Aquatic Ecosystem Health Assessment of the Athabasca River Mainstem and Tributaries Using Fish Health and Fish and Invertebrate Toxicological Testing

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Aquatic Ecosystem Health Assessment of the Athabasca River Mainstem and Tributaries Using Fish Health and Fish and Invertebrate Toxicological Testing

Mark McMaster[1], Joanne Parrott[1], Adrienne Bartlett[1], Francois Gagné[1], Marlene Evans[1], Gerald Tetreault[1], Heather Keith[2] and Jasmin Gee[2]

[1]Environment and Climate Change Canada
[2]Hatfield Consultants

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Foreword

Since February 2012, the governments of Alberta and Canada have worked in partnership to implement an environmental monitoring program for the oil sands region. In December 2017 both governments renewed their commitment to working together with Indigenous communities in the region by the signing the *Alberta-Canada Memorandum of Understanding (MOU) Respecting Environmental Monitoring in the Oil Sands Region*. The MOU establishes the foundation for an adaptive and inclusive approach to program implementation ensuring that the program is responsive to emerging priorities, information, knowledge, and input from key stakeholders and Indigenous peoples in the region.

The Oil Sands Monitoring Program is designed to enhance the understanding of the state of the environment and cumulate environmental effects as a result of oil sands development in the region though monitoring and publically reporting on the status and trends of air, water, land and biodiversity. Its vision is to integrate Indigenous knowledge and wisdom with western science to design, interpret, assess, report and govern the program.

Canada and Alberta have provided leadership to strengthen program delivery, and ensure that necessary monitoring and scientific activities meet program commitments and objectives. The oil sands industry provides funding support for the program under the Oil Sands Environmental Regulation (Alberta Regulation 226/2013). Key findings and results from the program inform regional resource management decisions and importantly, are considered as an objective source of scientific interpretation of credible environmental data.

A mandated cornerstone of the program is the public reporting of data, status and trends of environmental impacts caused by development of oil sands resources. The Oil Sands Monitoring Program *Technical Report Series* provides an objective, and timely, evaluation and interpretation of monitoring data and information collected across environmental media of the program. This includes reporting and evaluation of emission/release sources, fate, effects and transport of contaminants, landscape disturbance and responses across theme areas including atmospheric, aquatic, biotic, wetlands, and community based monitoring.

Executive Summary

In 2011, the Governments of Canada and Alberta designed a monitoring plan for surface water quality and quantity, air quality and biodiversity of the lower Athabasca River between Fort McMurray and its confluence with Lake Athabasca. This plan, known as the Joint Oil Sands Monitoring Plan (JOSM), included monitoring aquatic ecosystem health with a focus on wild fish health in the mainstem of the Athabasca River and its tributaries. The fish health program for JOSM concentrated on fish health endpoints developed through Canada's Environmental Effects Monitoring Programs for the pulp and paper and metal mining industries. Fish can be sensitive to multiple stressors, are critical components of aquatic ecosystems, and have significant social and economic value.

The objective of the fish component of the aquatic monitoring program was to provide necessary data and supporting information to address key questions regarding both environmental health of fish populations and fish health issues related to use and consumption. These included:

Environmental health

- What is the current status of fish health in the lower Athabasca Region?
- Are there existing differences in fish health among sites in the lower Athabasca Region?
- Are there any trends/changes in fish health relative to historical studies?

Human use

- What are contaminant levels in fish?

Cumulative effects

- Are there any predictive relationships between system drivers (including development stress)?
- Is there evidence of cumulative effects of development on fish in the lower Athabasca Region?

The objective of fish toxicological testing and in situ invertebrate bioassays was to answer the following questions:

- Which oil sands media contribute oil sands related chemicals (OSRCs) to the aquatic environment?
- What are important aquatic routes of exposure and potential effects in organisms under controlled conditions?

The report is divided into four sub-themes: mainstem fish health, tributary fish health, fish toxicology, and invertebrate toxicology. This assessment initiates the process of integrating fish health and toxicological information with other primary water themes (i.e., water quality, groundwater quality, regional hydrology and sediment dynamics modelling, and benthic macroinvertebrate assemblages.

Fish health sampling on the mainstem Athabasca River prior to the JOSM was limited to environmental effects monitoring studies conducted by pulp and paper mill discharges in the upper portions of the watershed and research studies conducted through the Program of Energy Research and Development in the late 1990s. Given limited fish health monitoring data, our JOSM investigations aimed to provide information needed to develop baseline fish health across the watershed (i.e., Phase 2 Plan (Environment Canada and Alberta Environment 2011) and Implementation Plan (Environment Canada and Alberta Environment 2012a). The JOSM mainstem program consisted

of large bodied fish health assessments at five stations (white sucker, *Catostomus commersoni*) and small bodied fish health assessments at nine stations (trout perch, *Percopsis omiscomaycus*) on the Athabasca River. Small bodied fish were included because they have reduced mobility and improve our ability to separate out sources or confounding factors. Additional species (walleye, *Sander vitreus*) were captured during the large bodied fish health assessments for determination of contaminants in a harvested fish species. The tributary fish health program using slimy sculpin (*Cottus cognatus*), conducted on five tributaries at nine stations, and other small bodied species (longnose dace, *Rhinichthys cataractae*; lake chub, *Couesius plumbeus*) were used to assess four other Athabasca River tributaries.

As part of the JOSM, the fish toxicology program in the oil sands expanded to include 18 river sediment sites over several years of study, up to 10 snow and freshet sites, and six groundwater sites. To our knowledge, oil sands area snow/freshet and groundwater had never been assessed in controlled exposures of fish in the lab. JOSM work using caged mussels (*Genus species*) and the freshwater amphipod crustacean, *Hyalella azteca*, was also unique in the oil sands area. *Hyalella* caging sites were selected based on actual fish health sampling sites to make results directly comparable.

White sucker were sensitive indicators of fish health in the system as we documented consistent changes in fish health downstream and within the oil sands deposit in 2011 and 2012. These differences were indicative of nutrient enrichment as white sucker had increased condition and increased levels of internal fat stores relative to fish upstream of the oil sands area. Environmental effects monitoring programs for pulp and paper and metal mining sectors also use adult fish surveys of sentinel species to evaluate potential of effluents to alter fish health (Environment Canada 1998, 2010, 2012b). In these programs, alterations in fish health endpoints within a sampling season relative to the reference location require confirmation in the following cycle. We confirmed responses in white sucker in the first two years of our studies. The third-year studies indicated changes occurring with fish within the deposit. These fish no longer had increased condition or internal fat stores and more closely resembled upstream reference fish. As differences between sites were reduced not increased, increased monitoring was not triggered. With a strong baseline developed, we recommend moving to sampling on a once-every-three-year cycle. The next sample period should evaluate fish health relative to year three of the baseline for confirmation of improvements.

The fish biomarker, EROD (ethoxyresorufin-O-deethylase) activity, was a good indicator of exposure to polycyclic aromatic related compounds (PAC) and indicated the potential for increased exposure to these compounds downstream of development. This was reflected best in PAC levels in white sucker liver tissue in both males and females, with increased PACs downstream of development. Levels were higher in male livers than females, a trend also demonstrated in walleye livers.

Although trout perch are less mobile than white sucker, they were less responsive to various conditions in the river. For female trout perch, no consistent effects were demonstrated within sites among years. Male data also failed to demonstrate consistent alterations in fish health endpoints among years, indicating that trout perch health was not affected by exposure to oil sand deposits or development. The data provide a good baseline of trout perch health that can be used to monitor the aquatic environment for change following increased development in the oil sands area as this species can be sampled throughout the mainstem Athabasca including areas where sampling white sucker is logistically very difficult.

Slimy sculpin appear to be sensitive indicators of fish health in tributaries of the Athabasca River as consistent changes were documented in downstream sections of the Steepbank River within the oil sands deposit in 2010 through 2013. These differences were indicative of exposure to PAC-related compounds, as slimy sculpin demonstrated increases in liver size with corresponding

induction of EROD activity. This species also demonstrated reductions in energy investment into reproductive development. Differences in liver size and gonad size were sometimes outside critical effect size limits. Steepbank River results were compared to studies conducted earlier by Tetreault et al. (2003b) on this system. Sufficient data were obtained to allow within-site predictions of fish health endpoints, and this type of analysis can be used to document change within a site over time. Ongoing studies at these sites will document reference site variability in slimy sculpin health endpoints, thus allowing for additional determination of change due to oil sands development. For other Athabasca River tributaries, it is recommended that additional annual collections (baseline data collection) be made to improve the capacity to detect change due to oil sands development.

Controlled exposures of fish to oil sands sediments from two river sites (Steepbank River lower site and Ells River lower site) indicated this exposure decreased embryo-larval fish survival. Also, exposure to snow from sites near mines and stacks decreased larval fish survival in the lab. However, freshet water collected from these same sites did not affect survival of larval fish, suggesting that dilution of contaminants in snow during spring freshet reduces exposure effects. Snow far from mines and stacks contained lower contaminant levels and exposure to this melt water did not affect larval fish survival in the lab.

Exposure to groundwater affected fish survival in the lab. Natural groundwater was more potent than groundwater collected close to tailings ponds. Low potency groundwater was found at sites outside of the oil sands area. This suggested that in rivers in the oil sands area, groundwater flowing through bitumen-containing substrates can dissolve oil sands related chemicals (OSRCs) in sufficient quantities to affect fish negatively in lab exposures.

There were no observed effects in *Hyalella* in situ exposures; however, differences were observed in natural benthic communities at the lower Steepbank and Ells River sites. This suggests that in situ methods with caged *Hyalella* were insensitive to environmental differences at these sites. Future application of the techniques should consider longer exposures and additional endpoints to improve bioassay performance.

Mussel caging results showed no changes in survival over three years of study. However, there were decreases in condition factor and increased signs of stress (decreased air survival time) in mussels caged in 2014 at several river sites (Athabasca River east side, Ells River lower site, and Steepbank River lower site).

Monitoring recommendations

The fish health component of the JOSM water program recommends that once sufficient baseline fish health data are obtained, fish health monitoring moves to a cyclical long-term monitoring program. For example, three years of fish health data are now available for the mainstem Athabasca and several tributary locations. These rivers can now be monitored using a three-year cyclical program with additional monitoring to be undertaken, if warranted, from tiered assessment approaches and effect thresholds developed from baseline data.

(1) Mainstem

- White sucker and trout perch fish health is now being monitored on a three-year cycle of data collections and analysis. Contaminant levels in walleye are also being measured following the three-year cycle established for white sucker. Sufficient data are now available to predict fish health within a site for the next monitoring period. These data can be used to trigger additional sampling requirements or to prompt detailed investigation of cause studies.
- An improved understanding of causal linkages among fish health, benthos, water quality, physical disturbance and other environmental variables is required.

(2) Tributaries

- Slimy sculpin fish health on the Steepbank River is monitored on a three-year cycle of data collection and analysis. Sufficient data are available to predict fish health within a site for the next monitoring period. Similarly, these data can be used to trigger additional sampling requirements or to initiate detailed investigation of cause studies as required. Other tributaries require additional years of baseline data collection before they can be transitioned to the long-term monitoring program.

- As with mainstem sites, improved understanding of causal linkages among fish health, benthos, water quality, physical disturbance and other environmental variables is required.

(3) Fish Toxicology

- Lab toxicological studies of fish were successfully implemented. Fathead minnow embryo-larval exposures were sensitive to elevated OSRCs (oil sands related chemicals) in some samples of sediments, snow, and groundwater.

- Fathead minnow toxicology studies have developed toxicological tools for use in detailed investigations as needed. Lab exposures of fish can be used in future to investigate specific effects of contaminant sources observed in the field, or to assess samples from areas of rivers here wild fish health or benthic communities are affected. Data collected to date provides a baseline of sediment effects on lab fish in controlled lab exposures. This baseline will be useful for making comparisons as development proceeds on currently undeveloped rivers (e.g., on Ells, Firebag, and Dover rivers and Alice Creek to the north).

- Sensitive fathead minnow laboratory tests developed and used to assess water samples and sediments from oil sands areas can be used in the future as a potential tool in testing end-pit-lake waters prior to their potential regulated release back to local rivers.

- In situ exposures of mussels and *Hyalella* were successfully implemented. Sensitivity of the organisms to river sites in the oil sands area varied. Mussel caging studies showed that survival was insensitive to exposure scenarios, but growth and stress endpoints were sensitive to exposure at several river sites. Moreover, *Hyalella* caging studies showed that these organisms were relatively insensitive to oil sands exposure, with no survival or growth effects seen at any river site.

- Invertebrate in situ exposure studies developed toxicological tools for use in detailed investigations. In situ mussel caging studies would be best used to investigate areas of rivers where changes in invertebrate communities have been observed.

- We suggest using these existing data for comparisons over time, as new developments begin near tributaries, or as baseline for future potential end-pit-lake water release. For the immediate future, we recommend that detailed in situ monitoring be discontinued, and be used only in focused studies with specific questions to answer.

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1. Introduction

These results from the Joint Oil Sands Monitoring Plan (JOSM) address the requirement to develop a comprehensive and robust biomonitoring program for fish and toxicity in fish and invertebrates. Fish health monitoring was conducted along the mainstem of the Lower Athabasca River (LAR) and its primary tributaries to assess spatial and temporal change in ecological condition within these aquatic habitats. The fish program for JOSM focused on fish health endpoints in select sentinel species as differences in growth, reproduction, condition and survival put fish at risk. Knowing this level of risk is important for managing aquatic ecosystems. By associating changes in fish health with invertebrate biodiversity, water and sediment chemistry and toxicology, and with physical habitat measurements, this program was designed to produce an integrated assessment that determines whether ecological effects are occurring in response to oil sands developments. Very little toxicological testing had been completed in this receiving environment prior to the initiation of JOSM. Thus, fish toxicological testing and in situ invertebrate bioassays were implemented as part of JOSM to assess which media contribute oil-sands-related chemicals (OSRCs) to the aquatic environment and, if ecological effects are documented, to identify the media most likely linked to these effects.

Three major historical sampling programs provided valuable baseline fish data: Alberta Oil Sands Environmental Research Program (AOSERP 1970-1980), Northern Rivers Basin Study (NRBS 1991-1996) and Regional Aquatic Monitoring Program (RAMP 1997-2011). The JOSM fish health sampling program, where possible and practical, followed historical sampling methods and sites to provide comparable data, although fish health endpoints were not often measured. Some of the most applicable historic data were collected as part of specific research programs conducted through funding from the Program of Energy Research and Development (PERD) in the late 1990s (Tetreault et al. 2003b). Data collected through the JOSM program will be compared to these historical datasets to provide a longer temporal perspective.

Given the limited fish health data for the LAR, our JOSM investigations were intended to provide information needed to assess monitoring designs identified in the Phase 2 Integrated Monitoring Plan for the Oil Sands (Environment Canada and Alberta Environment 2011). More importantly, work presented here attempts to answer the strategic questions identified in the Phase 2 plan. While the JOSM data collection provides baseline fish health information for the LAR, if the information produced is insufficient to address the questions below then monitoring adjustments will be made to address these concerns. Key questions for the environmental health of fish populations and fish health issues related to use and consumption include:

- What is the current status of fish health in the lower Athabasca Region?
- Are there existing differences in fish health among sites in the lower Athabasca Region?
- Are there any trends/changes in fish health relative to historical studies?
- What are contaminant levels in fish?
- Are there any predictive relationships between system drivers (including development stress) and variability within sites in fish responses?
- Is there evidence of cumulative effects of development on fish in the lower Athabasca Region?

JOSM results for toxicology assess several types of samples from various aquatic habitats within the oil sands area for their ability to cause effects in controlled exposures of fish and invertebrates. Sampling sites were aligned precisely with wild fish health study collection sites (for sediments), with atmospheric deposition collection sites (for snow), and with groundwater study collection sites. The toxicological testing and caging studies were designed to assess various oil sands media that may contribute related chemicals (OSRCs) to the aquatic environment.

Questions relating to fish toxicological testing and in situ invertebrate bioassays were as follows:

- Which oil sands media contribute oil sands related chemicals (OSRCs) to the aquatic environment?

- What are important aquatic routes of exposure and potential effects in organisms under controlled conditions?

The report is divided into sub-themes that describe work completed to date for fish health in the LAR mainstem and LAR tributaries, and toxicological testing in fish and invertebrates. It initiates the process of integrating fish health and toxicology information with other primary water themes (i.e., benthic invertebrate community, water quality, groundwater quality, regional hydrology and sediment dynamics modelling). This integration of water theme information, as well as further development of monitoring approaches, will be ongoing as will integration with the wildlife and air components of JOSM.

2. Mainstem Fish Health Sub-Theme

2.1 Introduction

Designing and implementing a 'world class' effects monitoring program, as called for in the Phase 2 Integrated Monitoring Plan (Environment Canada and Alberta Environment 2011), brings many challenges related to seasonal sampling, high flow events, shifting substrate, migratory fish species, low species richness, limited access and transportation on the river, habitat change upstream, and continual loss of reference areas to development. Also, the Athabasca River is affected by multiple human developments and is a large northern system where remote access is a challenge for sampling programs. Fish health studies on the mainstem Athabasca River (LAR) consisted of sampling two sentinel fish species at sites upstream outside of the oil sands deposit, sites within the deposit upstream of development, and in the deposit downstream of oil sands development (Fig. 1). The large bodied white sucker (*Catostomus commersoni*) was sampled during the fall of each year as a sentinel species because sucker species are known to demonstrate high site fidelity outside their spring spawning migration which can be tens of kilometers (Doherty et al. 2010). Sucker species are benthic feeders and provide potential linkages to invertebrate community bioassessment. A second sentinel fish species, the trout perch (*Percopsis omiscomaycus*), was included as a small bodied fish with reduced mobility that uses a smaller spatial area relative to the sucker species with unique foraging movements from deep waters during the day to shallow waters to feed at night. Previous fish health work on the mainstem Athabasca also used a sucker species and trout perch as sentinel species (Tetreault et al. 2003b).

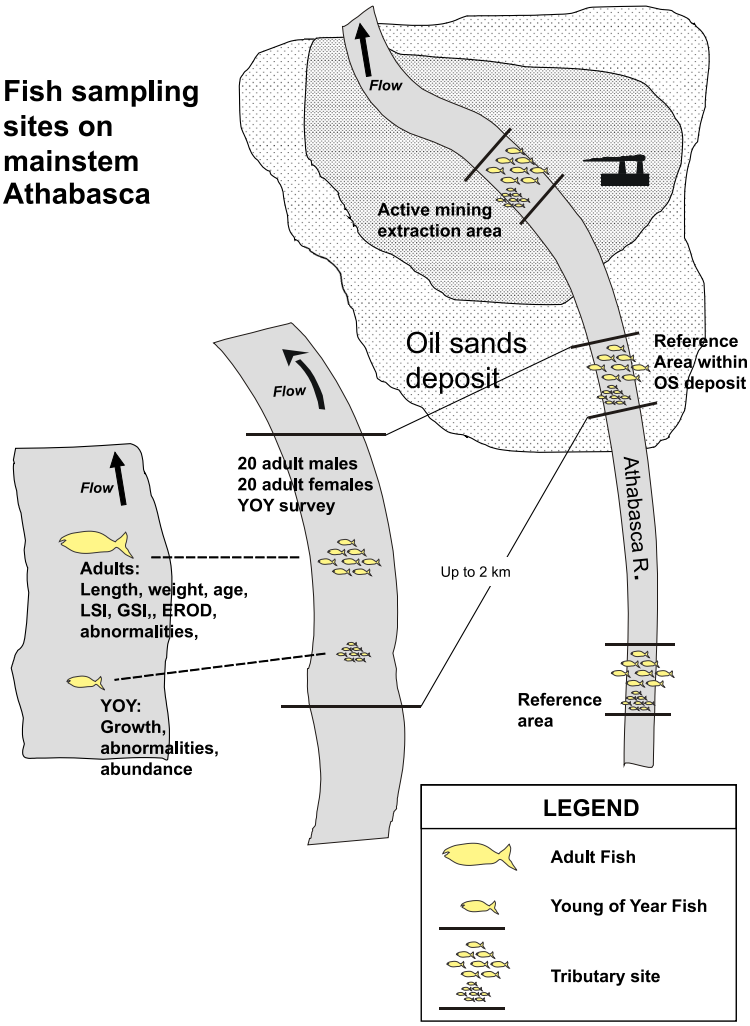

Figure 1. Schematic of oil sands sampling sites on mainstem Athabasca River.

Objectives

This 3-year JOSM study aimed to develop a comprehensive and robust fish health monitoring program for the LAR mainstem. Our studies examined whether methods developed for use in the pulp and paper and metal mining effluent regulations could be used to evaluate fish health in the Athabasca River. Baselines of fish health were developed for these sentinel species in the LAR mainstem; we also assessed the potential for development in the oil sands area to affect overall fish health using the sentinel species approach.

2.2 Methods

Study design

Adult fish survey sampling protocols under the Canadian Environmental Effects Monitoring (EEM) Programs for pulp and paper and metal mining sectors were used to assess fish health in the LAR mainstem and connecting tributaries. Study design for the fish health work, wherever possible, focused on collecting fish within the river system upstream, outside of the oil sands deposit as a reference site, within the deposit upstream of development, and within the deposit downstream of development (Fig. 1). The general study design for the fish health work was to collect 20 adult males and 20 adult females of the selected sentinel species for EEM endpoints (Environment Canada 2010). Three years of data were to be collected at each location to establish variability in EEM endpoints and used to obtain baseline data for assessment of further development and to develop predictive relationships of fish health. As Environment Canada initiated fish studies in 2009, these data were also included to develop the program and estimate variability in fish health endpoints in this system.

Sampling sites and endpoints

Fish in streams and rivers are biological indicators of fish health and important components of the aquatic ecosystem. We examined fish from within the LAR mainstem (Table 1, Fig. 2).

Sampling for fish health was conducted during fall (once in a calendar year) in the months September-October. On the Athabasca River, white sucker and longnose sucker (one year only) were sampled as the large bodied species, and trout perch were sampled as the small bodied species. Boat electrofishing (Smith-Root SR-20 electrofishing work boat) was used to collect white sucker for fish health and walleye for contaminant analysis at all sites. Removal of stunned fish was accomplished using dip nets (approx. 0.5-cm mesh size) followed by transportation to the on-site laboratory for processing. Trout perch were collected using a number of methods, including boat electrofishing where possible, back pack electrofishing for areas not accessible by the electrofishing boat, and bag seines at some locations. Detailed fish health assessments of individual fish included assessment of age, growth, condition, liver size and gonad size relative to body weight, and abnormality assessments, all EEM endpoints used in the monitoring plans (Environment Canada 1998, 2010, 2012b). Measurement of hepatic mixed-function oxygenase (MFO) activity as an indicator of exposure to pulp mill effluents, PCBs, PACs, and some pesticides using ethoxyresorufin-O-deethylase (EROD) methods (Munkittrick et al. 1995, Van den Heuvel et al. 1995) and abnormalities for histological evaluation were conducted (Blazer et al. 2009, Rafferty et al. 2009). Liver samples in white sucker were collected for liver tumour assessments while muscle and liver tissue were collected for contaminant (PACs and alkylated PACs) analyses. White sucker were also rated on visceral lipid stores using a subjective fat index ranging from 1-5 adapted from Munkittrick and Dixon (1988) (with 1 representing very little visceral lipids and 5 representing large amounts). Walleye were also collected from all LAR mainstem river sites (white sucker sites) for contaminant concentrations (PACs and alkylated PACs) in a fish consumed by the public.

Statistical methods

EEM Endpoints

<u>Within year comparisons</u>

For fish of the same species collected on the same river within a sampling year, ANCOVA was used to compare EEM endpoints of condition of the fish (length versus body weight relationships), gonadosomatic indices (gonad weight versus body weight), and liver somatic

Table 1. Athabasca River (AR) sites, species collected, samples collected and collection years. Site location identifications refer to fishing site location relative to Water Quality Monitoring sites M0 to M9. (DS = downstream; US = upstream).

Site Location	Site Type	Species Sampled	Samples Collected	Years Collected
Athabasca M0	Reference	White Sucker	Fish Health, Contaminants	2012, 2013
		Trout Perch	Fish Health, Contaminants	2013, 2014
		Walleye	Contaminants	2012, 2013
Poacher's Landing (AR DS M0)	Reference	White Sucker	Fish Health, Contaminants	2011, 2012, 2013
		Trout Perch	Fish Health Contaminants	2011, 2013, 2014
		Walleye	Contaminants	2011, 2012, 2013
Water Treatment (M2)	Deposit	Trout Perch	Fish Health, Contaminants	2009, 2010, 2011, 2013, 2014
Northlands (AR DS M3)	Deposit	White Sucker	Fish Health, Contaminants	2011, 2012, 2013
		Trout Perch	Fish Health, Contaminants	2009, 2010, 2011, 2013, 2014
		Walleye	Contaminants	2011, 2012, 2013
Suncor (AR US M4)	Development	White Sucker	Fish Health, Contaminants	2011, 2012, 2013
		Trout Perch	Fish Health, Contaminants	2009, 2010, 2011, 2013, 2014
		Walleye	Contaminants	2011, 2012, 2013
Muskeg (AR DS M4)	Development	White Sucker	Fish Health, Contaminants	2011, 2012, 2013
		Trout Perch	Fish Health, Contaminants	2009, 2010, 2011, 2013, 2014
		Walleye	Contaminants	2011, 2012, 2013
Ells (M7)	Development	Trout Perch	Fish Health, Contaminants	2009, 2011, 2014
Firebag (M8)	Far Field	Trout Perch	Fish Health, Contaminants	2010, 2013
M9	Far field	Trout Perch	Fish Health, Contaminants	2014

indices (liver weight versus body weight) among sites. For a system with multiple sites, pairwise comparisons were used to determine differences among sites. ANOVA was used to compare EEM endpoints of weight and length of fish among sites following checks for homogeneity of variances and normality of the data. Non-parametric Kruskal-Wallis analysis was used to compare MFO activity and the EEM endpoint of age in fish among sites.

Comparisons between years

Although the design of the first three years of the JOSM fish program was to generate data to develop baseline conditions for future development, it was important to determine if differences exist among sites within a year and if these differences were consistent between years of collection. EEM programs are designed to first evaluate site differences. In the next sampling

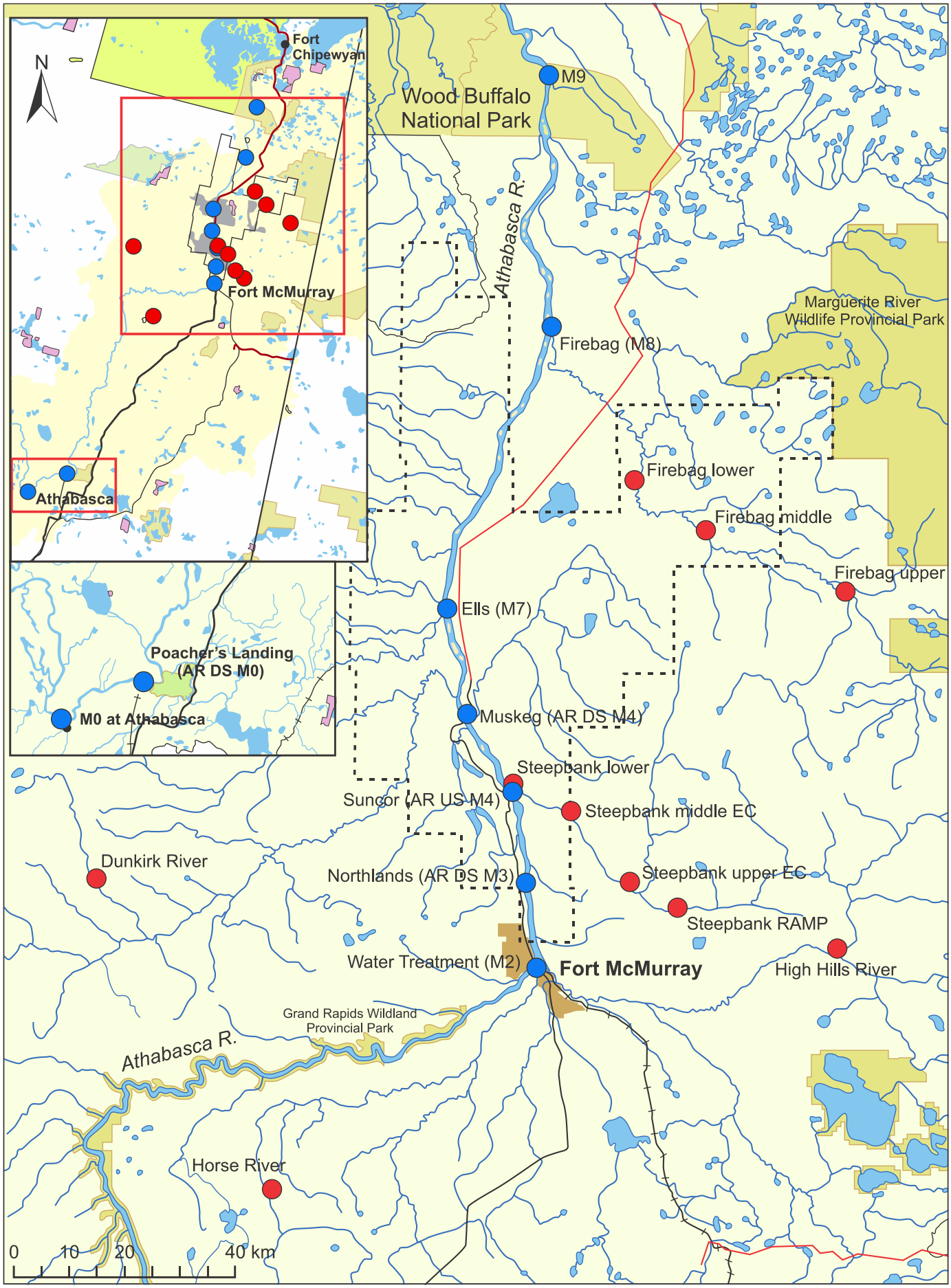

Figure 2. Map of fish collection sites.

period, the objective was to confirm responses seen in the previous year of sampling. As three years of data were collected, response patterns were compared among the three years of collections and assessments made as to whether the changes found were either getting better or getting worse.

Within site natural background variability

Through the EEM programs for pulp and paper and metal mining, critical effect sizes have been developed and were applied here for decision endpoints and for assessing natural background variability (Table 2) (Environment Canada 2010). For white sucker and trout perch data, average of the means for the upstream reference sites over time was calculated and critical effect sizes used from these means to assess change at downstream sites.

2.3 Results and Discussion

Athabasca Mainstem Fish Health

White sucker

Because of the mobility of large bodied white sucker, site selection was somewhat different than for water quality and other JOSM programs. Although studies have demonstrated that this species moves very little during the reproductive growing season of summer to late winter (Doherty et al. 2010), sites are more spatially distributed than for other endpoints (i.e., some sites are kilometres in length). In 2011, white sucker were collected from four sites on the Athabasca River (Table 1, Fig. 2). Male white sucker captured within the deposit were older, longer, heavier, and had increased condition relative to the upstream white sucker captured outside of the deposit ($p<0.05$) (Table 3). These fish also had increased internal fat storage in the body cavity and around the intestines and liver ($p<0.05$). Male white suck-

Table 2. Decision "triggers" for fish monitoring program (Environment Canada 2010). (GSI=gonadal-somatic index, LSI=liver-somatic index.)

Level	Trigger	Magnitude	Consequence
1	Effect detected	Statistical change	Seek confirmation
2	Confirmation of effect	Statistical change in same direction and endpoints	Increase spatial and temporal resolution around site of interest; add higher tier response variables
3	Exceeds critical effect size	25 % for GSI, LSI, weight-at-age, age; 10 % for condition; exceeds 2 SD for chemical/biochemical	Investigate potential source and cause of effect
Action Level	Exceeds critical effect size and is getting worse	Greater than 25 % for GSI, LSI, weight-at-age, age; 10 % for condition; exceeds 2 SD for chemical/biochemical, and getting worse	Recommend change in management strategy

Table 3. Male white sucker health parameters collected at sites on the Athabasca River (AR) during 2011-13. Values are means (±SE) and values that do not share a letter are significantly different. The numbers in brackets represents the sample size for the endpoint if different than n. (DS = downstream; US = upstream)

Male

Year	Site	Designation	n	Age	Length-at-age	Weight-at-age	Fat	Length	Weight	Condition	GSI	LSI
2011	AR DS M0	Reference	19	6.11 ± 0.32 (18)	a*	a	0.9 ± 0.2 (17) a	38.6 ± 0.6 a	727.3 ± 38.9 a	1.25 ± 0.03 a	5.49 ± 0.21 a	1.04 ± 0.03 ab*
	AR DS M3	Deposit	20	8.33 ± 0.55 (18)	a	ab	1.9 ± 0.3 (19) b	43.6 ± 0.6 b	1,276.3 ± 63.1 b	1.51 ± 0.02 b	5.30 ± 0.18 a	0.98 ± 0.05 a
	AR US M4	Develop	20	9.24 ± 0.57 (17)	b	ab	2.2 ± 0.3 b	43.9 ± 0.7 b	1,295.8 ± 70.6 b	1.50 ± 0.03 b	5.30 ± 0.18 a	1.21 ± 0.05 b
	AR DS M4	Develop	21	9.40 ± 0.61 (20)	b	b	2.6 ± 0.3 (19) b	45.0 ± 0.9 b	1,514.5 ± 99.9 b	1.61 ± 0.03 b	5.26 ± 0.21 (20) a	1.12 ± 0.04 (19) ab
2012	M0	Reference	16	6.00 ± 0.26	a	a*	0 ± 0 (15) a	36.9 ± 0.7 a	682.3 ± 30.5 a	1.36 ± 0.06 ab*	5.20 ± 0.26 a	0.99 ± 0.05 a*
	AR DS M0	Reference	19	6.11 ± 0.41	a	a	0.1 ± 0.1 ab	38.2 ± 0.7 a	745.5 ± 45.3 a	1.31 ± 0.02 a	4.96 ± 0.27 a	1.02 ± 0.03 a
	AR DS M3	Deposit	15	6.53 ± 0.76	a	ab	0.9 ± 0.4 b	39.2 ± 1.4 a	943.1 ± 106.1 a	1.47 ± 0.03 bc	5.42 ± 0.30 a	1.16 ± 0.08 ab
	AR US M4	Develop	18	8.56 ± 0.37	ab	bc	1.0 ± 0.2 c	42.8 ± 0.7 b	1,199.9 ± 73.5 b	1.50 ± 0.02 bc	4.93 ± 0.21 a	1.09 ± 0.05 ab
	AR DS M4	Develop	20	8.20 ± 0.43	b	c	1.4 ± 0.2 c	44.3 ± 0.7 b	1,401.5 ± 73.9 b	1.59 ± 0.02 c	5.50 ± 0.19 a	1.16 ± 0.05 b
2013	M0	Reference	13	6.00 ± 0.51	a	a	1.2 ± 0.2 ab	38.5 ± 0.7 a	756.5 ± 39.6 a	1.31 ± 0.02 a	6.97 ± 0.36 a*	1.04 ± 0.06 a
	AR DS M0	Reference	10	6.20 ± 0.49	ab	ab	1.9 ± 0.5 (8) d	39.6 ± 0.8 ab	893.4 ± 70.6 ab	1.41 ± 0.04 a	5.71 ± 0.42 ab	1.07 ± 0.08 a
	AR DS M3	Deposit	9	7.68 ± 0.58	a	a	1.0 ± 0.2 b	40.1 ± 0.8 ab	936.1 ± 64.9 ab	1.43 ± 0.03 a	5.27 ± 0.29 b	1.08 ± 0.05 a
	AR US M4	Develop	13	6.00 ± 0.65	a	a	1.2 ± 0.2 c	39.0 ± 1.0 a	850.0 ± 59.0 a	1.42 ± 0.05 a	4.67 ± 0.18 b	1.03 ± 0.05 a
	AR DS M4	Develop	22	7.50 ± 0.37	b	b	1.7 ± 0.3 (21) d	43.4 ± 0.8 b	1,252.0 ± 83.8 b	1.48 ± 0.03 a	5.16 ± 0.15 b	1.12 ± 0.05 a

er downstream of development also grew faster with increased length and weight at any given age ($p<0.05$; Table 3) relative to upstream reference males. Male white sucker within the deposit had increased hepatic mixed-function oxygenase activity using EROD methods relative to the upstream site ($p<0.001$), with no significant differences among sites within the deposit and downstream of development (Table 4).

In 2012, an additional reference location (M0 Athabasca) was added for fish health within the JOSM program (Table 1, Fig. 2). This site was used for water quality in the JOSM program and is upstream of the municipal wastewater discharge for the town of Athabasca. It serves as the upper most reference site for white sucker health as well as a reference location to evaluate the potential influence pulp mill discharge has on fish health at the AR DS M0 location. All male fish EEM endpoints were similar between the two upstream locations in 2012 (Table 3). Male white sucker downstream of development were older, longer and heavier than reference

Table 4. Ethoxyresorufin-O-deethylase (EROD) activity in male and female white sucker liver samples collected from sites on the Athabasca River (AR) during 2011-2013. Values represent the means (±SE), and same letters within a year are not significantly different. (DS = downstream; US = upstream).

Sex	Year	Location	EROD (pmol/min/mg)
Male	2011	AR DS M0	1.61 ± 0.17 a
		AR DS M3	7.14 ± 0.74 b
		AR US M4	10.42 ± 2.12 b
		AR DS M4	8.02 ± 1.24 b
	2012	M0	1.62 ± 0.18 a
		AR DS M0	1.55 ± 0.14 a
		AR DS M3	8.09 ± 1.25 b
		AR US M4	8.16 ± 1.17 b
		AR DS M4	9.03 ± 1.52 b
	2013	M0	2.28 ± 0.34 ac
		AR DS M0	1.36 ± 0.25 a
		AR DS M3	6.67 ± 1.61 bc
		AR US M4	13.11 ± 3.18 b
		AR DS M4	11.01 ± 2.25 b
Female	2011	AR DS M0	0.75 ± 0.05 A
		AR DS M3	2.19 ± 0.30 B
		AR US M4	4.66 ± 0.93 B
		AR DS M4	3.15 ± 0.32 B
	2012	M0	0.89 ± 0.09 A
		AR DS M0	0.87 ± 0.08 AC
		AR DS M3	4.45 ± 1.58 B
		AR US M4	6.34 ± 1.56 B
		AR DS M4	2.48 ± 0.40 BC
	2013	M0	0.72 ± 0.06 A
		AR DS M0	0.59 ± 0.05 A
		AR DS M3	2.84 ± 0.38 B
		AR US M4	5.29 ± 1.28 B
		AR DS M4	4.80 ± 1.19 B

fish, with increased internal fat stores and increased condition ($p<0.05$; Table 3). In 2012, the deposit site AR DS M3 was somewhat intermediate in most of the male white sucker health endpoints (Table 3). Similar to the white sucker collections in 2011, the major response pattern was one of nutrient enrichment as fish downstream of development were longer, heavier and had increased condition and internal fat stores. EROD activity was similar to 2011 as male white sucker within the deposit were induced relative to upstream reference males with no differences from deposit to downstream of development (Table 4).

Generally, in 2013, male white sucker collected within the deposit were comparable to upstream reference fish (Table 3). Male white sucker collected at the furthest downstream site, AR DS M4 were often significantly different than the M0 upstream reference site. However, these fish were not different than those collected downstream of the pulp mill discharge outside of the deposit at AR DS M0 (Table 3). Male white sucker EROD activity was still induced at all sites within the deposit ($p<0.001$; Table 4) similar to the two previous years. Male white sucker response patterns using EEM health endpoints and EROD as indicators of exposure show exposure appears to be very similar among the three years of baseline white sucker collections and fish health responses vary between years, with male white sucker downstream of development generally showing the most responses (Table 5). In 2011, males from the site within the deposit upstream of development were similar to fish collected downstream of development. In 2012, they were intermediate between upstream reference and downstream development. Male fish from all sites in 2013 were similar, demonstrating potential improvements in fish health within the deposit and downstream of development.

In 2011, female white sucker collected at all three sites within the deposit had increased condition factor and levels of internal fat around the intestines and liver ($p<0.05$; Table 6). Female white sucker at the furthest development downstream site (AR DS M4) were also longer, heavier and had increased growth rates, invested more energy into reproductive development, and had increased numbers of eggs relative to the females collected upstream outside of the deposit ($p<0.05$; Table 6). Female white sucker in 2011 demonstrated more of a graded MFO induction, with AR DS M0 being lowest, induced at AR DS M3, highest at the AR US M4 site, and reduced somewhat at the AR DS M4 location ($p<0.05$; Table 4).

In 2012, similar to male white sucker, all fish EEM health endpoints were comparable between the two upstream locations for female white sucker (Table 6). Female white sucker were older at the downstream locations within the deposit relative to both reference sites and generally longer, heavier and with increased condition similar to 2011, although only significant at the furthest downstream development site (AR DS M4) ($p<0.05$ Table 6). No significant differences in female white sucker growth or internal fat were found, although trends to increased internal fat were evident downstream in 2012 (Table 6). Female EROD activity was increased in all three locations within the deposit with no differences downstream of development, although levels appeared to be reduced somewhat at the AR DS M4 location (Table 4). Generally, in 2013, female white sucker collected within the deposit were similar to upstream reference fish (Table 6). Female white sucker were similar in all EEM health endpoints, although liver size did differ significantly between sites, no deposit or development relationship was evident. Similar to 2012, EROD activity was induced in female white sucker collected within the deposit with no differences in induction between the AR DS M3 site upstream of development and the two sites downstream of development (Table 4).

Similar to male white sucker, although exposure appears to be very similar among the three years of baseline white sucker collections, fish health responses vary between years with female white sucker downstream of development generally showing most responses (Table 7). In 2011 and 2012, female white sucker within the deposit upstream of development appear intermediate to the responses downstream of development. In 2013, females from all sites were similar, demonstrating potential improvements in fish health within the deposit and downstream of development.

Natural background variability

With three years of data (five collections from two upstream locations), it is possible to assess

Table 5. Summary of differences in fish parameters and EROD analysis among sites within the Athabasca River (AR) in male white sucker collected in September 2011-2013. "0" indicate no statistical difference from reference sites. Blue up arrows indicate a positive increase and red up arrows a negative increase. (Asterisk indicates interaction in ANCOVA; DS = downstream; US = upstream).

Year	Species	Site	Site Type	Sex	Age	Growth	Condition	GSI	LSI	EROD
2011	White sucker	AR DS M0	Reference	M						
		AR DS M3	Deposit		↑	0	↑	0	0	↑
		AR US M4	Development		↑	↑	↑	0	↑	↑
		AR DS M4	Development		↑	↑	↑	0	↑	↑
2012	White sucker	M0 / AR DS M0	Reference							
		AR DS M3	Deposit		0	0	↑	0	↑	↑
		AR US M4	Development		↑	↑	↑	0	0	↑
		AR DS M4	Development		↑	↑	↑	0	↑	↑
2013	White sucker	M0 / AR DS M0	Reference							
		AR DS M3	Deposit		0	0	0	*0	0	↑
		AR US M4	Development		0	0	0	0	0	↑
		AR DS M4	Development		0	0	0	0	0	↑

reference site variability and develop baselines for reference sites to compare differences in downstream fish to overall natural variability. As an example, we used male and female condition, which was consistent at the two upstream sites over the three-year period with no site differences within year. Using these data, we determined mean male white sucker condition at the upstream sites and then set upper and lower limits using both a critical effect size of 10% and two SDs of the mean. In 2011 and 2012, AR DS M4 male condition is above both the 10% critical effect size and two standard deviations of the reference mean (Fig. 3). Although condition is also higher in 2013, these data are within reference site variability. Female white sucker demonstrated very similar patterns (Fig. 4) in condition to males in the three years. Increased condition was found in females collected downstream of development (AR US M4 and AR DS M4) in both 2011 and 2012 (Fig. 4). The increases in 2011 were above the 10% critical effect size developed as part of national EEM programs as well as >2 standard deviations from reference site condition (Fig. 4). Females from the AR DS M3 site, within the deposit but upstream of major development, are intermediate in the two years and increased relative to upstream but still lower than development sites. In 2013, female condition factor is similar at all sites. This change is due to reductions in condition at the downstream sites, with reference site females staying the same (Fig. 4). Similar analysis can be completed with the other fish health endpoints, including age, growth, and gonado- and liver somatic indices.

Liver Assessments

It is known that PACs and related compounds can produce liver tumours in fish, specifically those with benthic feeding habits, which result from exposure to contaminated sediments. As part of the JOSM program, an assessment of liver tumour incidences in white sucker collected from the LAR mainstem was conducted and rates compared to both upstream reference locations and to additional reference baselines collected from Lake Superior sites (McMaster unpublished data). As rates are generally low, sample size requirements to detect change for these studies is high (n=100) (Blazer et al. 2009). White sucker liver samples from all sites collected in the 2011-2013 study years were sampled and processed for liver tumour analysis (Rafferty et al. 2009). Examination of tumour rates in white sucker collected in the LAR mainstem, for samples analyzed to date (2011), suggests that liver tumour rates are not elevated at any sites sampled on the Athabasca River (McMaster unpublished data), although it is clear from the EROD induction in fish within the deposit (Table 4) that these fish are exposed to inducing compounds. We will reassess this when the remaining samples are processed.

Table 6. Female white sucker health parameters collected at sites on the Athabasca River (AR) during 2011-13. Values are means ±SE and values that do not share a letter are significantly different. The numbers in brackets represents the sample size for the endpoint if different than n. (DS = downstream; US = upstream)

Female							Female						
Year	Site	Designation	n	Age	Length-at-age	Weight-at-age	Fat	Length	Weight	Condition	GSI	Fecundity	LSI
2011	AR DS M0	Reference	20	7.25+ 0.66 (16)	ab	a	0.94+ 0.14 (16) a	41.69+ 0.88 a	980.79+ 63.08 a	1.32 ± 0.02 a	5.50 ± 0.25 (17) ab	32,324 ± 2,195 (16) a	1.44 ± 0.05 (17) a
	AR DS M3	Deposit	19	7.93± 0.63 (15)	a	ab	2.37± 0.30 b	42.82± 0.58 a	1,162.95± 59.15 ab	1.46 ± 0.03 b	5.47 ± 0.24 ab	31,106 ± 1,520 a	1.36 ± 0.04 a
	AR US M4	Develop	21	7.38+ 0.42 (16)	ab	bc	2.70+ 0.27 (20) b	44.28+ 0.95 ab	1,395.48+ 99.82 bc	1.56 ± 0.04 b	5.23 ± 0.23 (20) a	35,214 ± 2,785 (17) a	1.42 ± 0.06 (20) a
	AR DS M4	Develop	20	8.95± 0.44	b	c	3.11± 0.33 (19) b	46.59± 1.03 b	1,656.34± 126.05 c	1.57 ± 0.04 b	6.64 ± 0.27 b	41,434 ± 3,069 b	1.52 ± 0.10 (19) a
2012	M0	Reference	20	6.55± 0.39	a	a	0.60± 0.11 ab	40.69± 0.62 a	890.72± 43.82 a	1.30 ± 0.02 a*	4.25 ± 0.16 a	33,530 ± 2,031 a	1.30 ± 0.05 a
	AR DS M0	Reference	18	6.61± 0.47	a	a	0.50± 0.15 ab	42.05± 0.80 ab	991.30± 50.69 ab	1.31 ± 0.02 a	4.55 ± 0.24 a	39,312 ± 2,969 a	1.40 ± 0.06 a
	AR DS M3	Deposit	18	7.44± 0.59	a	a	1.44± 0.33 b	43.17± 1.05 abc	1,189.37± 88.00 bc	1.43 ± 0.02 ab	4.60 ± 0.29 a	34,299 ± 2,476 a	1.41 ± 0.07 (17) a
	AR US M4	Develop	16	8.53± 0.48	a	a	1.56± 0.32 ab	45.44± 1.10 bc	1,441.89± 137.66 c	1.45 ± 0.04 ab	5.59 ± 0.42 a	46,262 ± 3,187 (15) a	1.45 ± 0.04 a
	AR DS M4	Develop	15	9.13± 0.51	a	a	2.07± 0.49 (14) ab	46.03± 0.84 c	1,536.69± 113.13 c	1.53 ± 0.04 b	5.06 ± 0.50 a	44,751 ± 3,508 a	1.53 ± 0.10 a
2013	M0	Reference	19	7.58+ 0.30	a	a	1.63+ 0.24 a	43.41+ 0.62 a	1,130.58+ 55.30 a	1.36 ± 0.03 a	5.33 ± 0.28 a	52,891 ± 3,688 (16) a	1.51 ± 0.06 ac
	AR DS M0	Reference	20	6.95± 0.35	a	a	1.37± 0.23 (19) a	43.06± 0.59 a	1,050.55± 45.71 a	1.30 ± 0.26 a	4.90 ± 0.22 a	43,397 ± 2,638 (19) a	1.29 ± 0.05 b
	AR DS M3	Deposit	19	7.21+ 0.36	a	a	1.11+ 0.21 (18) a	41.88+ 0.71 ab	994.56+ 58.25 a	1.33 ± 0.02 a	4.73 ± 0.26 a	33,898 ± 1,656 (18) a	1.36 ± 0.04 ab
	AR US M4	Develop	12	5.67± 0.57	a	a	1.00± 0.30 (10) b	40.03± 0.86 b	897.18± 69.67 a	1.37 ± 0.04 a	4.68 ± 0.49 a	32,832 ± 3,006 (11) a	1.31 ± 0.07 ab
	AR DS M4	Develop	16	7.13± 0.49	a	a	1.80± 0.34 (15) a	42.90± 0.93 ab	1,085.44± 91.31 a	1.34 ± 0.02 a	5.55 ± 0.18 a	37,042 ± 1,756 a	1.65 ± 0.05 c

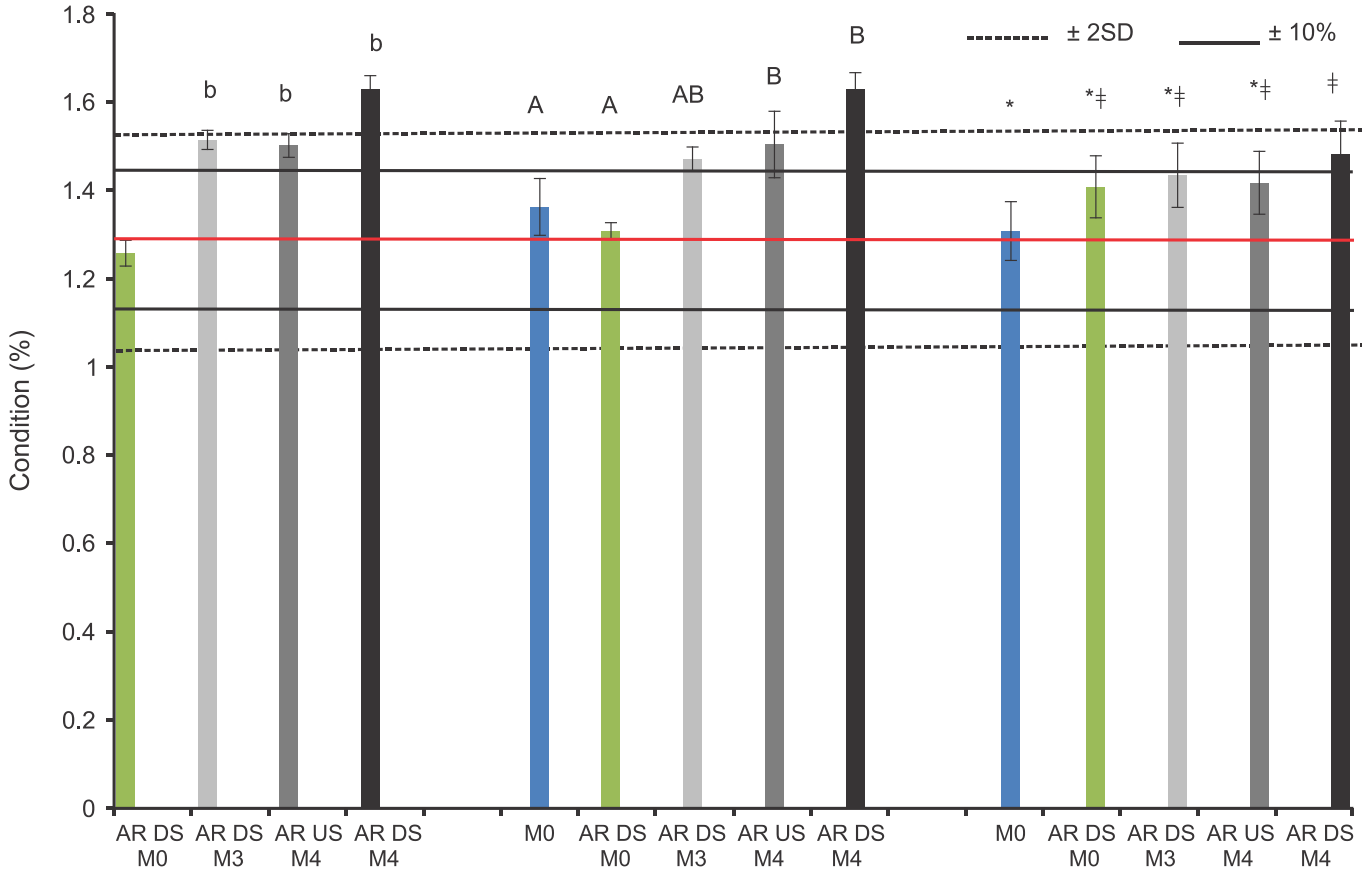

Figure 3. Male white sucker condition from sites collected on the Athabasca River (AR) in 2011-2013. Means ±SE with the critical effects size of 10 % and two standard deviations of the reference site means. (DS = downstream; US = upstream).

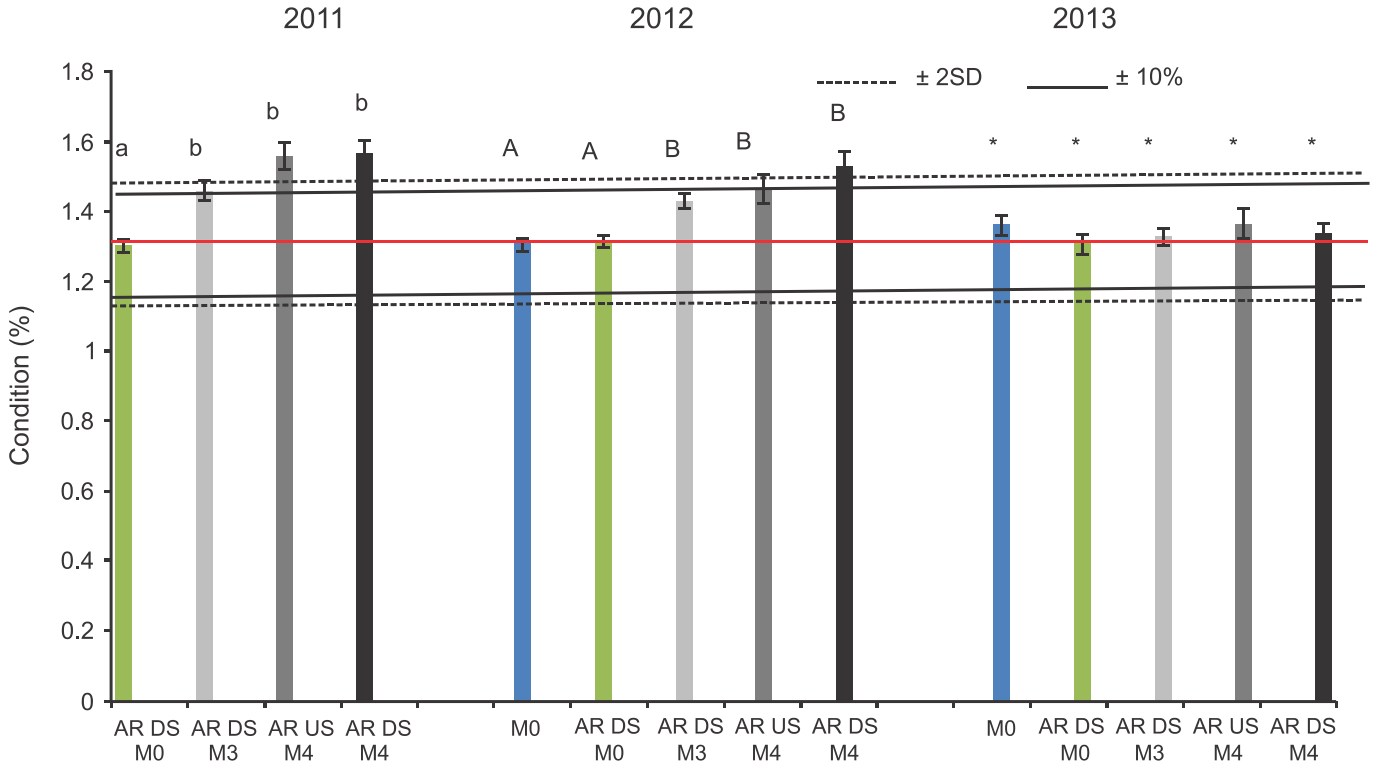

Figure 4. Female white sucker condition from sites collected on the Athabasca River (AR) in 2011-2013. Means ±SE with the critical effects size of 10 % and two standard deviations of the reference site means. (DS = downstream; US = upstream).

Contaminants in fish

PACs were measured in select fillet samples collected from female white sucker during 2012 and 2013. ΣPAC concentrations in female fillet were very low (11-19 ng/g wet weight) with slightly higher concentrations in 2013 than 2012 and at the AR DS M4 reach relative to the AR US M4 (Fig. 5). Alkylated PACs accounted for 89-93 % of ΣPAC, a slightly lower percentage than typically found in sediments (~95 %) in the oil sands area. Similar analysis was conducted on liver tissue taken from white sucker from the five locations in 2013 (Fig. 6). There was a general tendency in female white sucker liver tissue for ΣPAC concentrations to increase from the M0 (30 ng/g) to the AR DS M4 reach (75 ng/g); percent alkylated PACs increased from 61 % to 87 %. A somewhat similar pattern was observed for male liver tissue, with ΣPAC concentration increasing from 17 ng/g at the M0 reach to 229 ng/g at the AR US M4 reach; however, concentrations declined at the AR DS M4 reach (96 ng/g). Percent alkylated PACs increased from 59-75 % at the upstream M0 and AR DS M0 reaches to 85-90 % between AR DS M3 and AR DS M4 within the deposit. Overall, there appears to be a tendency for PAC concentrations to increase in white suckers from upstream of Fort McMurray to the oil sands development area, with the increase more apparent for alkylated PACs. An increase in the number of fish analyzed per site would strengthen the dataset for assessing trends/improvement in white sucker PAC concentrations.

Ten walleye from each location were also collected from the LAR mainstem for contaminant analysis. Only a minimum of samples from 2012 and 2013 have been processed from the two development sites (Fig. 7). ΣPAC concentrations in fillets were similar in male (6-15 ng/g) and female (10-16 ng/g) walleye from both locations and generally similar to female white sucker fillets; percent alkylated PACs accounted for 87-97 % of ΣPAC in 2012 but only 66-69 % in 2013. ΣPAC concentrations in walleye livers from the 2013 AR DS M4 location demonstrated

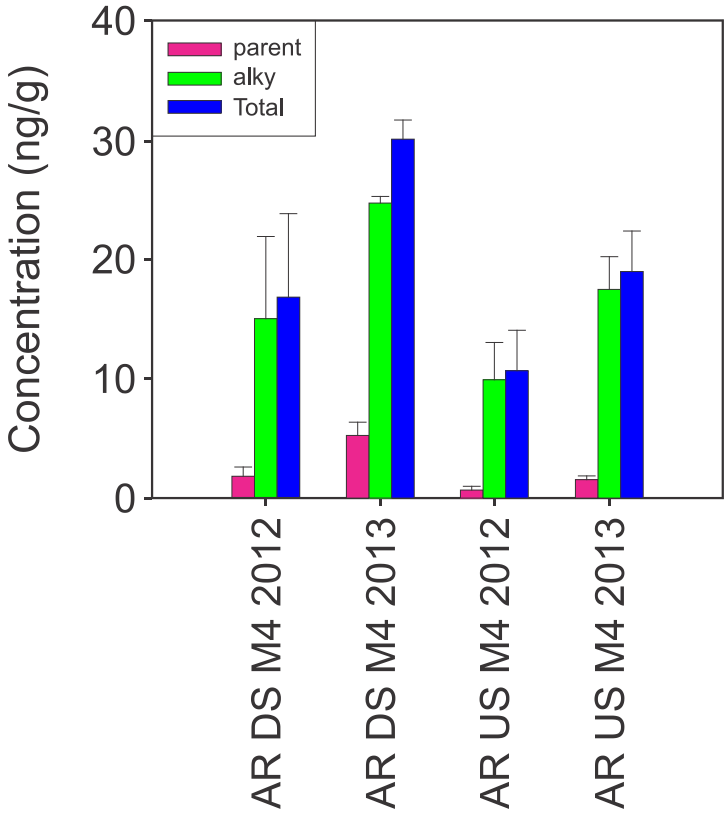

Figure 5. PAC concentrations (parent, alkylated and total) in muscle fillets of female white sucker collected in 2012-2013 from two sites on the Athabasca River (AR) downstream of oil sands development. (DS = downstream; US = upstream).

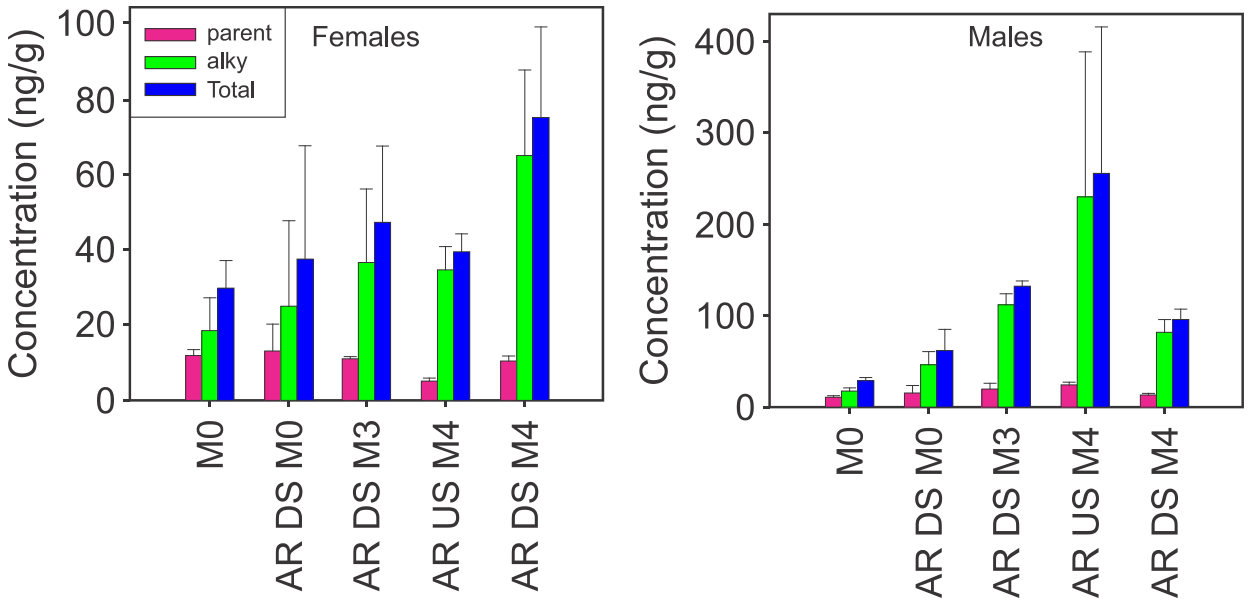

Figure 6. PAC concentrations (parent, alkylated and total) in liver of both female and male white sucker collected from the Athabasca River (AR) in the fall of 2013. (DS = downstream; US = upstream).

that males had substantially higher ΣPAC concentrations than females (344 ng/g versus 70 ng/g): alkylated PACs averaged 93 % in males and 83 % in females. These results need to be confirmed in the future with complete analysis of the samples collected for baseline measures.

White sucker summary

Overall, white sucker are sensitive indicators of fish health in the system as consistent changes in fish health were documented downstream within the oil sands deposit in 2011 and 2012. These differences are indicative of nutrient enrichment, as white sucker have increased condition and increased levels of internal fat stores. We confirmed responses in white sucker in the first two years of our studies. However, the third year of white sucker fish health studies indicated changes occurring with fish within the deposit, as condition factors were no longer different and improvements in excessive fat deposits in the body cavity were evident. The program has moved from three intensive years of baseline data collection to a three-year long-term monitoring cycle. We recommend evaluating whether improvements in fish health identified in year three of baseline monitoring are confirmed in the next sampling period in 2016. EROD activity was a good indicator of exposure to PAC-related compounds and indicates some potential increased exposure downstream of development. This was reflected best in PAC levels in white sucker liver tissue in both males and females with increased PACs downstream of development. Levels were higher in male livers than females, a trend also demonstrated in walleye livers. We have begun to evaluate site differences relative to overall upstream reference site variability and have documented change in fish collected in the deposit and downstream of industrial activity that exceed defined critical effects sizes. These differences, however, were very much improved in 2013. With three years of data at individual sites, we can define what is normal for that site over the three years of monitoring. To do this, a cumulative mean ±2 SD will be calculated and then used to make more meaningful predictions of future observations as more data are added (Arciszewski and Munkittrick 2015). These tools should be used to make predictions of fish health into the future and to identify change within a site and between sites.

Trout perch

LAR mainstem trout perch work was initiated in 2009 with five sites used for assessing fish health (Table 1, Fig. 2). In 2010, work was conducted in collaboration with the Regional Aquatic Monitoring Program (RAMP), with M7 being replaced with a site at the confluence of the Firebag River (M8) (Table 1, Fig. 2). In 2009 and 2010 only sites within the oil sands deposit were sampled with the water treatment plant (M2)

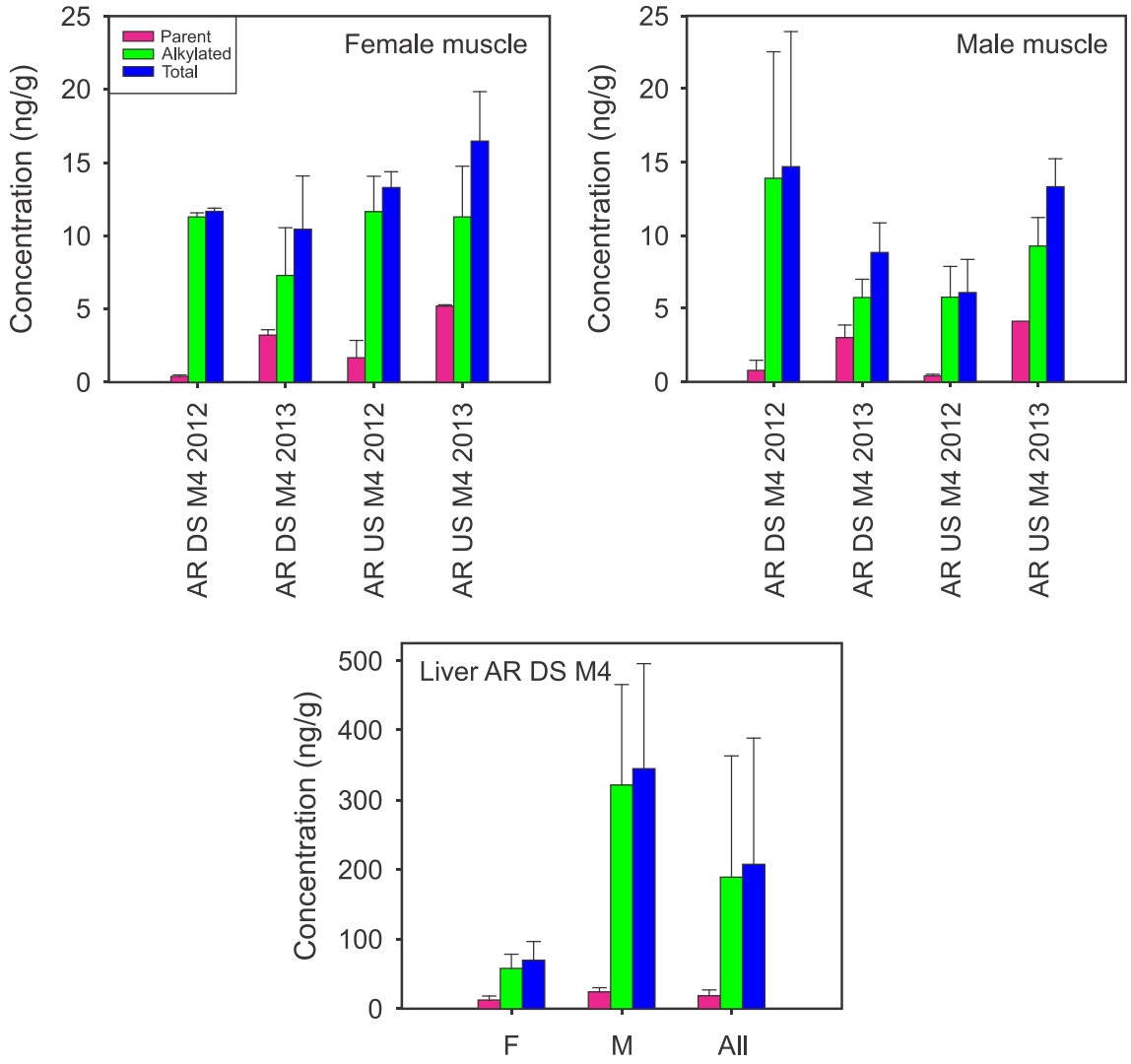

Figure 7. PAC concentrations (parent, alkylated and total) in walleye muscle (2 sites) and liver (1 site) collected on the Athabasca (AR) downstream of oil sands development. (US=upstream; DS=downstream; F=female; M=male).

as the upstream undeveloped site as reference for comparison purposes. Summaries of trout perch responses are found in Tables 8 (females) and 9 (males) demonstrating the overall trends in fish health across years within the Athabasca River relative to the upstream reference location used within year.

In 2009, male trout perch were similar among sites with the only site difference being increased condition in males at the AR US M4 location within the deposit downstream of development ($p<0.05$; Table 10). Although there was a trend of increased EROD activity in fish livers collected within the deposit and into development (highest levels at AR US M4), no significant differences were identified between sites (Table 11). Sampling in 2010 was then conducted to confirm the lack of site differences in 2009 and to further develop our baseline for trout perch health in the Athabasca River. Male trout perch in 2010 were similar among sites in most fish health parameters. Males from AR DS M4 were longer, heavier ($p<0.05$) and generally older but were of similar condition to males from the other sites. Male trout perch collected downstream of the Fort McMurray municipal sewage discharge (AR DS M3) had larger livers relative to the upstream M2 location ($p<0.05$; Table 10). EROD data are not available from the 2010 collections.

In 2011, we added an additional upstream reference site outside the oil sands deposit to determine if the deposit itself was the dominant factor in trout perch health examined in the previous two years (i.e., all sites similar within the oil sands deposit), and to further develop our

Table 7. Summary of differences in fish parameters and EROD analysis among sites within the Athabasca River (AR) in female white sucker collected in the September of 2011-2013. "0" indicate no statistical difference from reference sites. Blue up arrows indicate a positive increase, and red up arrows indicate a negative response relative to the reference sites. (DS = downstream; US = upstream).

Year	Species	Site	Site Type	Sex	Age	Growth	Condition	GSI	LSI	EROD
2011	White sucker	AR DS M0	Reference	F						
		AR DS M3	Deposit		0	0	↑	0	0	↑
		AR US M4	Development		0	↑	↑	0	0	↑
		AR DS M4	Development		0	↑	↑	0 ↑	0	↑
2012	White sucker	M0 / AR DS M0	Reference							
		AR DS M3	Deposit		0	0	0 ↑	0	0	↑
		AR US M4	Development		↑	0	0 ↑	0	0	↑
		AR DS M4	Development		↑	0	↑	0	0	↑
2013	White sucker	M0 / AR DS M0	Reference							
		AR DS M3	Deposit		0	0	0	0	0	↑
		AR US M4	Development		0	0	0	0	0	↑
		AR DS M4	Development		0	0	0	0	0	↑

Table 8. Summary of differences in fish parameters and EROD analysis among sites within the Athabasca River (AR) in female trout perch collected in September 2009-2014. "0" indicate no statistical difference from reference sites. Blue up arrows indicate a positive increase, red up arrows a negative increase and yellow down arrows indicate a decrease, relative to reference sites. (DS = downstream; US = upstream).

Year	Species	Site	Site Type	Sex	Age	Length	Weight	Condition	GSI	LSI	EROD
2009	Trout-perch	M2	Ref (Dep)	F							
		AR DS M3	Deposit			0	0	0	0	0	0
		AR US M4	Development			0	0	0	0	0	0
		AR DS M4	Development			0	0	0	0	0	0
		M7	Development			0	0	0	0	0	0
2010	Trout-perch	M2	Ref (Dep)								
		AR DS M3	Deposit		↑	↑	↑	0	↑	↑	NA
		AR US M4	Development		↑	↑	↑	0	↑	0	NA
		AR DS M4	Development		↑	↑	↑	0	↑	0	NA
		M8	Far Field		0	0	0	↓	↓	0	NA
2011	Trout-perch	AR DS M0	Reference								
		M2	Ref (Dep)			0	0	0	0	0	0
		AR DS M3	Deposit			0	0	0	↓	↑	↑
		AR US M4	Development			0	0	0	0	0	0
		AR DS M4	Development			0	0	0	0	↑	0
		M7	Development			0	0	0	0	0	↑
2013	Trout-perch	M0	Reference								
		AR DS M0	Reference		0	0	0	↑	0	0	0
		M2	Ref (Dep)		0	0	0	0	0	0	0
		AR DS M3	Deposit		0	0	0	0	0	0	↑
		AR US M4	Development		0	0	0	0	0	0	↑
		AR DS M4	Development		0	0	0	0	0	0	↑
		M8	Far Field		↓	0	0	0	↓	↓	↑
2014	Trout-perch	M0	Reference								
		AR DS M0	Reference		0	↓	↓	0	0	0	0
		M2	Ref (Dep)		0	0	0	0	0	↑	0
		AR DS M3	Deposit		0	0	0	0	0	0	0
		UP AR US M4	Development		0	0	0	0	0 ↓	0	0
		AR US M4	Development		0	0	0	0	0 ↓	0	0
		AR DS M4	Development		0	↓	↓	0	0 ↓	0	0
		M7	Development		0	0	0	0	0	↑	0
		M9	Far Field		0	↓	↓	0	0	0	0

Table 9. Summary of differences in fish parameters and EROD analysis among sites within the Athabasca River (AR) in male trout perch collected in September 2009-2014. "0" indicate no statistical difference from reference sites. Blue up arrows indicate a positive increase, red up arrows a negative increase and yellow down arrows indicate a decrease, relative to reference sites. (DS = downstream; US = upstream).

Year	Species	Site	Site Type	Sex	Age	Length	Weight	Condition	GSI	LSI	EROD
2009	Trout-perch	M2	Ref (Dep)	M							
		AR DS M3	Deposit			0	0	0	0	0	0
		AR US M4	Development			0	0	↑	0	0	0
		AR DS M4	Development			0	0	0	0	0	0
		M7	Development			0	0	0	0	0	0
2010	Trout-perch	M2	Ref (Dep)								
		AR DS M3	Deposit		0	0	0	0	0	↑	NA
		AR US M4	Development		0	0	0	0	0	0	NA
		AR DS M4	Development		0	↑	↑	0	0	0	NA
		M8	Far Field		0	0	0	0	↓	0	NA
2011	Trout-perch	AR DS M0	Reference								
		M2	Ref (Dep)			0	0	0	0	0	0
		AR DS M3	Deposit			↓	0	0	0	0	↑
		AR US M4	Development			0	0	0	0	0	0
		AR DS M4	Development			0	0	0	0	0	0
		M7	Development			0	0	↓	0	↓	↑
2013	Trout-perch	M0	Reference								
		AR DS M0	Reference		0	0	0	↑	0	0	0
		M2	Ref (Dep)		0	0	0	0	0	0	0
		AR DS M3	Deposit		0	0	0	0	0	0	0
		AR US M4	Development		0	0	0	0	0	0	0
		AR DS M4	Development		0	↑	0	0	0	↑	0
		M8	Far Field		0	0	0	0	0	0	0
2014	Trout-perch	M0	Reference								
		AR DS M0	Reference		0	0	0	0	0	0	0
		M2	Ref (Dep)		0	0	0	0	0	0	0
		AR DS M3	Deposit		↓	0	0	0	0 ↓	0	0
		UP AR US M4	Development		0	0	0	0	0 ↓	0	0
		AR US M4	Development		0	0	0	0	0 ↓	0	0
		AR DS M4	Development		0	0	0	0	0	0	0
		M7	Development		0	0	0	0	0	0	0
		M9	Far Field		0	0	0	0	↑	0	0

understanding of baseline trout perch health in the system. Male trout perch were similar among sites in 2011 as well, with the only response being reduced condition ($p<0.05$) and suggestions of reduced gonadosomatic indices in males collected at the AR M7 location at the Ells River confluence (Table 10). There were no site differences in male trout perch health endpoints between the new upstream reference site outside of the deposit and the deposit reference site located at M2. However, male EROD activity indicated significant induction in males collected at the AR DS M3 and the M7 location relative to the AR DS M0 reference site outside of the deposit ($p<0.05$; Table 11)

In 2013, similar to the white sucker collections, an additional reference location was added at the M0 Athabasca location outside the deposit and upstream of the pulp mill discharge at AR DS M0 (Fig. 2). Male trout perch again were very similar among all sites, except at site AR DS M0 where condition increased relative to all other sites (Table 10, significant interaction). The only other significant difference was an increase in liver size in male trout perch from the downstream AR DS M4 location relative to the AR DS M0 and M2 locations (Table 10). EROD activity demonstrated no deposit related induction in male trout perch as induction again was rather low and demonstrated some variability within sites (Table 11).

In 2014, nine sites were sampled for trout perch health including all sites sampled in previous years as well as a site further downstream, M9 at the 27th baseline within Wood Buffalo National Park (Fig. 2). Male trout perch in 2014

Table 10. Male trout perch collected on the Athabasca River (AR) during 2009-2014. Values represent means ±SE and values with different letters are significantly different. (DS = downstream; US = upstream).

Male

Year	Site	N	Age	Length (mm)	Weight (g)	GSI	LSI	Condition
2009	M2	21		71.6 ± 1.7	4.15 ± 0.29 ab	1.61 ± 0.07	1.19 ± 0.06	1.10 ± 0.01 a
	AR DS M3	20		71.8 ± 1.5	4.11 ± 0.24 ab	1.48 ± 0.09	1.45 ± 0.09	1.09 ± 0.01 a
	AR US M4	20		73.6 ± 1.2	4.67 ± 0.21 a	1.77 ± 0.08	1.36 ± 0.10	1.16 ± 0.02 b
	AR DS M4	20		67.5 ± 1.8	3.40 ± 0.22 b	1.41 ± 0.06	1.29 ± 0.04	1.06 ± 0.01 a
	M7	20		70.9 ± 1.2	3.78 ± 0.17 ab	1.59 ± 0.05	1.33 ± 0.04	1.06 ± 0.02 a
2010	M2	20	2.8 ± 0.2 ab	66.8 ± 1.4 b	3.27± 0.20 b	2.48 ± 0.11 a	1.65 ± 0.07 a	1.07± 0.02
	AR DS M3	20	3.2 ± 0.2 ab	65.0 ± 1.5 b	3.09 ± 0.24 b	2.08 ± 0.11 a	2.00 ± 0.08 b	1.10 ± 0.01
	AR US M4	20	3.3 ± 0.2 ab	67.3 ±2.1 b	3.33 ± 0.32 b	2.29 ± 0.13 a	1.57 ± 0.08 a	1.07 ± 0.01
	AR DS M4	20	3.6 ± 0.2 b	74.1 ± 1.7 c	4.39 ± 0.26 c	2.68 ± 0.11 a	1.68 ± 0.06 ab	1.05 ± 0.01
	M8†	13	2.5 ± 0.2 a	57.5 ± 2.2 a	2.14 ± 0.31 a	1.50 ± 0.14 b	1.71± 0.08 a	1.07 ± 0.02
2011	AR DS M0	20		66.2 ± 1.0 ab	3.17 ± 0.13 ab	2.7 ± 0.08 ab	1.31 ± 0.04	1.08 ± 0.02 a
	M2	21		70.3 ± 1.5 a	3.67 ± 0.21 a	2.8 ± 0.09 ab	1.48 ± 0.05	1.03 ± 0.02 ab
	AR DS M3	20		62.8 ± 1.8 b	2.69 ± 0.23 b	2.48 ± 0.1 ab	1.59 ± 0.09	1.04 ± 0.02 ab
	AR US M4	20		68.2 ± 1.5 ab	3.44 ± 0.21 a	2.9 ± 0.13 ab	1.44 ± 0.06	1.06 ± 0.01 a
	AR DS M4	20		68.1 ± 1.4 ab	3.36 ± 0.22 ab	2.97 ± 0.11 a	1.51 ± 0.1	1.04 ± 0.01 ab
	M7	20		68.8 ± 0.8 a	3.31 ± 0.19 a	2.64 ± 0.09 b	1.36 ± 0.04	1.01 ± 0.01 b
2013	M0	20	3.8 ± 0.2	66.4 ± 2.0 b	3.50 ± 0.30	2.13 ± 0.13	1.39 ± 0.04 ab	1.12 ± 0.02*
	AR DS M0	20	4.4 ± 0.4	67.3 ± 2.3 ab	3.97 ± 0.34	2.01 ± 0.10	1.29 ± 0.04 a	1.25 ± 0.02
	M2	21	4.52 ± 0.2	72.0 ± 2.1 ab	4.07 ± 0.29	2.31 ± 0.15	1.29 ± 0.06 a	1.05 ± 0.01
	AR DS M3	20	3.40 ± 0.3	69.1 ± 1.5 ab	3.76 ± 0.26	1.95 ± 0.11	1.46 ± 0.05 ab	1.10 ± 0.01
	AR US M4	20	4.0 ± 0.3	71.7 ± 1.3 ab	4.15 ± 0.20	2.1 ± 0.14	1.36 ± 0.05 ab	1.10 ± 0.01
	AR DS M4	20	4.0 ± 0.2	73.3 ± 0.9 a	4.20 ± 0.12	2.13 ± 0.07	1.56 ± 0.07 b	1.07 ± 0.02
	M8	21	3.1 ± 0.2	71.6 ± 0.9 ab	3.99 ± 0.13	2.43 ± 0.11	1.34 ± 0.04 ab	1.08 ± 0.01
2014	M0	23	3.6 ± 0.2 b	66.9 ± 1.9	3.53 ± 0.31	1.01 ± 0.06*	1.16 ± 0.05*	1.12 ± 0.02
	AR DS M0	20	3.8 ± 0.2 b	62.9 ± 1.9	3.09 ± 0.30	0.85 ± 0.09	1.07 ± 0.05	1.18 ± 0.02
	M2	20	4.1 ± 0.2 b	69.7 ± 2.0	4.13 ± 0.38	1.08 ± 0.10	1.39 ± 0.05	1.13 ± 0.02
	AR DS M3	20	2.8 ± 0.2 a	69.3 ± 2.2	4.00 ± 0.37	0.65 ± 0.06	1.17 ± 0.04	1.15 ± 0.02
	Up AR US M4	20	4.0 ± 0.3 b	68.5 ± 2.1	3.86 ± 0.34	0.66 ± 0.07	1.17 ± 0.04	1.14 ± 0.01
	Down AR US M4	19	4.1 ± 0.3 b	70.4 ± 1.9	4.22 ± 0.33	0.73 ± 0.05	1.03 ± 0.04	1.17 ± 0.02
	AR DS M4	24	4.5 ± 0.3 b	66.2 ± 1.6	3.37 ± 0.26	0.83 ± 0.08	1.12 ± 0.05	1.13 ± 0.01
	M7	20	3.6 ± 0.3 ab	65.6 ± 2.2	3.35 ± 0.36	1.11 ± 0.11	1.33 ± 0.06	1.11 ± 0.01
	M9	21	4.0 ± 0.2 b	64.8 ± 1.7	3.16 ± 0.26	1.31 ± 0.14	1.25 ± 0.05	1.12 ± 0.01

Table 11. Ethoxyresorufin-O-deethylase (EROD) activity in male and female trout perch liver samples collected from sites on the Athabasca River (AR) during 2009-2014. Values represent the means ±SE with similar letters within a year not significantly different. (DS = downstream; US = upstream).

Sex	Year	Location	EROD (pmol/min/mg)
Male	2009	M2	0.16 ± 0.02 a
		AR DS M3	0.20 ± 0.04 a
		AR US M4	0.35 ± 0.10 a
		AR DS M4	0.14 ± 0.02 a
		M7	0.31 ± 0.14 a
	2011	AR DS M0	0.41 ± 0.09 ac
		M2	0.50 ± 0.09 bc
		AR DS M3	1.21 ± 0.61 b
		AR US M4	0.72 ± 0.33 bc
		AR DS M4	0.62 ± 0.12 bc
		M7	0.96 ± 0.21 b
	2013	M0	0.51 ± 0.20 a
		AR DS M0	0.23 ± 0.02 a
		M2	0.34 ± 0.06 a
		AR DS M3	0.64 ± 0.18 a
		AR US M4	0.36 ± 0.03 a
		AR DS M4	0.54 ± 0.08 a
		M8	0.28 ± 0.04 a
	2014	M0	0.24 ± 0.03 ab
		AR DS M0	0.20 ± 0.02 b
		M2	0.28 ± 0.03 ab
		AR DS M3	0.27 ± 0.02 ab
		UP AR US M4	0.23 ± 0.03 ab
		DOWN AR US M4	0.37 ± 0.05 a
		AR DS M4	0.30 ± 0.05 ab
		M7	0.29 ± 0.03 ab
		M9	0.30 ± 0.04 ab
Female	2009	M2	0.18 ± 0.02 A
		AR DS M3	0.25 ± 0.07 A
		AR US M4	0.32 ± 0.10 A
		AR DS M4	0.24 ± 0.06 A
		M7	0.15 ± 0.03 A
	2011	AR DS M0	0.28 ± 0.04 AC
		M2	0.37 ± 0.04 BC
		AR DS M3	0.75 ± 0.19 B
		AR US M4	0.43 ± 0.12 BC
		AR DS M4	0.30 ± 0.03 BC
		M7	1.52 ± 0.45 B
	2013	M0	0.16 ± 0.02 B
		AR DS M0	0.27 ± 0.04 AB
		M2	0.34 ± 0.06 AB
		AR DS M3	0.50 ± 0.18 A
		AR US M4	0.48 ± 0.26 A
		AR DS M4	0.37 ± 0.04 A
		M8	0.36 ± 0.06 A
	2014	M0	0.19 ± 0.03 A
		AR DS M0	0.20 ± 0.03 A
		M2	0.26 ± 0.03 A
		AR DS M3	0.25 ± 0.02 A
		UP AR US M4	0.19 ± 0.02 A
		DOWN AR US M4	0.24 ± 0.05 A
		AR DS M4	0.43 ± 0.09 A
		M7	0.39 ± 0.10 A
		M9	0.25 ± 0.03 A

demonstrated reductions in gonadal development at three consecutive sites on the river, AR DS M3 (sewage), and the upstream AR US M4 site and at the AR US M4 site, which also could be dominated by sewage effluent from the town of Fort McMurray (Table 10; significant interaction between sites and gonad to weight relationship). The UP AR US M4 site was added to get additional information on trout perch health upstream and downstream of a water intake location. Fish sampled within Wood Buffalo National Park demonstrated increases in gonadal investment relative to upstream reference sites. As in previous years, no consistent significant differences were found in EROD induction in male trout perch sampled on the Athabasca River (Table 11).

In 2009, female trout perch were all very similar among sites with some differences between sites downstream of development, but not relative to the M2 reference location (Table 12). EROD activity in female trout perch was quite low, similar to males with some indications of increases downstream of the sewage input and oil sands development, but differences were not significant (Table 11). In 2010, female trout perch collected downstream of the city of Fort McMurray (AR DS M3, AR US M4 and AR DS M4) were generally older, longer and heavier with increased investment in gonadal development ($p<0.05$; Table 12 significant interaction in GSI visual examination of data demonstrates site differences), but no differences in condition were found. Females collected at the M8 Firebag confluence were much smaller and sample sizes were low at this location.

In 2011, female trout perch from the AR DS M3 location had reduced investment in gonadal development and increased liver sizes relative to the AR DS M0 location, but were similar to the M2 upstream site within the deposit (Table 12). EROD activity demonstrated significant site differences as AR DS M3 and AR M7 females were induced relative to the new upstream site, AR DS M0, which is outside the deposit (Table 11). EROD activity in female trout perch in 2013 was induced at all sites within the deposit downstream of Fort McMurray relative to the M0 site upstream outside the deposit (Table 11). Although these fish demonstrate some relative exposure, no consistent fish health responses were evident as only AR M8 females had reduced gonadal development and liver size relative to upstream reference fish (Table 12). In 2014 with the largest range of sites sampled, no significant differences were seen in EROD activity in female trout perch (Table 11). The only interpretable responses to exposure were seen in female trout perch at the AR DS M4 location where fish were shorter, lighter and had reduced investment in reproductive development ($p<0.05$; Table 12). Females from the UP AR US M4, AR US M4 and AR DS M4 locations also had indications of reduced investment in reproductive development similar to males (Table 12).

Natural background variability

Inter-annual variation and change relative to reference sites over time were evaluated using the multi-year data for male condition factor for the LAR mainstem. The two reference groupings for male condition factor are different inside the deposit (1.1 %) versus outside the deposit (1.2 %) (Fig. 8). Dotted lines for both reference means represent the critical effects size of 10 % used in the two federal EEM programs. In 2014, condition of males from all sites are above the deposit running average but are still within the 10 % critical effect size. Upstream reference condition factor means are clearly influenced by the unusual condition in male trout perch from the AR DS M0 location in 2013. These responses should be examined further (sampled again in 2015) and require evaluation in terms of other external factors, such as water temperature and pulp mill effluent, that may be influencing these health endpoints. Similar analysis should be completed on other endpoints for trout perch health (growth, gonadosomatic and liver somatic indices) to evaluate natural background variability, change over time, and the significance of external factors involved when change occurs.

Trout perch summary

Although trout perch are less mobile than the white sucker, they appear to be less responsive to the various conditions in the river. EEM programs for the pulp and paper and metal mining sectors also use adult fish surveys of sentinel species to evaluate potential of effluents to alter fish health (Environment Canada 2010, 2012b). In these programs, alterations in fish health endpoints within a sampling season relative to the reference location require confirmation in the following cycle. For trout perch, no consistent effects were demonstrated within a

Table 12. Female trout perch collected on the Athabasca River (AR) during 2009-2014. Values represent means ±SE and values with different letters are significantly different. (DS = downstream; US = upstream).

Year	Site	N	Age	Length (mm)	Weight (g)	GSI	LSI	Condition
2009	M2	20		77.0 ± 2.0	5.27 ± 0.42	5.82 ± 0.22*	1.71 ± 0.1ab	1.11 ± 0.02 ab
	AR DS M3	21		78.1 ± 1.8	5.64 ± 0.34	5.80 ± 0.17	1.68± 0.11a	1.11 ± 0.02 ab
	AR US M4	20		75.6 ± 1.4	5.08 ± 0.28	5.52 ± 0.16	1.90 ± 0.07b	1.16 ± 0.01 b
	AR DS M4	24		75.5 ± 1.2	4.69 ± 0.22	5.83 ± 0.15	1.69 ± 0.07ab	1.08 ± 0.01 a
	M7	20		77.4 ± 1.0	4.95 ± 0.18	6.02 ± 0.15	1.68 ± 0.04ab	1.06 ± 0.01 a
2010	M2	20	2.2 ± 0.1 a	64.2 ± 2.0 a	3.04 ± 0.27 a	4.60 ± 0.23*	2.18 ± 0.1	1.11 ± 0.02*
	AR DS M3	28	4.1 ± 0.1 b	79.1 ± 2.4 b	5.79 ± 0.50 b	6.49 ± 0.21	2.57 ± 0.11	1.10 ± 0.02
	AR US M4	25	4.6 ± 0.2 c	79.4 ± 2.3 b	5.90 ± 0.50 b	6.57 ± 0.25	2.17 ± 0.08	1.11 ± 0.01
	AR DS M4	20	4.0 ± 0.2 b	80.8 ± 2.0 b	5.92 ± 0.40 b	5.92 ± 0.28	2.16 ± 0.1	1.09 ± 0.01
	M8†	9	2.3 ± 0.2 a	59.4 ± 2.6 a	2.27 ± 0.34 a	3.54 ± 0.83	1.81 ± 0.07	1.04 ± 0.01
2011	AR DS M0	20		70.0 ± 1.4	3.83 ± 0.24	6.76 ± 0.15 a	1.74 ± 0.06a	1.09 ± 0.02
	M2	20		73.4 ± 1.4	4.27 ± 0.22	6.62 ± 0.17 ab	2.00 ± 0.08ab	1.06 ± 0.01
	AR DS M3	21		73.4 ± 1.6	4.35 ± 0.27	6.21 ± 0.15 b	2.25 ± 0.07bc	1.06 ± 0.01
	AR US M4	20		74.6 ± 1.1	4.56 ± 0.22	6.77 ± 0.18 ab	2.01 ± 0.09ab	1.08 ± 0.02
	AR DS M4	20		74.4 ± 1.6	4.56 ± 0.32	6.47 ± 0.18 ab	2.43 ± 0.12c	1.08 ± 0.01
	M7	20		72.1 ± 1.3	3.95 ± 0.23	6.09 ± 0.11 ab	1.84 ± 0.05a	1.04 ± 0.02
2013	M0	20	4.8 ± 0.3 a	77.4 ± 1.8	5.39 ± 0.37	6.19 ± 0.20*	2.05 ± 0.09*	1.13 ± 0.02 a
	AR DS M0	20	4.8 ± 0.3 a	75.5 ± 1.9	5.46 ± 0.42	5.70 ± 0.23	1.78 ± 0.08	1.22 ± 0.02 b
	M2	17	5.1 ± 0.3 ac	74.8 ± 2.0	4.74 ± 0.33	6.27 ± 0.29	1.86 ± 0.09	1.10 ± 0.01 a
	AR DS M3	20	4.2 ± 0.3 a	74.1 ± 1.5	4.55 ± 0.25	5.61 ± 0.31	2.00 ± 0.08	1.10 ± 0.01 a
	AR US M4	20	4.2 ± 0.2 ab	75.3 ± 1.2	4.80 ± 0.24	5.76 ± 0.19	1.91 ± 0.06	1.11 ± 0.01 a
	AR DS M4	20	4.2 ± 0.2 ab	77.6 ± 1.1	5.21 ± 0.21	5.90 ± 0.18	1.92 ± 0.07	1.11 ± 0.01 a
	M8	18	2.9 ± 0.2 d	72.1 ± 1.4	4.18 ± 0.23	5.41 ± 0.41	1.71 ± 0.11	1.09 ± 0.02 a
2014	M0	20	4.9 ± 0.3	81.8 ± 3.0 a	6.77 ± 0.73 a	4.19 ± 0.24*	1.25 ± 0.05b	1.14 ± 0.02*
	AR DS M0	20	4.0 ± 0.3	67.3 ± 2.6 b	3.92 ± 0.41 b	3.16 ± 0.27	1.13 ± 0.07b	1.20 ± 0.02
	M2	20	5.0 ± 0.4	79.0 ± 3.1 ab	6.21 ± 0.65 a	3.49 ± 0.30	1.70 ± 0.07a	1.16 ± 0.01
	AR DS M3	20	4.1 ± 0.3	82.2 ± 2.9 a	7.12 ± 0.74 a	3.01 ± 0.19	1.34 ± 0.06ab	1.20 ± 0.02
	Up AR US M4	20	4.2 ± 0.4	74.5 ± 2.5 ab	5.19 ± 0.56 a	2.58 ± 0.24	1.20 ± 0.05b	1.19 ± 0.01
	Down AR US M4	20	4.8 ± 0.4	72.8 ± 2.6 ab	4.88 ± 0.53 ab	2.89 ± 0.30	1.09 ± 0.06b	1.17 ± 0.01
	AR DS M4	20	4.2 ± 0.3	67.6 ± 2.6 b	3.70 ± 0.46 b	2.59 ± 0.24	1.23 ± 0.08b	1.12 ± 0.01
	M7	20	4.5 ± 0.2	80.0 ± 2.5 a	6.04 ± 0.51 a	4.00 ± 0.24	1.62 ± 0.08a	1.12 ± 0.01
	M9	20	3.9 ± 0.2	64.3 ± 1.7 b	3.14 ± 0.26 b	2.82 ± 0.24	1.39 ± 0.06ab	1.14 ± 0.02

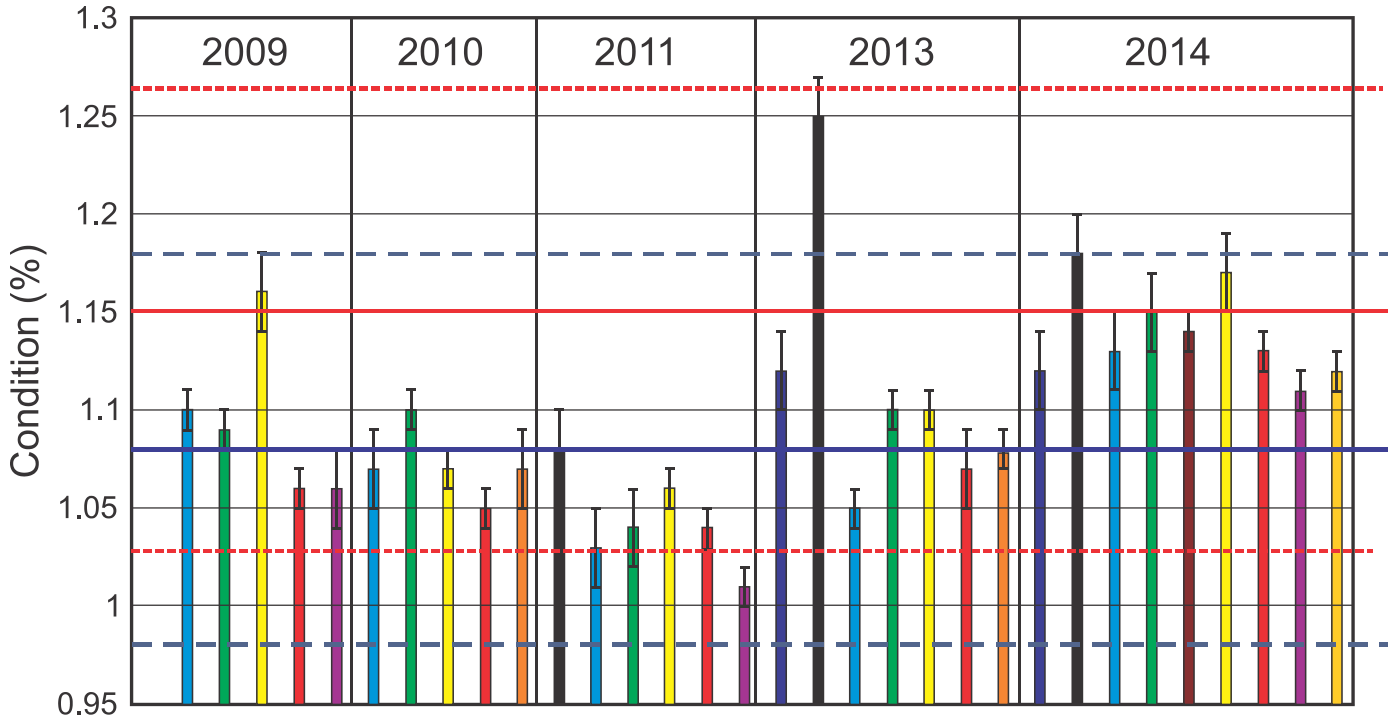

Figure 8. Male trout perch condition over five years of sampling on the Athabasca River. Values represent means ±SE. Light blue – M2; green – AR DS M3; yellow – AR US M4; red – AR DS M4; purple – M7; orange – M8; black – AR DS M0; dark blue – M0; brown – Up AR US M4; olive – M9. Blue horizontal bars represent mean ± critical effect size of 10 % of five M2 sampling years, red horizontal bars represent mean ± critical effect size of 10 % of M0 and AR DS M0 upstream samples.

site between years (no effect confirmation). Examination of the data also fails to demonstrate consistent alterations in fish health endpoints between years indicating no measurable alterations in trout perch health during these years that could be related to exposure to oil sand deposits or development. These data provide a good baseline of trout perch health that can be used to monitor the aquatic environment for change following increased development in the oil sands area and to understand variability and predictability of various EEM endpoints.

Contaminants in trout perch

As part of the trout perch sampling campaigns, frozen trout perch were archived for both contaminant analysis and development of additional endpoints for assessing change in the oil sands area using parasite communities in various tissues of trout perch (Blanar et al. 2016). Female trout perch collected from various locations along the Athabasca River in 2013 were analyzed for PACs to investigate spatial variability relative to developments. Analyses were based on the carcasses after the gonads, liver and aging structure were removed. ΣPAC concentrations were substantially higher (62-146 ng/g) and alkylated PACs ranged from 65-74 % indicating proportionally more parent PACs than in walleye and white sucker (Fig. 9). Highest levels were identified in females from the M2 and lower M8 locations but a signature related to development was not apparent.

2.4 Summary and Conclusions

During JOSM investigations of the LAR mainstem for fish health, considerable progress was made on answering the questions posed in the Phase 2 Integrated Monitoring Plan for the Oil Sands (Environment Canada and Alberta Environment 2011). Each of the main questions is listed below along with a summary of our state of knowledge and the extent to which JOSM questions were answered through three years of monitoring.

- What is the current status of fish health in the lower Athabasca Region?

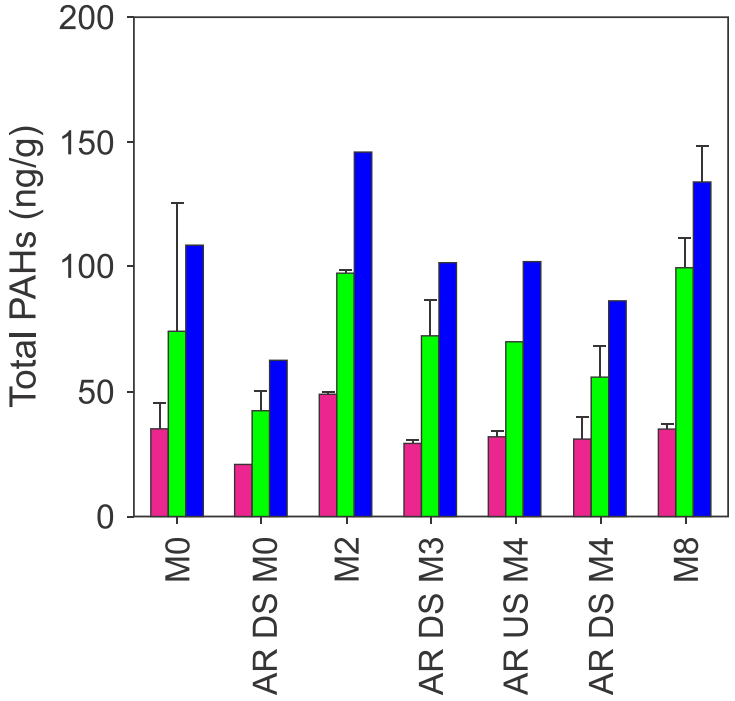

Figure 9. PAC levels in female trout perch collected from the Athabasca River (AR) during the fall of 2013. Values represent the mean ±SE Parent PACs – pink; alkylated PACs – green; total PACs – blue. (DS = downstream; US = upstream).

Fish health studies on the LAR mainstem documented change in white sucker health within the deposit downstream of development. These responses are indicative of nutrient enrichment as fish grow faster and store more energy. Fish within the deposit are clearly exposed to PACs as white sucker livers have significantly increased enzyme activity within the deposit, with some increased induction downstream of development. Trout perch appear to be less sensitive to development; however, it is clear sites outside the deposit are required to understand the influence of the deposit and further development on fish health.

- Are there existing differences in fish health among sites in the lower Athabasca Region?

As discussed above, the three years of baseline data on fish health clearly demonstrate site differences in these endpoints in the LAR mainstem, including documentation of increased condition, liver size and fat storage in white sucker. With a better understanding of reference site variability and expected change within site, we have an improved ability to predict normal fish health and to identify significant change.

- Are there any trends/changes in fish health relative to historical studies?

Presently, we are collecting historical data for comparison to our detailed baseline fish health data. Now that we have a good baseline for fish health on the lower Athabasca mainstem, we can use these data to determine if fish health has changed with time.

- What are the contaminant levels in fish?

We have begun to measure PACs in fish tissue from the fish health studies. Similar to EROD induction, preliminary analyses indicate that fish collected within the deposit have increased levels of PACs in both muscle and liver tissue (white sucker). It is clear from our studies that additional analysis of walleye samples is required to improve the contaminant baseline for fish consumed by humans.

- Are there any predictive relationships between system drivers (including development stress) and variability within sites in fish responses?

Baseline studies of fish health provide predictive relationships between the influence of the oil sands deposit and increased development and fish health endpoints in white sucker collected in the lower Athabasca Region. Further long-term data collection is required to improve this relationship, but sufficient data are now available to make predictions of fish health within specific sites during follow-up studies. Initial evaluation of the relationship among water temperature and condition, growth, gonadal development and liver size at these sites is in progress. This information should allow better predictions of fish health within and between sites with the potential to reduce measurement variability, thereby improving our understanding of factors controlling fish health endpoints.

- Is there evidence of cumulative effects of development on fish in the lower Athabasca Region?

Recent developments of adaptive monitoring frameworks that support cumulative effects assessments have identified four steps towards cumulative effects assessment (CWN and COSIA 2016).

Step 1. *Develop a consistent set of monitoring indicators that will best support effective cumulative effects monitoring program* (near term — includes defining effective 'baseline' monitoring).

Step 2. *Develop a series of monitoring triggers for endpoints that allows the monitoring program to be adapted, while maintaining sufficient consistency to allow subsequent steps* (mid-term focus is on refining effectiveness and robustness of monitoring approach).

Step 3. *Develop a series of relationships that links drivers to responses* (mid to long term increased focus on ability of monitoring to support adaptive management needs).

Step 4. *Develop a cumulative effects assessment model* (long term—enables more effective planning and management decisions).

Clearly, from the steps identified above our fish health studies on the lower Athabasca Region have moved forward in the first three steps. Further work is needed in step two, refining effectiveness and robustness of monitoring approach, and in step three, developing predictive relationships that link drivers to responses. When sufficient information is obtained, a cumulative effects assessment model for fish health may be developed.

3. Tributary Fish Health Sub-Theme

3.1 Introduction

As noted for the LAR mainstem fish program and the Phase 2 Integrated Monitoring Plan (Environment Canada and Alberta Environment 2011), challenges with monitoring tributary fish included access issues (e.g., requirement for helicopter use), seasonal sampling needs, high flow events, shifting substrate, migratory fish species, low species richness, habitat change upstream on some tributaries, and continual loss of reference areas to development. Fish health studies on Athabasca River (LAR) tributaries consisted of sampling a single sentinel fish species at sites upstream, outside of the deposit, at sites in the deposit upstream of development, and at sites in the deposit downstream of oil sands development wherever possible (Fig. 1). As small bodied fish are generally the only year-round adult inhabitants of these tributaries, the most abundant species within a tributary was chosen for the fish health assessment for that specific tributary. Wherever possible, additional reference tributaries were selected and sampled to increase our understanding of variability in fish health endpoints for the selected species.

Objectives

This 3-year JOSM study aimed to develop a comprehensive and robust fish health monitoring program for tributaries of the LAR mainstem. The study examined the capacity of the methods developed in the pulp and paper and metal mining EEM regulations to evaluate fish health in tributaries of the Athabasca River. We used sentinel species to develop baselines of fish health for various tributaries and to begin assessing the potential for development in the oil sands area to affect overall fish health.

3.2 Methods

Study design

Adult fish survey sampling protocols under the Canadian EEM programs for the pulp and paper and metal mining sectors were used to assess fish health in the tributaries of the lower Athabasca River (Environment Canada 2010). Study design for the fish health work aimed to collect fish within the river system upstream, outside of the oil sands deposit as a reference site, within the oil sands deposit upstream of development, and within the oil sands deposit downstream of development (Fig. 1). The general study design for fish health work was to collect 20 adult males and 20 adult females of the selected sentinel species for EEM endpoints (Environment Canada 2010). Fish were collected using backpack electrofishing techniques, generally with three netters downstream of the backpack unit creating a wall of nets to capture fish shocked and swept downstream by the current. Three years of data were to be collected at each location to establish variability in EEM endpoints, provide a baseline for future comparisons, and begin to develop predictive relationships for fish health. Environment and Climate Change Canada initiated tributary fish studies in 2010 and these data were included to develop the program and to estimate variability in fish endpoints in these systems.

Sampling sites and endpoints

Fish health in streams and rivers serves as a biological indicator and is an important component of aquatic ecosystems. We examined fish health within tributaries of the mainstem Athabasca River (Table 13, Fig. 2). Sampling for fish health was conducted during fall (once in a calendar year) in September-October. In tributaries on the east side of the Athabasca River, the slimy sculpin (*Cottus cognatus*) was the most abundant species. On the west side of the river, lake chub (*Couesius plumbeus*) were sampled on the Ells and Dover rivers and in Alice Creek, and longnose dace (*Rhinichthys cataractae*) on the MacKay River; these data are not included here as additional years of sampling are required. Detailed studies on slimy sculpin using monthly collections and mark and recapture studies have determined very good site fidelity (Brasfield et al. 2013). For the first year of collection on the Steepbank River, three sites were sampled including an upper site outside of the influence of the deposit (U EC), a mid-site within the deposit upstream of development (M EC), and a lower site (L) within the deposit downstream of development (Fig. 2). These sites were identical to those sampled by (Tetreault et al. 2003b) and can be used for historical comparisons. In 2012 and onward, a fourth site was added further up-

Table 13. Athabasca River Tributary sites, species collected, samples collected and collection years.

Site Location	Species Sampled	Samples Collected	Years Collected
Steepbank RAMP	Slimy Sculpin	Fish Health, Contaminants	2012, 2013
Steepbank Upper EC	Slimy sculpin	Fish Health, Contaminants	2010, 2011, 2012, 2013,
Steepbank Middle EC	Slimy sculpin	Fish Health, Contaminants	2010, 2011, 2012, 2013
Steepbank Lower	Slimy sculpin	Fish Health, Contaminants	2010, 2011, 2012, 2013
Firebag Upper	Slimy sculpin	Fish Health, Contaminants	2010, 2012, 2013, 2014
Firebag Middle	Slimy sculpin	Fish Health Contaminants	2010, 2012, 2013
Firebag Lower	Slimy sculpin	Fish Health, Contaminants	2010
Dunkirk River	Slimy sculpin	Fish Health, Contaminants	2012, 2013, 2014
Horse River	Slimy sculpin	Fish Health, Contaminants	2012, 2013, 2014
High Hills River	Slimy sculpin	Fish Health, Contaminants	2013, 2014

stream to increase reference site comparisons (RAMP). On the Firebag River three sites were sampled (U - upper, M - middle, L- lower) and additional reference sites were added on the Dunkirk, Horse and High Hills rivers increasing our understanding of reference site variability. On the Ells, Dover and MacKay rivers, sites were chosen again to represent upper sites outside the influence of the deposit, middle sites within the deposit, and lower sites downstream of development, if it existed at the time. Two sites were also sampled on Alice Creek as baseline data for potential development north near Lake Claire. Detailed population health assessments of individual fish included assessment of age, growth, condition, liver size, gonad size, and abnormalities (EEM endpoints). We also measured hepatic mixed-function oxygenase (MFO) activity using ethoxyresorufin-O-deethylase (EROD) methods (Munkittrick et al. 1995, Van den Heuvel et al. 1995) as an indicator of exposure to PACs and related compounds and abnormalities for histological evaluation (Blazer et al. 2009, Rafferty et al. 2009). Body samples were also collected for contaminant (PAC) analysis.

Statistical methods

EEM Endpoints

Within year comparisons

For fish of the same species collected on the same river within a sampling year, ANCOVA was used to compare the condition of fish (length versus body weight relationships), the gonadosomatic indices of fish (gonad weight versus body weight), and the liver somatic indices of fish (liver weight versus body weight) among sites within a river. Pairwise comparisons were used, if site differences existed, to determine which were different when multiple sites existed on that system. ANOVA was used to compare weight and length of fish among sites. Non-parametric Kruskal Wallis was used to compare MFO activity and age in fish among sites.

Comparisons between years

Although the design of the first three years of the JOSM fish program was to generate data to develop baseline conditions for future develop-

ment, it was important to determine if differences existed among sites within a year, and if these differences were consistent between years of collection. Within a year comparisons were made and then response patterns in endpoints were compared between years to determine whether responses were consistent between years (effects confirmed), getting better or getting worse. Through the EEM programs for pulp and paper and metal mining, critical effect sizes have been developed and were applied here for decision endpoints (Table 2). For slimy sculpin data, average of the means for the upstream reference sites over time was calculated and critical effects sizes used to assess change at downstream sites.

Within site natural background variability

New methods are continually being developed to help make decisions on data collected on fish health endpoints (Arciszewski and Munkittrick 2015). Data for slimy sculpin were examined over time using these new methods to help define triggers and decision criteria with the data collected in the JOSM program. For sculpin data, we have begun to examine these methods using our data and some of the historical data collected in 1999-2000, where sites overlapped with our JOSM sampling.

Thresholds used in this analysis were adapted over time to incorporate new information from additional years of data collection at those sites. Through this serial adaptation, site means provide more meaningful predictions of future observations as more data are added (Arciszewski and Munkittrick 2015). A cumulative mean (CM) ±2SD for condition factor (K), gonadosomatic indices (GSI), and liver somatic indices (LSI) by sex was calculated for each site to determine the range of variability (background) of these three parameters among years within a site. This approach is based upon development of the primary trigger for locations using the site-specific running and historical grand mean ±2SD, which can be used to make predictions of future observations when data are normally distributed; a mean ±2SD encapsulates 95% of observations from an assumed normal distribution (Arciszewski and Munkittrick 2015). For example, the Steepbank River upper EC site has multiple years of data. From the means of a given parameter for the first three years of data (i.e., 2010+2011+2012) the CMi+2SD is calculated. That CM+2SD range delineates the 'predicted range' for the next year's mean +SE data (i.e., 2013). If the data for the next year (i.e., 2013) fall within the 'predicted range' then the prediction is considered valid and the 2013 data are included to calculate the new CMi+j for the next sampling cycle (e.g., 2010+2011+2012+2013). This new CM±2SD range delineates the 'predicted range' for the next year's mean ±SE data (i.e., 2014). Hence, the 'predicted range' is an evolving range that encompasses both historical and recent data to provide the expected and predictive range for further sampling cycles. If a sampling cycle generates (i.e., 2014) a mean that falls outside the predictive range then that year is not included to generate a new CM (e.g., CM_{i+j+k}) and range, and the CMi±j is used for the next sampling cycle (i.e., 2015) to confirm the parameter is outside of the 'predicted range.' If the parameter continues to be outside the predicted range then this would confirm deviation from the established background variability and further actions should be considered to investigate the cause of this deviation.

Tributary surface water temperature

Since 2012, in the month of June, Hobo Tidbit temperature recorders were deployed at each of the sites (Athabasca and tributaries) followed by collection and data recovery the following September. The data among years analyzed were standardized to Julian Day 172 (June 21st) to 250 (September 7th). During this period, the mean daily temperature was summarized within sites among years, and the number of degree days was calculated. The temperature of the surrounding environment is a major environmental factor that has a significant influence on the physiological status of fish and their ability to obtain and utilize resources for growth and development (Trudgill et al. 2005, Chezik et al. 2014). The number of degree days (DD) is a method to account for the temperature of the surface water among years or sites. The calculation of degree days (DD) is as follows:

Degree days (DD) = $((T_{max}+T_{min})/2)-T_o$; where T_{max} = max daily temperature; T_{min} = minimum daily temperature; and T_o = minimal development threshold for growth for that species.

3.3 Results and Discussion

Tributary fish health

Temperature

Surface water temperatures within each Athabasca River tributary demonstrate both temporal and spatial variability. For example, in the Steepbank and Firebag rivers, water temperature gradually increases from the uppermost site to the lower reaches on a consistent basis (Table 14). However, there is a larger gradient in the Steepbank River than in the Firebag River among sites, with the Firebag upper site warmer than the Steepbank reference sites. Reference sites on the Horse and High Hills rivers have thermal ranges similar to those of the upper Steepbank River sites, whereas the Dunkirk temperature is higher and comparable to those of the lower reaches of the other tributaries. Although this information has not been incorporated into the interpretation of fish health responses to date, the capacity to understand variability in fish health endpoints will enable better predictions for fish health in the future.

Tributary slimy sculpin (Steepbank, Firebag, Dunkirk, High Hills and Horse rivers)

In 2010, slimy sculpin were collected from three sites on the Steepbank River (upstream of the deposit – Steepbank upper EC, within the deposit upstream of development – Steepbank mid EC and in the deposit adjacent to surface mining activity – Steepbank L), as well as three sites on the Firebag River (upper, mid and lower) (Tables 13; Fig. 2). Currently, there are no surface mining projects in the Firebag River catchment, although there are in situ (Steam-Assisted Gravity Drainage or SAGD) operations in the upper portion of the sub-watershed. Data trends and summaries within rivers for slimy sculpin health responses were used to evaluate health and to demonstrate whether responses are consistent within rivers over time (Tables 15, 16). The summary figures were developed using the detailed evaluations in Tables 17-19

Table 14. Degree-days (sum of mean daily temperature above 4 °C) for water temperatures collected between June 21st and September 7th from 2012-2014 for tributaries on the Athabasca River near Fort McMurray. NA = indicates probe not deployed or retrieved from that site.

Mean Daily Temperature				
Site	2012	2013	2013	Mean
Steepbank RAMP	15.563	15.436	15.371	15.457
Steepbank Upper	15.906	15.666	15.997	15.857
Steepbank Mid	16.579	NA	16.898	16.738
Steepbank Lower	18.807	18.184	17.754	18.248
Firebag Upper	17.446	17.257	18.228	17.643
Firebag Mid	18.283	17.887	NA	18.085
Firebag Lower	18.504	18.231	19.038	18.591
Horse	17.552	16.250	17.403	17.068
Dunkirk	18.298	NA	18.177	18.238
High Hills	NA	15.932	16.797	16.365
Daily Degree Days				
Site	2012	2013	2013	Mean
Steepbank RAMP	918.476	907.691	904.719	910.295
Steepbank Upper	947.995	928.589	952.595	943.059
Steepbank Mid	1002.700	NA	1025.277	1013.988
Steepbank Lower	1138.431	1183.835	1094.382	1138.883
Firebag Upper	1071.871	1054.595	1131.844	1086.103
Firebag Mid	1138.431	1157.556	NA	1147.994
Firebag Lower	1153.476	1131.564	1190.881	1158.640
Horse	1082.884	854.682	1065.310	1000.958
Dunkirk	1136.357	NA	1123.391	1129.874
High Hills	NA	955.017	1042.247	998.632

reported below. Given that habitat is different at the downstream location on the Steepbank River as it runs through the oil sands deposit, fish capture was difficult and sample sizes were lower than expected. In 2010, female sculpin at the lower Steepbank site had reduced condition ($p<0.026$), indications of reduced investment in reproductive development (Table 17), and increased EROD activity (Table 18). In 2011, however, there were no differences in condition, liver size was increased at both mid and lower sites ($p<0.001$) (Table 17) and EROD was still induced at the lower Steepbank site (Table 18). In 2012, female sculpin at the lower site again had reduced gonadal development ($p<0.001$; Table 17) similar to 2010 and trends to increased liver size at the lower site but differences in liver size were not significant. Steepbank M female sculpin had induced EROD activity (Table 18) but livers from the lower site were compromised (thawed accidentally). Female sculpin in 2013 demonstrated most responses relative to the reference upstream females, with females from the lower site having slightly reduced condition ($p<0.001$)

Table 15. Summary of differences in fish parameters and EROD analysis among sites within tributaries of the Athabasca River in female slimy sculpin collected in September 2010-2014. "0" indicates no statistical difference from reference sites. Blue up arrows indicate a positive increase, red up arrows a negative increase and yellow down arrows indicate a decrease, relative to in-stream reference sites. (U=upper; M=middle; L=lower)

Year	Species	Site	Site Type	Sex	Length	Weight	Condition	GSI	LSI	EROD
2010	Slimy Sculpin	Steepbank U EC	Reference	F						
		Steepbank M EC	Deposit		↑↑	0	0	0	0	0
		Steepbank L	Development		↑↑	↑	↓	0	0	↑
		Firebag U	Reference							
		Firebag M	Reference		0	0	0	0	0	0
		Firebag L	Reference		0	0	0	0	0	0
2011	Slimy Sculpin	Steepbank U EC	Reference							
		Steepbank M EC	Deposite		0	0	0	0	↑	0
		Steepbank L	Development		↓	↓	0	0	↑	↑
2012	Slimy Sculpin	Steepbank RAMP	Reference							
		Steepbank U EC	Reference							
		Steepbank M EC	Deposit		↑	↑	0	0	0	↑
		Steepbank L	Development		↓	↑	↓	0	0	NA
		Firebag U	Reference							
		Firebag M	Reference		0	0	0	0	0	↑
		Dunkirk River	Reference		↑	↑	↑	0	0	0
		Horse River	Reference		↓	↓	↓	0	0	0
2013	Slimy Sculpin	Steepbank RAMP	Reference							
		Steepbank U EC	Reference							
		Steepbank M EC	Deposit		0	↓	0	↓	↑	0
		Steepbank L	Development		0	↓	↓	↓	0	↑
		Firebag U	Reference							
		Firebag M	Reference		↑	↑	0	0	0	0
		Dunkirk River	Reference		0	0	0	0	0	0
		High Hills	Reference		0	0	0	0	0	0
		Horse River	Reference		↓	↓	↓	0	↓	0
2014	Slimy Sculpin	Firebag U	Reference							
		Horse River	Reference		↓↓	↓↓	↓↓	↓↓	↑↑	0
		Dunkirk River	Reference		↓↓	↓↓	↓↓	↓↓	↑↑	↑
		High Hills	Reference		↓	↓	↓	↓	↑	0

Table 16. Summary of differences in fish parameters and EROD analysis among sites within tributaries of the Athabasca River in male slimy sculpin collected in September 2010-2014. "0" indicates no statistical difference from reference sites. Blue up arrows indicate a positive increase, red up arrows a negative increase and yellow down arrows indicate a decrease, relative to in-stream reference sites. (U=upper; M=middle; L=lower)

Year	Species	Site	Site Type	Sex	Length	Weight	Condition	GSI	LSI	EROD
2010	Slimy Sculpin	Steepbank U EC	Reference	M						
		Steepbank M EC	Deposit		↑↑	↑↑	0	0	0	0
		Steepbank L	Development		↑↑	↑↑	↑	0	0	↑
		Firebag U	Reference							
		Firebag M	Reference		0	0	0	0	0	0
		Firebag L	Reference		0	0	0	0	0	0
2011	Slimy Sculpin	Steepbank U EC	Reference							
		Steepbank M EC	Deposit		0	0	↑	0	0	0
		Steepbank L	Development		0	0	0	0	↑	↑↑
2012	Slimy Sculpin	Steepbank RAMP	Reference							
		Steepbank U EC	Reference							
		Steepbank M EC	Deposit		↑↑	↑↑	0	0	↑	↑
		Steepbank L	Development		↑↑	↑↑	↓	0	↑	NA
		Firebag U	Reference							
		Firebag M	Reference		0	0	0	0	0	↑
		Dunkirk River	Reference		0	0	0	0	0	0
		Horse River	Reference		↓	↓	↓	0	0	0
2013	Slimy Sculpin	Steepbank RAMP	Reference							
		Steepbank U EC	Reference							
		Steepbank M EC	Deposit		0	0	0	0	0	0
		Steepbank L	Development		0	0	0	↓	↑	↑↑
		Firebag U	Reference							
		Firebag M	Reference		↑	↑	0	↓	0	0
		Dunkirk River	Reference		0	0	0	0	0	0
		High Hills	Reference		0	0	0	0	0	0
		Horse River	Reference		↓	↓	↓	0	0	0
2014	Slimy Sculpin	Firebag U	Reference							
		Horse River	Reference		↓	↓	↓	0	0	0
		Dunkirk River	Reference		↓↓	↓↓	↓↓	0	↑	0
		High Hills	Reference		↓	↓	↓	0	↑	0

but reduced investment in reproductive development ($p<0.001$) (Table 17) and confirmed induction of EROD activity ($p<0.001$) (Table 18). Females from the mid-site were intermediate in 2013, with reduced gonad size ($p<0.026$) and increased liver size. Overall, female slimy sculpin collected on the Firebag River were similar and these data were used in the analysis below examining fish health across multiple reference locations similar to the additional reference locations of the Dunkirk, Horse and High Hills rivers.

Male slimy sculpin in 2010 had increased condition at the lower Steepbank site ($p<0.013$; Table 19) and induced EROD activity ($p<0.001$) (Table 18). In 2011, condition increased in Steepbank mid fish ($p<0.010$) but not at the lower site, although increased EROD activity (Table 18) and increased liver size were evident in slimy sculpin collected at the lower development location. In 2012, increases in liver size were confirmed at the Steepbank lower site ($p<0.001$) (Table 19). EROD (Table 18) was higher in males from the mid location relative to both upstream Steep-

Table 17. Mean (±SE) of female slimy sculpin health parameters among sites within tributaries of the Athabasca River collected in September 2010-2014. Statistical comparisons are conducted within tributary and within year. Differences among sites within years are denoted by different lowercase letters and symbols. Asterisks indicate when there was an interaction in the data analysis. (U=upper; M=middle; L=lower).

Year	Species	Site	Designation	n	Length		Weight		Condition		GSI		LSI	
2010	Slimy Sculpin	Steepbank U. EC	Reference	20	7.38 + 0.14	a	4.37 + 0.29	a	1.06 ± 0.02	a	2.16 ± 0.11	a	2.05 ± 0.10	a
		Steepbank M. EC	Exposed	15	7.98 + 0.17	b	5.72 + 0.40	b	1.1 ± 0.02	a	1.85 ± 0.11	b	2.57 ± 0.19	a
		Steepbank L.	Exposed	4	7.70 + 0.41	ab	4.63 + 0.89	ab	0.97 ± 0.04	b	1.74 ± 0.30	ab	2.25 ± 0.28	a
		Firebag U.	Reference	17	7.89 + 0.19	a	5.27 + 0.42	a	1.04 ± 0.02	a	1.62 ± 0.07	a	1.95 ± 0.17	a
		Firebag M.	Exposed	20	8.27 + 0.25	a	6.02 + 0.62	a	1.00 ± 0.02	a	1.80 ± 0.07	a	1.80 ± 0.12	a
		Firebag L.	Exposed	9	8.11 + 0.23	a	5.61 + 0.48	a	1.03 ± 0.03	a	1.68 ± 0.16	a	2.35 ± 0.26	a
2011	Slimy Sculpin	Steepbank U. EC	Reference	21	7.68 + 0.15	a	4.40 + 0.23	a	0.96 ± 0.01	*a	1.89 ± 0.07	a	1.32 ± 0.07	a
		Steepbank M. EC	Exposed	20	6.79 + 0.21	b	3.41 + 0.33	b	1.04 ± 0.01	b	1.71 ± 0.06	a	2.00 ± 0.11	b
		Steepbank L.	Exposed	20	7.23 + 0.16	ab	3.78 + 0.28	ab	0.98 ± 0.02	ab	1.90 ± 0.09	a	2.30 ± 0.14	b
		Firebag U.	Reference	21	7.51 + 0.18		4.23 + 0.31		0.97 ± 0.02		1.48 ± 0.04		1.47 ± 0.08 (20)	
		Muskeg L.	Exposed	16	7.14 + 0.11		3.71 + 0.19		1.00 ± 0.02		1.73 ± 0.10		2.24 ± 0.11	
2012	Slimy Sculpin	Steepbank U RAMP	Reference	23	6.41 + 0.12	a	2.49 + 0.13	a	0.93 ± 0.01	a	2.20 ± 0.10	*a	2.07 ± 0.11 (22)	a
		Steepbank U. EC	Reference	20	6.59 + 0.13	ab	2.89 + 0.18	ab	1.00 ± 0.03	a	1.95 ± 0.07	a	1.96 ± 0.16	a
		Steepbank M. EC	Exposed	20	6.94 + 0.14	b	3.24 + 0.19	b	0.95 ± 0.01	a	1.93 ± 0.13	a	2.08 ± 0.10	a
		Steepbank L.	Exposed	19	7.46 + 0.14	bc	4.00 + 0.26	bc	0.93 ± 0.02	a	1.59 ± 0.05	b	2.67 ± 0.18	a
		Firebag U.	Reference	20	7.81 + 0.10	ǂ	4.72 + 0.21	ǂ	0.98 ± 0.02	ǂ	1.69 ± 0.06	ǂ	1.79 ± 0.10	ǂ
		Firebag M.	Exposed	20	7.62 + 0.18	ǂ	4.30 + 0.28	ǂ	0.96 ± 0.02	ǂ	1.70 ± 0.05	ǂ	1.99 ± 0.10	ǂ
		Horse River	Reference	20	7.21 + 0.13	#	3.42 + 0.15	#	0.91 ± 0.02	#	1.65 ± 0.04	*	2.46 ± 0.18	*
		Dunkirk River	Reference	21	7.79 + 0.11	$	4.70 + 0.22	$	0.98 ± 0.01	$	1.82 ± 0.11		2.41 ± 0.13	
		Muskeg L.	Exposed	2	7.00 + 1.60		4.33 + 2.71		1.07 ± 0.04		1.20 ± 0.76		2.12 ± 0.35	
2013		Steepbank U RAMP	Reference	26	7.16 + 0.11	a	3.78 + 0.18	ab	1.01 ± 0.02	ab	2.25 ± 0.07 (20)	a	2.85 ± 0.11 (20)	*ab
		Steepbank U. EC	Reference	20	7.14 + 0.07	a	4.25 + 0.11	a	1.04 ± 0.02	a	2.26 ± 0.05	a	2.53 ± 0.14	a
		Steepbank M. EC	Exposed	21	7.04 + 0..09	a	3.60 + 0.13	b	1.02 ± 0.02	ab	1.81 ± 0.05 (20)	b	2.99 ± 0.10 (20)	b
		Steepbank L.	Exposed	15	7.20 + 0.14	a	3.60 + 0.21	b	0.96 ± 0.03	b	1.40 ± 0.06	c	2.96 ± 0.18	ab
		Firebag U.	Reference	24	7.75 + 0.11	^	4.68 + 0.19	^	0.99 ± 0.02	ǂ	1.76 ± 0.04 (20)	ǂ	2.10 ± 0.06 (20)	ǂ
		Firebag M.	Exposed	15	9.19 + 0.12	ǂ	8.14 + 0.30	ǂ	1.04 ± 0.02	ǂ	1.87 ± 0.17 (14)	ǂ	2.03 ± 0.09	ǂ
		Horse River	Reference	20	6.30 + 0.07	#	1.75 + 0.04	#	0.70 ± 0.02	*#	1.82 ± 0.07	*#	1.75 ± 0.15	#
		Dunkirk River	Reference	20	7.35 + 0.16	$	3.95 + 0.25	$	0.98 ± 0.02	$	2.12 ± 0.06	#	2.72 ± 0.10	$
		High Hills	Reference	20	6.98 + 0.06	%	3.33 + 0.09	%	0.98 ± 0.02	$	1.96 ± 0.08 (19)	#	3.15 ± 0.17	$
2014		Firebag U.	Reference	20	8.86 + 0.10	ǂ	7.94 + 0.34	ǂ	1.12 ± 0.02	ǂ	2.05 ± 0.20	*	1.81 ± 0.14	ǂ
		Horse River	Reference	20	6.89 + 0.10	#	3.32 + 0.14	#	1.01 ± 0.01	$	1.86 ± 0.11		2.48 ± 0.14	ǂ$
		Dunkirk River	Reference	20	7.96 + 0.16	$	5.32 + 0.34	$	1.04 ± 0.02	$	1.91 ± 0.07		2.80 ± 0.11	$
		High Hills	Reference	20	7.74 + 0.07	$	4.60 + 0.16	$	0.99 ± 0.02	$	1.72 ± 0.05		2.87 ± 0.64	ǂ$

Table 18. Mean (±SE) 7-ethoxyresorufin-o-deethylase (EROD) activity in female and male slimy sculpin collected from tributaries of the Athabasca River near the Athabasca Oil Sands deposit from 2010-2013. Differences among sites within years and sex ($p<0.05$) are denoted by different lowercase letters. (U=upper; M=middle; L=lower).

Sex	Year	Site	n	EROD (mean ± SE)		Sex	Year	Site	n	EROD (mean ± SE)	
F	2010	Steepbank U	19	0.54 ± 0.07	ab	M	2010	Steepbank U	12	0.65 ± 0.09	a
		Steepbank M	13	2.68 ± 0.84	a			Steepbank M	11	1.52 ± 0.26	a
		Steepbank L	4	5.35 ± 1.62	c			Steepbank L	8	3.32 ± 0.74	b
		Firebag U		NA				Firebag U		NA	
		Firebag M	11	0.98 ± 0.10	b			Firebag M	15	0.71 ± 0.16	ab
		Firebag L	1	2.18 ± 0.00	d			Firebag L	2	1.54 ± 0.55	c
	2011	Steepbank U	21	1.40 ± 0.12	a		2011	Steepbank U	17	2.11 ± 0.35	a
		Steepbank M	20	5.70 ± 2.76	b			Steepbank M	20	2.66 ± 0.50	a
		Steepbank L	20	24.64 ± 3.10	c			Steepbank L	20	18.95 ± 3.30	b
		Firebag U	21	1.20 ± 0.12	a			Firebag U	21	1.33 ± 0.20	c
		Muskeg L	16	12.78 ± 1.85	ab			Muskeg L	21	11.80 ± 1.70	b
	2012	Steepbank U	16	30.03 ± 4.85	a		2012	Steepbank U	13	29.49 ± 4.41	a
		Steepbank M	20	79.98 ± 16.18	b			Steepbank M	15	67.10 ± 11.57	ab
		Firebag U	20	15.36 ± 2.21	a			Firebag U	19	21.22 ± 1.25	b
		Firebag M	18	29.904± 3.16	a			Firebag M	6	25.72 ± 7.63	c
	2013	Steepbank RAMP	20	2.16 ± 0.36	ab		2013	Steepbank RAMP	20	2.15 ± 0.45	a
		Steepbank U	20	1.39 ± 0.11	a			Steepbank U	19	1.79 ± 0.24	a
		Steepbank M	20	3.05 ± 0.63	b			Steepbank M	10	2.46 ± 0.26	b
		Steepbank L	15	21.85 ± 4.26	c			Steepbank L	11	25.62 ± 3.26	c
		Firebag U	20	0.70 ± 0.06	d			Firebag U	12	0.75 ± 0.07	d
		Firebag M	15	1.54 ± 0.26	a			Firebag M	9	0.97 ± 0.13	d
		Horse	19	0.67 ± 0.09	ad			Horse	20	0.45 ± 0.05	e
		High Hills	20	0.93 ± 0.07	d			High Hills	8	1.10 ± 0.13	d
		Dunkirk	20	3.90 ± 0.49	b			Dunkirk	20	5.06 ± 0.72	b

Table 19. Mean (±SE) of male slimy sculpin health parameters among sites within tributaries of the Athabasca River collected in September 2010-2014. Statistical comparisons are conducted within a tributary and within a year. Differences among sites within years are denoted by different lowercase letters and symbols. Asterisks indicate when there was an interaction in the data analysis. (U=upper; M=middle; L=lower).

Year	Species	Site	Designation	n	Length		Weight		Condition		GSI		LSI	
2010	Slimy sculpin	Steepbank U EC	Reference	14	7.91 ± 0.16	a	5.71 ± 0.48	a	1.11 ± 0.04	a	2.01 ± 0.11	a	1.31 ± 0.09	a
		Steepbank M EC	Exposed	10	8.58 ± 0.20	b	7.35 ± 0.66	ab	1.13 ± 0.03	ab	2.24 ± 0.09 (9)	a	1.45 ± 0.17	a
		Steepbank L	Exposed	8	9.24 ± 0.21	b	9.31 ± 0.73	b	1.16 ± 0.03	b	2.03 ± 0.13	a	1.61 ± 0.12	a
		Firebag U	Reference	23	8.06 ± 0.13	a	5.72 ± 0.28	a	1.07 ± 0.02	a	2.07 ± 0.06	a	1.22 ± 0.06	a
		Firebag M	Exposed	22	8.39 ± 0.14	a	6.28 ± 0.37	a	1.04 ± 0.02	a	1.97 ± 0.06	a	1.07± 0.08	a
		Firebag L	Exposed	7	8.57 ± 0.08	a	7.04 ± 0.32	a	1.12 ± 0.03	a	2.08 ± 0.12	a	1.56 ± 0.16	b
2011	Slimy sculpin	Steepbank U EC	Reference	17	7.61 ± 0.25	a	4.64 ± 0.48	a	1.00 ± 0.01	a	1.98 ± 0.08	a	1.08 ± 0.07	a
		Steepbank M EC	Exposed	20	7.30 ± 0.22	a	4.40 ± 0.50	a	1.06 ± 0.02	b	1.92 ± 0.06	a	1.14 ± 0.08	a
		Steepbank L	Exposed	21	7.35 ± 0.11	a	4.10 ± 0.19	a	1.02 ± 0.02	ab	2.04 ± 0.08	a	1.58 ± 0.10	b
		Firebag U	Reference	20	7.49 ± 0.07		4.17 ± 0.13		0.99 ± 0.02		2.06 ± 0.09		1.00 ± 0.07	
		Muskeg L	Exposed	21	7.88 ± 0.13		5.02 ± 0.30		1.00 ± 0.02		2.06 ± 0.09		1.20 ± 0.08	
2012	Slimy sculpin	Steepbank RAMP	Reference	20	6.44 ± 0.12	a	2.58 ± 0.16	a	0.95 ± 0.01	*ab	1.93 ± 0.13	a	1.18 ± 0.04	a
		Steepbank U EC	Reference	20	6.96 ± 0.12	b	3.35 ± 0.17	b	0.98 ± 0.02	ab	1.92 ± 0.14	a	1.09 ± 0.06	a
		Steepbank M EC	Exposed	20	6.96 ± 0.10	b	3.41 ± 0.14	bc	1.00 ± 0.01	a	1.72 ± 0.10	a	1.44 ± 0.06	b
		Steepbank L	Exposed	21	7.58 ± 0.14	c	4.25 ± 0.29	c	0.94 ± 0.02	b	1.64 ± 0.06	a	1.90 ± 0.13	c
		Firebag U	Reference	20	8.01 ± 0.13		5.35 ± 0.24		1.03 ± 0.02		2.02 ± 0.13		1.15 ± 0.07	
		Firebag M	Exposed	11	7.81 ± 0.17		4.90 ± 0.30		1.02 ± 0.02		1.97 ± 0.05		1.59 ± 0.12	ǂ
		Horse River	Reference	16	7.04 ± 0.18	#	3.37 ± 0.27	#	0.93 ± 0.02	#	1.62 ± 0.10	#	1.20 ± 0.09	#
		Dunkirk River	Reference	18	8.96 ± 0.18	$	7.57 ± 0.52	$	1.03 ± 0.01	$	1.93 ± 0.06	#	1.33 ± 0.06	#
2013	Slimy sculpin	Steepbank RAMP	Reference	20	7.45 ± 0.11	a	4.24 ± 0.20	a	1.01 ± 0.02	a	2.10 ± 0.05	ab	1.29 ± 0.05	a
		Steepbank U EC	Reference	22	7.81 ± 0.12	a	5.19 ± 0.22	a	1.08 ± 0.01	a	2.37 ± 0.12	a	1.25 ± 0.05	a
		Steepbank M EC	Exposed	10	7.35 ± 0.12	a	4.14 ± 0.20	a	1.04 ± 0.03	a	2.29 ± 0.09	a	1.40 ± 0.09	a
		Steepbank L	Exposed	12	7.83 ± 0.38	a	5.64 ± 1.19	a	1.05 ± 0.05	a	1.82 ± 0.16	b	1.97 ± 0.12	b
		Firebag U	Reference	12	7.90 ± 0.18	^	5.12 ± 0.36	^	1.02 ± 0.02	^	2.05 ± 0.13	^	1.43 ± 0.08	ǂ
		Firebag M	Exposed	9	9.08 ± 0.23	ǂ	8.49 ± 0.59	ǂ	1.13 ± 0..05	ǂ	1.77 ± 0.25	ǂ	1.45 ± 0.05	ǂ
		Horse River	Reference	20	6.62 ± 0.12	#	2.20 ± 0.14	#	0.75 ± 0.02	#	1.53 ± 0.12	#	1.07 ± 0.07	#
		Dunkirk River	Reference	20	7.49 ± 0.14	$	4.33 ± 0.25	$	1.01 ± 0.02	$	2.06 ± 0.05	#	1.40 ± 0.06	#
		High Hills	Reference	8	7.34 ± 0.17	$	3.78 ± 0.22	$	0.95 ± 0.02	$	2.04 ± 0.10	#	1.40 ± 0.08	#
2014	Slimy sculpin	Firebag U	Reference	8	8.95 ± 0.28	ǂ	8.84 ± 0.79	ǂ	1.22 ± 0.03	ǂ	1.88 ± 0.18	ǂ	1.12 ± 0.12	ǂ#
		Horse River	Reference	20	7.45 ± 0.10	#	4.15 ± 0.15	#	1.00 ± 0.01	#	1.97 ± 0.07	ǂ	1.14 ± 0.06	#
		Dunkirk River	Reference	20	8.32 ± 0.12	$	6.31 ± 0.31	$	1.08 ± 0.02	$	1.96 ± 0.13	ǂ	1.39 ± 0.06	ǂ
		High Hills	Reference	16	8.18 ± 0.13	$	5.97 ± 0.32	$	1.08 ± 0.02	$	1.69 ± 0.04	ǂ	1.21 ± 0.06	ǂ#

bank locations. Again, livers from fish collected at the lower site were also compromised. In 2013, males from the furthest downstream site had reduced gonadal development ($p<0.013$), increased liver sizes ($p<0.001$; Table 19), and induced EROD activity, confirming some of the responses demonstrated in 2012 (Table 18). As with females, male slimy sculpin collected on the Firebag River were quite similar and these data will be used in the analysis below examining fish health across multiple reference locations similar to the additional reference locations (Dunkirk, Horse and High Hills).

The magnitude of the difference in EROD activity from 2010-2013 reflects changes to the assay analysis and is not a reflection of the reduction of exposure of fish to PACs over time. Also, it must be noted that the 2012 analysis was conducted at Simon Fraser University (C. Kennedy pers. comm.), which involved a refined protocol that accounts for the discrepancy in the magnitude of EROD levels among years of collections for JOSM.

Critical effect sizes (CES) and expected thresholds and ranges (±2SD)

Through the JOSM program, collections have occurred at many sites for three years for the purpose of gathering sufficient data to assess spatial and temporal variability of selected endpoints and to develop baselines for reference sites to distinguish site differences observed in downstream fish from overall natural variability (Arciszewski and Munkittrick 2015). Based on a minimum of three years of data, mean values for condition, gonado- and liver somatic indices were calculated for slimy sculpin by sex. From these data, upper and lower limits of both the critical effect sizes (CES) and ±2SD of the mean were calculated for each of the three endpoints. For condition, CES was set at ±10 % and for gonado- and liver somatic indices CES was set at ±25 % of the mean (Environment Canada 2010). The CES thresholds were derived from Steepbank River upper EC reference sites (2010-2013) but excluded the 1999 and 2000 historical data to reflect the most current state of oil sands mining activity.

Female slimy sculpin CES

In terms of anticipated energy storage (±10 % condition) of 2012 female sculpin, fish from all sites demonstrate condition within the CES range derived from the first three years of collections from the Steepbank upper EC site (Tables 17, 20). When incorporating 2013 condition data, female sculpin from the Horse River site fell below the CES while those from all other sites remained within the CES (Tables 17, 20).

Table 20. Calculated regional thresholds for mean (standard deviation; +2SD) condition factor (K), gonado- (GSI) and liver somatic (LSI) indices of female slimy sculpin from the Steepbank River EC upstream (U) location. Thresholds were established by evaluating the variability of a minimum of three cumulative years of data (a= 2010 + 2011 + 2012; b = a + 2013).

Site	Cumulative Years	Parameter	Mean	-2SD	+2SD	Critical Effect Size	
						(-)	(+)
Steepbank U EC	3a	K	1.002	0.903	1.102	0.902	1.103
		GSI	2	1.722	2.278	1.8	2.2
		LSI	1.775	0.978	2.573	1.6	1.953
	4b	K	1.0123	0.922	1.103	0.911	1.113
		GSI	2.065	1.721	2.408	1.858	2.271
		LSI	1.963	0.97	2.956	1.766	2.159

When CES for LSI (±25 %) was considered, female LSI from all sites, except for those from the Firebag upper site, exceeded the predicted cumulative CES for female sculpin in 2012. The main driver of these results is the low LSI value (1.32±0.07) of the 2011 Steepbank upper EC site for female fish (Tables 17, 20). These exceedances persisted for some of the sites (Dunkirk, High Hills, Steepbank RAMP, upper EC, Mid and L) when the cumulative CES included 2013 data, but the LSI of Horse River female fish fell below the calculated cumulative CES (Tables 17, 20).

In terms of anticipated energy allocation (±25 % gonadosomatic index) of 2012 female sculpin, fish from the Horse, Firebag upper and mid sites fell below the predicted cumulative CES, while all other sites remained within the CES (Tables 17, 20). In 2013, GSI of female sculpin from the Horse and Firebag upper sites again fell below the new cumulative CES, but so did fish from both the Steepbank mid and lower sites. Only female sculpin from the High Hills reference location fell below the CES for GSI, while all other sites remained within the CES (Tables 17, 20).

Male slimy sculpin CES

In terms of energy storage (condition) of male sculpin from 2012-2014, there were no observations of male sculpin that fell below the predicted cumulative CES for condition (Tables 19, 21). Although there were no exceedances in 2012, male sculpin from the Horse River exceeded the CES for condition in 2013, as did fish from the Firebag upper site in 2014 (Tables 19, 21). For the other endpoint related to energy storage, liver somatic index (±25 % LSI), there again were no observations of male sculpin that fell below the predicted cumulative CES. In 2012, male sculpin from the Steepbank lower, Firebag mid and Dunkirk sites exceeded the CES (Tables 19, 21). Male sculpin from all sites exceeded the predicted cumulative LSI CES in 2013. Lastly, in 2014, only the LSI of male sculpin from the Dunkirk site exceeded the calculated CES from 2013 (Tables 19, 21).

For evaluation of energy utilization (gonadosomatic index; GSI) there were deviations from the established CES of 25 % among sites and years. In 2012, GSI of male sculpin from the Steepbank mid and lower sites as well as those from the Horse were below the calculated GSI CES, and there were no locations that exceeded the CES (Tables 19, 21). In 2013, again male sculpin from the Steepbank lower and Horse sites, along with those from the Firebag mid sites were below the calculated CES. In that same year, the GSI of fish from both the Steepbank upper EC and mid sites exceeded the cumulative CES. GSI of male sculpin from the High Hills sites in 2014 was below the CES calculated up to 2013, while there were no other site differences (Tables 19, 21).

For all of these changes relative to reference, detailed examination of temperature data presented above should be made to evaluate the potential influence of altered temperature profiles on fish health endpoints.

Female slimy sculpin expected ranges

Expected ranges by sex (cumulative mean±2SD) of energy storage of fish (condition; liver somatic index (LSI)) and energy utilization (gonadosomatic index (GSI)) were calculated to determine the range of variability (background) of these three parameters among years within a site. For slimy sculpin, the cumulative mean±2SD for each parameter was set by the Steepbank River upper reference site incorporating the 2010+2011+2012 data (example shown here if for female GSI, Fig. 10). In 2012, the GSI of female fish from the Steepbank L, Horse, Firebag upper and mid sites was all below the expected range (Fig. 10). The GSI of female sculpin from the Muskeg lower site was also well below the predicted GSI range; however, this is potentially due to the low sample size (n=2) at this site due to challenging sampling conditions resulting in large data variability. In 2013, the expected range increased somewhat and only the Steepbank lower site fell well below the predicted GSI range similar to that site in 2012. GSI values from all sites sampled in 2014 were within the expected range (Fig. 10).

Male slimy sculpin expected ranges

The expected ranges for condition, LSI and GSI were also determined for male sculpin incorporating the Steepbank River upper reference site data from 2010+2011+2012. In the case of male slimy sculpin, LSI values are provided

Table 21. Calculated regional thresholds for mean (standard deviation (SD); + 2 standard deviations of the mean), condition factor (K), gonado- (GSI) and liver somatic (LSI) indices of male slimy sculpin from the Steepbank River EC upstream (U) location. Thresholds were established by evaluating the variability of a minimum of three cumulative years of data (a= 2010 + 2011 + 2012; b = a + 2013).

Site	Cumulative Years	Parameter	Mean	-2SD	+2SD	Critical Effect Size	
						(-)	(+)
Steepbank U EC	3a	K	1.029	0.892	1.166	0.926	1.132
		GSI	1.97	1.88	2.061	1.773	2.167
		LSI	1.162	0.899	1.425	1.046	1.279
	4b	K	1.041	0.919	1.163	0.937	1.145
		GSI	2.071	1.661	2.482	1.864	2.278
		LSI	1.185	0.952	1.418	1.066	1.303

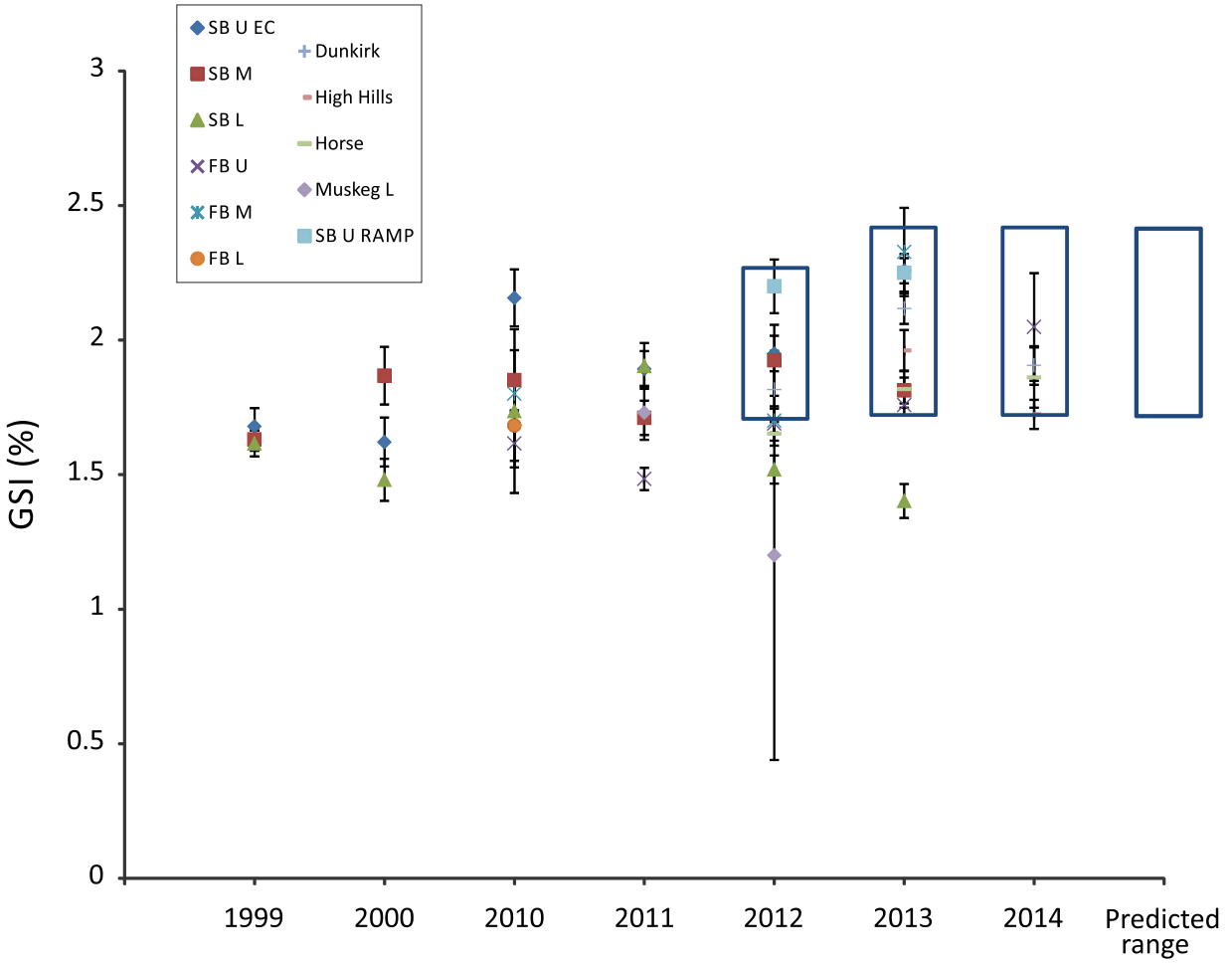

Figure 10. Expected range (blue rectangles) of the cumulative mean (+2SD) of the gonadosomatic index of female slimy sculpin set by Steepbank River upstream (U) EC reference site to define the predicted range in successive years. The predicted ranges do not incorporate the 1999 + 2000 historical data. (U=upper; M=middle; L=lower).

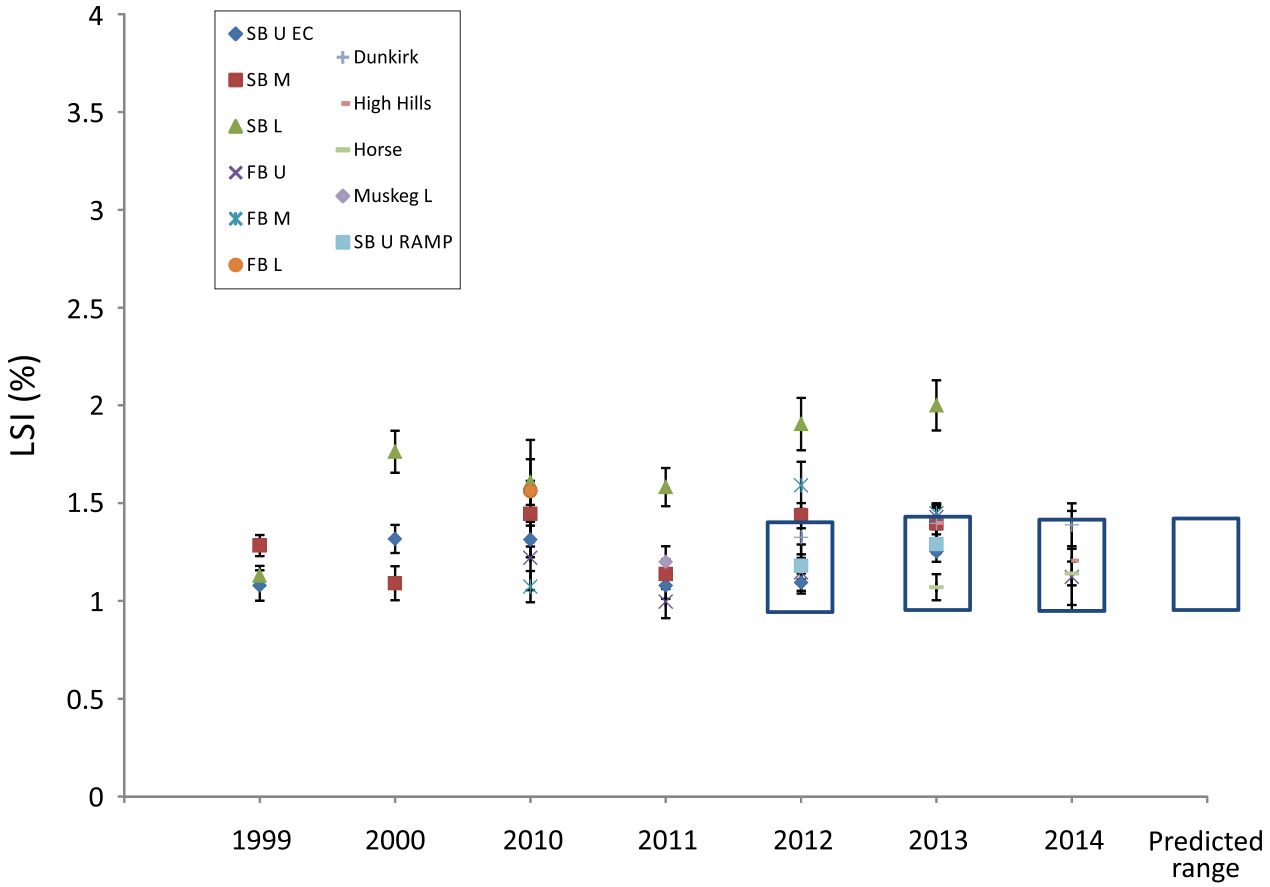

Figure 11. Expected range (blue rectangles) of the cumulative mean (+2SD) of the liver somatic index of male slimy sculpin set by Steepbank River upstream (U) EC reference site to define the predicted range in successive years. The predicted ranges do not incorporate the 1999 + 2000 historical data. (U=upper; M=middle; L=lower).

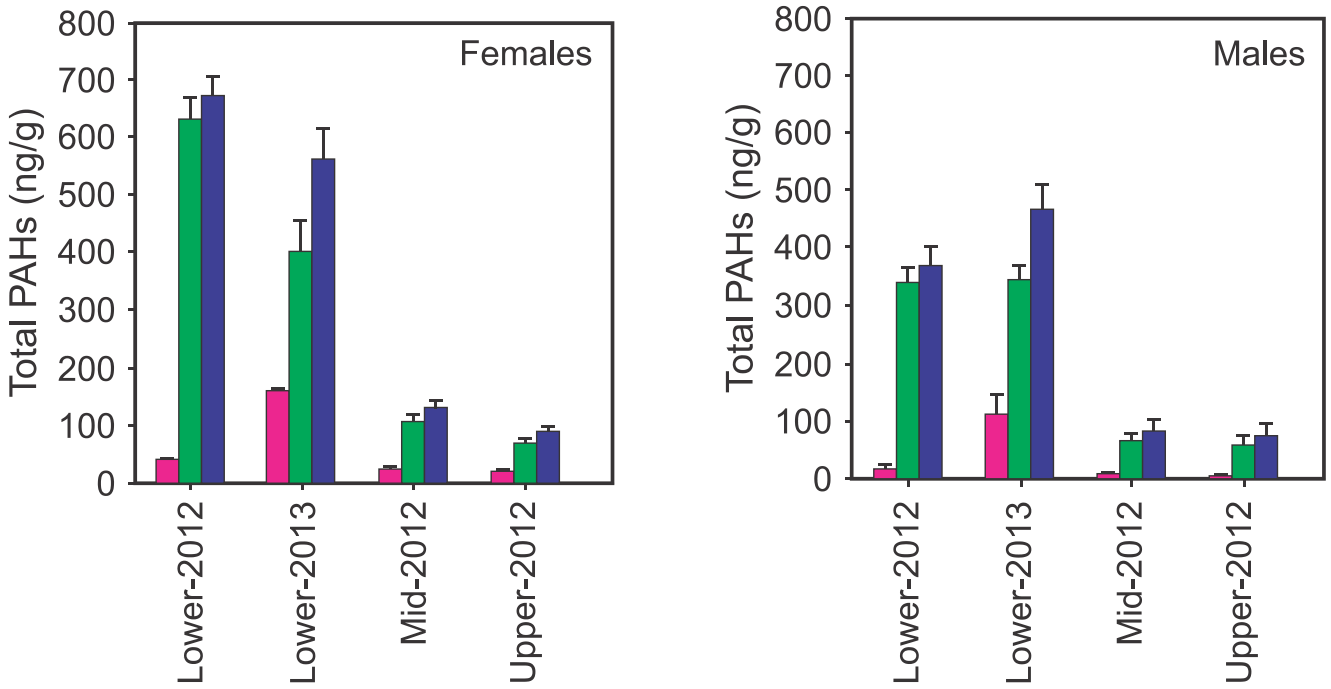

Figure 12. PAC levels in slimy sculpin collected from the Steepbank River during the fall of 2012-13. Sites represent the Steepbank lower site (lower and RAMP lower – same site), Steepbank mid site (MC Mid), Steepbank upper site (MC Upper) and a site further upstream (RAMP Upper). Values represent the mean ±SE Parent PACs – pink; alkylated PACs – green; total PACs – blue

here as an example of the use of this technique (Fig. 11). Liver size in male slimy sculpin collected from the Steepbank lower site exceeded the expected range in 2012 and 2013 (Fig. 11). In 2012, male fish from the Steepbank and Firebag mid sites also exceeded the expected range. The LSI of males from both Firebag sites (U and M) exceeded the expected range in 2013, as did those from Steepbank lower site, which were significantly larger than livers from males at all other sites. The 2014 data demonstrated no exceedances among sites sampled that year for male slimy sculpin LSI as they were all reference locations.

Contaminants in slimy sculpin

PACs were measured in select samples collected from slimy sculpin during 2012 and 2013. Levels in whole bodies of fish collected from the two upper Steepbank reference sites are lowest, slightly higher levels are found in fish collected from the mid site within the deposit, and much higher levels occurred in fish collected at the lower site downstream of development in both 2012 and 2013 (Fig. 12). Levels were slightly higher in the bodies of female sculpin and overall values were much higher than large bodied fish and the smaller bodied trout perch collected on the LAR mainstem.

Slimy sculpin summary

Overall, slimy sculpin appear to be sensitive indicators of fish health in the system as consistent changes in fish health were documented downstream within the oil sands deposit in 2010 through 2013. These differences are indicative of exposure to inducing compounds; given slimy sculpin demonstrate increases in liver size with corresponding induction of EROD activity. These fish often also demonstrate reductions in energy investment to reproductive development. Differences are sometimes outside critical effect sizes developed through years of data collection from environmental effects monitoring programs. We were also able to compare these results from the Steepbank River to studies conducted earlier by Tetreault et al. (2002). Also, we now have sufficient data to allow within-site predictions of fish health endpoints and can use this type of analysis to document change within sites over time. Studies will continue at these sites on a three year cycle, documenting reference variability in slimy sculpin health endpoints allowing for additional determination of change due to oil sands development. For other tributaries to the Athabasca, it is recommended that more years of baseline information be collected to allow sufficient power to detect change due to oil sands development.

3.4 Summary and Conclusions

During JOSM investigations of the LAR tributaries for fish health, considerable progress was made on answering questions posed in the Phase 2 Integrated Monitoring Plan for the Oil Sands (Environment Canada and Alberta Environment 2011). Each of the main questions is listed below along with a summary of our state of knowledge and the extent to which JOSM questions were answered through three years of monitoring.

- What is the current status of fish health in the Lower Athabasca Region?

Fish health studies on the Steepbank River, which is a major tributary to the mainstem Athabasca River, documented changes in slimy sculpin within the deposit downstream of development. These changes included increased liver size, reduced condition and reduced gonadal development. These responses are indicative of exposure to inducing compounds as MFO activity followed a very similar pattern to PAC body burdens. Additional years of data collection are required on other Athabasca River tributaries before current status can be determined with sufficient levels of confidence.

- Are there existing differences in fish health among sites in the Lower Athabasca Region?

As discussed above, the three years of baseline fish health on the Steepbank River clearly demonstrate some differences in fish health endpoints among sites on tributaries of the Athabasca. With a better understanding of reference site variability and expected change within site, we have a much better ability to predict normal fish health and to identify thresholds of change to trigger studies evaluating cause of the potential change within these systems.

- Are there any trends/changes in fish health relative to historical studies?

Now that we have a good baseline for fish health on the Steepbank River, we can use these data to compare to historical studies examining fish health on this system (Tetreault et al. 2002). Although there was some development on the Steepbank River during these earlier studies, development has increased significantly on this system since the original studies and we should be able to determine whether increased development resulted in increased alterations in fish health in this system. With the addition of multiple reference locations within the region, differences due to development can be compared to overall reference site variability allowing for a better understanding of the overall changes due to oil sands development.

- What are contaminant levels in fish?

We have begun to measure PACs in fish tissue from the fish health studies on some of the tributary sites. This is the first time PAC body burdens have been determined on these small bodied fish from these sites. Preliminary analysis indicates that similar to MFO induction, fish collected within the deposit have increased levels of PACs in muscle tissue. These contaminant concentrations can now be used as a baseline for fish tissue levels to monitor change over time with increased development.

- Are there any predictive relationships between system drivers (including development stress) and variability within sites in fish responses?

Fish health baseline studies clearly indicate some predictive relationships between development stress and fish health endpoints in slimy sculpin collected in the Steepbank River. Additional long-term data collections are required to further develop this relationship, but sufficient data are now available to make predictions of fish health within site during follow-up years of study. We have also begun to evaluate the relationship between water temperature at these sites and our fish health endpoints. This information should allow better predictions of fish health within and among sites, reducing variability in our understanding of factors controlling health endpoints.

- Is there evidence of cumulative effects of development on fish in the Lower Athabasca Region?

Recent work developing adaptive monitoring frameworks in support of cumulative effects assessments identified four steps in this progression (CWN and COSIA 2016) (see LAR mainstem fish above).

Clearly from the steps identified, our fish health studies on the Steepbank River have moved forward in the first three steps with further work needed in step two, refining effectiveness and robustness of monitoring approach, and in step three, developing predictive relationships that link drivers to the responses. Clear changes in fish health are apparent downstream of development within the deposit. Before developing a cumulative effects assessment model for fish health and further development in the oil sands region continued work in this direction is required. Also, additional years of baseline fish health data on more tributaries to the mainstem Athabasca River are needed. Knowledge of the influence of input from all tributaries flowing through the oil sands deposit is required to define the cumulative effects of development on the LAR mainstem.

4. Fish Toxicology Testing Sub-Theme

4.1 Introduction

Joint Oil Sands Monitoring (JOSM) results for fish toxicology address the requirement to assess several types of samples from various aquatic habitats within the oil sands area for their ability to cause effects in controlled exposures of fish. Fish toxicological testing was designed to assess various oil sands media that may contribute oil sands related chemicals (OSRCs) to the aquatic environment. By assessing these samples in the lab, information can be obtained on important aquatic routes of exposure and potential effects in organisms under controlled conditions. The fish toxicology program for JOSM focused on exposures in embryo-larval fathead minnows because early-life-stage tests using this species have been shown previously to be sensitive to OSRCs.

Aquatic toxicity testing of environmental samples from the oil sands area provides a direct measure of the effects in fish caused by a sample or a site, and the potency of the sample/site relative to others. By associating patterns of toxicity on fish in a controlled setting, this program was designed to determine if there is potential for impact on wild fish, and to determine which pathways and environmental media are contributing OSRCs. This information will allow us to understand exposure routes and pathways through which any observed ecological effects are occurring in response to oils sands developments.

Fish toxicity studies were first used to assess oil sands river sediments in 1999-2002 (Tetreault et al. 2003a, Colavecchia et al. 2004, 2006). The species used in these earlier studies were common lab fish test species (embryo-larval fathead minnow, *Pimephales promelas*) as well as wild fish (embryo-larval white sucker, adult slimy sculpin), and the sediments tested were collected from sites on Athabasca River tributaries (Ells and Steepbank rivers). These studies documented the effects of natural oil sands sediment in the fathead minnow, a commonly-used model fish test species (Ankley and Villeneuve 2006), and in species of relevance to the Athabasca River area that are not commonly tested in the lab. The number and type of samples in these earlier studies were limited (usually 2-3 river sediment samples), and they focused entirely on oil sands river sediments. The studies provided background information and a solid basis for determining what types of fish tests would respond to OSRCs.

As part of JOSM, the earlier embryo-larval fathead minnow studies were expanded in 2010 to 2014 to include 18 river sediment sites over several years, with many sites sampled yearly. These earlier studies were also used to design exposure methodologies for assessing other types of oil sands samples (melted snow, freshet, and groundwater). In the JOSM studies presented here, up to 10 snow and freshet sites, and six groundwater sites were assessed. To our knowledge, oil sands area snow/freshet and groundwater have never been assessed in controlled exposures of fish in the lab, so these exposures present unique results.

Given limited fish toxicity data for the oil sands area, our JOSM investigations were intended to provide information over a large number of sites, over several years, as identified in the Phase 2 Integrated Monitoring Plan for the Oil Sands (Environment Canada and Alberta Environment 2011). Addressing these questions provides information on the relative contribution of various environmental media and OSRC-exposure pathways in rivers in the oil sands area. The answer to these questions determines if important change has occurred since earlier studies, and describes the exposure pathways of most importance at the current time. The specific fish toxicology questions include:

- Which oil sands media contribute oil sands related chemicals (OSRCs) to the aquatic environment?
- What are the important aquatic routes of exposure and potential effects in organisms under controlled conditions?

The toxicology work is divided into fish and invertebrate subthemes that describe the work completed for toxicological assessments in the Athabasca River and its tributaries. The synthesis component integrates findings from fish toxicology and invertebrate caging studies with findings from wild fish health, invertebrate com-

munity, aerial snow deposition, and groundwater studies. The assessment initiates the process of integrating fish toxicology and invertebrate caging information with other primary water themes (wild fish population and health, benthic invertebrate biomonitoring, aerial deposition, and groundwater quality).

Objectives

The objective of the fish toxicological testing is to support and inform the wild fish health monitoring program. Information on whether sediments, groundwater, or snow and freshet are toxic in controlled lab exposures of fish can help to make clear which pathways are important for wild fish health and exposures. Also, toxicological results, in themselves, contribute to baseline data for future site-specific comparisons such as potential permitted release of end-pit-lake waters back to rivers in the oil sands area.

4.2 Methods

Study design

The objective of the fish toxicological testing is to produce information on whether sediments, groundwater, or snow and freshet were toxic in controlled lab exposures of fish. Toxicological testing of fish was conducted at Environment and Climate Change Canada in Burlington, Ontario. Samples were collected from the oil sands area and shipped to the lab, where controlled exposures of fathead minnow embryos and larvae were performed. Sampling sites were aligned precisely with wild fish health study collection sites (for sediments), with atmospheric deposition collection sites (for snow), and with groundwater study collection sites.

Tributary sediments

Tributary sediments were assessed to determine if they were a potential pathway by which OSRCs could affect fish. Contaminants in sediments could arise naturally from river sediments containing oil sands bitumen, or could arise anthropogenically from erosion and particle deposition. Other potential anthropogenic contributions to sediments were dusts from mining and coke piles, and particle deposition from stack emissions.

Controlled sediment exposures of fish in the lab to 1-25 g sediment/L were our approximation of the 'worst-case' natural exposure of wild fish to sediments. In the ambient environment, exposure of fish to sediment-derived compounds would sometimes be low, as the sediment surface was weathered. In these cases the water would contain low concentrations of sediment-derived compounds. In cases of erosion of sediments, exposure to sediment-derived compounds would be increased, as fresh sediment was exposed to water flowing over it. In the erosional setting, concentrations of sediment-derived compounds in water would be higher, and exposure of wild fish would be higher. Total Suspended Solids (TSS) in water from the Athabasca River have a mean of 87.78 mg TSS/L (95% C.I. to 98.45, full range of values – 1 to 2310 mg/L) (Alexander et al., 2017, Chambers et al., 2018). To mimic this in the lab we replaced sediments daily, which is a 'worst-case' exposure scenario. Frequently in the ambient environment, exposure to sediments would be lower. Although there would be more sediment volume, larger water volumes would flow over it making the top layer more inert if it remained undisturbed. This would be the case in a non-erosional environment. However, during periods of high water flow or sediment erosion in oil sands rivers (during ice break up or summer high flow storm events) bottom sediment would be eroded and mixed into the water column. In this case, there would be increased contact of sediment with water, and likely increased concentrations of sediment-derived OSRCs in the water. Our in-lab fish exposures sought to replicate this worst-case erosional event where exposure to sediment and sediment-derived OSRCs in the water column would be the highest.

Sediment samples from tributary sites (Fig. 13) were assessed in the lab for the capacity to affect fish survival and growth. For assessment of tributary sediments, study sites were selected on tributaries (Alice Creek, and Dunkirk, Ells, Firebag, Horse, High Hills, and Steepbank rivers) upstream of the bitumen deposit, inside the deposit, and inside the deposit close to mining and upgrading activities wherever possible (Table 22). These study sites were identical to the sites where wild fish health was assessed (see Fig. 2) and where invertebrate communities were assessed on tributaries. The exception to this was that we also assessed sediments collected from three sites on the Muskeg River.

Sediments

Collected from…
Upper, Middle, & Lower sites on tributaries

Collection sites

- **2010 – 9 sediments**
- **2011 – 8 sediments**
- **2012 – 13 sediments**
- **2013 – 13 sediments (same as 2012)**
- **2014 – 16 sediments (same as 2013 + 3 far d/s in Delta)**

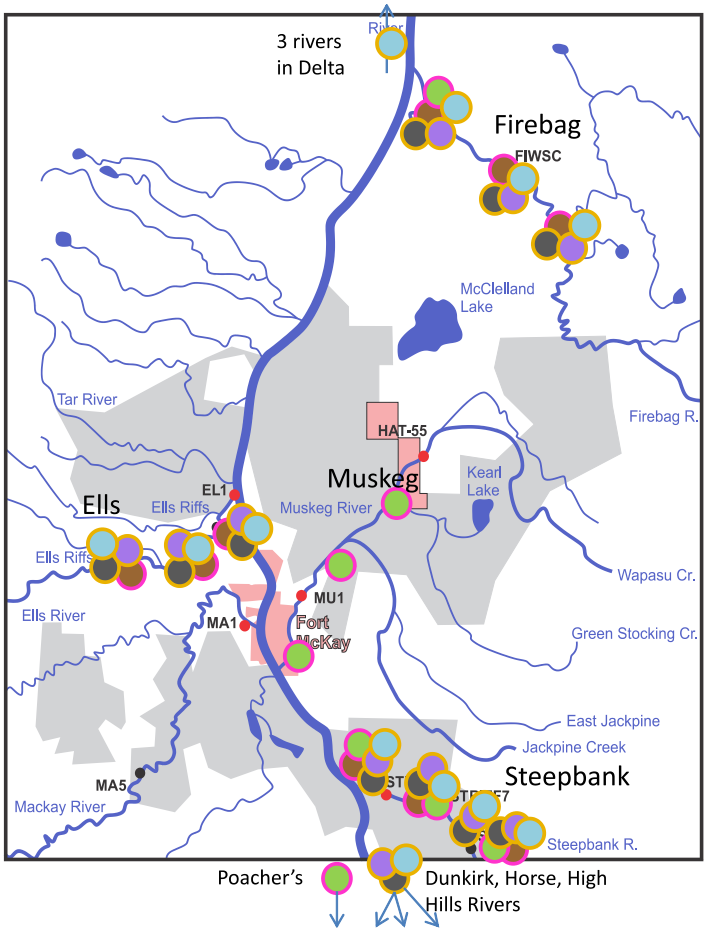

Figure 13. Map of sediment sampling sites from 2010-2014. River sediments were sampled and brought back to the lab to test for effects in exposed embryo larval fathead minnows.

Snow and freshet

Snow and freshet samples were assessed to determine if aerial deposition was a potential pathway by which OSRCs could affect fish. Sources of contaminants in snow would be largely anthropogenic, and would be deposited over 4-5 months (during the autumn and winter) in dusts from mining and coke piles, and in particles from stack emissions.

For snow and freshet sampling, study sites were chosen to be either near to, or far from, industry mining and upgrading activities (Fig. 14). Samples were taken from sites on the LAR mainstem and the Ells and Steepbank rivers (Table 23). Sites were coordinated with atmospheric sampling in 2011, 2012, and 2013 and with the Ells and Steepbank tributary Representative Sub-basin Studies (REPS) in 2013 and 2014. All snow samples were taken over ice on the river, and freshet samples were taken directly from the river as the ice was melting, so that results would represent snow that entered the river when snow melt occurred, or actual freshet water (see Table 23).

Tests were designed to determine if these snow and freshet samples had the potential to affect fish survival and growth in the lab. While fish do not live in 100 or 50 or 25 % melted snow, we tested the samples of pure snow in the lab to assess whether the mix of compounds in the snow had the potential to affect fish. The relevance of any observed effects from the snow exposures would be addressed in the testing of freshet water, which is what fish would be exposed to in the real environment. The freshet samples represented the realistic situation when the snow melts in the spring and mixes with river waters. Freshet water was always tested at 100 % as this was the realistic concentration that wild fish would be exposed to.

Groundwater

Groundwater was assessed to determine if they were a potential pathway for OSRCs to affect

Table 22. Site names, latitude and longitude for sediment collection sites on the Athabasca River and its tributaries. All collections were in September or October in the year indicated.

Site Name	Latitude	Longitude	Site Name	Latitude	Longitude
2010 sediment sites			**2013 sediment sites**		
Steepb'k Lower	57.023333	-111.47475	Ells Lower	57.2645	-111.71642
Steepb'k Mid	56.991472	-111.33569	Ells Mid	57.238333	-111.76942
Steepb'k Upper	56.863639	-111.12594	Steepb'k Lower	57.023333	-111.47475
Ells Lower	57.2645	-111.71642	Steepb'k Mid	56.991472	-111.33569
Ells Mid	57.238333	-111.76942	Steepb'k Upper	56.863639	-111.12594
Ells Upper	57.23025	-111.88919	Steepb'k RAMP		
Firebag Lower	57.516056	-111.11286	Ells Upper	57.23025	-111.88919
Firebag Mid	57.436361	-110.89308	Firebag Lower	57.516056	-111.11286
Firebag Upper	57.335222	-110.47619	Firebag Mid	57.436361	-110.89308
2011 sediment sites			Firebag Upper	57.335222	-110.47619
Steepb'k Lower	57.023333	-111.47475	Dunkirk	56.85942	-112.71147
Steepb'k Mid	56.991472	-111.33569	Horse	56.36135	-112.17625
Firebag Lower	57.516056	-111.11286	High Hills	56.75400	-110.50872
Steepb'k Upper	56.863639	-111.12594	**2014 sediment sites**		
Muskeg Lower	57.133494	-111.59874	Ells Upper	57.23025	-111.88919
Muskeg Mid	57.332616	-111.37369	Firebag Upper	57.335222	-110.47619
Muskeg Shell Up	57.192068	-111.57227	Firebag Mid	57.436361	-110.89308
2012 sediment sites			Firebag Lower	57.516056	-111.11286
Ells Lower	57.2645	-111.71642	Steepb'k RAMP	56.82175	110.98392
Ells Mid	57.238333	-111.76942	Steepb'k Upper	56.863639	-111.12594
Steepb'k Lower	57.023333	-111.47475	Steepb'k Mid	56.991472	-111.33569
Steepb'k Mid	56.991472	-111.33569	Steepb'k Lower	57.023333	-111.47475
Firebag Lower	57.516056	-111.11286	Ells Lower	57.2645	-111.71642
Steepb'k Upper	56.863639	-111.12594	Ells Mid	57.238333	-111.76942
Steepb'k RAMP	56.82175	110.98392	Dunkirk	56.85942	-112.71147
Ells Upper	57.23025	-111.88919	Horse	56.36135	-112.17625
Firebag Mid	57.436361	-110.89308	High Hills	56.75400	-110.50872
Firebag Upper	57.335222	-110.47619	Athabasca		
Dunkirk	56.85942	-112.71147	Alice Creek Upper		
Horse	56.36135	-112.17625	Alice Creek Mid		
High Hills	56.75400	-110.50872	Steepb'k Mid	56.991472	-111.33569
			Firebag Lower	57.516056	-111.11286

fish. Contaminants in groundwater could arise naturally from contact of the groundwater with bitumen, or could arise anthropogenically from tailings pond leaching and mixing with natural groundwater. Similar to snow, fish do not live in 100, 50 or 25 % groundwater. However, we tested a range of concentrations (3 % to 100 %) of groundwater in the lab to determine if the mixture of compounds in groundwater had the potential to affect fish. In the real environment, fish eggs or newly hatched fry in the sediment may be exposed to groundwater slowly entering river systems. Free-swimming larval fish would be exposed to groundwater that was much more dilute as it would have been mixed with river water.

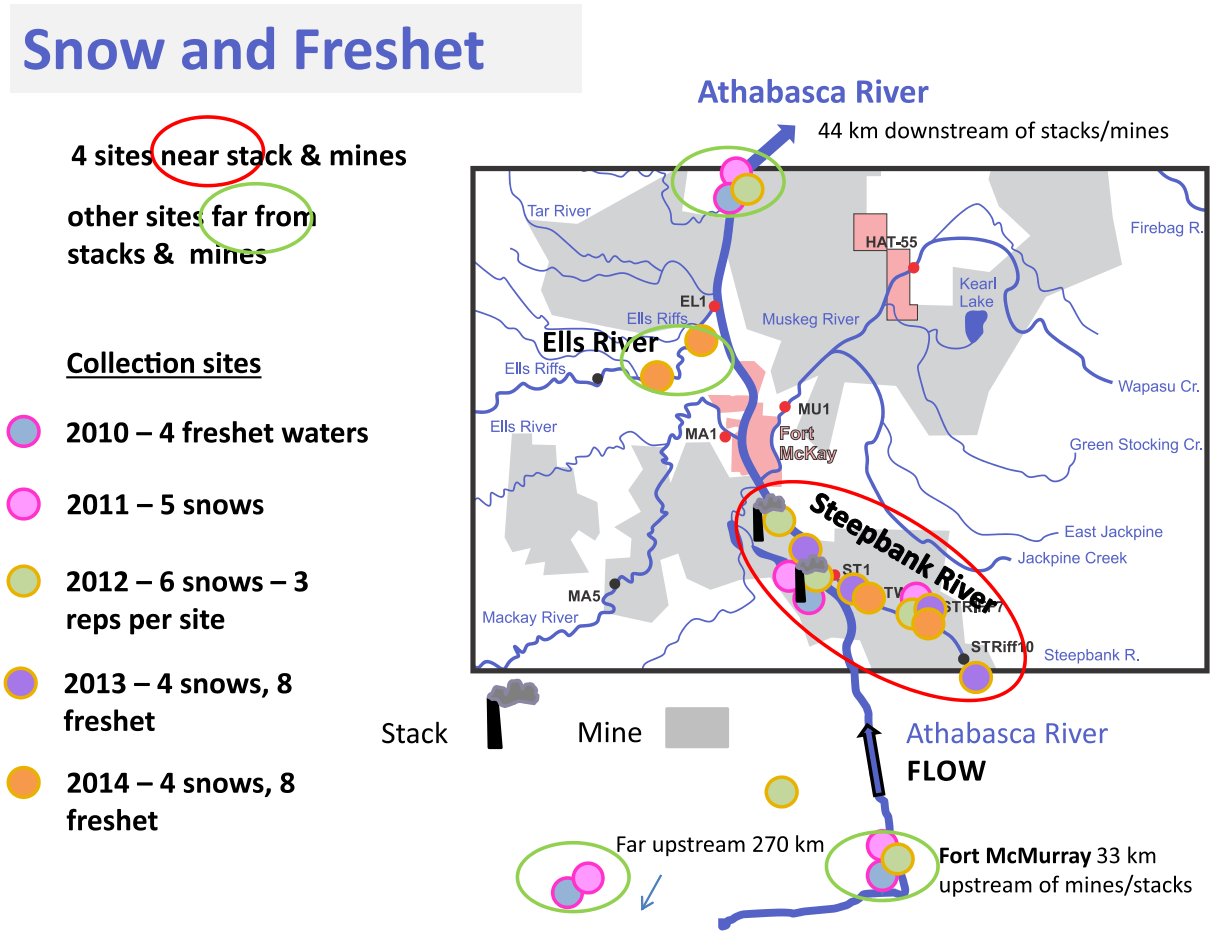

Figure 14. Map of snow and freshet sampling sites from 2010-2014. Snow and freshet were sampled and brought back to the lab to test for effects in exposed embryo larval fathead minnows.

Groundwater samples were chosen to be near tailings ponds on the LAR mainstem, or far from the influence of tailings ponds at natural sites on the Athabasca River or on the Ells River (see Table 27). Groundwater was assessed in the lab for the ability to affect fish survival and growth. Groundwater was tested at exposure concentrations of 3, 6, 12, 25, 50, or 100 %. Fish exposure solutions were prepared daily and allowed to equilibrate for 24 h at 25 °C in the dark prior to embryo/fish transfer in the screen-bottomed glass egg cup.

Laboratory exposures

Fathead minnow embryos were exposed to oil sands environmental samples (river sediments, groundwater, snow melt or freshet) for 19-21 days. Detailed methods for the fathead minnow sediment tests have been published previously (Colavecchia et al. 2004). Differences and improvements in the method included daily renewal of water and sediment. The exposure period covered the sensitive windows of embryo-differentiation, hatching, and early development of 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. Exposure solutions were renewed daily so that there was a relatively constant concentration of any OSRC throughout the 21-day exposure. The endpoints assessed in the eggs and larval fish were survival, growth, and deformities. Deformities were assessed at hatch as in Marentette et al (2015), 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).

All sediments were tested at 1, 5, and 25 g sediment/L of lab water in a glass beaker. Water was added to sediment in a beaker, and the solution

Table 23. Site names, latitude and longitude, dates of collection, and concentrations tested, for snow and freshet collection sites on the Athabasca and Steepbank rivers.

Site Name and Date of collection. **Concentration tested in lab exposures of fish is also shown**	**Year**	**Latitude**	**Longitude**
2010 - March 23	2010		
Athabasca River water under ice (Freshet)			
CA1-100%	23-Mar-10	55.09031	112.88161
AR1-100%	23-Mar-10	56.72042	111.40644
AR6-100%	23-Mar-10	57.01728	111.47944
AR15-100%	23-Mar-10	57.42483	111.64478
2011 - Feb 26 to Mar 3	2011		
Snow on Athabasca River or Steepbank River			
CA1-100%	28-Feb-11	55.090306	112.881611
AR1-100%	26-Feb-11	56.726406	111.401569
AR6-25, 50, 100%	27-Feb-11	57.017278	111.479444
ST2-25, 100%	03-Mar-11	56.992917	111.376033
AR15-100%	02-Mar-11	57.424833	111.644778
2012 March 6 to 9	2012		
Snow on Athabasca River or Steepbank River			
AR1- 25, 50, 100%		56.722028	111.402944
AR15- 25, 50, 100%		57.427806	111.646972
AR6-25, 50, 100%		57.013583	111.478222
AR7- 25, 50, 100%		57.045500	111.507556
CA1-25, 50, 100%		55.088444	112.883611
ST2- 25, 50, 100%		56.993667	111.378194
2013 March 3 to 4	2013		
Snow on Athabasca River or Steepbank River			
AR6-25, 50%		57.012900	111.476826
ST2-25, 50%		56.993607	111.378369
AR6-100%		57.012900	111.476826
ST2-100% = Steep Mid		56.993607	111.378369
ST1-100% = most upstream site on Steepbank R that particles were seen		56.9913078	111.337481
ST3-25, 50, 100% = Steep Low		57.0236863	111.472961
Freshet from Steepbank River on April 4 and May 1			
Steep Up Apr-100%		56.863583	111.117528
Steep Low Apr-100%		57.023083	111.475667
Steep Up May-100%		56.863917	111.125611
Steep Low May-100%		57.023639	111.473500
2014 March 10	2014		
Snow on Ells River or Steepbank River			
Ells Low-100%		57.28796	111.70936
Ells Mid-100%		57.24559	111.73741
Steep Mid-25, 50, 100%		56.99163	111.33330
Steep Low-25, 50, 100%		57.02332	111.46257
Freshet from Ells River or Steepbank River between ice (pure snowmelt) or under ice (snowmelt mixed with river water) – March 16 to 17			
Ells 2A -100%		57.24559	111.73741
Ells 3A -100%		57.28690	111.70333
Steep 2A -100%		56.99163	111.33330
Steep 3A -100%		57.02332	111.46257

was allowed to equilibrate for 24 h at 25 °C. Fathead minnow eggs and larval fish were held above the sediment in a nylon mesh-bottomed glass egg cup. The cup facilitated daily transfer of the embryos or larvae to a new exposure beaker. Beakers were covered and solutions were aerated gently, and changed daily.

For fathead minnow exposures to water-based, oil-sands samples (groundwater, snow, freshet), exposure methods and assessment of embryos and larval fish are identical to that of Marentette et al. (2015). Snow exposures were to 25, 50, and 100 % melted snow. Freshet water was always tested at 100 % with no dilution. Groundwater was tested at 3, 6, 12, 25, 50, and 100 %.

All dilution waters for sediments, snows, and groundwater were fish-quality water from Environment and Climate Change Canada's Aquatic Life Research Facility in Burlington. This dilution water was from the City of Burlington and was dechlorinated, charcoal filtered, and UV sterilized. This water was also used as control water exposures for each series of fathead minnow exposures.

Sediment sampling

Sediments were sampled using a stainless steel shovel to load 15-20 L of sediment into food-grade polyethylene bags inside a 20 L pail with a lid. Sediments were held in 4 °C refrigerated coolers, and shipped from Fort McMurray by refrigerated truck. Sediments were stored in cold rooms for three to nine months until fish toxicity testing began. Storage time did not affect sediment potency measured as ability to decrease survival of larval fathead minnows. In several repeat fish exposures to sediments collected in September 2013, potency was unchanged up to two years after collection. Aliquots of sediment 1, 5, or 25 g, were weighed into glass scintillation vials and kept in the fridge. Daily fish exposure solutions were prepared by adding the vial contents to a 1-L beaker and mixing sediment into the water by filling the beaker with dechlorinated, charcoal-filtered lab water. The sediment-water beaker was allowed to equilibrate for 24 h at 25°C in the dark, before the fathead minnow eggs or larvae in the egg cup were transferred into it. Volatile compounds in the sediment would have been lost during the equilibration period. However, we wanted to assess the chronic toxicity of compounds that remained in the sediment during the 21-d exposure, so we accepted that some of the more volatile, acutely toxic compounds in the sediment may have been lost.

Snow and freshet sampling

Snow was sampled in March of each year. A 100 m^2 site far from the landing-circle disturbance of the helicopter blade (i.e., >30m distance) was delimited, and snow was sampled from five 1 m by 1 m areas (from the corners and centre of the large square). GPS location was taken from the centre of the sampling area. Snow was sampled by stainless steel shovels, and loaded into double food-grade polyethylene bags inside 37 L plastic totes with lids. Snow samples were taken from the surface snow to the depth of the frozen ice surface over the river. The snow was kept at -20 °C until toxicity tests were performed.

Freshet water was collected at the same locations as winter snow samples by pumping water into stainless steel canisters transported at 4 °C to Burlington. Before toxicity exposures, the melted snow was amended with ions (see Table 24), as pure snow was toxic to fish due to the lack of ions in the water. These salt additions prevented fish death from the ion-poor water.

Groundwater sampling

Groundwater was sampled at three locations near a tailings pond on the Athabasca River, two of which were influenced by oil sands process affected water (OSPW). In addition, two locations previously reported as containing a high concentration of natural bitumen-derived chemicals (due to passage through the natural oil sand deposit) and not affected by existing tailings ponds, were sampled. Finally, a single groundwater location was sampled outside the oil sands formation. At all of these sites, groundwater with upward directional flow was sampled via a drive-point at 0.5 to 1 m below the river bed. Groundwater was collected in a 20 L stainless steel canister, with subsequent storage and transportation to the laboratory at 4 °C. Groundwater samples were centrifuged to remove particulates then stored at 4 °C until fathead minnow tests were ready to start.

Table 24. Salts added to melted snow to provide ions necessary to support embryo-larval fish survival and growth.

Salts to add	mM Required	mg Salt/L
CaCl2.2H2O	0.4455	65.5
CaSO4.2H2O	0.4455	76.7
NaHCO3	0.5176	43.5
NaBr	0.0100	1.03
KCl	0.0294	2.19
MgSO4.7H2O	0.4059	100.1

Final mg/L for each ion	
SO4	81.79
Ca	35.71
Mg	9.87
Cl	32.63
Na	12.13
K	1.15
HCO3	25.37
Br	0.799

Statistical approach

Replicate exposure beakers (3-4 per concentration, 6-12 per control water) contained 30 newly-fertilized fathead minnow eggs (or 20 eggs from 2014 forward). Beakers and eggs were randomly assigned treatments and randomly assigned locations in incubators. All exposure solutions and sediments were changed daily. The 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.

Statistical comparison of these endpoints to those of controls (lab water exposed embryos and larvae) was performed via ANOVA to assess whether there was an overall treatment effect. After that, 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 sediment exposure concentrations (e.g., comparing mean % survival of control larvae to mean % survival in larvae exposed to 5 g/L Ells River lower sediment).

4.3 Results and Discussion

In the fish toxicology studies, lab fish were exposed under controlled conditions to various

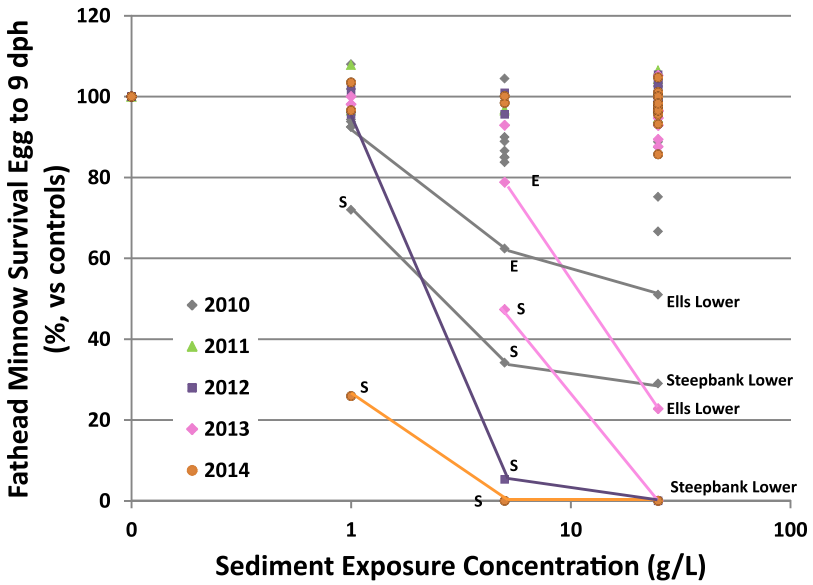

Figure 15. Fathead minnow embryo-larval survival after exposure to various river sediments from tributaries of the Athabasca River. Steepbank and Ells lower sediments are noted on the plot, as these sediment samples consistently caused lower survival in fish exposed in the lab over several different sampling years.

samples from rivers in the oil sands area to assess which pathways of exposure were important for wild fish and invertebrates. The components we tested were sediments, snow/freshet, and groundwater.

Tributary sediments

Tributary sediments containing natural bitumen can affect fish in lab exposures. Most tributary sediments caused no effects in embryo-larval fathead minnows exposed in the lab at up to 25 g/L (the highest concentration tested) (i.e., >80% survival). However, sediments from the lower sites on two tributaries, Steepbank lower and Ells lower, did significantly reduce survival of fathead minnow larvae at concentrations of 1, 5 and 25 g/L and this effect was seen over several years (Table 25, Fig. 15). Potencies varied year to year (Table 25). Sediments collected from the Steepbank lower site in 2011 caused no effects, while those collected in 2010, 2012, 2013, and 2014 consistently affected survival of larval fish in the lab. Ells lower sediments collected in 2010 and 2013 affected fish survival, but sediments collected from this site in 2011, 2012, and 2014 did not. Overall, most samples of sediment collected from the Steepbank lower site and some samples of sediment collected from the Ells lower site over five years (from 2010-2014) showed the same patterns of effect.

The embryo-larval fathead minnow were sensitive to the OSRCs in oil sands sediments. It was important to expose embryos and larvae for 21 days to the sediment, as often we saw very few effects on the eggs during an exposure, but effects manifested as the eggs hatched and as the larvae were exposed for another 16 days (Table 25). For example, results from exposure to 2010 Steepbank lower sediment (at 25 g/L)

Table 25. Mean survival (% surv and SD) of fathead minnow eggs, fry, and larvae over 20-21 day exposures to oil sands sediments (at 0, 1, 5, and 25 g sediment/L). Survival is shown until hatch (day 5 of test), until 8-9 dph (day 13 or 14 of test), and until 15-16 dph (day 20 or 21 of test). Abbreviations are dph = days post hatch. Values in bold with asterisks are significantly different from controls, p≤0.050 (Bonferroni's p value with separate variances from two-sample t-tests comparing treatment means).

2010 sediment data													
		Hatch				**8 dph**				**15 dph**			
Site Name		g sediment per L											
		0	**1**	**5**	**25**	**0**	**1**	**5**	**25**	**0**	**1**	**5**	**25**
Control water	surv	88.3				87.2				72.9			
	sd	8.4				9.0				10.8			
Steepb'k Lower	surv		88.9	94.4	92.2		85.6	54.4	**44.4***	53.3	53.3	35.4	**16.7***
	sd		7.7	3.9	5.1		5.1	30.2	6.9	6.1	6.1	29.8	3.3
Steepb'k Mid	surv		93.3	90.0	92.2		88.9	75.6	65.6		69.2	49.6	37.0
	sd		3.3	5.8	5.1		10.7	6.9	24.1		28.9	12.6	23.2
Steepb'k Upper	surv		86.7	93.3	93.3		82.2	91.1	84.4		66.0	83.1	72.0
	sd		5.8	3.3	6.7		11.7	1.9	7.7		13.1	10.7	18.7
Control water	surv	93.3				83.3							
	sd	0.0				9.4							
Control water	surv	78.3				65.0							
	sd	10.1				10.5							
Ells Lower	surv		80.0	76.7	66.7		60.0	**22.2***	**18.9***			no data	
n=1 beaker for 1 g/L	sd			13.3	5.8			5.1	7.7				
Ells Mid	surv		86.7	72.2	68.9		80.0	54.4	43.3			no data	
n=1 beaker for 1 g/L	sd			7.7	21.7			17.1	23.1				
Ells Upper	surv		96.7	77.8	77.8		90.0	57.8	66.7			no data	
n=1 beaker for 1 g/L	sd			8.4	5.1			5.1	8.8				
Control water	surv	93.9				88.9				67.1			
	sd	4.9				5.8				9.0			
Firebag Lower	surv		92.2	91.1	90.0		83.3	75.6	77.8		64.8	57.3	45.7
	sd		1.9	12.6	6.7		3.3	9.6	15.0		6.9	14.0	20.0
Firebag Mid	surv		93.3	94.4	83.3		82.2	80.0	78.9		65.8	63.9	49.0
	sd		0.0	1.9	3.3		13.5	11.5	5.1		16.1	21.1	15.1
Firebag Upper	surv		88.9	90.0	94.4		82.2	75.6	87.8		68.1	64.1	64.3
	sd		6.9	8.8	1.9		3.8	8.4	5.1		7.6	12.5	5.7

2011 sediment data		Hatch				8 dph				15 dph			
Site Name		g sediment per L											
		0	1	5	25	0	1	5	25	0	1	5	25
Control water	surv	92.2				88.7				86.8			
	sd	5.2				5.7				7.9			
Steepb'k Lower	surv		97.8	95.6	94.4		95.6	85.6	90.0		95.6	81.9	87.9
	sd		1.9	5.1	5.1		1.9	10.7	3.3		1.9	12.6	1.8
Steepb'k Mid	surv		94.4	94.4	97.8		85.6	85.9	94.4		85.6	85.9	90.4
	sd		1.9	1.9	3.8		10.2	6.6	1.9		10.2	6.6	2.9
Control water	surv	97.7				94.8				84.3			
	sd	1.8				4.9				7.4			
Firebag Lower	surv				96.6				89.8				80.1
	sd				6.0				7.0				7.3
Steepb'k Upper	surv				94.4				92.2				80.1
	sd				1.9				3.8				9.3
Control water	surv	94.4				89.5				84.3			
	sd	4.0				7.0				5.4			
Muskeg Lower	surv				95.6				89.0				87.1
	sd				1.9				4.9				8.0
Muskeg Mid	surv				93.3				93.3				91.3
	sd				0.0				0.0				3.6
Muskeg Shell Up	surv				90.0				86.8				82.9
	sd				6.7				8.7				7.6

2012 sediment data		Hatch				9 dph				16 dph			
Site Name		g sediment per L											
		0	1	5	25	0	1	5	25	0	1	5	25
Control water	surv	94.2				94.2				94.2			
	sd	7.4				7.4				7.4			
Ells Lower	surv		95.0	96.7	100.0		95.0	95.0	93.3		95.0	95.0	93.3
	sd		8.7	5.8	0.0		8.7	5.0	11.5		8.7	5.0	11.5
Ells Mid	surv		96.7	98.3	95.0		96.7	90.0	93.3		96.7	90.0	93.3
	sd		5.8	2.9	5.0		5.8	17.3	7.6		5.8	17.3	7.6
Steepb'k Lower	surv		95.0	**50.0***	**1.7***		90.0	**5.0***	**0.0***		84.2	**1.7**	**0.0***
	sd		5.0	13.2	2.9		5.0	8.7	0.0		9.6	2.9	0.0
Control water	surv	99.2				98.3				98.3			
	sd	2.0				4.1				4.1			
Steepb'k Mid	surv				100.0				100.0				93.3
	sd				0.0				0.0				11.5
Firebag Lower	surv				98.3				98.3				98.3
	sd				2.9				2.9				2.9
Control water	surv	94.4				89.5				84.3			
	sd	4.0				7.0				5.4			
Steepb'k Upper	surv				90.0				86.7				82.8
	sd				3.3				3.3				7.5
Steepb'k RAMP	surv				95.6				92.3				92.3
	sd				1.9				1.8				1.8
Ells Upper	surv				95.6				92.2				92.2
	sd				3.8				8.4				8.4
Firebag Mid	surv				98.9				94.4				90.3
	sd				1.9				6.9				9.6
Firebag Upper	surv				93.2				89.8				89.8
	sd				6.9				3.6				3.6
Dunkirk	surv				95.6				93.3				89.5
	sd				1.9				5.8				12.4
Horse	surv				91.1				87.8				85.9
	sd				5.1				1.9				4.6
High Hills	surv				92.1				87.6				85.7
	sd				4.0				2.1				4.6

2013 sediment data													
		Hatch				**9 dph**				**16 dph**			
Site Name		g sediment per L											
		0	**1**	**5**	**25**	**0**	**1**	**5**	**25**	**0**	**1**	**5**	**25**
Control water	surv	96.0				95.1				95.1			
	sd	5.6				6.1				6.1			
Control water	surv	93.3				88.3				88.3			
	sd	7.6				12.6				12.6			
Ells Lower	surv		90.0	85.0	**50.0***		88.3	75.0	**21.7***		88.3	75.0	**18.3***
	sd		8.7	17.3	13.2		7.6	17.3	16.1		7.6	17.3	10.4
Ells Mid	surv			96.7	95.0			88.3	91.7			88.3	88.5
	sd			2.9	5.0			16.1	2.9			16.1	2.6
Steepb'k Lower	surv		91.7	81.7	**8.3***		86.7	**45.0***	**0.0***		83.5	**40.0***	**0.0***
	sd		2.9	12.6	2.9		7.6	8.7	0.0		3.0	10.0	0.0
Control water	surv	97.5				95.1				93.5			
	sd	2.7				3.0				4.9			
Steepb'k Mid	surv				90.0				90.0				84.3
	sd				5.0				5.0				14.4
Steepb'k Upper	surv				100.0				100.0				100.0
	sd				0.0				0.0				0.0
Steepb'k RAMP	surv				91.7				91.7				85.7
	sd				7.6				7.6				14.0
Ells Upper	surv				93.5				91.8				91.8
	sd				5.6				7.6				7.6
Firebag Lower	surv				90.0				88.3				88.3
	sd				8.7				7.6				7.6
Firebag Mid	surv				91.7				85.0				85.0
	sd				2.9				10.0				10.0
Firebag Upper	surv				91.7				91.7				91.7
	sd				10.4				10.4				10.4
Dunkirk	surv				93.3				83.3				80.0
	sd				5.8				20.8				17.3
Horse	surv				91.7				90.0				90.0
	sd				2.9				5.0				5.0
High Hills	surv				90.0				88.3				85.7
	sd				5.0				7.6				12.1

2014 sediment data													
		Hatch				**9 dph**				**16 dph**			
Site Name		g sediment per L											
		0	**1**	**5**	**25**	**0**	**1**	**5**	**25**	**0**	**1**	**5**	**25**
Control water	surv	93.2				90.7				89.0			
	sd	7.7				7.5				6.0			
Ells Upper	surv				91.7				88.3				88.3
	sd				2.9				5.8				5.8
Firebag Upper	surv				93.2				88.0				88.0
	sd				2.8				8.2				8.2
Firebag Mid	surv				91.7				86.7				83.8
	sd				2.9				2.9				6.8
Firebag Lower	surv				91.4				95.0				95.0
	sd				8.0				7.1				7.1
Steepb'k RAMP	surv				98.3				91.7				91.7
	sd				2.9				2.9				2.9
Steepb'k Upper	surv				93.3				88.3				85.0
	sd				7.6				10.4				5.0
Steepb'k Mid	surv				86.3				77.7				77.7
	sd				8.1				13.6				13.6
Control water	surv	99.2				96.6				96.6			
	sd	2.0				4.3				4.3			
Steepb'k Lower	surv		93.3	**13.3***	**0.0***		**25.0***	**0.0***	**0.0***		**21.7***	**0.0***	**0.0***
	sd		5.8	7.6	0.0		13.2	0.0	0.0		12.6	0.0	0.0
Ells Lower	surv		100.0	100.0	100.0		100.0	95.0	90.0		100.0	95.0	90.0
	sd		0.0	0.0	0.0		0.0	0.0	5.0		0.0	0.0	5.0
Ells Mid	surv		96.7	98.3	96.7		93.3	96.7	93.3		93.3	93.3	93.3
	sd		2.9	2.9	2.9		2.9	2.9	5.8		2.9	2.9	5.8
Control water	surv	99.2				98.3				98.3			
	sd	2.0				4.1				4.1			
Dunkirk	surv				95.0				95.0				95.0
	sd				5.0				5.0				5.0
Horse	surv				98.3				96.7				96.7
	sd				2.9				2.9				2.9
High Hills	surv				98.3				98.3				98.3
	sd				2.9				2.9				2.9
Alice Creek Mid	surv				98.3				95.0				95.0
	sd				2.9				5.0				5.0
Alice Creek Lower	surv				98.3				96.7				96.7
	sd				2.9				2.9				2.9

show normal survival (92 %) from egg to hatch, but just 44 % and 17 % survival from egg to 8 and 15 dph, respectively. The extension of the exposure past the egg stage often increases sensitivity and provides valuable information. Studies of fish embryos that ended at hatch (Greeley Jr et al. 2014) may have missed sediment effects that would have manifested themselves later as the fry developed and grew.

Snow and freshet

Snow sampled from sites close to upgraders and mines affected larval fathead minnow survival (Fig. 16). Snow collected on the Athabasca River or on the Steepbank River close to mining and upgrading sites caused reduced survival in the lab at concentrations of 25, 50, or 100 %. Larval survival was significantly reduced when

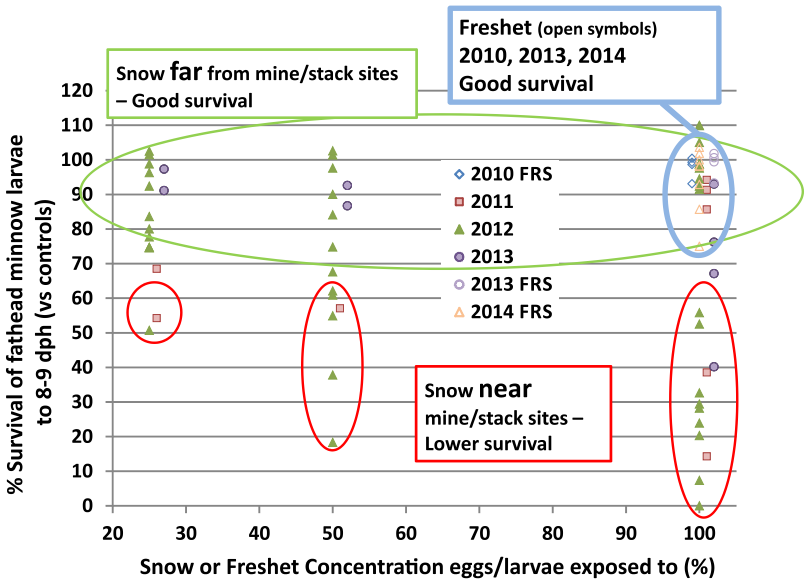

Figure 16. Fathead minnow embryo-larval survival after exposure to melted snow or freshet water collected from the Athabasca, Ells and Steepbank rivers. Exposures were to 25, 50, and 100 % melted snow or 100 % freshet. Some points are jittered to allow overlapping data points to be seen. Different coloured symbols show various snow and freshet sampling years from 2010-2014.

Table 26. Survival (%, and SD) of fathead minnow embryos and larvae in melted snow (25, 50 or 100%) or 100% freshet. Exposures were from fertilized egg to 7-9 days post hatch or 15-16 days post hatch (dph). All solutions were renewed daily. Bold values with asterisks are significantly different from controls ($p \leq 0.050$, Bonferroni's adjusted p value with separate variances comparing treatment means using two-sample t-tests).

	% survival from egg to hatch	sd	% survival from egg to 8 dph	sd	% survival from egg to 15 dph	sd
2010	Athabasca River water under ice (Freshet)					
Lab Water Control	92.9	8.8	90.4	7.7	85.1	9.0
CA1-100%	94.2	3.2	90.8	7.9	86.5	11.8
AR1-100%	91.7	10.0	89.2	8.8	87.9	11.2
AR6-100%	88.3	8.8	84.2	15.0	82.1	19.0
AR15-100%	94.1	5.1	90.0	11.9	88.8	14.2
n=8 ctrl n=4 all trts 30 eggs per beaker						

	% survival from egg to hatch	sd	% survival from egg to 7 dph	sd
2011	Snow on Athabasca River or Steepbank River			
Lab Water Control	86.7	9.4	77.9	11.7
Deionized H2O	**0.0***	0.0	**0.0***	0.0
D.I Amended with Salt	86.7	5.8	74.4	5.1
CA1-100%	86.7	10.0	66.7	12.0
AR1-100%	86.7	0.0	73.3	4.7
AR6-25%	90.0	3.3	**53.3***	8.8
AR6-50%	83.3	0.0	**44.4***	16.4
AR6-100%	87.8	1.9	**30.0***	15.3
ST2-25%	87.8	6.9	42.2	20.1
ST2-100%	77.8	13.5	**11.1***	13.9
AR15-100%	87.8	3.8	71.1	13.5

n=6 ctrl n=3 all trts
30 eggs per beaker

	% survival from egg to hatch	sd	% survival from egg to 8 dph	sd	% survival from egg to 15 dph	sd
2012	Snow on Athabasca River or Steepbank River					
Lab Water Control	92.3	4.7	83.1	5.5	65.9	11.5
Salt Control	93.6	3.5	80.9	7.4	63.5	12.2
AR1-100%	91.6	6.5	77.7	6.3	58.0	9.7
AR1-50%	93.3	3.3	88.9	1.9	85.0	4.6
AR1-25%	95.5	1.9	89.8	3.6	87.8	2.0
AR15-100%	93.4	4.5	81.6	6.1	58.7	13.9
AR15-50%	96.7	5.8	90.0	3.3	84.0	3.1
AR15-25%	96.7	3.3	89.0	2.0	83.1	7.5
AR6-100%	89.9	5.6	**24.8***	9.8	**5.55***	5.32
AR6-50%	92.9	5.8	**48.9***	5.1	**17.7***	12.7
AR6-25%	91.8	3.7	**59.6***	10.6	**31.5***	11.8
AR7-100%	91.5	5.5	**37.6***	10.5	**13.8***	9.8
AR7-50%	94.3	3.1	**67.0***	8.7	**41.1***	9.0
AR7-25%	92.7	3.9	72.9	10.2	53.8	13.7
CA1-100%	96.0	1.7	85.3	4.3	66.8	13.0
CA1-50%	88.9	7.7	85.6	7.7	78.7	19.5
CA1-25%	91.1	3.8	86.7	5.8	79.0	7.5
ST2-100%	85.4	13.4	**8.15***	4.78	**2.22***	1.92
ST2-50%	89.0	7.5	**37.4***	10.6	**17.2***	7.4
ST2-25%	89.6	5.6	**57.4***	8.3	31.7	14.3

average of 3 snows collected per site
n=6 ctrl n=3 all trts
30 eggs per beaker

Site Name and % exposure	% survival from egg to hatch	sd	% survival from egg to 9 dph	sd	% survival from egg to 16 dph	sd
2013	Snow on Athabasca River or Steepbank River					
Lab Water Control	99.4	1.4	93.6	5.2	90.5	6.7
Salt Control	98.9	2.7				
AR6-25%	97.8	1.9	91.1	1.9	91.1	1.9
AR6-50%	98.9	1.9	86.7	6.7	**84.6***	4.0
ST2-25%	98.9	5.1	85.3	10.5	83.6	12.9
ST2-50%	97.8	1.9	81.1	10.7	72.3	13.3
Lab Water Control	98.9	1.7	91.1	5.4	88.0	6.3
AR6-100%	94.4	1.9	61.1	17.1	**53.9***	13.7
ST2-100%	93.3	6.7	36.7	31.8	**29.0***	21.7
Lab Water Control	96.1	5.3	86.0	8.8	83.4	11.7
ST1-100%	93.3	3.3	80.0	6.7	78.2	7.3
ST3-25%	94.4	5.1	92.2	6.9	85.6	5.1
ST3-50%	93.3	0.1	80.8	5.5	73.9	11.2
ST3-100%	97.8	3.8	65.6	13.9	**58.7***	10.2
	Freshet from Steepbank River in April and May					
Lab Water Control	97.8	2.7	91.7	5.9	85.5	5.5
Steep Up Apr-100%	97.8	1.9	92.2	3.8	84.1	6.1
Steep Low Apr-100%	97.8	1.9	85.6	1.9	79.9	6.1
Steep Up May-100%	95.6	1.9	91.1	3.8	87.3	10.5
Steep Low May-100%	96.7	0.0	93.3	3.3	89.2	7.9
n=6 ctrl n=3 all trts 30 eggs per beaker						

Site Name and % exposure	% survival from egg to hatch	sd	% survival from egg to 9 dph	sd	% survival from egg to 16 dph	sd
2014	Snow on Ells River or Steepbank River					
Lab Water Control	95.0	4.5	94.2	4.9	94.2	4.9
Ells Low-100%	96.7	5.8	95.0	8.7	95.0	8.7
Ells Mid-100%	90.0	10.0	88.3	7.6	88.3	7.6
Steep Mid-25%	90.0	5.0	78.3	10.4	78.3	10.4
Steep Mid-50%	90.2	4.6	**50.9***	8.0	**46.9***	1.6
Steep Mid-100%	**58.3***	10.4	**1.67***	2.89	**1.67***	2.89
Steep Low-25%	96.7	5.8	90.0	8.7	90.0	8.7
Steep Low-50%	93.5	2.6	91.8	2.8	91.8	2.8
Steep Low-100%	82.1	10.9	**50.7***	5.2	**47.1***	4.5
	Freshet sampled between ice (btwn=pure snowmelt) or under ice (undr=snowmelt mixed with river water)					
Lab Water Control	96.7	4.1	93.3	4.1	93.3	4.1
Ells Mid btwn-100%	91.7	7.6	91.7	7.6	91.7	7.6
Ells Mid undr-100%	91.7	10.4	86.7	7.6	86.7	7.6
Ells Low btwn-100%	98.4	2.7	95.1	5.0	95.1	5.0
Ells Low undr-100%	98.3	2.9	96.7	2.9	96.7	2.9
Steep Mid btwn-100%	96.7	2.9	91.7	2.9	91.7	2.9
Steep Mid undr-100%	93.3	5.8	93.3	5.8	93.3	5.8
Steep Low btwn-100%	78.3	10.0	70.0	15.0	70.0	15.0
Steep Low undr-100%	88.3	12.0	85.0	10.0	80.0	7.1
n=6 ctrl n=3 all trts 20 eggs per beaker						

exposed to snow from these three near-mining sites (ST2, AR6 and AR7, see red ellipses on Fig. 16, and data in Table 26). Significant reductions in survival were seen in larval fish exposed to snow melt from sites AR6 and ST2 in 2011, 2012, 2013, and 2014. For example, exposure to 100 % melted snow collected from AR6 and ST2 (in 2012) resulted in only 6 % and 2 % larval fish survival until 15 dph (Table 26). Snow collected from AR7 in 2012 also negatively affected larval fish survival (with 14 % of larval fish surviving until 15 dph in the 100 % exposure group). In 2013 and 2014 we increased the sites on the Steepbank River to assess not only ST2, but also Steepbank upper and lower snow (as these sites coordinated with wild fish sampling sites and sediment sampling sites). In 2013, significant negative effects on larval fish survival were seen in snow collected from ST2 and lower sites (with 29 % and 59 % survival until 16 dph for the 100 % exposure group), and in 2014 significant negative effects on survival were seen in larval fish exposed to snow collected from Steepbank mid and lower sites (with 2 % and 47 % survival until 16 dph for the 100 % exposure group) (Table 26).

The embryo-larval fathead minnows were sensitive to the OSRCs in oil sands snow. It was important to expose embryos and larvae for 21 days to the snow samples as often we saw very few effects on fathead minnow egg survival or hatching success. Effects were seen after hatch in larval fathead minnows during the early larval growing period. For example, the results from exposure to 2012 AR6 snow melt at 25 % show normal survival (92 %) from egg to hatch, but just 60 % and 32 % survival from egg to 8 and 15 dph, respectively (Table 26). Similar to larval exposures to oil sands sediments, it appeared that longer exposure times (up to 21 days) were necessary to see the full effects of the snow on larval survival.

Snow collected far from upgraders and far from active mining sites did not affect larval fathead minnow survival (see green ellipse in Fig. 16). These sites (AR1, CA1, 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. 16; Table 26). Exposure to snow collected from sites CA1, AR1, and AR15 in 2011 and 2012 caused no significant changes in larval fathead minnow survival, compared to control fish (Table 26). Similarly, snow collected from the Ells lower and mid sites in 2014 caused no significant effects on larval fathead minnow survival, with 95 % and 88 % survival until 16 dph, respectively, for fish exposed to 100 % snow melt (Table 26).

We emphasize that the data for the effects on survival of fish in the lab from exposure to pure snow collected near mining sites and upgraders should be interpreted with caution. It shows

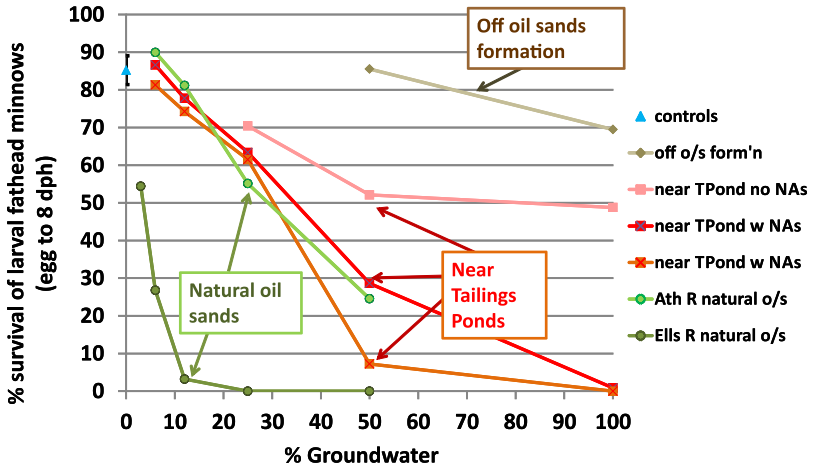

Figure 17. Fathead minnow embryo-larval survival after exposure to groundwater at dilutions of 3 to 100 %). Groundwater was collected near tailings ponds (red arrows AR7, AR10, AR11), and at sites far from tailings ponds (at natural oil sands sites Ells Mid and AR132, green arrows), which allowed assessment of natural groundwater contamination in the region. One groundwater was collected off the oil sands formation (brown arrow, site AR128).

Table 27. Fathead minnow embryo and larval survival in different dilutions (3-100 %) of groundwater collected from the oil sands area in 2012 and 2013. For 2012, data show mean survival (%) and standard deviation (SD) until hatch, and until 8 and 15 days post-hatch (dph). n=3 replicate beakers per exposure concentration, n=6 for control water, 30 eggs per beaker. For 2013, data show mean survival (%) and SD until hatch (5 days exposure). n=3 replicate plates per exposure concentration, n=9 for control water. There were at least 20 eggs per plate for each treatment concentration and control. All solutions were renewed daily. Bold values with asterisks are significantly different from controls (p≤0.050, Bonferroni's adjusted p value with separate variances comparing treatment means using two-sample t-tests).

2012 Groundwaters

Hatch

Site Name and Characteristics		% exposure concentration						
		0	3	6	12	25	50	100
Pooled Controls	surv	93.1						
	sd	5.6						
Expt 1 controls	surv	90.6						
	sd	5.7						
Expt 2 controls	surv	95.6						
	sd	4.6						
AR128 - off oil sands formation	surv						91.1	81.1
	sd						6.9	10.2
AR10 - near Tailings Pond	surv					93.2	84.4	80.2
	sd					3.5	7.7	18.2
AR7 - near Tailings Pond	surv			91.1	95.6	94.4	87.8	66.7
	sd			5.1	5.1	9.6	8.4	31.8
AR11 - near Tailings Pond	surv			88.9	88.8	93.3	90.0	**0.0***
	sd			5.1	3.7	8.8	11.5	0.0
AR132 - Athabasca R. natural oil sands	surv			97.8	91.1	87.8	91.1	
	sd			3.8	5.1	6.9	1.9	
Ells Mid - Ells River natural oil sands	surv		93.3	91.1	87.8	83.3	**6.7***	
	sd		5.8	3.8	6.9	12.0	11.5	

Egg to 8 dph

		% exposure concentration						
		0	3	6	12	25	50	100
Pooled Controls	surv	85.3						
	sd	9.3						
Expt 1 controls	surv	85.0						
	sd	9.4						
Expt 2 controls	surv	85.6						
	sd	10.0						
AR128 - off oil sands formation	surv						85.6	70.0
	sd						8.4	17.6
AR10 - near Tailings Pond	surv					70.7	**52.2***	50.6
	sd					16.8	8.4	24.3
AR7 – near Tailings Pond	surv			86.7	77.8	**63.3***	**27.8***	**1.1***
	sd			6.7	8.4	6.7	16.8	1.9
AR11 - near Tailings Pond	surv			81.1	74.1	61.1	**7.8***	**0.0***
	sd			1.9	5.2	23.6	6.9	0.0
AR132 - Athabasca R. natural oil sands	surv			90.0	81.1	**55.6***	**24.4***	
	sd			8.8	3.8	13.9	16.4	
Ells Mid - Ells River natural oil sands	surv		**54.4***	**26.7***	**3.3***	**0.0***	**0.0***	
	sd		13.9	8.8	3.3	0.0	0.0	

Egg to 15 dph

		% exposure concentration						
		0	3	6	12	25	50	100
Pooled Controls	surv	64.8						
	sd	13.2						
Expt 1 controls	surv	68.7						
	sd	8.0						
Expt 2 controls	surv	60.8						
	sd	16.9						
AR128 - off oil sands formation	surv						72.4	54.7
	sd						12.8	25.5
AR10 - near Tailings Pond	surv					50.6	**40.9***	45.0
	sd					20.0	1.5	27.7
AR7 – near Tailings Pond	surv			70.0	55.6	**41.6***	**22.2***	**0.0***
	sd			11.1	19.7	4.0	15.0	0.0
AR11 - near Tailings Pond	surv			72.4	61.1	44.2	**4.4***	**0.0***
	sd			2.7	9.1	12.4	5.1	0.0
AR132 - Athabasca R. natural oil sands	surv			78.1	75.7	**24.1***	**8.9***	
	sd			10.3	3.6	15.1	6.9	
Ells Mid - Ells River natural oil sands	surv		**26.4***	**14.4***	**0.0***	**0.0***	**0.0***	
	sd		6.3	8.4	0.0	0.0	0.0	

2013 Groundwaters

Hatch

Site Name and Characteristics		% exposure concentration						
		0		6	12	25	50	100
Pooled Controls	surv	96.9						
	sd	4.6						
Expt 1 controls	surv	98.9						
	sd	2.2						
Expt 2 controls	surv	95.0						
	sd	5.6						
AR7 - near Tailings Pond	surv				96.7	85.0	88.3	70.0
	sd				5.8	18.0	7.6	13.2
AR11 - near Tailings Pond	surv				91.7	**76.7***	**76.7***	**70.0***
	sd				10.4	7.6	2.9	8.7
AR132 - Athabasca R. natural oil sands	surv				98.3	98.3	85.0	**8.3***
	sd				2.9	2.9	18.0	7.6
Ells Mid - Ells River natural oil sands	surv			95.0	98.3	91.7	**11.7***	
	sd			5.0	2.9	7.6	5.8	

that the compounds accumulated in some snows over the 4-5 month period are able to affect fish survival. However, it does not indicate environmental relevance of the deposition, as exposure of wild fish will be to the melting snow mixed with large volumes of flowing river waters. This is why we tested the effects of actual freshet water sampled over several years.

Since fish in the wild are not exposed to pure melted snow, it was important to determine if river water collected during freshet could affect fish in the lab. We collected freshet water in 2010, 2013, and 2014. In all cases, river water collected during freshet did not significantly affect fathead minnow embryo-larval survival, even when exposure was to 100 % freshet (see blue ellipse in Fig. 16). For example, in 2010, survival after exposure to 100 % freshet ranged from 82-89 %, in 2013 it ranged from 80-89 %, and in 2014 survival ranged from 70-97 % (Table 26). During freshet, dilution of the snow melt with river water could ameliorate the negative effects of snow on larval fish survival.

Groundwater

Natural groundwater and those close to tailings pond sites contained compounds that affected fish survival in lab exposures. There were differences in potency, but almost all groundwater samples caused some decreased survival in fathead minnow embryos and larvae exposed to 3-100%. Groundwater from natural sites (Ells mid) affected fathead minnow survival at concentrations as low as 3 % groundwater. Groundwater from the two natural sites (Ells mid, and Athabasca River site 132) was more potent than or equally potent to groundwater from sites close to tailings ponds (Fig. 17, Table 27). Exposure until 15 dph to 3 % groundwater from Ells mid or 25 % groundwater from AR132 resulted in only 24-26 % survival. Groundwater from sites close to tailings ponds (AR7 and AR11 sites on the Athabasca River near tailings pond 1) also contained compounds that decreased larval fathead minnow survival at concentrations of 12-25 % groundwater. Exposure until 15 dph to 50 % AR7 or AR11 groundwater resulted in 22 and 4 % survival, respectively (Table 27). Groundwater collected off the oil sands formation (AR128) did not significantly decrease fathead minnow larval survival at concentrations of 100 %. This was the least potent groundwater collected.

To assess the significance of the effects of groundwater on survival of wild fish embryos or larval fish, results should be compared to measurement of groundwater flow and dilution with river waters at these sites. What the groundwater exposures of embryo larval fathead minnow lab results do indicate is that natural groundwater within the oil sands formation and those close to tailings ponds can contain compounds that decrease embryo larval fish survival.

4.4 Summary and Conclusions

Controlled fish exposures showed that exposure to oil sands sediments from two river sites (Steepbank and Ells rivers lower sites) could decrease embryo-larval fish survival. In addition, exposure to snow from sites near mines and stacks decreased larval survival. However, freshet water collected from these same sites did not affect survival of larval fish. This suggests that dilution of contaminants in snow as it melts in spring and mixes with river water has a protective effect. Exposure to snow far from mines and stacks did not affect larval fish survival in the lab.

Exposure to groundwater affected fish survival in the lab. Natural groundwater collected within the deposit was more potent than groundwater collected close to tailings ponds. Low potency groundwater was found at a site outside of the oil sands area. This suggests that in rivers in the oil sands area, groundwater flowing through bitumen-containing substrates can dissolve OSRCs in quantities sufficient to negatively affect fish in lab exposures.

5. Invertebrate In Situ Bioassays Sub-Theme

5.1 Introduction

In situ exposures, in which laboratory cultures or organisms collected from reference sites are caged in the field, are more environmentally realistic than laboratory experiments, are more controlled than invertebrate community analyses, and can provide efficient, pertinent information on biological effects under field conditions (Chappie and Burton Jr. 1997, Burton Jr. et al. 2005). In-situ techniques are also useful as early warning indicators of higher level effects (e.g., population changes), as responses observed in short-term single-species in situ exposures can be reflective of long-term impacts at community and ecosystem levels (Maltby et al. 2002). In situ methods can overcome some of the limitations encountered by traditional assessments in the laboratory and the field (e.g., known exposure dose), and provide unique and complementary data to more standardized research approaches (Burton Jr et al. 2005).

Our JOSM work using caged mussels and *Hyalella* is also unique in the oil sands area. To our knowledge from literature searches, invertebrate caging studies previously had not been attempted in rivers within the oil sands. Mussel caging techniques have been used extensively for the past 25 years for sites receiving municipal effluents, pulp and paper effluents and in harbours with anthropogenic contaminant inputs (Gagné and Blaise 2009, Benedicto et al. 2011, Marigómez et al. 2013). Mussel caging techniques have never been used in assessment of rivers in the oils sands area. In situ studies with *Hyalella* have been used to determine impacts of urban and agricultural runoff (Tucker and Burton Jr. 1999), assess stressor exposure and effects (Burton Jr et al. 2005), measure bioaccumulation of metals in rivers affected by metal mining (Borgmann et al. 2007), and evaluate effects of pesticides (Bartlett et al. 2015). *Hyalella* caging studies had never been employed in the oil sands area.

The in situ caging focused on invertebrate exposures of *Hyalella* and mussels, as these species have previously been shown to respond to many diverse chemical mixtures (PACs, metals, pesticides, pulp mill effluents, municipal wastewater effluents). These species also are useful as they have limited ability to metabolize many chemicals relative to fish (Porte and Albaigés 1994; Livingstone 1998), so their tissues accumulate chemicals from the environment that they are exposed to during the controlled caging. The two selected invertebrate species are also hardy organisms that will survive caging without daily feedings, which is important in remote oil sands river areas. In addition, both test species are native to the Athabasca River region, so the results of these caging studies are environmentally relevant.

The specific invertebrate caging questions include:

- Which oil sands media contribute oil sands related chemicals (OSRCs) to the aquatic environment?
- What are the important aquatic routes of exposure and potential effects in organisms under controlled conditions?

Objectives

Hyalella and mussel in-situ studies were conducted to examine exposure and potential effects in organisms under controlled conditions. We assessed whether there was evidence of impairment in *Hyalella azteca* (a freshwater amphipod crustacean) and *Anodonta grandis simpsoniana* (a freshwater mussel) from environmental exposure to oil sands areas influenced by both natural and anthropogenic sources (i.e., sites near mining activity) compared to environments influenced primarily by natural sources (i.e., sites in the natural deposit but upstream from mining activity, as well as sites outside of the natural deposit such as Long Lake, north of Edmonton). Site selection for *Hyalella* caging in the tributaries was coordinated with those chosen for community assessments of benthic invertebrates and wild fish, as well as water and sediment samples collected for laboratory tests of invertebrates and fish. This design allows better linking of results from in situ bioassays to ecological effects monitored in natural populations of benthos and fish and laboratory observations from invertebrate and fish exposures.

5.2 Methods

Study design

Toxicological testing of invertebrates was conducted in the field using in situ methods, with wild-collected *Hyalella* and mussels caged at several locations in the Athabasca River and its tributaries. *Hyalella* caging sites were associated with wild fish health collection sites, and with sites where sediment was collected for fish toxicological testing. Mussel caging sites were along the Athabasca River (AR1, M2, M3, M5, M6) and Clearwater River (CL1), and on two tributaries (Ells mouth close to M7, and Steepbank mouth close to M3, as well as Steepbank lower, mid, and upper sites). In situ bioassays were conducted in fall (September-October) of 2012, 2013, and 2014.

Hyalella azteca for in situ exposures were collected from a wetland within the Athabasca River watershed but outside the area of oil sands development and activity (56° 30′ 52.3″N, 111° 16′ 03.1″W). Exposure sites along the Athabasca River tributaries were coordinated with wild fish and benthic community studies, and consisted of three sites on the Ells River, three sites on the Firebag River, and four sites on the Steepbank River (Table 28). *Hyalella* were randomly counted into groups of 20, and each group of 20 was then randomly assigned to an exposure cage. Five replicate cages were deployed per site, each cage containing 20 *Hyalella*, one piece of cotton gauze as a substrate, and ground fish food flakes. Cages were removed two weeks after deployment and survival and size of *Hyalella* were assessed. Caged Hyalella tissues were frozen at -80 °C for subsequent analysis of contaminant bioaccumulation. Passive samplers (SPMD, POCIS) were deployed with *Hyalella* cages to measure organic contaminants in the water. Additional details on methods for invertebrate in situ bioassays can be found in Bartlett et al. (2016).

Freshwater mussels were collected upstream of the industrial oil sands extraction area (Clearwater River near the confluence of the Athabasca River) and Long Lake (outside the oil sands area in the Athabasca River basin, approximately 300 km upstream of Fort McMurray; Table 29). Mussels from the Clearwater River were used for caging at all oil sands sites. These were the reference mussels for comparison with all oil sands caged sites. Mussels from Long Lake were used as "cage controls" to determine if the cages impacted the mussels. Mussels were collected by hand and transported back to Fort McMurray for handling and cage preparation. They were kept in aerated water in coolers for no longer than two days before cage immersion.

Exposure sites along the Athabasca River tributaries were coordinated with some of the wild fish and benthic community studies (Fig. 18). The sites were on the Ells River near the confluence of the Athabasca River, sites on the west and east side of the Athabasca River at the oil sands extraction site (Down W, Down E), 1-3 sites on the Steepbank River (for 2013 only), one upstream site near Fort McMurray (Ups) and one site on Long Lake. Mussels between 4 and 8 cm in length were selected (to minimize the weight to length variation) and randomly placed in cages. Cages contained 30-40 mussels; one cage was deployed per site. Cages consisted of a netted cylinder attached to a cement block at each end to ensure cages were secure in sandy environments.

Mussels were removed five weeks after deployment. Survival, growth (wet weight; for 2014 only) and air time survival (days; for 2014 only) of mussels were assessed. Some mussels were frozen on dry ice and sent to the laboratory for biomarker of toxic stress analyses. Grab samples at the beginning and end of mussel cage deployment were collected to measure general water quality and inorganic contaminant analyses in the water and mussels. More details on methods for the mussel in situ bioassays can be found in Gagné et al. (2002). This methodology was based on a standardized ASTM assay methodology developed for mussel caging (Salazar and Salazar 1995).

Statistical approach

Endpoints assessed for the caged *Hyalella* were percent survival and average body size (wet weight/amphipod). Statistical comparisons of these endpoints among sites for each year were done using a one-way analysis of variance (ANOVA) to assess whether there was an overall site effect, followed by Tukey's honestly-significant-difference (HSD) post hoc tests to determine differences between individual sites.

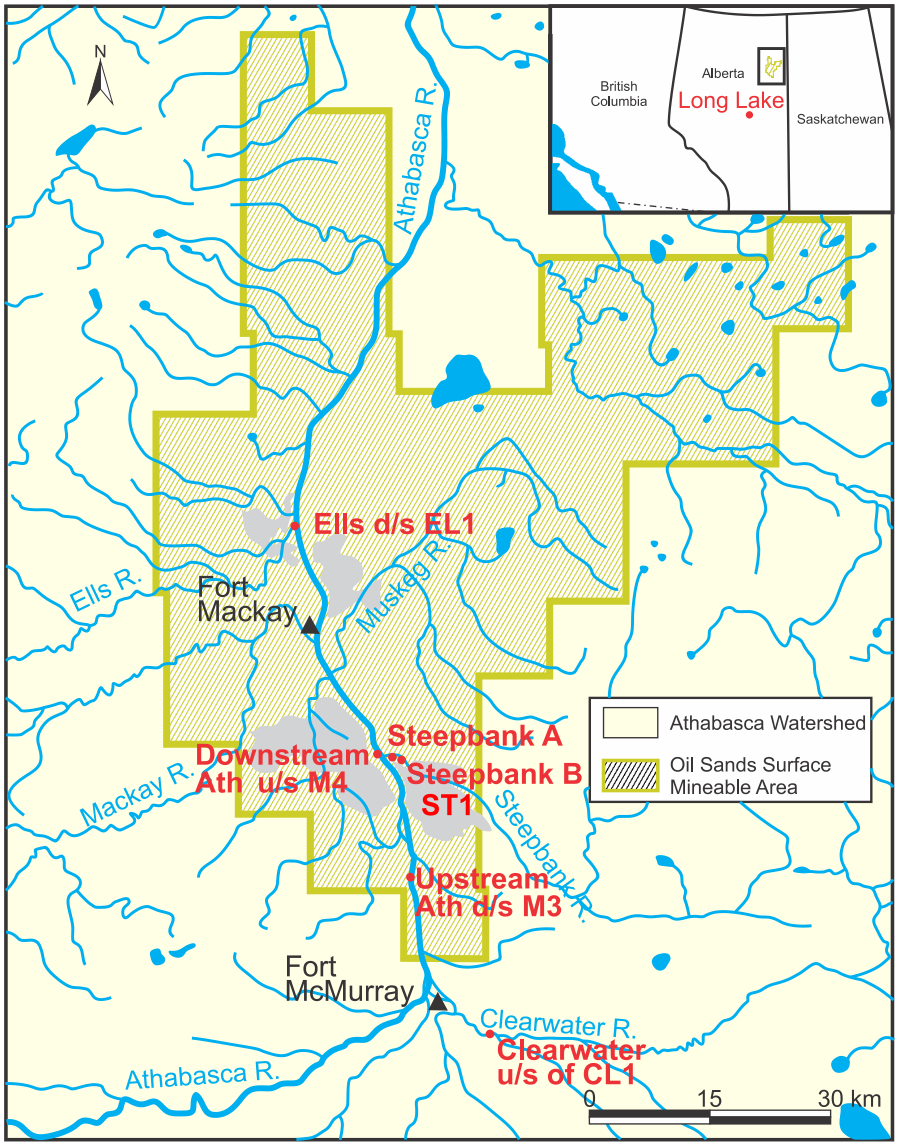

Figure 18. Mussel caging sites in autumn 2014.

Endpoints assessed in the caged mussels were percent survival (all years) and size, mussel weight, shell length, condition factor (mussel weight/shell length), and air survival time (in 2014 only, calculated as the mean time required for mussels to die, as indicated by the shell opening). Statistical comparisons of endpoints in caged mussels between sites for each year were completed using an ANOVA to assess whether there was an overall site effect, followed by least square difference (LSD) post hoc test to determine statistical variations among sites.

5.3 Results and Discussion

No differences in survival or body size of caged *Hyalella* were attributable to natural and/or anthropogenic sources of oil sands chemicals at any of the 10 sites in Athabasca River tributaries. Survival was >90 % for all years at all sites with the exception of the Steepbank middle (85 %) and Firebag lower (67 %) sites in 2012 (Fig. 19). Although differences in survival from 2012 exposures were statistically significant (ANOVA; p=0.036), the lower survival at these two sites was likely due to a high amount of sediment present in the cages at the end of the two-week exposure, which resulted in physical effects on survival (i.e., *Hyalella* were buried in the cages and likely suffocated). Survival of caged *Hyalella* in 2013 and 2014 was not statistically significant between sites (ANOVA; p=0.97 and 0.71, respectively). Average size of amphipods at all sites showed <10 % difference within the same exposure year, and was not statistically significant between sites (ANOVA; p=0.07, 0.12, and 0.56 for the years 2012, 2013, and 2014, respectively) (Fig. 20).

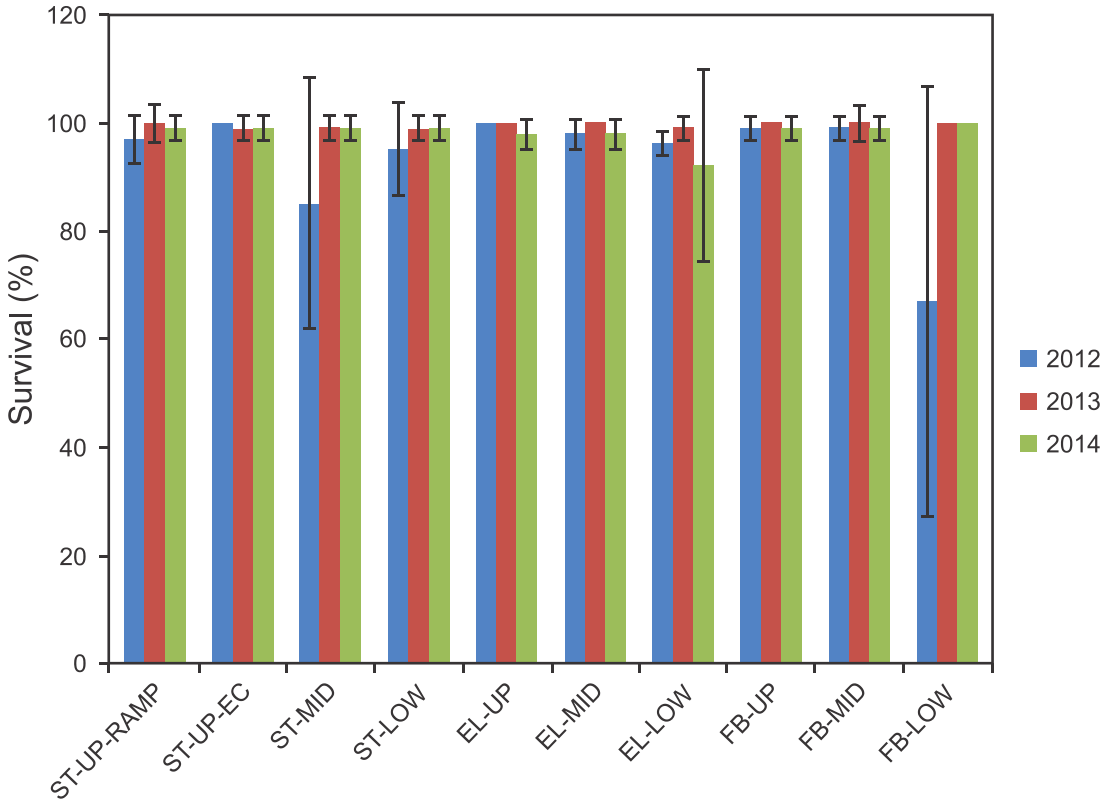

Figure 19. Survival of *Hyalella azteca* exposed in situ in three Athabasca River tributaries for two weeks in September-October of 2012, 2013, and 2014. Caged exposures were conducted at four sites on the Steepbank River (ST), three sites on the Ells River (EL), and three sites on the Firebag River (FB). Each bar represents the mean percent survival (out of 20 amphipods per cage) of five cages per site. Error bars are standard deviations.

Mussels caged at all sites on the Athabasca River in 2012 and 2013 showed no changes in survival during a five-week caging period. Mussels were caged in 2014 for a longer period (six weeks in September-October) and condition factors and stress responses were measured. No changes in mortality were observed in 2014 at any of the sites in the Athabasca and Steepbank rivers. However, there were changes in condition factors and in their ability to cope with air-stress. Mussels caged on the east side of the Athabasca River near the Steepbank River confluence, in the Steepbank River and in the Ells River in 2014 survived significantly shorter times in air (6-10 days) compared to mussels located at the west side of the Athabasca River (at the oil sands development area of Suncor), upstream site (located downstream of Fort McMurray), Clearwater River and a site outside the oil sands development area in the Athabasca River basin (Long Lake near Edmonton) (Fig. 21). Mean air survival time was 14.7 days for sites outside the oil sands development area. Exposure to some sites downstream of oil sands development area resulted in significantly lower survival time of mussels in air. This suggests that these oil sands-exposed mussels are less able to cope with stress. The condition factor (mussel weight/shell length) ratio was also significantly lower at sites downstream of the oil sands development area compared to the Clearwater River sites (upstream sites which are still in the natural oil sands deposits, Fig. 22). Condition factors (measured after the air-survival-time test) were significantly lower in mussels at the east side of the oil sands development area, in the Steepbank and Ells river sites.

5.4 Summary and Conclusions

There were no observed effects in the *Hyalella* in-situ exposures. However, Culp et al. (2018) showed that there were differences in the natural benthic communities between the upper and lower sites of the Steepbank and Ells rivers. From this discrepancy between in situ and natural benthic responses, we surmise that in situ methods with

Table 28. Site names and locations for in situ bioassays with *Hyalella azteca* conducted in three Athabasca River tributaries in 2012, 2013, and 2014. RAMP represents a site used by the Regional Aquatic Monitoring Program and EC represents the Environment and Climate Change Canada upstream site.

Site Name	Latitude (N)	Longitude (W)
Upper Steepbank RAMP	56.821722	110.999278
Upper Steepbank EC	56.863700	111.126230
Middle Steepbank	56.991390	111.332720
Lower Steepbank	57.020880	111.477420
Upper Ells	57.226840	111.970660
Middle Ells	57.242890	111.737350
Lower Ells	57.284890	111.701040
Upper Firebag	57.334750	110.473590
Middle Firebag	57.434340	110.892240
Lower Firebag	57.516790	111.111690

Table 29. Site names and locations for in situ bioassays with mussels conducted in 2014.

Site Name	Latitude (W)	Longitude (N)
Long Lake	54.580373	-113.646063
Clearwater	56.69495	-111.29983
Upstream OS- Athabasca River (AR1)	56.87745	-111.4336
Downstream OS at Suncor	57.02105	-111.49327
Steepbank A	57.0201667	-111.4805667
Steepbank B	57.02087	-111.47933
Ells River	57.30518	-111.67367

caged *Hyalella* were unable to detect changes shown at the benthic community level. If the in situ bioassay is used in the future, we suggest that longer exposures and additional endpoints be used to improve bioassay performance. It is also possible that this species was not sensitive to OSRCs and consideration should be given to caging organisms from the tributaries (e.g., larval mayflies) that may be more effective.

Mussel caging results showed no changes in survival over the three years of study. However, there were decreases in condition factor and increased signs of stress (decreased air survival time) in mussels caged in 2014 at several river sites (Athabasca River east side, Ells River lower site and Steepbank River lower site).

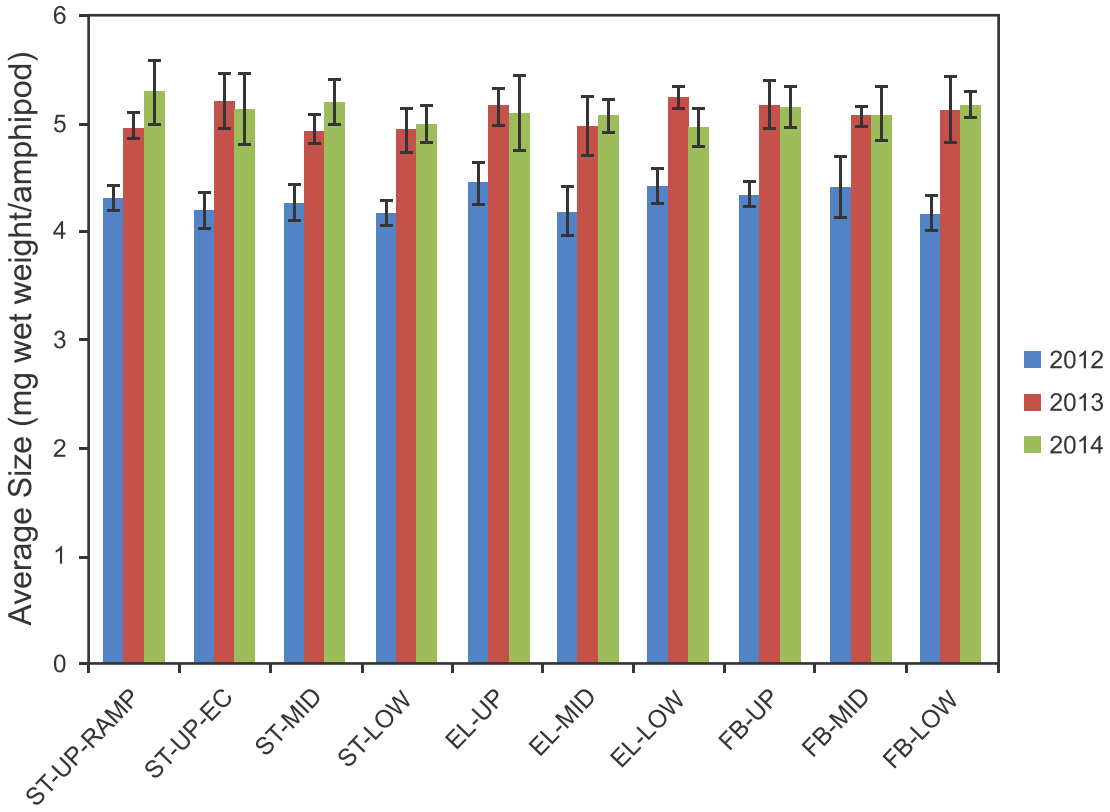

Figure 20. Average body size of *Hyalella azteca* exposed in situ in three Athabasca River tributaries for two weeks in September-October of 2012, 2013, and 2014. Caged exposures were conducted at four sites on the Steepbank River (ST), three sites on the Ells River (EL), and three sites on the Firebag River (FB). Each bar represents the mean amphipod size (total amphipod wet weight per cage/number of surviving amphipods per cage) of five cages per site. Error bars are standard deviations.

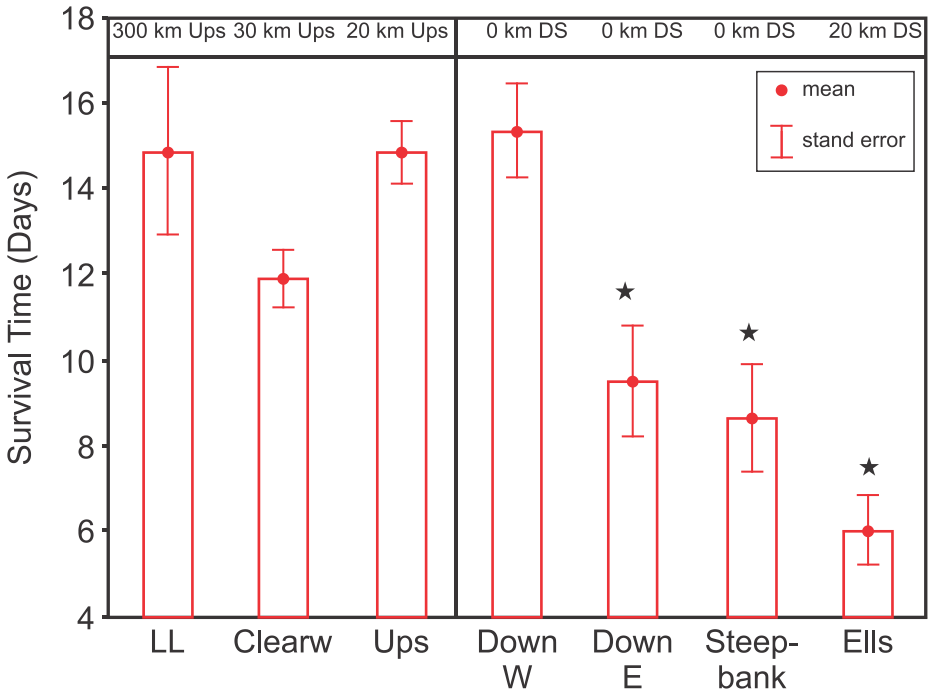

Figure 21. Mussels caged in 2014 for six weeks showed decreased air survival time at sites on the Athabasca River east side (Down E), Steepbank River (Steepbk), and Ells River (Ells). Comparisons were made with air survival time for reference site caged mussels from the Clearwater River (Clearw), and upstream on the Athabasca River (Ups). Mussels caged on the Athabasca River west (Down W) side did not have different air survival times than reference site mussels. Long Lake (LL) mussels were assessed for cage effects only.

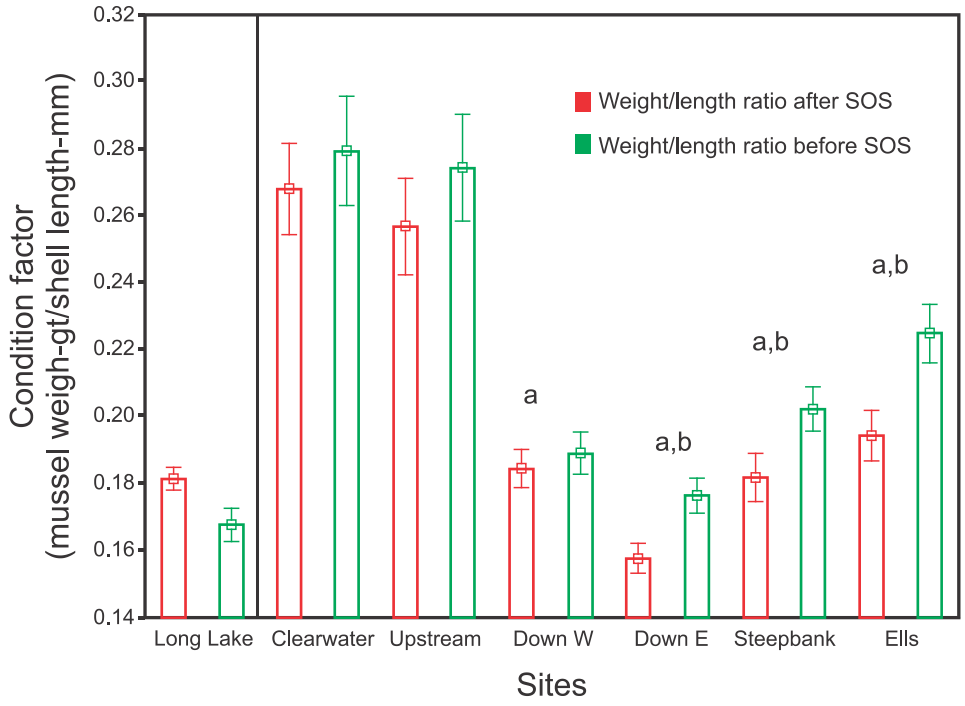

Figure 22. Mussels caged in 2014 for six weeks showed decreased condition factor at sites on the Athabasca River west side (Down W), Athabasca River east side (Down E), Steepbank River (Steepbank), and Ells River (Ells). Comparisons were made with condition factor for reference site caged mussels from the Clearwater River (Clearwater). There was no difference in mussels caged at the site upstream on the Athabasca River (Upstream). The letter 'a' denotes a significant difference from the Clearwater River and 'b' denotes a difference between the before and after SOS (stress on stress) response. Long Lake (LL) mussels were assessed for cage effects only.

6. Assessment and Linkages

The overall strategy of the aquatic biotic response monitoring program in the oil sands was to assess status and trends of ecological effects of physical landscape disturbance and contaminants from oil sands developments on aquatic ecosystem structure and function. Our program used fish health and invertebrate community structure as tools for this assessment and was designed to show whether there are changes in wild fish health and invertebrate communities downstream of industrial development. If effects in wild fish health at certain locations are seen, then several questions were asked including the following:

- Are effects similar in invertebrates?
- Are the response patterns similar?
- Are contaminants in fish and invertebrates demonstrating similar patterns?
- Can these changes be linked to indicators of exposure?
- What is causing the effects in wild fish and invertebrates?

The answers may involve looking at data from several components, including fish health, contaminants, toxicology, invertebrate bioassessments, sediment chemistry, and deposition/pathways.

White sucker are sensitive indicators of fish health in the system as consistent changes in fish health were documented downstream within the oil sands deposit in 2011 and 2012. These differences are indicative of nutrient enrichment as white sucker have increased condition and increased levels of internal fat stores. We confirmed responses in white sucker in the first two years of our studies; however, the third year of white sucker fish health studies indicated changes occurring with fish within the deposit as condition factors were no longer different and improvements in excessive fat deposits in the body cavity were evident. The program has moved from three intensive years of baseline data collection to a three-year, long-term monitoring cycle. We recommend evaluating whether improvements in fish health identified in year three of baseline monitoring are confirmed in the next sampling period in 2016. EROD activity was a good indicator of exposure to PAC-related compounds and indicates some potential increased exposure downstream of development. This was reflected best in PAC levels in white sucker liver tissue in both males and females with increased PACs downstream of development. Levels were higher in male livers than females, a trend also demonstrated in walleye livers. We have begun to evaluate site differences relative to overall upstream reference site variability and have documented change in fish collected in the deposit and downstream of industrial activity that exceed the defined critical effects sizes. These differences, however, were very much improved in 2013. With three years of data at individual sites, we can define normal ranges for sites over the three years of monitoring. To do this, a cumulative mean ±2SD will be calculated and then used to make more meaningful predictions of future observations as more data are added (Arciszewski and Munkittrick 2015). These tools should be used to make predictions of fish health into the future and to identify change both within site and between sites.

Although trout perch are less mobile then white sucker, they appear to be less responsive to the various conditions in the river. For trout perch of both sexes, no consistent effects were demonstrated within a site between years (no confirmation of effect), indicating no deposit or development related alterations in trout perch health during these study years. The data provide a good baseline of trout perch health that can be used to monitor the aquatic environment for change following increased development in the oil sands area. Although this species was less responsive, it should be continued in the long term monitoring program as it can be monitored throughout the watershed where sampling of white sucker is logistically impractical.

Alterations in benthic community structure were also demonstrated between sites sampled within the mainstem LAR. Benthic macroinvertebrate assemblages at upstream reaches (M0 to M2) had more intolerant EPT species and fewer tolerant oligochaete worms compared to middle river reaches (sites M3 to M7C) as reported in Culp et al. (2018). This supports responses demonstrated in white sucker sampled from these same general areas.

6.1 Environmental Health

- What is the current status of fish health in the Lower Athabasca Region?

The LAR mainstem fish health assessments indicate that the middle and lower reaches of the study area exposed to Municipal Sewage Effluents (MSE) from Fort McMurray and oil sands development show warning signals that may be associated with environmental stress. This was evident in white sucker more so than the smaller bodied trout perch. Similar signs of stress on one of the most developed tributaries, the Steepbank River, as slimy sculpin also demonstrated exposure to PAC related compounds.

- Are there existing differences in fish health among sites in the Lower Athabasca Region?

There are differences in fish health among sites in the LAR and its major tributaries studied to date. With this detailed baseline defined, it is recommended that fish health be followed in these areas over time for additional change, as part of the long-term fish monitoring program.

- Are there any trends/changes in fish health relative to historical studies?

Previous fish health related studies on two of the major tributaries identified alterations in fish health within the deposit and downstream of development. Our studies confirm these changes and recommend continued monitoring to ensure fish health is not declining further as part of the long-term monitoring program for fish.

6.2 Human Use

- What are contaminant levels in fish?

We have identified increased levels of PACs in fish collected within the oil sands deposit and downstream of development. Additional samples are being analyzed to further our understanding of differences between upstream reference areas outside of the oil sands deposit and downstream in developed regions. This will provide a solid baseline with which to compare future development in the area.

6.3 Cumulative effects

- Are there any predictive relationships between system drivers (including development stress) and variability within sites in fish responses?

We have begun to develop predictive relationships between fish health endpoints and water temperature during the reproductive growing season. This should reduce variability in our endpoints allowing for clearer evaluation of site differences in fish health.

- Is there evidence of cumulative effects of development on fish in the lower Athabasca Region?

Until predictive relationships are developed and tested, direct evidence of cumulative effects of development on fish is not possible.

7. Theme Assessment

7.1 Wild Fish Health and Toxicology for Key Biota Sub-themes

Impacts of industrial development

Evidence of potential effects related to industrial development was seen in our studies with observation of a decrease in survival of larval fish exposed to snow from sites near oil sands mining activities. However, environmental impact of the melted snow near oil sands sites is minimal as river water collected during freshet did not affect fathead minnow embryo-larval survival. From this repeatable, multi-year snow and freshet data we can say that although snow collected close to mining activities does contain OSRCs that can affect lab fish, the freshet caused no negative effects in larval fish in the lab. Fish collected from the lower site on the Steepbank River consistently demonstrated alterations in fish health relative to sites upstream outside the oil sands deposit similar to results from the laboratory exposures to sediment from this site. White sucker collected on the mainstem Athabasca River also demonstrated alterations in health downstream of development. White sucker collected from sites within the deposit upstream of development often demonstrated responses that were intermediate between upstream reference and lower industrial sites.

Identification of reference condition

The fish health data for the LAR mainstem and many of its tributaries provide a baseline for fish health parameters, such as condition, gonadosomatic index, and liver somatic index. These baseline fish health parameters will be useful for comparing as development proceeds on some rivers (Firebag, Dover, Mackay and Alice). Using upstream, outside of deposit reference locations, we have begun to develop reference conditions, including natural variability in the fish health endpoints. This reference condition mean can be used to evaluate fish health endpoints from sites collected within the oil sands area.

JOSM data also provide baseline data for fish tissue contaminants for the LAR mainstem and tributaries in the oil sands mining region. These will be used in future for retrospective comparisons as development in the area increases, or as release to rivers of treated oil sands process waters (or end-pit-lake waters) is permitted.

We now have baseline data for the LAR for fish health and tissue contaminants. There are differences in fish health among sites in the LAR and major tributaries studied to date. With this detailed baseline defined, it will be possible to follow fish health and tissue contaminants in these areas over time to assess if change is occurring.

Data collected from fish toxicology studies provide a baseline of effects in fish from controlled lab exposures to sediments, snow melt and freshet, and groundwater. This toxicity baseline will be useful for comparison as development proceeds in the future on these currently undeveloped rivers (e.g., on Ells, Firebag, Dover, Mackay and Alice rivers).

The data from mussel caging studies provide baseline information for future comparisons as development increases in the mainstem and on some tributaries. Baseline caging data will also be useful if regulated releases of oil sands process waters are allowed in some areas. Comparisons of future mussel caging findings to current results will allow us to determine if releases have contributed additional OSRCs to river systems sufficient to change mussel health.

7.2 Integration with other Themes

We have linked findings of the four themes in fish health and fish and invertebrate toxicological testing with the findings of other themes, grouped by site.

LAR Mainstem

Fish health assessment in the LAR mainstem showed that white sucker demonstrated consistent changes in fish health downstream within the oil sands deposit in the first two years of study. These differences were indicative of nutrient enrichment, with increased condition, growth and levels of internal fat stores. Often, intermediate responses were demonstrated downstream of the Fort McMurray municipal sewage discharge location, with increased

responses downstream of development. However, the third year of fish health studies indicated changes occurring with fish within the deposit, suggesting recovery in fish health, which should be confirmed in the long-term monitoring taking place in the 2016 sampling year. EROD activity is a good indicator of exposure to PAC-related compounds with induction within the deposit downstream of the sewage discharge, with some potential increased exposure downstream of development. This was reflected best in PAC levels in white sucker liver tissue in both males and females with increased PACs downstream of development. Fish PAC levels were also confirmed with SPMD deployments at similar sites.

LAR mainstem benthic bioassessments also confirmed alterations in the middle river reaches between M3 and M7 with early warning signs of environmental stress. Similarly, there is a need to tease apart the combined effects of nutrient and contaminant stressors present within this river reach.

Analyses of snowpack and lake sediment cores collected from 2011-2014 also demonstrated that deposition of contaminants, including PACs, was most elevated close to major developments. These areas overlapped our fish and benthic study sites demonstrating alterations in health and community endpoints. Further work is required to link these aspects with water quality, groundwater recharge and overall ecosystem function.

Steepbank River lower site

Fish health assessment at the Steepbank River lower site showed slimy sculpin had decreased gonad size, increased liver size, increased MFO enzymes in liver, and increased (alkylated) PACs in tissues. These findings were similar to those of benthic communities from the Steepbank River lower site, which were significantly different from invertebrate communities at other river sites.

These findings in the field were duplicated in situ and in the lab: caged mussels from this site had significantly decreased air survival time and condition factors compared to caged mussels from upstream sites on the Athabasca River. Sediments from the Steepbank River lower site caused decreased survival in embryo-larval fathead minnows in the lab, compared to survival in larval fish exposed to sediment from most other sites.

Exposure to PACs is also documented at this site through results from SPMDs, which showed that there was high exposure to PACs and alkylated PACs at the Steepbank River lower site (Hewitt and Frank unpublished data).

Taken together, results suggest that bituminous sediments at the Steepbank River lower site contribute increased PACs and alkylated PACs to the water column. These compounds induce slimy sculpin MFO enzymes, and may be involved in increased liver size, and decreased investment of energy to gonad size. Compounds in sediments may also affect the benthic community, decreasing abundance and species richness.

The Steepbank River lower site can also be used to link atmospheric deposition theme studies to the fish toxicological exposures to snow. Atmospheric deposition of PACs and alkylated PACs in snow pack is highest at these sites, and accumulated compounds can affect larval fathead minnow in the lab. As the freshet from this same site was non-toxic, fish toxicological studies have shown that environmental impact of deposited contaminants is negligible.

Ells River lower site

Benthic community assessments of the Ells River lower site showed that communities were significantly lower in abundance and diversity compared to other tributary sites. Although we have initiated our collection of baseline data for fish health in the Ells River, additional years of collections are required. Also, exposure to PACs and alkylated PACs was high in the lower Ells River, as shown by concentrations of these compounds in SPMDs (Hewitt and Frank unpublished data).

This was similar to our findings in situ and in the lab: response patterns of caged mussels from the Ells River lower site showed decreased air survival time and condition factors compared to caged mussels from upstream sites on the Athabasca River. Sediments from the Ells River lower site caused decreased survival in embryo-larval fathead minnows in the lab, compared to survival of larval fish exposed to sediment from most other sites.

Results from invertebrate communities (diversity and abundance) and assessment of the toxicity of sediment in the lab, suggest that sediments are the source of the elevated PACs in the water column, and that the sediments from the Ells River lower site contain enough PACs to affect mussels exposed in situ.

7.3 Future Research Needs

Contaminant levels in fish

We have identified increased levels of PACs in fish collected within the oil sands deposit and downstream of development. Additional samples (especially walleye, which is most often consumed by local communities) should be analyzed to further our understanding of the variability of concentrations and differences between upstream reference areas outside of the oil sands deposit and downstream in developed regions.

Linking observed effects to exposures

At the mainstem and tributary sites, we require research linking observed effects to exposures. Future research should be focused on improving our understanding of causal linkages between fish health, benthos, water quality, physical disturbance and other environmental variables.

Cumulative effects

We have begun to develop predictive relationships between fish health endpoints and water temperature during the reproductive growing season. This work should be continued and assessed over several years to see if predictors are robust. We are also using new techniques to predict fish health in future sampling campaigns using our existing baseline data within a site and between reference sites, sites within deposit upstream of development and those downstream of development. These tools allow us to identify change within a site more quickly and to adapt our long-term monitoring program design to address change.

7.4 Monitoring Recommendations

Once sufficient baseline fish health data are obtained (three years of data), fish health monitoring should move to a cyclical long-term monitoring program. For example, three years of fish health data are now available for the mainstem Athabasca and some tributary locations. Monitoring of these rivers could be changed to a three-year cyclical program with additional monitoring undertaken if warranted by using tiers and triggers developed using the baseline data.

Mainstem

White sucker are sensitive indicators of fish health as consistent changes (such as increased condition factor, growth and internal fat stores) were documented downstream within the oil sands deposit in 2011 and 2012. We recommend moving to a three-year, continuing, long-term monitoring cycle to evaluate whether changes identified in year three of our monitoring (normal condition factor) are confirmed in the next sampling period in 2016.

Although trout perch are less mobile than white sucker, they appeared to be less responsive to the various conditions in the river. It is recommended that the trout perch monitoring be moved to a three-year, continuing, long-term monitoring cycle. The data provide a good baseline of trout perch health that can be used to monitor the aquatic environment for change following increased development in the oil sands area. Initial evaluation of the relationship among water temperature and condition, growth, gonadal development and liver size at these sites is in progress. This information should allow better predictions of fish health within and between sites with the potential to reducing measurement variability, thereby improving our understanding of factors controlling fish health endpoints.

Tributaries

Slimy sculpin appear to be sensitive indicators of fish health as consistent changes (decreased gonad size, increased liver size, increased MFO enzymes in liver, and increased (alkylated) PACs in tissues) were documented downstream within the oil sands deposit in 2010 through 2013 in the Steepbank River. There are sufficient data to allow within-site predictions of fish health endpoints and this type of analysis can be used to document change within a site over time and to predict fish health in future monitoring cycles. It

is recommended that studies continue at these sites on a three-year long-term monitoring schedule, documenting reference variability in slimy sculpin health endpoints allowing for additional determination of change due to oil sands development.

For other tributaries to the Athabasca (Ells, Firebag, Dover, MacKay and Alice rivers), it is recommended to collect additional years (three years) of baseline information to allow sufficient power to detect changes due to oil sands development. Initial evaluation of the relationship among water temperature and condition, growth, gonadal development and liver size at these sites is also in progress. This information should allow better predictions of fish health within and between sites with the potential to reduce measurement variability, thereby improving our understanding of factors controlling fish health endpoints.

Fish health in lower Athabasca region (LAR)

There are differences in fish health among sites in the LAR and major tributaries studied to date. With this detailed baseline defined, it is recommended that fish health in these areas be followed over time for additional change.

Fish toxicology and in situ invertebrate exposures

It is recommended to discontinue intensive fish toxicology studies and in situ mussel caging studies. *Hyalella* caging studies are not recommended as these organisms or exposure times were not sensitive to OSRCs. The data from these lab fish exposures and mussel caging studies provide baseline information for future comparisons as development increases on some tributaries. These intensive site-specific toxicology and caging studies may be triggered back in to investigate causative pathways. They could be used in future, for example, if information is needed about a particular site where fish health or benthic community changes are consistently seen.

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