring melt water collections occurred over several years and the toxicity findings were repeatable.

5. Conclusions

Controlled lab exposures showed that major ion-augmented melted snow from sites near (~7 km) oil sands facilities decreased larval fish survival, while exposure to melted snow far from (~25 km) mines and stacks did not affect fish. Aerial deposition of fugitive dust particles and aerosols from oil sands mines, coke piles, and stacks can result in
snowmelt that is toxic to larval fish. Observed toxicity corresponded with the quantity of parent PAHs and alkylated PAHs deposited in the near-industry snows over the 5 month winter period. Spring melt water collected from the same sites near oil sands industries over several years did not affect survival of larval fish. Based on our studies with fathead minnows, this suggests that the dilution of the contaminants in snow as it melts in the spring and mixes with river water is currently sufficient to confer a protective effect for larval fish in local rivers.

Acknowledgement


References


