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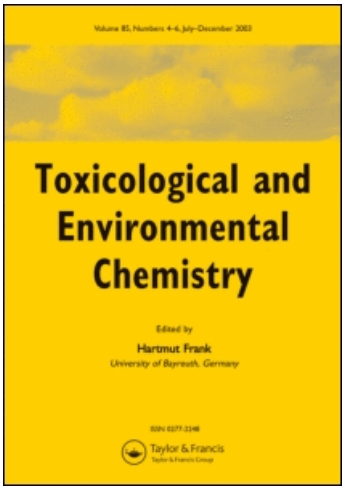

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Extraction of crude oil from oil sand produces solid (sand) and liquid (water with suspended fine particles) tailings materials, called oil sands process-affected materials (OSPM). These waste materials are stored on the mine site due to a "zero discharge" policy and must be reclaimed when operations end. The liquid tailings materials are known to contain naphthenic acids and polycyclic aromatic hydrocarbons and have high pH and salinity. One method of reclamation is the "wet landscape" approach, which involves using oil sands tailings materials to form wetlands that would mimic natural wetland ecological function. This study investigated the effects of wetlands formed with oil sands tailings materials on the survival and growth of wood frog (*Rana sylvatica)* larvae. In spring 2007, *in-situ* caging studies were completed in 14 wetlands that were of four different classes; young or old, reference or reclaimed. Tadpole survival was different between types of wetlands, with young tailings-affected wetlands ($\leq$7 years old) having 41.5%, 62.6%, and 54.7% higher tadpole mortality than old tailings-affected ($>$7 years old), young reference, and old reference wetlands, respectively. Since old wetlands created from OSPM showed effects on tadpoles similar to those of reference wetlands, which had markedly lower toxicity than young tailings-affected wetlands, we provide evidence that wetlands, at least 7 years old, can sustain amphibian life.

Keywords: oil sands; wood frog; toxicology; bioindicator

Introduction

The demand for oil worldwide is increasing while reserves of oil are decreasing. As a result, production of oil from unconventional oil deposits, such as Alberta's oil sands, is expanding rapidly. At the time of this study, there were three companies using surface mining to excavate oil sand from which bitumen (heavy crude oil) is extracted. Oil sands production accounts for approximately 25% of Canada's total crude oil production, and this percentage is expected to increase as more companies begin production (Leung, MacKinnon, and Smith 2003).

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Crude oil that is extracted from oil sand, is dense and highly viscous. In the ground bitumen is associated with sand, fine clay particles, and water (Speight 2007). Surface mining of oil sands involves clearing land, digging out raw oil-impregnated sand with excavation equipment, and transporting the oil sand with heavy haul trucks to the extraction facilities (Squires 2005). Bitumen is separated from the associated materials by a hot water floatation process (Clark and Pasternak 1932). This produces large quantities of solid (sand) and liquid (process-water containing suspended fine particles) wastes, called tailings or oil sands process-affected materials (OSPM), which are stored on mine sites in large dyked tailings ponds. Quagraine, Peterson, and Headley (2005) state that by the year 2025 as much as one billion cubic meters of OSPM may be stored in tailings ponds. Government legislation requires the mine sites to be returned to a state similar to that of the area before the production began (Madill et al. 2001). One strategy adopted by the oil sands industry to achieve this reclamation, the "wet landscape approach", involves the use of stored tailings material to form lakes, ponds, and other types of "constructed wetlands". Wetlands are expected to cover 20–40% of the final reclamation landscape (Bendell-Young et al. 2000; Gentes et al. 2006; Siwik et al. 2000).

Since these constructed wetlands are meant to form a working ecosystem over time, they must be able to support communities of wildlife that would naturally inhabit the region. This may be problematic since OSPM and some of its constituents can be toxic to many organisms such as plants (Renault et al. 1998), amphibian larvae (Pollet and Bendell-Young 2000), mammals (Rogers et al. 2002), fish (Nero et al. 2006; van den Heuvel et al. 2000), aquatic invertebrates (Leung, MacKinnon, and Smith 2003), and birds (Gentes et al. 2006; Smits et al. 2000). It is thought that increased concentrations of salts, polycyclic aromatic hydrocarbons, and naphthenic acids (NAs) are the main cause of the toxicity to wildlife (Gentes et al. 2007; Nero et al. 2006). However, the OSPM toxicity is expected to diminish over time (Leung, MacKinnon, and Smith 2003). Little is known about the effects of OSPM on amphibians, and chronic, low-grade toxicity may interfere with the formation of a stable ecosystem that can support amphibian life.

Amphibians such as the wood frog (*Rana sylvatica*), which is native to the northern boreal regions, are expected to live in and around wetlands formed with OSPM. The larvae (tadpoles) of the wood frog are entirely aquatic until they complete metamorphosis. Amphibians and their larvae have been used as model subjects in many types of toxicological and ecological studies including investigations of the effects of pesticides (Cooke 1972, 1981; Materna, Rabeni, and LaPoint 1995), industrial wastes (Huang et al. 2007; Snodgrass et al. 2004), and oil sands tailings water (Pollet and Bendell-Young 2000). Recently, the use of confining amphibians in a wetland of interest (*in-situ* caging) has been recognized as a practical method for toxicology studies. Caging reduces variables associated with the field collection of wild specimens (predation, stress/unreliability of capture, variations in diet, and unknown confounding elements) while still exposing amphibians to realistic environmental conditions such as light, temperature, and water chemistry (Harris, Bishop, and McDaniel 2001). For these reasons *R. sylvatica* larvae are excellent subjects for an *in-situ* toxicological study of wetlands formed with OSPM. This study is intended to provide information on the viability of using wetlands based on OSPM, as part of a reclamation strategy that could be implemented by oil sands companies in northern Alberta.

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During the course of this study, survival, growth, and developmental endpoints of indigenous *R. sylvatica* larvae were assessed to determine the ecological sustainability of the wetlands and whether this sustainability held true for wetlands of different ages. Triglyceride concentration in the larvae was also measured to reveal possible alterations in energy storage or use by amphibians exposed to OSPM, which may reduce their ability to flourish in such ecosystems.

Materials and methods

Study sites, experimental animals, and experimental design

Different numbers of wetlands of the four classes (young OSPM, old OSPM, young reference, and old reference) were studied for several reasons. First, wetlands were selected if they were already included in another large-scale collaborative ecological study of wetlands formed with OSPM. Studies were conducted at various trophic levels, frogs being one of these. Second, larger number of OSPM wetlands is available, while only few young reference sites exist on site. Clearly, because of the impossibility of ''control'' wetlands, we use ''reference'' wetlands which have never received direct or indirect input of tailings materials, and could be man-made or naturally occurring. Finally, only wetlands located on a protected mine site were chosen to prevent unwanted human disturbances. These were three limiting factors resulting in the use of unequal numbers of wetlands representing the four classes. This also led to poor replication among wetland classes. In the final analyses, one wetland (Natural Wetland) and a few single enclosures were excluded due to severe disruption by environmental factors.

The study investigated 14 sites (Table 1), seven reference sites (no OSPM), and seven OSPM-impacted sites. Table 1 gives the data on the presence of possible invertebrate predators to tadpoles. However, being caged, tadpoles could not be affected by direct predation. Wetlands were located 40 km north of the city of Fort McMurray, Alberta, on oil sands mine sites. Exact coordinates of each wetland are provided in Table 2. All cages were placed directly in the wetland to be studied. All wetlands were categorized by age. Wetlands were considered ''young'' if they were 7 years old or less and ''old'' if they were older than 7 years. The designation of young versus old wetlands was based on the need to be consistent among researchers in our collaborative study. The initial research that led to this designation was that of Leonhardt (2003), which found that zoobenthic invertebrate abundance and richness started to reach a plateau when wetlands were aged approximately 8 years and reached a maximum when wetlands were near 15 years of age (Table 3).

Four enclosures were placed in each wetland and each enclosure contained 50 tadpoles. Gosner staging is a method for describing the developmental progress of anuran embryos and larvae based on anatomical features. Tadpoles of Gosner stages 23–25 (Gosner 1960), which is the formation of the operculum, were collected from Bill's Lake, an on site wetland unaffected by OSPM. The study started on 11 May 2007 and was ended enclosure by enclosure with the shortest study duration being 52 days from the start and the longest being 75 days. The end date was determined based on daily observations, when greater than 75% of tadpoles in an enclosure reached Gosner stage 42, emergence of fore-limbs, and considered to be metamorphic climax. All animals were collected and euthanized for further

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Table 1. Wetland description including size and presence of possible aquatic predators by genus.

Wetland	OSPM status	Age	Area (m^2)	*Dytiscus*	*Notonecta*	*Aeshna*	*Dolomedes*	*Lethocerus*
Bill's Lake	Reference	Old	5821					
Peat Pond	Reference	Young	6624		X			
Golden Pond	Reference	Young	5677		X			
Mike's Pond	OSPM	Young	16,920		X			
Test Pond 5	OSPM	Old	675					
Test Pond 9	OSPM	Old	3732		X	X		
West Interceptor Ditch Wetland	Reference	Old	3155		X	X		
South West Sands Storage (Flood Wetland)	OSPM	Young	11,954					
Test Pond 14 (Shallow Wetland)	Reference	Old	35,000		X	X	X	
Natural Wetland	OSPM	Old	12,227	X	X	X	X	
High Sulfate Wetland	Reference	Old	2394		X	XX		
Weir 1	Reference	Old	56,419					
4 m CT – no peat zone	OSPM	Young	4006					
4 m CT – peat zone	OSPM	Young	4006			X		

Notes: X denotes presence of a genus. Shaded cells indicate no data available.

Table 2. Coordinates of wetlands studied.

Wetland name	Coordinates
Bill's Lake	56°59′54.06″N 111°36′9.79″W
Peat Pond	56°59′37.45″N 111°37′24.81″W
Golden Pond	56°59′50.28″N 111°37′28.44″W
Mike's Pond	57°6′39.49″N 111°40′49.79″W
Test Pond 5	57°5′4.60″N 111°41. ′40.41″W
Test Pond 9	57°5′3.58″N 111°41′32.20″W
West Interceptor Ditch Wetland	57°6′35.34″N 111°41′36.52″W
South West Sands Storage (Flood Wetland)	56°58′26.00″N 111°47′40.00″W
Test Pond 14 (Shallow Wetland)	57°4′52.53″N 111°41′27.91″W
Natural Wetland	56°58′50.27″N 111°30′35.31″W
High Sulfate Wetland	56°59′50.03″N 111°33′10.32″W
Weir 1	56°58′36.76″N 111°27′57.26″W
4 m CT – no peat zone	56°59′28.07″N 111°31′55.42″W
4 m CT – peat zone	56°59′28.42″N 111°31′54.14″W

Table 3. Water chemistry (ranges over duration of study – if no range, a single measurement).

Wetland	pH	Conductivity ($\mu s\,cm^{-1}$)	Naphthenic acids ($mg\,L^{-1}$)	Dissolved oxygen ($mg\,L^{-1}$)	NH_4 ($mg\,L^{-1}$)
Bill's Lake	6.96–7.80	349–626	0.56–0.80	10.10	0.14–0.37
Peat Pond	7.56–8.23	915–1200	0.14–1.10	7.50	0.12–0.56
Golden Pond	8.09–8.79	1090–1218	0.16–3.20	8.00	0.15
Mike's Pond	8.22–8.86	3450–4610	12.80–35.80	9.00–11.10	BDL-0.68
Test Pond 5	8.21–9.26	1687–2250	4.60–14.80	8.10–12.60	BDL-2.10
Test Pond 9	8.66–9.21	1640–2140	14.00–29.00	7.50–11.50	BDL-0.41
Test Pond 14[a]	7.46–8.59	312–450	0.60–1.30	6.30–8.50	BDL-0.83
WID[b]	7.89–8.08	548–617	0.24–1.50	8.40	0.58
SWSS[c]	8.08	4130	40.80	N/A	N/A
Natural wetland	8.16–9.10	1140–1660	19.4–70.7	10.60	0.66
HS[d]	7.68–8.57	2950–3740	7.0	8.16	0.45
4m CT – peat[e]	8.13–8.62	2690–2700	31.4–39.9	7.10	BDL
4m CT – no peat[e]	8.09	2770	30.90	7.30	0.24

Notes: [a]Also called Shallow Wetland. [b]WID – West Interceptor Ditch Wetland. [c]Flood Wetland on South West Sands. [d]High Sulfate Wetland. [e]Two different areas of the same wetland – one area peat amended, one area no peat amendment.

sampling. Completing the analyses in the manner we chose assumes that tadpoles were all of the same age at the start of the study. Tadpoles were likely to be of the same age based on the breeding phenology of *R. sylvatica*, which is characterized by highly synchronized breeding and hatching of eggs. In a study by Petranka and Thomas (1995), 81% of the eggs found were laid within three days of each other and hatched relatively synchronously. Tadpoles were fed boiled lettuce *ad libitum*. Uneaten lettuce was removed and replaced with new lettuce every second day.

Air temperature was collected from Environment Canada website for the area surrounding the oil sands. Water temperature data, although more relevant than air temperature, were unfortunately not complete due to equipment failure. Nevertheless, air temperature does give a reasonable indication of influences on local wetlands (Figure 1).

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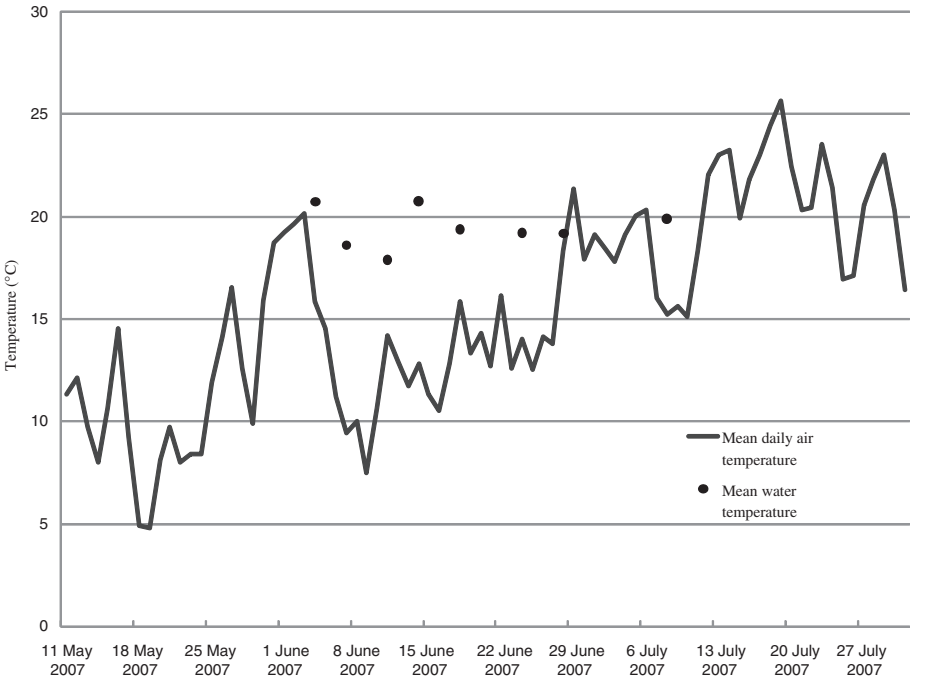

Figure 1. Mean daily air temperature over entire study period shown as line. Dots show mean water temperature of all wetlands for available dates.

Enclosure design

Enclosure design (Figure 2) was adopted and modified from several sources (Harris, Bishop, and McDaniel 2001; Materna, Rabeni, and LaPoint 1995). The design used in this study consisted of a rectangular wooden frame measuring $76 \times 44.5 \times 44.5\,cm^3$. A wooden framed lid, secured in place with removable nails, also measured $76 \times 44.5\,cm^2$. The sides and bottom of the rectangular frame and the associated lid were covered with 1600 µm fiberglass mesh. Wooden stakes were also attached to the frame allowing the enclosures to be secured in the wetlands. Black landscaping cloth was attached to one side of the lid and draped over other side of the enclosure, acting to shade the developing larvae. When placed in wetlands, approximately three quarters of the enclosures' total height was submerged. The initial density of tadpoles in each mesocosm was approximately 0.44 tadpoles L^{-1} or 16.9 tadpoles m^{-2} of pond bottom. This density is much lower than can be found naturally in wetlands. For instance, Petranka and Thomas (1995) estimated that tadpole densities reach 2000 tadpoles m^{-2} of pond bottom, while Biesterfeldt, Petranka, and Sherbondy (1993) reported that densities of tadpoles ranged from 577 to 8931 m^{-2} in several different wetlands.

Wood frog survival and growth

Tadpole survival was measured and reported as the percentage of tadpoles surviving out of the original 50 individuals, after 52 days. Enclosures were checked every second day and dead or moribund tadpoles were removed.

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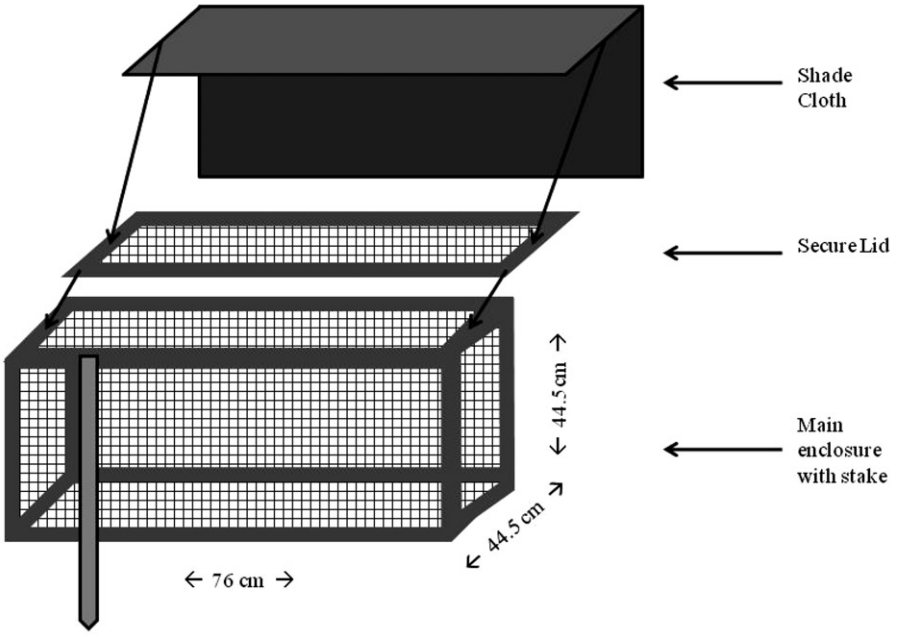

Figure 2. Enclosure design showing landscaping cloth used as shade cloth, enclosure with lid covered with 1600 μm mesh screen and a stake for securing the enclosure to the bottom of the wetland.

Tadpole weight and length were measured immediately following euthanasia at the termination of the field study. Termination of the study for each enclosure was completed when tadpoles reached metamorphic climax [stage 42 (Gosner 1960)]. Length (snout to tail) was measured with a Westward electronic caliper (Acklands-Grainger, Fort McMurray, AB, Canada). Body weight was measured with a Sartorius TE313S scale (max 310 g, $d = 0.001$) (Sartorius AG, Goettingen, Germany) after drying excess moisture from the tadpole by gentle blotting with tissue paper and placing the tadpole on a piece of laboratory film.

Whole body triglyceride extraction and measurement

The method for triglyceride extraction was adapted from Brasfield et al. (2004) and Gupta et al. (2008). Whole tadpoles and livers removed from selected tadpoles were frozen in liquid nitrogen (Praxair, Saskatoon, SK, Canada) immediately following the collection and measurement of body weight and body length. A total of five tadpoles were sampled from each enclosure for this analysis. Once returned to the Toxicology Centre in Saskatoon, SK, samples were transferred to an −80°C freezer until analyzed. During sample homogenization and extraction, all materials (scissors, forceps, glass tubes, etc.) and buffers were placed on ice. Homogenization buffer consisting of 1 mM 6-propyl-2-thiouracil (Sigma Aldrich, Oakville, ON, Canada) in 95% ethanol was made prior to extraction and stored at −20°C in a glass bottle. The first step in both extraction processes was to remove the tadpoles from

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cryovials and place them in plastic weigh boats (VWR International, Mississauga, ON, Canada). A volume of homogenization buffer equal to that of the tadpole was then added (e.g., for a tadpole that weighs 500 mg, 500 μL of buffer is added). Tadpoles were finely minced using scissors and transferred along with the buffer to a $16 \times 100\,mm^2$ glass culture tube (VWR International, Mississauga, ON, Canada). Another volume of homogenization buffer equal to the volume of the tadpole was then added to the tadpole homogenate in the glass tube. Further homogenization was completed using a Tissue Tearor (BioSpec Products, Inc., Bartlesville, OK, USA) in three bursts of 10 s each. Next, samples were vortexed vigorously for 1 min then stored on ice. Each sample was then centrifuged at 2900 rpm at 4°C for 10 min in an Eppendorf 5810 R centrifuge and swinging bucket rotor [model A-4-62 (Eppendorf Canada, Mississauga, ON, Canada)]. Supernatant was carefully removed and transferred to a clean glass tube. Another volume equal to twice the volume of the tadpole was then added to the remaining pellet, which was then re-suspended by vortexing for 1 min. The re-suspended homogenate was then centrifuged again, as described above. The resulting supernatant, which contained ethanol used in the extraction, thyroid hormones, and triglycerides was again carefully poured off and combined with the previous supernatant. This sample was then evaporated under a stream of nitrogen in a water bath at 50°C so that the final volume was equal to that of the initial tadpole. Once the desired volume was reached, the extract was divided into as many 150 μL aliquots as possible and stored at −80°C until assays could be completed.

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Statistical analysis

Based on the knowledge that as OSPM-affected wetlands get older there is an expected reduction in toxicity due to bioremediation, we grouped the wetlands according to their age (either young or old) and OSPM status (OSPM or no OSPM). We assumed that wetlands of the same group (young OSPM-affected, old OSPM-affected, young reference, old reference) have similar characteristics, such as the concentration of NAs. This method of grouping allowed us to complete the two-way ANOVA described below to test the hypothesis that there would be an interaction between age and OSPM status of wetlands. We predicted that older OSPM-affected wetlands would be similar to reference wetlands and much less toxic than young OSPM-affected wetlands. Statistics were carried out using SPSS statistical software package (Version 16.0.1, SPSS Inc., Chicago, IL, USA). The level of significance was $p < 0.05$. All data were tested for the normality and equality of variances assumptions using the Shapiro–Wilks test and Levene's test, respectively. If assumptions of the normality or homogeneity of variances were violated, data presented as percentages were arcsin square root transformed, and regular, numeric data were log 10 transformed. If assumptions were met after transformation, a two-way ANOVA was completed. Age of wetlands (young or old) and OSPM status (OSPM or no OSPM) of the wetlands were the two factors in the two-way ANOVA. If assumptions of normality and homogeneity of variances were violated, the Scheirer–Ray–Hare extension of the Kruskal–Wallis test [a non-parametric variation of a two-way ANOVA test (Sokal and Rohlf 2003)] was performed as in Rickwood et al. (2008).

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Results

Survival was much lower in young wetlands containing OSPM than all other classes of wetlands. Old OSPM-containing wetlands showed survival similar to that of both young and old reference wetlands. There was a significant interaction effect between the factors of age and OSPM status. Treatment effects of age and OSPM status were also found to have significant effect [(two-way ANOVA, interaction $F_{1,44} = 28.838$, $p < 0.001$, age $F_{1,44} = 19.498$, $p = 0.004$, OSPM $F_{1,44} = 9.191$, $p < 0.001$) (Figure 3)] indicating that survival was not affected independently by these factors. Young OSPM-affected wetlands had 41.5%, 62.6%, and 54.7% lower tadpole densities than old OSPM-affected, young reference, and old reference wetlands, respectively, because of differential survival, all mesocosms having started with 50 animals.

Tadpoles that were raised in young OSPM-containing wetlands were heavier than those raised in the other classes of wetlands. For the body weight of tadpoles, there again was a significant interaction effect between the wetland's age and OSPM status but no effects of age or OSPM status alone [(two-way ANOVA, interaction $F_{1,38} = 6.307$, $p = 0.016$, age $F_{1,38} = 0.607$, $p = 0.441$, OSPM $F_{1,38} = 1.136$, $p = 0.293$) (Figure 4)]. For body length, a significant interaction between treatment effects was detected, but not for treatment effects alone [(two-way ANOVA, interaction $F_{1,38} = 23.664$, $p < 0.001$, age $F_{1,38} = 0.010$, $p = 0.920$, OSPM status $F_{1,38} = 0.020$, $p = 0.890$) (Figure 5)].

There was a significant difference in triglyceride concentrations of tadpoles raised in different wetland types due to an interaction between the factors of age and OSPM status, but not based on treatment effects alone [(two-way ANOVA, interaction $F_{1,33} = 4.546$, $p = 0.041$, age $F_{1,33} = 0.113$, $p = 0.739$, OSPM status $F_{1,33} = 0.140$, $p = 0.711$) (Figure 6)]. Tadpoles raised in old reference wetlands had the highest concentrations of triglycerides followed by young OSPM-affected wetlands.

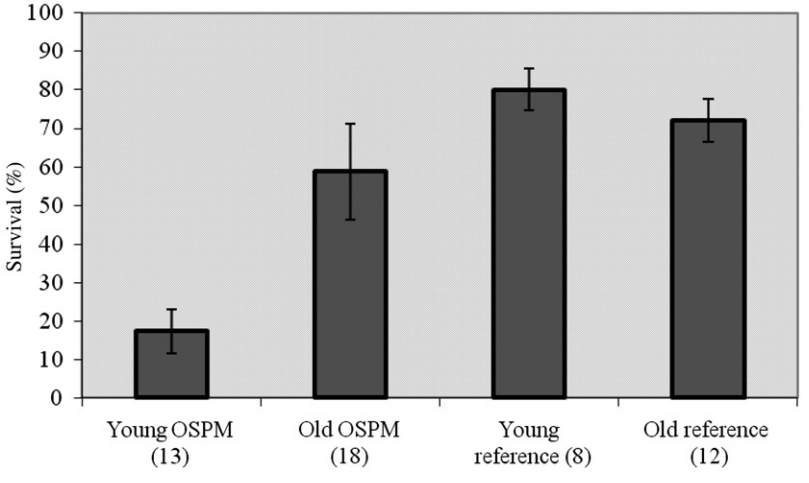

Figure 3. Mean survival (% ±SE) per mesocosm *R. sylvatica* tadpoles raised in different wetlands grouped by the factors of age (young or old) and OSPM status (OSPM containing or reference). The number of enclosures per type of wetland is shown in parentheses (*n*), differences were considered significant when $p < 0.05$. A significant difference due to a treatment interaction, as well as age and OSPM status effects alone was detected (two-way ANOVA, interaction $p < 0.001$, age $p = 0.004$, OSPM status $p < 0.001$).

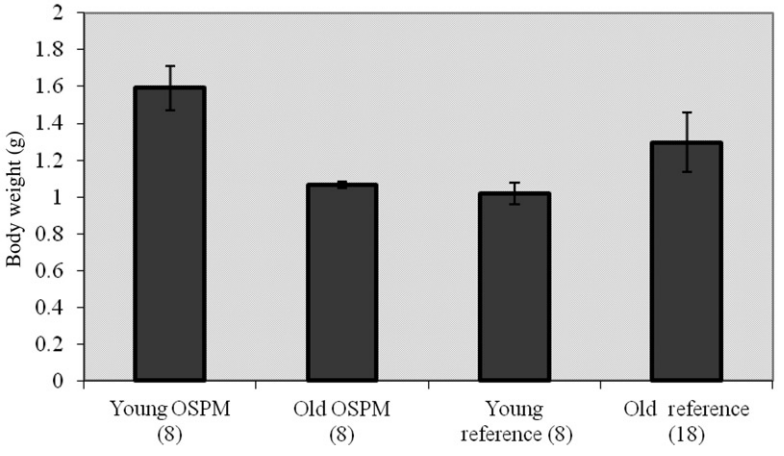

Figure 4. Mean body weight (g ± SE) of *R. sylvatica* tadpoles raised in different wetlands grouped by the factors of age (young or old) and OSPM status (OSPM containing or reference). The number of enclosures per type of wetland is shown in parentheses (*n*), differences were considered significant when $p < 0.05$. A significant difference due to an interaction between treatment effects was detected, but not treatment effects alone (two-way ANOVA, interaction $p = 0.016$, age $p = 0.441$, OSPM status $p = 0.293$).

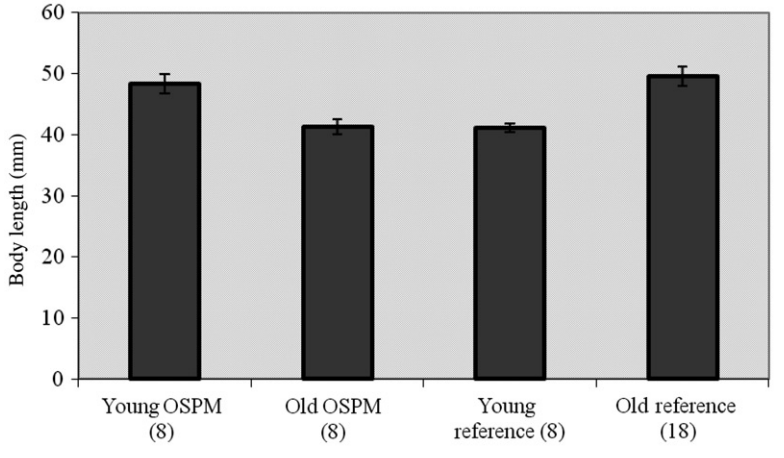

Figure 5. Mean body length (mm ± SE) of *R. sylvatica* tadpoles raised in different wetlands grouped by the factors of age (young or old) and OSPM status (OSPM containing or reference). The number of enclosures per type of wetland is shown in parentheses (*n*), differences were considered significant when $p < 0.05$. A significant difference due to an interaction between treatment effects was detected, but not for treatment effects alone (two-way ANOVA, interaction $p < 0.001$, age $p = 0.920$, OSPM status $p = 0.890$).

Young reference sites and old OSPM sites were similar to each other and had the lowest concentrations of triglycerides.

Discussion

The lowest survival of tadpoles occurred in wetlands that contained young OSPM. These results support those of Pollet and Bendell-Young (2000), who found

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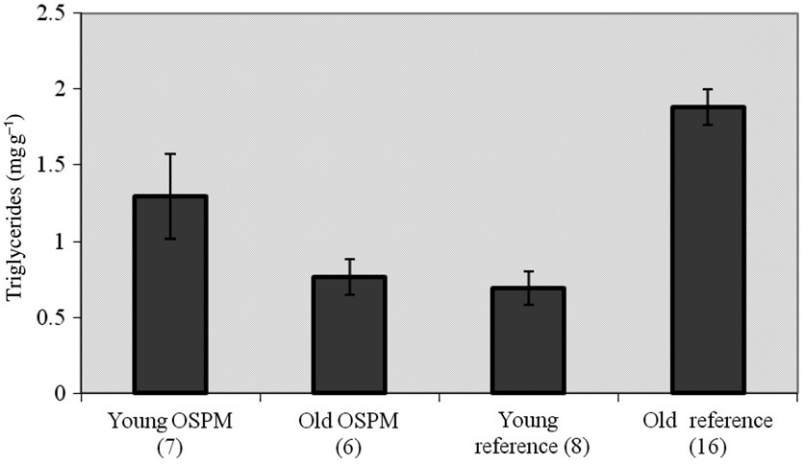

Figure 6. Mean whole-body triglyceride concentrations ($mg\,g^{-1} \pm$ S.E.) of *R. sylvatica* tadpoles raised in different wetlands grouped by the factors of age (young or old) and OSPM status (OSPM containing or reference). The number of enclosures per type of wetland is shown in parentheses (n), differences were considered significant when $p < 0.05$. A significant difference due to an interaction between treatment effects was detected, but not treatment effects alone (two-way ANOVA, interaction $p = 0.041$, age $p = 0.739$, OSPM status $p = 0.711$).

amphibians raised in OSPM-affected sites had the highest mortality. Our results also showed similar survival between old OSPM and young and old reference wetlands, which was much higher than the young OSPM sites in all cases. These results strongly suggest that toxicity from OSPM is diminishing over time. However, it is not known which components of the OSPM are causing the toxic effects. Many previous studies (Holowenko, MacKinnon, and Fedorak 2001; Rogers et al. 2002; Quagraine, Peterson, and Headley 2005) have shown that a large part of the toxicity from OSPM (mainly tailings water) is a result of NAs. NAs are complex mixtures of naturally occurring carboxylic acids that are of several different molecular weights, chain lengths, and may have several ring structures.

Other possible sources of OSPM toxicity are from high levels of salinity (Nero et al. 2006; Renault et al. 1998), which has been shown to be toxic to amphibians (Christy and Dickman 2002; Rios-Lopez 2008). The PAHs may also contribute to more toxicity. Studies have shown that natural aging of OSPM-affected wetlands can reduce toxicity (Quagraine, Peterson, and Headley 2005, references there in). Research has shown that microbes can degrade PAHs (Madill et al. 2001) and NAs (Herman et al. 1994; Lai et al. 1996), which is thought be an important factor in the reduction of the toxicity of OSPM over time (Madill et al. 2001). Toor et al. (2008) have shown that NAs degrade over time, with the majority of the NAs degraded being those of lower molecular weight and lowest number of rings. This causes a reduction in the acute toxicity of tailings water, yet some chronic toxicity remains and is thought to be due to remaining NAs with high molecular weight and more ring structures. The results of this study will be of considerable interest to oil sands companies since young tailings material is toxic to *R. sylvatica* larvae.

These findings indicate that amphibians are unlikely to survive in wetlands constructed from young OSPM, and these wetlands should somehow be made inaccessible

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to frogs to avoid potentially severe detrimental effects on populations of native amphibians in the area. On the encouraging side, this work presents solid evidence, through low mortality rates among tadpoles caged both in more mature OSPM and reference wetlands, that amphibian populations may be able to form part of sustainable communities in wetlands containing more mature OSPM.

Growth (length and weight) of tadpoles is a very important endpoint since it has been shown that the survival of juvenile frogs is positively related to the size at metamorphosis (Morey and Reznick 2001). The body size among tadpoles was highest in those raised in young OSPM-affected wetlands. This was unexpected because normally organisms dealing with toxic insult are smaller, expending more energy towards detoxification efforts, and having lower resources to commit to growth (Berven 1990; Morey and Reznick 2001). The larger size of the surviving tadpoles was most likely due to three factors: (i) much lower density than those in other mesocosms, (ii) their fitness advantage conferred through some genetic advantage that allowed them to survive the same level of toxicity that killed the vast majority of their enclosure mates, and (iii) they were significantly older at the time of metamorphic climax, meaning they had more time to grow. Eaton et al. (2005) found that increased densities of tadpoles resulted in smaller size at the time of metamorphosis. Many other researchers have also discussed similar correlations (Berven 1990; Morey and Reznick 2001). Food limitation is the mechanism that is most often used to explain density-dependent effects on growth and survival (Kupferberg 1997). However, these animals were provided a steady, unlimited, supplementary diet of boiled lettuce. Therefore, population density and related energy expenditure and social stressors in acquiring food would have supported higher body mass in the most toxic treatment groups. Several studies observe that tadpoles, which cannot complete metamorphosis, continue to grow (Allen 1929; Shi 2000). There is the recognition of high plasticity of tadpole development, which results in considerable size variation within populations as well as amongst different populations (Morey and Reznick 2001).

Triglycerides are a main form of energy storage in many types of animals including fish (Bennett 2006; Bennett and Janz 2007), and are a common biomarker used in ecotoxicology studies (Owen, Sogge, and Kern 2005). Our goal was to use total body triglyceride concentration as one indicator of overall condition and energy storage or consumption associated with the exposure to OSPM. Triglyceride concentrations were different among wetlands. In parallel with the findings regarding body mass, tadpoles from the most toxic wetlands had higher triglyceride concentrations, most similar to those from old reference sites. The old reference sites are expected to best support the local fauna, but the advantages from lower tadpole densities in wetlands with highest mortality, and longer growth periods because of delayed metamorphosis would support increased triglyceride reserves for the same reasons discussed earlier (Petranka and Thomas 1995).

Conclusions

Survival of tadpoles was generally poor in wetlands that were young and contained OSPM. Over the longer term this could compromise sustainable amphibian populations from forming in wetlands constructed on oil sands mine sites. However, if the OSPM wetlands are allowed to mature for a sufficient period of time, which this study suggests is at least 7 years, toxicity appears to decrease to a

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level comparable with reference wetlands as shown by the survival rates of tadpoles in wetlands formed with more mature OSPM. It was beyond the scope of this study to determine what caused the reduction in OSPM toxicity over time; but as discussed earlier, it is known that some known toxic constituents of OSPM, such as NAs and PAHs, can be degraded by microorganisms, which helps with decreasing toxicity of OSPM-containing wetlands. For a more complete evaluation of the ability of wetlands formed with OSPM to support amphibian populations, longer term, reproductive studies that follow juveniles through reproductive adulthood at 2 years of age for wood frogs, should be completed under field conditions.

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