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Plasma proteome profiles of White Sucker (*Catostomus commersonii*) from the Athabasca River within the oil sands deposit☆

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ABSTRACT

There are questions about the potential for oil sands related chemicals to enter the Athabasca River, whether from tailing ponds, atmospheric deposition, precipitation, or transport of mining dust, at concentrations sufficient to negatively impact the health of biota. We applied shotgun proteomics to generate protein profiles of mature male and female White Sucker (*Catostomus commersonii*) that were collected from various sites along the main stem of the Athabasca River in 2011 and 2012. On average, 399 ± 131 (standard deviation) proteins were identified in fish plasma from each location in both years. Ingenuity Pathway Analysis software was used to determine the proteins' core functions and to compare the datasets by location, year, and sex. Principal component analysis (PCA) was used to determine if variation in the number of proteins related to a core function among all male and female individuals from both sampling years was affected by location. The core biological functions of plasma proteins that were common to both sampling years for males and females from each location were also estimated separately (based on Ingenuity's Knowledge Base). PCA revealed site-specific differences in the functional characteristics of the plasma proteome from white sucker sampled from downstream of oil sands extraction facilities compared with fish from upstream. Plasma proteins that were unique to fish downstream of oil sands extraction were related to lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism, endocrine system disorders, skeletal and muscular development and function, neoplasia, carcinomas, and gastrointestinal disease.

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1. Introduction

The Athabasca River runs through the McMurray Geologic Formation (McMF) in Northern Alberta, which contains 97% of Canada's crude oil reserves, and is one of the largest bitumen deposits in the world (CAPP, 2013). Approximately 45% of Athabasca oil sands production involves open pit mining of bitumen (CAPP, 2013). The subsequent extraction and refining of bitumen generate waste in the form of tailings and oil sands process-affected water (OSPW) which is held in tailings ponds. Discharges of OSPW from tailings ponds are not permitted, however there is public concern that OSPW (or its components) may find their way to the Athabasca River accidentally, or via an alternative indirect process such as atmospheric transport. There is also concern that large volume water withdrawals by oil sands extraction and refining facilities (OSERFs) may negatively impact the surrounding environment (Gosselin et al., 2010). Recent assessments of oil sands industry impacts have recommended increased monitoring of water quality and biological impacts (as summarized by Miall (2013b) and Schindler (2013)).

The toxicological and biological effects of OSPW have been assessed by multiple studies in whole fish, fish cell lines, and at the level of gene expression. Observed effects from those studies include endocrine system disruption (He et al., 2012; Kavanagh et al., 2012; Van den Heuvel et al., 2012), oxidative stress (Gagne et al., 2012; Wiseman et al., 2013), and changes to immunological function (McNeill et al., 2012; MacDonald et al., 2013; Wiseman et al., 2013). However, the chemical composition of OSPW is notoriously complex, containing undefined mixtures of napthenic acids, polyaromatic hydrocarbons, and heavy metals (Headley et al., 2013). Additionally, the composition of OSPW will change depending on the source of bitumen and the extraction process. Thus, the development of quantitative chemical analyses to detect OSPW in surface waters for the purposes of water quality monitoring presents significant analytical challenges. Despite this, analytical methods have been developed to identify some industry specific contaminants (Headley et al., 2013), and a recent study has detected the chemical signatures of certain OSPW components in upward flowing groundwater beneath the Athabasca River, suggesting that OSPW may reach the river system via groundwater (Frank et al., 2014). However, at the present time, there is no chemical evidence directly demonstrating that OSPW is present in the Athabasca River (Miall, 2013a).

There may, however, be indirect sources of stress to the Athabasca River watershed due to oil sands activities (Giesy et al., 2010). Increased levels of polycyclic aromatic hydrocarbons (PAHs), metals, and metalloid elements that have been detected in the Athabasca River water

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near to OSERFs have been variably associated with discharges, airborne deposition and snow melt (Kelly et al., 2009, 2010; Hodson, 2013; Kurek et al., 2013; Schindler, 2014). Recent modeling data suggests that airborne PAH emissions in the Athabasca oil sands region may exceed values originally estimated by impact assessments (Parajulee and Wania, 2014). Historical assessments of the water levels and water flow have indicated that there is great year-to-year variability in the water level of the Athabasca River (Monk et al., 2012) and strong evidence that water flow has decreased since oil sands extraction began in the 1970s. Models and fish fence data have demonstrated that water level variations in the Athabasca River may be affecting recruitment of wild fish (Paul, 2013; Schwalb et al., 2015). Thus, high volume water withdrawals from the Athabasca River might also be a potential source of stress to wild fish, although the RSC Oil Sands expert panel report (Gosselin et al., 2010) deemed that the current water management plan eliminated that concern.

Alberta has monitored water quality in the oil sands region since the early 1970s. The surface water quality management framework sets ambient limits and triggers for a suite of indicators, and requires a management response if these triggers or limits are exceeded (Alberta Government, 2016). Independent stressor monitoring studies in wild fish have reported lower mercury concentrations in walleye and lake whitefish sampled from the Athabasca River in 2011 compared to 1984 (Evans and Talbot, 2012). More recently, PAH biotransformation products have been measured in the bile of fish collected from the Athabasca River between summer 2011 and spring 2012, where there were higher concentrations in fish from Fort McMurray and Fort McKay (Ohiozebau et al., 2015). Naphthenic acids (NAs) have not been detected in the tissue of wild fish sampled from the Athabasca River (Young et al., 2011). Relatively few studies have directly assessed the health of wild fish in the Athabasca River within the McMF, likely due to the rather limited available chemical data in fish tissues (as reviewed above). Slimy sculpin (*Cottus cognatus*) and pearl dace (*Semotilus margarita*) sampled from Athabasca River tributaries close to OSERFs had elevated hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity at two sites, one within the bitumen deposit and one adjacent to OSERFs, when compared to EROD activity in fish from a reference site (Tetreault et al., 2003). Despite images in the media of fish from the Athabasca River with visible tumors (CBC, 2014), there are no available tumor incidence data for wild fish populations from the McMF reaches of the Athabasca River available in the peer-reviewed literature. Recently however, Schwalb et al. (2015) reviewed current and 40 years of historical fish health, migration, and population data and found that declines in fish migration and health have occurred in the Athabasca River in the oil sands region, and could be due to oil sands activities but may also reflect changes in hydrology and habitat due to drought-induced climate events, urban development, and landscaping.

The proteome is the entire complement of proteins synthesized by a genome, cell, tissue, or organism at a particular time under a given set of conditions. In shotgun proteomics, complex protein mixtures are digested into peptides which are then sequenced using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) in a bottom-up approach. Protein profiling involves the large-scale identification of the proteome for the purpose of differential comparison. Due to the randomized nature of shotgun proteomics, it is possible to gain understanding of an organism's health in a discovery approach, by comparative protein profiling. One of the benefits of the plasma proteome is that it yields many subset proteomes from multiple organs — more than a traditional biomarker study would be capable of, including signaling and immunological response, which are typically difficult to isolate to a single organ system (Anderson and Anderson, 2002). Plasma also introduces the potential for non-lethal sampling for ecological and effects based monitoring programs. Finally, the discovery aspect of shotgun plasma proteomics can be used to inform effects-based monitoring programs by helping to identify appropriate endpoints to monitor in wild fish, thus enabling cost savings by more focused resource allocation.

We previously demonstrated the utility of unlabeled protein profiling of the White Sucker (*C. commersonii*) plasma proteome as a tool for effects based monitoring by detecting distinct differences in proteome responses related to tumor formation of White Sucker sampled from an exposed, recovering, and a reference site (Simmons et al., 2012). For the present study, we used the same shotgun approach to profile plasma proteins, and then functionally characterized the plasma proteome of White Sucker from three sites within the exposed bitumen area of the McMF: one upstream and two downstream of the OSERFs. Our primary objective was to test whether the plasma proteome of *C. commersonii* from the Athabasca River within the McMF varied among sampling locations. Secondly, if the plasma proteome varied among locations, we wished to identify potential health effects based on the expressed proteome that might be considered as endpoints for incorporation into future monitoring or research programs.

2. Materials and methods

As part of a broader fish health program, sexually mature male and female *C. commersonii* were collected at the same time of day (during daylight) during September 2011 and September 2012 by electrofishing. Fish were sampled from the Athabasca River downstream of Fort McMurray but upstream of oil sands development (US1), a location downstream of the Syncrude/Suncor oil sands development (DS1), and a location downstream of the Syncrude/Suncor oil sands development area and also downstream of the Muskeg river, a major tributary flowing through more recent oil sands developments (Shell Albian Sands/Jackpine Mine & Imperial Oil) (DS2) (Fig 1) (see Supplementary material Table S1 for detailed site coordinates). For the present study, 10 males and 10 females from each site were analyzed for the plasma proteome and associated morphological endpoints: we analyzed males and females separately to assess whether effects were sex-specific.

All fish were anaesthetized with tricaine methanesulfonate (Animal Care Protocol AU1122) in a bath of water taken from each sampling location. Briefly, blood was drawn by caudal puncture using a chilled, heparinized, and sterile 21-gauge needle and syringe, and then ejected after removal of the needle into a cold microcentrifuge tube. Blood was then stored on ice for no longer than 1 h. Plasma was separated from the blood samples by centrifugation (9300 $\times g$, 4 min) at 4 °C, transferred to cryogenic vials, flash frozen in liquid nitrogen, and subsequently stored at −80 °C. Gonads and liver were removed by dissection, fork length (±0.1 cm), body weight (±1.0 g), liver and gonad weights (±0.01 g) were recorded (see Supplemental material Tables S2 and S3 for morphological data).

Stored plasma collected from individual fish were thawed on ice and transferred into a low-retention micro-centrifuge. Plasma samples were reduced and acetylated using tris-2-carboxyethylphosphine hydrochloride solution (TCEP, Sigma-Aldrich, Oakville, ON) and iodoacetamide (IAM, Sigma-Aldrich, Oakville, ON), and then digested in 10% v/v formic acid (Sigma-Aldrich, Oakville, ON) at 120 °C for 30 min (an 8 hour shorter period than is typically used for enzymatic digestion). The resulting peptide mixtures in each tube were then evaporated to near dryness using centrifugal evaporation (Savant Instruments, Inc., model AES1010-120) and then re-suspended by vortex in HPLC solvent. Finally, the tubes were centrifuged for 10 min at 15,000 $\times g$ to remove debris, and the supernatant was transferred from each tube into chromatography vials for subsequent analysis by HPLC-MS/MS.

Peptides were separated by Agilent 1260 Infinity Binary LC on a Zorbax, 300SB-C18, 1.0 × 50 mm 3.5 μm column with a 50 min gradient with 5% acetonitrile 0.1% formic acid, and 95% acetonitrile 0.1% formic acid (Simmons et al., 2012). The Agilent 6520 Accurate-Mass Quadrupole Time of Flight (Q-TOF) was used as the detector in tandem to the Agilent 1260 system in Auto MS/MS (scan) mode with 10 precursors per cycle and active exclusion. Instrumental settings are listed in Supplementary material Table S4. Plasma vitellogenin (Vtg) was quantified

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