Chronic toxicity of oil sands tailings pond sediments to early life stages of fathead minnow (*Pimephales promelas*)

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**ABSTRACT**

In this study fathead minnow (*Pimephales promelas*) embryo-larval stages were exposed to two oil sands tailings pond sediments which had previously been shown to decrease the survival of embryo-larval larval stages of walleye (*Sander vitreus*) and northern pike (*Esox lucius*). Fathead minnow are standard test species and we wanted to compare their sensitivity to the other two species. Fathead minnow larvae were exposed for 20 days (5 days in the egg stage and 15 days in the larval stage) with daily renewal of sediments and waters. Sediments contained polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs (APAHs). Results from an earlier study showed that Sediment 1 contained 173 μg/g total PAHs + APAHs (97 % alkylated), and sediment 2 contained 401 μg/g total PAHs + APAHs (95 % alkylated). Fathead minnow larvae exposed to oil sands tailings pond sediments had decreased survival, decreased weight, and increased deformities. Fathead minnow survival was unaffected at the embryo stage and at hatch. Most deaths occurred at the larval stages 1–8 days after hatching, showing the importance of exposing the fish for at least a week after hatch. Toxicity was seen at 0.2 g/L of sediment, which was equivalent to the addition of 35 and 80 μg total PAHs + APAHs to 1 L of overlying water for sediment 1 and 2, respectively. When compared to embryo-larval northern pike and walleye results from previous studies, all three species of fish responded more strongly to sediment 2 compared to sediment 1. For effects on lethality, fathead minnow were equally sensitive to pike, but walleye were 5–28 times more sensitive to the lethal effects of the sediments compared to both fathead minnow and pike. The study (and comparisons to our previous studies) shows the difference in sensitivity between a model laboratory species (fathead minnow) and some species of wild fish that are highly relevant to the oil sands area of Alberta.

**1. Introduction**

Oil sands refining requires large quantities of water that is re-used and stored on-site in tailings ponds. Oil sands tailings pond waters contain chemicals found in the bitumen (salts, metals, naphthenic acids, and polycyclic aromatic hydrocarbons (PAHs)) (Small et al., 2015). PAHs, especially alkylated PAHs (APAHs), are rich in bitumen, and in oil sands tailings pond waters these exist as oily films and bitumen slicks on the surface of the tailings ponds. The PAHs and APAHs can also adsorb onto fine clay particles and settle in bottom sediments of the oil sands tailings pond. As a result, the oil sands tailings ponds contain a thick layer of sediment (residual bitumen and fine clay particles), which serves as a barrier against the oil sands tailings pond water leaching (Ferguson et al., 2009). This oil sands tailings pond sediment contains high concentrations of PAHs and alkylated PAHs, which can decrease larval fish survival and growth (Raine et al., 2017, 2018) similar to the effects of PAHs and APAHs from crude oil in marine fish species (Brette et al., 2017; Carls et al., 2008; Incardona et al. 2004, 2014, 2015).

Fathead minnow (*Pimephales promelas*) embryo-larval exposures have been successfully used to study the toxicity of oil sands naphthenic acids, river sediments, and snowmelt (Colavecchia et al., 2004; Marentette et al., 2015a,b; Parrott et al., 2018). Embryo-larval exposures are advantageous because the embryological and larval stages are sensitive to toxicant exposure (Embry et al., 2010; Hodson, 2017; Lillicrap et al., 2016). Survival and growth of hatched larvae can also be assessed, and the duration of the exposures is substantially shorter than full lifecycle tests, making these methods more practical when testing large numbers of samples. The fathead minnow embryo-larval assay was therefore used to examine lethal and sublethal effects after exposure to two oil sands tailings pond sediments from an oil sands mine in northern Alberta.
The toxicity of oil sands two tailings pond sediments has been assessed in embryo-larval walleye (*Sander vitreus*) and northern pike (*Esox lucius*) (Raine et al., 2017, 2018). The two oil sands tailings pond sediments were toxic to larval stages of walleye and pike at concentrations as low as 0.04 g wet weight sediment/L (equivalent to 16 µg total PAHs + APAHs/L). Here we expose embryo-larval fathead minnow to the same two oil sands tailings pond sediments, and assess their survival, deformities at hatch, and growth. We also compare the relative sensitivities of the three fish species to the oil sands tailings pond sediments.

The fathead minnow 3-week chronic static-renewal exposure assay is used in our laboratory for assessment of sediments as well as pure compounds. It is a relevant assay that is useful for environmental risk assessments. The endpoints of survival, growth, and deformities are clear and interpretable in terms of fish population survival and health. When effects are manifest at the sensitive embryo-larval stages (such as with sediments from oil sands tailings ponds), this 20-21-day chronic exposure is our method of choice, and can be a surrogate for lengthy and difficult fish lifecycle exposures.

Fathead minnows are an environmentally-relevant species and are found in tributaries of the Athabasca River (Bond and Machniak, 1979; Tripp and McCart, 1979). They are also used extensively in aquatic toxicity testing (Ankley and Villeneuve, 2006). Because routine toxicity tests employ fathead minnow, questions on the relevance of the results to other fish species present in the ecosystem are warranted. So we wanted to compare their relative sensitivity to two previously tested species (walleye and northern pike) that are rarely used in toxicity testing, as they are difficult to culture and their embryo-larval stages are available for a short time in May each year. All three fish species are present in the Athabasca River or in local tributaries. However, the health of walleye and northern pike are of special concern to sport angler and local Aboriginal groups, thus the relative sensitivity of the standard laboratory test species (fathead minnow) was important to assess in comparison to these two wild fish species.

In particular, First Nations communities in the Athabasca River area are concerned with the impacts on walleye and pike (as they value these fish and consume them). So the relative sensitivity of these species is important to scientists who study the oil sands, to oil sands regulators, and to First Nations people living in the area downstream of the oil sands. The study, and the species comparisons, are also of value to a wider group of scientists who study freshwater oil spills.

2. Methods

2.1. Sediment collection and exposure beaker preparation

Sediments were collected from two locations in one active oil sands tailings pond during September 2009 as described in (Raine et al., 2017). Fathead minnow embryo-larval exposures to oil sands tailings pond sediments occurred in Jan–Feb 2010, and walleye and northern pike embryo-larval exposures reported previously (Raine et al. 2017, 2018) occurred in May 2010 and May 2011, respectively. Oils sands tailings pond sediments were stored at 4 °C in the dark (in food-grade polyethylene bags inside plastic pails with lids) until fish exposures began. Subsamples of sediment were analyzed for PAHs, alkyl PAHs, and naphthenic acids in May 2010, as described previously (Raine et al., 2017). One week prior to the fathead minnow embryo-larval exposures, aliquots of wet sediment (1 g, 0.2 g, and 0.04 g) were weighed into individual scintillation vials and kept in the freezer. Normally for sediment exposures, we assess 1, 5, and 25 g sediment/L. For these oil sands tailings pond sediments, there was complete lethality at 1 g sediment per L, so we continued reducing the concentrations in a geometric series, by dividing the previous concentration by 5. Thus the concentrations tested were 0.04, 0.2, and 1.0 g sediment/L. Daily, vials of sediment were withdrawn, thawed in the fridge, and used to prepare new fish exposure solutions. Sediment exposure solutions were prepared the day prior to exposure and placed in a 25 °C incubator to warm and equilibrate. Daily solution changeovers involved the transfer of eggs/larval fish and egg cups to a new equilibrated beaker. Changeovers of solutions were always done 2 h after fish feeding, to maximize the removal of fish waste and excess food. In more recent exposures of fathead minnow embryos and larvae to sediments, we have used river sediments (Upper Ells River, Upper Steepbank River) from outside of the oil sands area as reference sediments (Droppo et al., 2019; McMaster et al., 2018). Sediment controls are necessary when sediment loading to beakers is high (e.g. 35 g sediment/L) (Prosser et al., 2017), however we have previously shown that reference river sediments in the oil sands area had no effects on fathead minnow survival at 25 g/L (Raine et al., 2017). In the present exposures of oil sands tailings pond sediments, we did not utilize a reference sediment, as sediment loading was very low (up to 1 g sediment/L).

2.2. Fathead minnow embryo-larval tests

Exposures to oil sands tailings pond sediments were conducted during January and February of 2010. All exposures of fathead minnows oil sands tailings pond sediments, and sampling and euthanasia methods, were conducted under an approved animal use protocol (#0917) from the Department of Fisheries and Oceans/Environment Canada Joint Animal Care Committee for the Canada Centre for Inland Waters (Burlington, ON, Canada), operated under the approval of the Canadian Council of Animal Care. Fathead minnow early-life stage assays were performed in accordance with OECD TG 210 (1992) guidelines but typically ended at 15 days post-hatch (dph) which was at day 20 of exposure. Typically in our laboratory the test is run until 15–16 dph, for a total of 20–21 days of exposure. For most chemicals and sediments, toxicity occurs within the first week after hatch. The test is terminated at 15–16 days post-hatch, as running to 28 dph (as in OECD TG 210 (1992)) would not be possible with the static-renewal procedures we use.

Eggs were collected from an in-house fathead minnow breeding culture, and were less than 24 h post-fertilization (24 hpf) at the start of the exposure. Our exposure encompasses all the embryonic stages after 24 h post-fertilization (for fathead minnow this 24 hpf is at the end of the neurula stage, and before heartbeat and movements start at 30 hpf or 1.25 dph). Replicates were started with eggs from at least 3 breeding groups to maximize genetic diversity and variability, so in total eggs from 12 breeding groups were used to start the exposures. There were 30 eggs per 1 L beaker, and 4 replicates of each sediment exposure concentration, and 8 replicates of controls (water only). Temperature was maintained by housing all beakers within an incubator set to 25 °C with 16 h light and 8 h dark photoperiod. Temperature, pH, conductivity, dissolved oxygen and free ammonia were measured in fish exposure beakers several times during the test (Supplemental Data Table S1). All parameters were within acceptable limits over the test periods.

The sediment and water are renewed daily to mimic a “worst case” scenario. With daily water and sediment renewal we are able to keep the exposures relatively constant over the 21 d exposure. Static-renewal exposures began at 0 days post-fertilization (dpf) and ended at 15 dph, for a total exposure of 20 days (with hatch at 4–5 dpf). Eggs and larvae were reared in aerated 1-L glass beakers (with 1 L test solution) in egg cups (glass cylinders with fine Nitex nylon mesh) to facilitate daily transfer between old and new solutions. Fish exposure solutions and beakers were renewed daily over the 20-day exposure. Mesh-bottomed egg cups were replaced and washed at hatch on day 5, and at first larval fish sampling on day 13 of the exposure (which was 8 days post hatch).

Nominal test concentrations for sediment exposures were: 0.04, 0.2, and 1 g wet weight sediment/L, diluted with laboratory water. Control groups consisted of eggs and larvae exposed to laboratory water only. Laboratory water was from the municipal system (City of Burlington), that was dechlorinated, charcoal-filtered, and UV-sterilized.

Embryos and larvae were inspected each day for mortalities, and any fish showing deformities were recorded and removed. Severely deformed
and/or immobile larvae, or those with severe necrosis (seen under the microscope as tissue damage and dissolution), but still with heartbeats, were described, removed, and euthanized in tricaine methane sulfonate (250 mg/L). Larvae were fed 20 μL/fish of a newly-hatched brine shrimp slurry daily (mean density of 6 nauplii/μL). Feeding was 2 h prior to the daily solution and sediment changeover. All newly-hatched larvae were removed and assessed for deformities at 0 dph (hatch). Deformities assessed were spinal, pericardial edema, yolk sac edema, craniofacial, small head, sub-epidermal bubbles, small eyes, short body, and hemorrhages. The number of larvae in the beaker was randomly culled at day 13 of the test (8 dph) to a maximum of 15 larvae. The culled individuals were assessed for total length (in mm; to 0.01 mm, at 6.3x magnification), wet weight (in mg; to 0.01 mg), and condition factor (CF; calculated as wet weight/total length3). At day 20 of the test (15 dph) all remaining surviving larvae were euthanized and similarly assessed (length and wet weight measured, and condition factor calculated).

Survival was calculated at several time points during each test. Formulas are described below:

- Survival from ‘egg to hatch’ = # larvae hatching on day 5/# eggs in replicate beaker at the start.
- Survival from ‘egg to 8 dph’ = # larvae alive on day 8 post-hatch (prior to the cull)/# eggs in that replicate beaker at the start.
- Survival from ‘8 dph to 15 dph’ = # larvae alive on day 15 post-hatch/# larvae left after the cull on day 8 post-hatch (usually 15 larvae).
- Survival from ‘egg to 15 dph’ (overall percent survival for the entire exposure until 15 dph or 20 days post-fertilization, dpf) = % survival ‘egg to 8 dph’ x % survival ‘8 dph to 15 dph’/100

2.3. Measurement of PAHs, alkylated PAHs and naphthenic acids in sediments

Sediment PAHs, alkylated PAHs and naphthenic acids in sediments were previously reported in (Raine et al., 2017).

2.4. Statistics

All statistical analyses were performed using SYSTAT (version 11.0, Systat Software Inc., San José, CA, USA). Egg and fish survival were expressed as % laboratory water control, to account for control mortality during the 20-day tests. Control mortality over the 20-day exposures was never below 68 % from the start of the test egg phase to day 20 of the test (15 dph). Growth comparisons were done on mean weight, length, or condition factor calculated for each beaker (replicate). Data were assessed for normality using the Shapiro-Wilk Normality Test. Survival data were 88 % normal and growth data were 93 % normal, so no transformations were necessary and parametric statistics were appropriate. Analysis of Variance (ANOVA) was used to compare untransformed survival data, and mean length, weight, and condition factor (CF) data for each sediment concentration to the water controls. Where ANOVAs showed significant differences among treatments (i.e., p ≤ 0.05), two-sample t-tests were used to compare mean survival/weight/length/CF for individual sediment concentrations to the mean survival/weight/length/CF of water controls. Two sample t-tests using Bonferroni’s adjusted p values (with pooled variances) were used to denote levels of significance for the comparison of each sediment concentration mean with the control mean. LC50s were calculated using a polynomial model on % survival (as a % control survival) for each replicate beaker and exposure concentration of the sediment.

3. Results

3.1. Water quality during fathead minnow sediment exposures

Water quality was measured three times over the 20 day exposure in each beaker containing eggs or larval fish. Overall water quality was similar to control water, with high O2 and low NH3: Mean dissolved oxygen was 7.7–8.4 mg/L, temperature was 23.1–23.5 °C, pH was 8.04–8.33, free ammonia was 0.000–0.006 mg/L and conductivity was 323–342 μS/s. Detailed water quality parameters by sediment concentration are in Supplemental Data Table S1.

3.2. Control egg and larval survival

Control fathead minnow survival was above acceptable survival rates over the 20-day exposure. Survival of fathead minnow eggs in laboratory water until hatch was between 79.8 (±10, standard deviation, s.d.) % for controls in sediment 2 test and 85.3 (±8.2 s.d.) % for controls in sediment 1 test. Survival over the entire 20-day test (from the egg stage to the end of the exposure at 15 dph) was between 75.4 (±8.0 s.d.) % and 68.0 (±9.5 s.d.) % for the controls in sediment 1 and 2 tests, respectively. This is considered to be good control survival for fathead minnow embryos, compared to the acceptable control survival of 52.5 % (OECD, 2013). The OECD fathead minnow early life stage test sets the survival acceptability standard at 70 % from egg to hatch, and then 75 % from hatch to 28 dph, so overall from the egg stage to 28 dph, the acceptable survival is 52.5 % (OECD, 2013). Although our tests went to only 15 dph, survival was good. Raw data for embryo and larval control survival per replicate is in Supplemental Data Table S2.

3.3. Survival of fathead minnow embryos and larvae in oil sands tailings pond sediments

Exposure to oil sands tailings pond sediments caused decreased survival in fathead minnows over time (Fig. 1). Sediment 2 was more potent than sediment 1. Lethal concentrations for the two sediments that caused fathead minnow larval survival to drop to 50 % of control survival
Fathead minnows exposed to the oil sands tailings pond sediments had high rates of deformities at hatch at sediment concentrations of 1 g/L (sediments 1 and 2) and 0.2 g/L (sediment 2) (Fig. 2). Most deformities were observed at hatch, and 89% (sediment 1) and over 97% (sediment 2) of the deformities were yolk sac edema and pericardial edema combined. Fig. 3 shows a fathead minnow with yolk and pericardial edema (top fish, exposed to sediment 2 at 1 g/L) compared to a control fish (bottom).

### 3.4. Deformities in fathead minnow embryos and larvae in oil sands tailings pond sediments

There were some effects on growth of larval fish exposed to oil sands tailings pond sediments. Sediment 1 caused significantly decreased length at 8 dph with exposure to 0.2 g sediment per L (Table 2, Supplemental Data Fig. S3). There were no significant changes in weight or condition factor (CF) in any of the exposures to Sediment 1. There were no decreases in growth seen at 15 dph (except for the two surviving fish from 1 g/L sediment 1 exposure that were larger than all other fish).

Exposure to 0.04 g/L sediment 2 caused decreased length and CF at 8 dph (Table 2). Sediment 2 exposure at 8 dph significantly decreased length at 0.2 g/L and CF at 1 g/L. Sediment 2 (0.04 g/L) caused no significant changes in growth at 15 dph compared to controls. Higher concentration caused complete mortality. All growth data per replicate are in Supplemental Data Table S3.

### 4. Discussion

In this study we investigated the effects of oil sands tailings pond sediments on fathead minnow embryo-larval survival, growth, and deformities. Consistent with previous results on walleye and pike larvae, decreased survival (especially in larval stages), decreased growth, and increased deformities at hatch were observed, and sediment 2 exhibited a greater potency than sediment 1. The suite of abnormalities observed in larval fish exposed to these two oil sands tailings pond sediments is consistent with well-known PAH and APAH toxicity in fish early life stages (Carls et al., 1999; Couillard, 2002; Edmunds et al., 2015; Incarmona and Scholz, 2016; Jung et al., 2015; Marty et al., 1997). Since APAH and PAH levels were higher in Sediment 2, this explains the higher potency of this oil sands tailings pond sediment in fathead minnow larvae.

The data from this study was generated 9 years ago, and several advances in the field have been made with fish exposed to mixtures of PAHs since that time. However, there have not been many studies of the PAHs (LC50s, expressed as, g wet weight sediment/L) were calculated at three exposure times: egg until hatch, egg until 8 dph, and egg until 15 dph (Table 1). Raw data for embryo and larval survival per replicate in the two sediment exposures is in Supplemental Data Table S2.

### 3.5. Growth of larval fathead minnows exposed to oil sands sediments

| Sediment | LC50 expressed as g wet weight sediment/L (r² value) for each exposure duration |
|----------|--------------------------------------------------------------------------------|
|          | At hatch | 8 dph | 15 dph |
| sediment 1 | N/D | 0.567 (r² = 0.93) | 0.515 (r² = 0.93) |
| sediment 2 | 0.922 (r² = 0.49) | 0.118 (r² = 0.87) | 0.097 (r² = 0.88) |

* No effect on egg survival or hatching.
To do this we compared the three species in detail. However, we wanted to compare their overall sensi-
tivities (as reported in Raine et al., 2017) with sediment 1 having lower concentrations than sediment 2. Sediment 1 contained 173 μg/g total PAHs + APAHs (97 % alkylated), and sediment 2 contained 401 μg/g total PAHs + APAHs (95 % alkylated). Alkylated PAHs dominated both sediments, with 95–97 % of the total PAHs being alkylated. Patterns of PAHs in oil sands tailings pond sediments were very similar, with increasing concentrations of alkylated PAHs as the substituents progressed from C1 to C2 to C3 for naphthalenes, phenanthrenes, dibenzothiophenes, fluorenes and chrysenes. Individual PAH and alkylated PAH profiles in the two oil sands tailings pond sediments are in (Raine et al., 2017).

Comparisons of sub-fractions of the mixture would have been appropriate if we wanted to assess mechanisms of toxicity across these species in detail. However, we wanted to compare their overall sensiti-
tivities to the mixture of PAHs and APAHs that are relevant to oil sands. To do this we compared the three fish species exposed to the entire sediment (mixture of contaminants), as this is relevant to natural bitumen-containing oil sands river sediments.

4.1. Concentrations of PAHs and alkylated PAHs in sediments

Concentrations of PAHs and alkylated PAHs differed in the two sediments (as reported in Raine et al., 2017) with sediment 1 having lower concentrations than sediment 2. Sediment 1 contained 173 μg/g total PAHs + APAHs (97 % alkylated), and sediment 2 contained 401 μg/g total PAHs + APAHs (95 % alkylated). Alkylated PAHs dominated both sediments, with 95–97 % of the total PAHs being alkylated. Patterns of PAHs in oil sands tailings pond sediments were very similar, with increasing concentrations of alkylated PAHs as the substituents progressed from C1 to C2 to C3 for naphthalenes, phenanthrenes, dibenzo-
thiophenes, fluorenes and chrysenes. Individual PAH and alkylated PAH profiles in the two oil sands tailings pond sediments are in (Raine et al., 2017).

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tivities to the mixture of PAHs and APAHs that are relevant to oil sands. To do this we compared the three fish species exposed to the entire sediment (mixture of contaminants), as this is relevant to natural bitumen-containing oil sands river sediments.

4.2. Concentrations of naphthenic acids in sediments

Concentrations of naphthenic acids were low and similar in both sediments. Naphthenic acid concentrations were consistent for both sediments (9.3 and 10.2 μg/g wet weight of sediment for sediment 1 and sediment 2, respectively, even though PAH and alkylated PAHs concentra-
tions differed between the two sediments (Raine et al., 2017). Sediment 1 contained 14.5 mg NA/g dry weight and sediment 2 contained 15.9 mg NA/g dry weight. Water content was 36 % of sediment 1 and 54.9 % of sediment 2 (Raine et al., 2018).

4.3. Deformities in fathead minnow fry at hatch

Deformities at hatch in fathead minnow embryos exposed to the two oil sands tailings pond sediments were predominantly yolk edema combined with pericardial edema. Over 95 % of the deformed fish had these two deformities combined. Other deformities seen along with these were craniofacial deformities (small head, eye deformities) and multiple, sub-
epidermal swellings and hemorrhages. These deformities are similar to those seen in walleye and pike exposed to these same two sediments (Raine et al. 2017, 2018). The suite of deformities we observed is similar to those seen in embryo-larval stages of marine fish species exposed to the mixture of PAHs and APAHs found in crude oil (Carlss et al., 1999; Couillard, 2002; Edmunds et al., 2015; Incardona et al., 2005; Incardona and Scholz, 2016; Jung et al., 2015; Marty et al., 1997).

The sensitivity of fish embryos to PAHs and APAHs is stage specific. Experiments with haddock show that early crude oil exposure just after embryo fertilization causes more effects, especially craniofacial malformations (Sorhus et al., 2016). Our exposures began with eggs 16–24 h post fertilization, which is at the epiboly and neurula stage for fathead minnows, and so along with pericardial edema and yolk-sac edema, craniofacial deformities were also common in our oil sands tailings pond sediment exposures.

4.4. Toxicity of oil sands tailings pond sediments over time

The pattern of delayed toxicity for these sediments emphasizes the importance of running longer embryo-larval fish bioassays. Note that in Fig. 1 for most sediment exposure concentrations there was good survival past hatch, and it was not until the period after hatch until 8 dph that the full effects of the sediments were observed. This is important, as in some countries there is a move to end fish bioassays just after the fish hatch, mainly for ethical considerations (Embry et al., 2010). While the use of fewer fish in toxicity testing is important, the generation of meaningful data for assessment of sediments is the ultimate goal of these tests. In this case, if the test had ended at hatch, the two oil sands tailings pond sediments would have had no effect (sediment 1) or much reduced effect (sediment 2) in our fathead minnow bioassays.

Similar results of delayed toxicity were seen in embryo-larval walleye and pike exposed to these same two sediments (Raine et al. 2017, 2018). Delayed toxicity was also observed fathead minnow embryos and larvae exposed to natural bitumen-rich oil sands river sediments (Colavecchia et al. 2004, 2007; McMaster et al., 2018; Vignet et al., 2019), and oil
4.5. Species sensitivities

We have previously exposed northern pike (Esox lucius, in May 2011) and walleye (Sander vitreus, in May 2010) to these same oil sands tailings pond sediments (Raine et al., 2017, 2018). Comparisons of species sensitivity show that pike and fathead minnow are similar in sensitivity, and that larval walleye are from 5 to 28 times more sensitive (Table 3). This sensitivity difference may be caused by the longer embryonic period in walleye (18 days), compared to pike and fathead minnow (9 days and 5 days, respectively). In these tests, both fathead minnow and northern pike were exposed for 5 d during the embryonic period, while walleye were exposed for 18 d.

The study provides strong evidence that walleye are more sensitive to these oil sands toxicants compared to fathead minnow. Also this study (and our previous studies) show that Northern pike and fathead minnow are equally sensitive. This is relevant to resource managers in the oil sands area, as almost all of the laboratory testing of oil sands waters (waters from end pit lakes, tests of oil sands process water remediation effectiveness) is done and will be done in future in fathead minnows. The relatively larger surface area (relative to egg volume) of the walleye eggs may have allowed for increased PAH + APAH uptake from the sediments (Raine et al., 2018). Edmunds et al. (2015) found a relationship between egg size and differences in crude oil vulnerability for three species. Mahi mahi (Coryphaena hippurus) had the largest egg diameter (1.4 mm) relative to amberjack (1.2 mm) and bluefin and yellowfin tunas (1 mm). The rank order of vulnerability (tunas > amberjack > mahi mahi) corresponds to respective egg surface-volume ratios of 6.7, 5.4, and 4.3 (Edmunds et al., 2015). However, some data from the present exposures does not support this egg-volume-to-surface-area theory, as fathead minnow eggs are small (1.2–1.3 mm) similar to walleye eggs, so the egg-size theory cannot explain the differences in sensitivity between these two species in our tests. Other researchers suggest that differences in metabolic capacity may explain the differences in sensitivity of embryo-larval fish to PAHs + APAHs in crude oils. Embryonic spotted sea bass (Lateolabrax maculates) had higher metabolic capacity and higher lipid content, and this was thought to cause their lower sensitivity to Iranian heavy crude oil compared to embryonic olive flounder (Paralichthys olivaceus) (Jung et al., 2015).

Table 3

| Sediment tested and species of fish | Length of exposure (days exposed as eggs, total days exposed from egg to end of test) | Egg to hatch LC50 (g wet weight sediment/L) | Egg to end of exposure LC50 (g wet weight sediment/L) |
|-----------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------|--------------------------------------------------|
| Fathead minnow                    | 5 d, 20 d                                                                            | N/D — no effects                          | 0.515 (2nd order poly r² = 0.93)                  |
| Walleye                           | 18 d, 31 d                                                                           | N/D — no effects                          | 0.018 (2 pts : 3rd order poly)                    |
| Northern pike                     | 9 d, 20 d                                                                            | 1.16 (2nd order poly r² = 0.92)           | 0.45 (2nd order poly r² = 0.98)                  |
| Fathead minnow                    | 5 d, 20 d                                                                            | 0.922 (2nd order poly r² = 0.49)          | 0.097 (2nd order poly r² = 0.88)                 |
| Walleye                           | 18 d, 31 d                                                                           | 0.20 (3 pts : 2nd order poly)             | 0.019 (2 pts : 2nd order poly)                    |
| Northern pike                     | 9 d, 20 d                                                                            | 0.36 (2nd order poly r² = 0.87)           | 0.12 (exp eqn r² = 0.98)                         |

* Hatched at 9 days post-fertilization (dpf), exposure was for 5 days, from 4 dpf to 9 dpf.
sensitivity of walleye embryos (relative to fathead minnows) to this group of contaminants. Studies on the relative sensitivities of important local fish species can be used to inform management decisions when oil sands tailings ponds are reclaimed, and end pit lakes are connected with local surface waters.

Declarations

Author contribution statement

Joanne L Parrott: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jason C Raine, Mark E McMaster, L. Mark Hewitt: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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Fig. 4. Mean % survival (±standard deviation, s.d.) of fathead minnow embryos and larvace plotted against the total concentration of PAHs (PAHs + APAHs, μg/L) in the sediments added to each exposure beaker. Exposures were for 20 days to oil sands tailings pond sediments at sediment concentrations of 0 (controls), 0.02, 0.4, and 1.0 g/L of sediment per L water. These added sediment concentrations were equivalent to 7, 35, 173 μg TPAH for sediment 1 (red dotted line), and 16, 80, and 401 μg TPAH for sediment 2 (purple dashed line), per L added to each beaker of water. Control points (blue circles) had 0 μg/L added TPAHs, but points are shown on figure for comparison. The estimated EC50s (for TPAH added to the beakers) are 35 μg TPAH/L for sediment 1 and 80 μg TPAH/L for sediment 2.

5. Conclusions

Embryo-larval fathead minnow exposed to PAHs and APAHs in sediments from oil sands tailings ponds had decreased survival and increased deformities in 20-day chronic exposures. Mortality was delayed, and occurred mainly in the period from hatch to 8 days post-hatch. Compared to previous exposures of northern pike and walleye to these same two oil sands tailings pond sediments, fathead minnow were similar in sensitivity to northern pike. Walleye were 5- to 28-fold more sensitive to the two oil sand tailings pond sediments than both fathead minnow and northern pike.

Similar to walleye and pike, fathead minnow are environmentally-relevant to the area, so there is a need to determine their sensitivities to tailings pond sediments and the mixture of PAHs and APAHs they contain. Furthermore, it is likely that fathead minnow will be the species used to evaluate future treatments and remediation options for oil sands process waters. This work therefore defines the relative sensitivity of this model/relevant species (the fathead minnow) compared to other local species (walleye and pike) that are important as a food source and as a sport fish. This work allows risk assessors to account for the increased
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