



# Spatial assessment of major and trace element concentrations from Lower Athabasca Region Trout-perch (*Percopsis omiscomaycus*) otoliths

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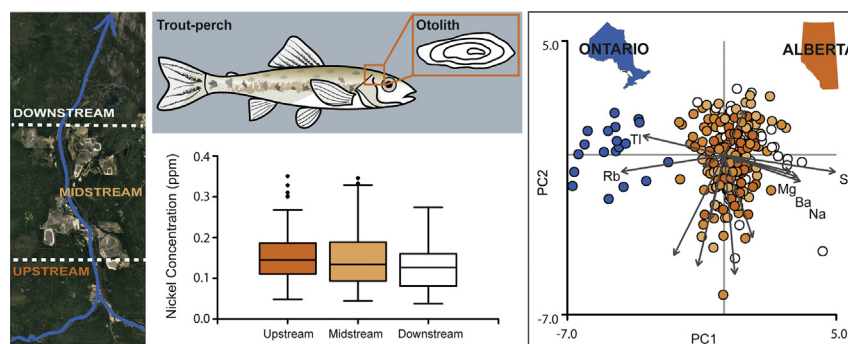
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## HIGHLIGHTS

- Approximately 1/3 of elements analysed in otolith were below limits of detection.
- Cu, Li and Pb differed among Lower Athabasca Region locations.
- Patterns of variation did not support impacts of industrial footprint.
- Differences between Alberta and Ontario otoliths likely reflect geology.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The Lower Athabasca Region (LAR) is home to the largest bitumen deposit in Alberta, and has seen industrial development related to the extraction and processing of bituminous sands since the late 1960s. Along with industrial and economic growth related to oil sands development, environmental concerns have increased in recent decades, including those about potential effects on fish. We measured major and trace element concentrations in Trout-perch otoliths from the Athabasca and Clearwater Rivers in the LAR, to illustrate spatial variations and identify possible industrial impacts. Both laser ablation ICP-MS and solution-based ICP-MS methods were employed. Of the trace elements enriched in bitumen (V, Ni, Mo and Re), only Ni and Re were above the limits of detection using at least one of the methods. The only significant differences in element concentrations between upstream and downstream locations were found for Li, Cu, and Pb which were more abundant upstream of industry. For comparison and additional perspective, otoliths from the same fish species, but taken from the Batchawana River in northern Ontario, were also examined. The fish from Alberta yielded greater concentrations of Ba, Bi, Li, Mg, Na, Re, Sc, Th and Y, but the Ontario fish had more Cr, Rb and Tl, likely because of differences in geology.

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## 1. Introduction

The Lower Athabasca Region (LAR) in northeastern Alberta is home to one of the largest bitumen reserves in the world. This region has seen

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industrial development related to the extraction and processing of bituminous sands since the late 1960s, with development increasing in recent decades (reviewed in Schindler, 2013). Along with this industrial and economic growth, recent decades have seen increases in environmental concerns surrounding regional water quality (e.g. Schindler, 2013), with potential impacts on aquatic biota (e.g. Schwalb et al., 2015) and consequences for human health (e.g. Ohiozebau et al., 2017).

The Athabasca River, its tributaries, and Lake Athabasca support a rich fish community, including many large-bodied migratory species, some of which are important for both subsistence and recreation in the region. Such large-bodied migratory fish species (e.g. walleye, *Sander vitreus*, northern pike, *Esox lucius*, Arctic grayling, *Thallymus arcticus*, lake whitefish, *Coregonus clupeaformis*, suckers, *Catostomus* spp.) overwinter in Lake Athabasca and migrate up the Athabasca River during the open water season, to occupy it or its tributaries and fulfil a number of life-history requirements such as spawning and rearing (Schwalb et al., 2015).

The large and growing body of literature on water quality in the Athabasca oil sands region has presented differing views on the amounts, distribution and source of various elements including potentially toxic metals found in the Athabasca River and its watershed (Donner et al., 2017; Javed et al., 2017; Kelly et al., 2010; Shotyk et al., 2017). Potential sources of various pollutants, including, but not limited to metals, have been suggested to include oils sands activities (i.e. bitumen upgrading (Kelly et al., 2009; Kurek et al., 2013; Timoney and Lee, 2011), seepage from tailings ponds (Golder Associates, 2009; Sun et al., 2017)) and naturally occurring inputs (Miall, 2013; Shotyk et al., 2017; Sun et al., 2017). Of the large suite of metals and other elements analysed along the Athabasca River by Shotyk et al. (2017), the authors found that concentrations of Ag, Cd, Pb, Sb and Tl were low and not significantly different downstream of industrial activity compared to upstream. Conversely, the authors found that V, Ni, Mo, and Re, which are known to be enriched in bitumen (Hitchon and Filby, 1983; Selby and Creaser, 2005), were elevated downstream of industrial activity. The authors noted, however, that since natural saline inputs also increased downstream, it was unclear whether the elevated bitumen-associated elements were the result of anthropogenic or natural inputs.

Regardless of the source, the potential for elevated concentrations of some potentially toxic elements to have detrimental impacts on fish in the LAR remains a concern. While data on various aspects of fish populations and individual fish health in the LAR have been collected annually or semi-annually since 1997 (Hatfield Consultants Partnership, 2009), few published studies have assessed spatial or temporal variation in fish characteristics with respect to industrial activities and/or natural saline inputs. An analysis assessing historical changes in hydrology, water chemistry and migratory fish community composition and health was conducted using existing data on the LAR (Schwalb et al., 2015). The authors concluded that the data reflected significant ecological degradation since the 1970s; however, they also noted that determining the causal relationships requires further study. In a study comparing metal concentrations in the liver of white suckers captured from the Muskeg River downstream of Athabasca oil sands development with that of white suckers captured at an upstream reference lake site, Arens et al. (2017) found elevated levels of Cd, Cu, Ni and Se in the liver tissues of fish collected downstream. Unfortunately, the comparison of fish samples from river and lake systems characterized by different biogeochemical processes limits the inferences that can be made on these findings.

Small bodied fish have limited home ranges and consequently are known to reflect site-specific conditions (Spafford, 1999). As such, the study of small bodied fish collected from discrete sites along a river system provides the opportunity to assess spatial variation in metal exposure relative to an industrial footprint. Trout-perch are a small bodied fish whose utility as a sentinel species has been demonstrated in the LAR (Spafford, 1999).

Otoliths are small, primarily calcium carbonate structures that form extracellularly within the inner ear of the fish (Carlström, 1963; Degens et al., 1969). Otoliths are metabolically inert so that otolith material is not reabsorbed after deposition, and formation occurs continually in concentric layers of proteins and calcium carbonate about the nucleus (reviewed in Campana, 1999; Campana and Neilson, 1985). Because of these characteristics, some elements, primarily those that are non-essential to fish (e.g. Sr, Ba, Pb), have been used to infer environmental concentrations. Non-essential elements such as Ba and Sr directly replace Ca during uptake, although their success at uptake for incorporation into calcium based structures is dependent upon environmental calcium concentrations (Loewen et al., 2016). As such, otolith microchemistry has been used widely in stock discrimination, identification of nursery habitat, determination of migration history, and temporal reconstruction of environmental condition and thermal history (Campana, 1999). Otolith microchemistry has also been used to assess the exposure of fish to anthropogenic activities, such as mine tailings (Arnold et al., 2015; Friedrich and Halden, 2008, 2010; Palace et al., 2007), urban contamination (Andronis et al., 2017), and oil and gas activities and spills (Granneman et al., 2017; Lopez-Duarte et al., 2016; Nelson et al., 2015; Nowling et al., 2011).

We measured element concentrations in Trout-perch otoliths from the main stem of the Athabasca and Clearwater Rivers in the LAR, including sites upstream and downstream of industrial development. To help view the measured concentrations in perspective and to provide additional context, otoliths from the same fish species were obtained for study from a river in northern Ontario located in a different geological setting. The primary objectives of this study were to (1) determine which elements were quantifiable in the otoliths using state-of-the-art analytical technologies, (2) assess whether element concentrations varied among locations along LAR rivers with varying proximity to industrial development, and (3) determine whether element concentrations in otoliths from fish varied between different geological settings (the LAR versus northern Ontario). Emphasis was placed on the elements known to be enriched in bitumen (i.e. Ni, Mo, Re, V) as well as the potentially toxic trace elements such as Cd and Pb which have generated so much concern (Kelly et al., 2010).

## 2. Methods

### 2.1. Study area

Trout-perch were captured from 19 sites in the Lower Athabasca Region, Alberta: 15 sites on the Athabasca River between Fort McMurray and the Firebag River, and 4 sites along the Clearwater River between the confluences with the Christina and Athabasca Rivers (Table 1, Fig. 1). Fish were primarily sampled during October 2014, however an additional sampling event occurred in August 2015 to supplement sample numbers. Site selection was based on terrain conductivity presented by Gibson et al. (2013), and an additional terrain conductivity survey conducted in 2014 by WorleyParsons along the Clearwater River and the Athabasca River upstream of Fort McMurray. Higher terrain conductivity is thought to reflect potential high salinity inputs from natural and/or industrial sources (Shotyk et al., 2017). Sites were selected to represent high conductivity sites upstream, downstream and proximal (hereafter called midstream) to industrial activity on the Athabasca and Clearwater Rivers, and also reference sites with low conductivity. Trout-perch were also obtained from the Batchawana River in northern Ontario in 2011 and 2012.

The geology along the Athabasca River in the LAR from approximately Fort McMurray to the Firebag River is characterized by the Devonian Waterways formation comprising shales and limestones. Overlaying this is the Cretaceous McMurray Formation which holds the bulk of the bituminous sands in the region and is made up of shale and interbedded shale and sandstone (Conly et al., 2002). The Clearwater Formation overlies the bituminous-rich McMurray formation and

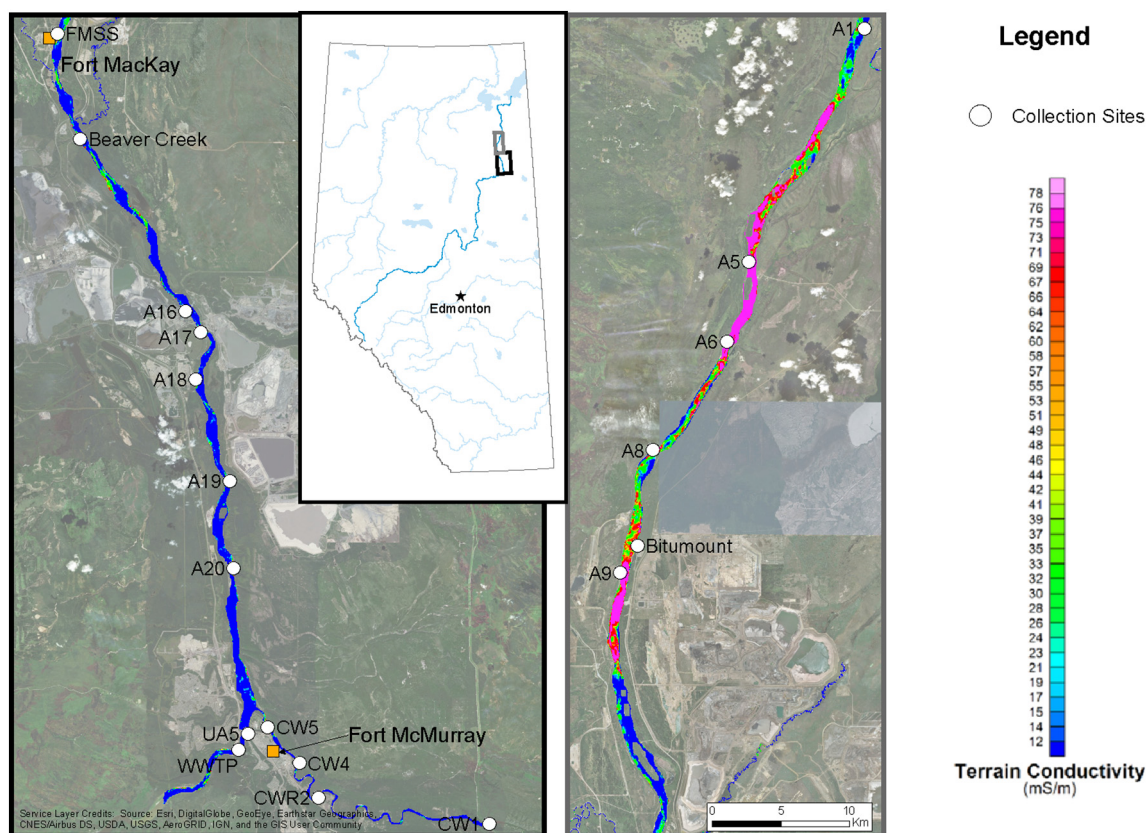
**Table 1**

Site name, latitude and longitude, river name, and sampling date. The number of otoliths analysed from each site (n, including outliers) and the mean fork length (FL, mm) and standard deviation are presented separately for laser ablation inductively coupled mass spectrometry (LA-ICP-MS) and solution based inductively coupled mass spectrometry (SO-ICP-MS) methods.

Site name	Latitude (°N)	Longitude (°W)	River name	Date sampled	LA-ICP-MS		SO-ICP-MS	
					n	Mean FL (mm) ± SD	n	Mean FL (mm) ± SD
A1	57.725220	111.374860	Athabasca	Oct 2014	12	63.02 ± 10.66	6	64.33 ± 10.03
A5	57.572170	111.513720	Athabasca	Aug 2015	19	45.47 ± 12.56	3	50.33 ± 3.21
A6	57.519500	111.539944	Athabasca	Oct 2014	8	58.50 ± 11.80	–	–
A8	57.448000	111.628220	Athabasca	Oct 2014	11	64.20 ± 11.00	3	57.00 ± 3.61
Bitumount	57.385270	111.645990	Athabasca	Oct 2014	9	69.22 ± 9.01	3	66.00 ± 3.46
A9	57.367360	111.666690	Athabasca	Oct 2014	12	68.46 ± 6.35	3	68.00 ± 4.58
FMSS	57.189640	111.627440	Athabasca	Oct 2014	11	70.00 ± 13.52	3	61.67 ± 2.08
Beaver Creek	57.120782	111.598790	Athabasca	Oct 2015	12	49.83 ± 7.12	–	–
A16	57.008210	111.470640	Athabasca	Oct 2014	7	62.38 ± 14.17	3	61.67 ± 4.51
				Aug 2015	10	61.60 ± 14.29	3	53.33 ± 3.06
A17	56.994920	111.452370	Athabasca	Oct 2014	12	67.58 ± 11.42	3	58.00 ± 2.65
A18	56.963800	111.457750	Athabasca	Oct 2014	12	68.83 ± 14.17	3	62.33 ± 3.21
A19	56.897480	111.416360	Athabasca	Oct 2014	11	75.00 ± 8.60	5	77.00 ± 6.40
A20	56.840250	111.411460	Athabasca	Oct 2014	6	69.77 ± 10.80	6	69.83 ± 10.72
				Aug 2015	10	63.70 ± 14.18	3	50.33 ± 0.58
UA5	56.731721	111.393190	Athabasca	Oct 2014	10	80.30 ± 9.05	3	81.00 ± 3.61
WWTP	56.721219	111.404257	Athabasca	Oct 2014	10	81.05 ± 5.32	–	–
CW5	56.736061	111.369354	Clearwater	Oct 2014	12	59.67 ± 7.66	–	–
CW4	56.712800	111.331765	Clearwater	Oct 2014	12	76.92 ± 8.09	–	–
CWR2	56.690169	111.308400	Clearwater	Oct 2014	13	73.44 ± 11.39	4	70.75 ± 14.41
CW1	56.673330	111.105840	Clearwater	Oct 2014	12	71.00 ± 14.90	3	58.33 ± 5.03
ON	47.03391	84.47960	Batchawana	Jul 2011	7	58.00 ± 4.85	2	57.00 ± 1.41
				Jul 2012	13	67.00 ± 7.26	4	55.00 ± 2.45

comprises primarily marine shale, and a small amount of bituminous sands (Conly et al., 2002). This formation is overlain by the Grand Rapids Formation. In addition to the exposed McMurray Formation that appears sporadically along the Athabasca and Clearwater Rivers in the

LAR (Conly et al., 2002), inputs of saline water have also been identified (Gibson et al., 2013). Calcium concentrations in waters from the main stem of the Athabasca River within the study reach vary seasonally from approximately 25 to 45 mg/L (Gue et al., 2018; Shoty et al.,



**Fig. 1.** Map of Lower Athabasca Region indicating fish collection sites in 2014 and 2015 (Table 1). Terrain conductivity depicted below the confluence between the Athabasca and Clearwater Rivers were provided by Gibson et al. (2013). Terrain conductivity for the Clearwater and Athabasca Rivers upstream of their confluence were obtained for the present study by WorleyParsons in 2014.



2017; Tondy, 2017). Although the Clearwater River is found in a similar geological setting, it has a clearly different chemical composition relative to the Athabasca River, with greater concentrations of Na, Fe, and Mn, and lower concentrations of Ca, Mg, Ba, Cd, Cu and Sr (Tondy, 2017). In the Clearwater River, Ca concentrations range between 18 and 50 mg/L (Gue et al., 2018).

The Batchawana River, Ontario, is 95 km long and drains an area of approximately 1233 km<sup>2</sup> with a natural flow regime. The river sits on the Canadian Shield and flows through the Batchawana greenstone belt, flowing over sedimentary bedrock in the north-eastern section, then volcanic bedrock in the central section of the river, and finally approaches glaciolacustrine deposits near the mouth where it drains into Lake Superior (Ontario Parks, 2006). Calcium concentrations ranged between approximately 5 and 11 mg/L (DFO, unpublished data).

## 2.2. Field collection

Fish were captured using a 20 ft Smith-Root electrofishing boat (Model SR-20H) equipped with a 7.5 GPP electrofishing system, by conducting multiple transects of approximately 1 km in length starting from the site locations (Fig. 1, Table 1). A subset of up to 20 Trout-perch were sacrificed, frozen, and shipped back to the University of Alberta for processing. Otoliths were also obtained from Trout-perch sampled from the Batchawana River near Sault Ste. Marie, Ontario in 2011 and 2012, for comparison (Kelly et al., 2017). These fish were captured using a backpack electrofisher. Otoliths from the Batchawana River Trout-perch were obtained opportunistically from an unrelated study where archived otolith samples were retained (Kelly et al., 2017).

## 2.3. Otolith microchemistry

In the lab, fish were defrosted, measured for fork length and weight, and sagittal otoliths were extracted, cleaned with deionized water, and allowed to air dry overnight. One otolith from a subset of Trout-perch from each site was embedded in Buehler Epothin™ 2 epoxy resin. A Nikon dissection microscope and an ultrafine permanent marker were used to mark the nucleus of the otolith, and the intended sectioning line. Each embedded otolith was sectioned using a Buhler Isomet™ slow-speed saw. The sections were embedded with epoxy into acrylic tube rings. The face of the ring with exposed otolith sections was polished by hand using 3M™ aluminum oxide lapping film with a series of grits (30 µm, 9 µm 0.5 µm), and a final Masterprep™ polishing solution, applied to a Metaserv™ 250 Grinder-polisher (polishing wheel). Polished rings were cleaned ultrasonically in deionized water.

## 2.4. Electron microprobe

As an internal standard for laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) data processing, accurate Ca concentrations were obtained for a subset of otoliths using a JEOL JXA-8900R electron microprobe. In addition to back-scattered-electron images and secondary-electron-images, quantitative compositional data were acquired using wavelength-dispersive spectrometry and Probe for EPMA software (Donovan et al., 2015). Four elements (Na, Sr, Ca, and Mn) were measured using the following conditions: 15 kV accelerating voltage, 20 nA beam current, and 5 µm beam diameter. Total count times of 30 s were used for both peaks and backgrounds. The X-ray lines, analyzing crystals, and standards were: Na Kα, TAP, rhyolite USNM 72854; Sr Lα, TAP, strontianite (intensity data aggregated from two spectrometers, Donovan et al., 2011); Ca Kα, PET, calcite; Mn Kα, LiF, Mn-Cu alloy (Jarosewich et al., 1980; Jarosewich and MacIntyre, 1983; Jarosewich and White, 1987). Time-dependent intensity corrections for Na and Ca were carried out (peak count times divided into six intervals) with Probe for EPMA software following Nielsen and Sigurdsson (1981). X-ray intensity data were reduced following Armstrong (1995). Carbon dioxide concentrations calculated using

stoichiometry were included in the data reduction, and recalculated following the convergence of the matrix corrections. The calculated average limits of detection (LOD) at the 99% confidence level were: Na 350, Sr 160, Ca 90, and Mn 190 ppm. The calcite standard was analysed periodically as a check on the precision of the quantitative analysis routine (Jarosewich and MacIntyre, 1983).

## 2.5. Trace element analysis by in situ LA-ICP-MS

Otolith trace element concentrations were obtained from a total of 251 polished sections (see Table 1 for sample sizes by site) using in situ laser ablation (RESOLUTION M-50 193 nm excimer laser system) coupled with sector field inductively coupled plasma-mass spectrometry (Thermo Element XR) (LA-SF-ICP-MS) at the Arctic Resources Laser Ablation ICP-MS facility at the University of Alberta. The mass spectrometer was operated in low mass resolution mode ( $M/\Delta M = \text{ca. } 300$ ). The otoliths were ablated using 75 µm craters, a 20 Hz repetition and the laser energy at the target (fluence), regulated at 3–4 J/cm<sup>2</sup> at a discrete spot on the ventral edge of the section. An analysis comprised 30 s of background gas collection followed by 40 s of ablation. Ablated aerosols were entrained in a He cell gas flow (1000 mL/min) and subsequently mixed with Ar (0.8 mL/min) prior to entering the ICP-MS torch. The ICP-MS was operated at 1300 W with a torch depth of 3.5 mm. Argon and He gas flow, torch position and focusing potentials were optimized in order to achieve optimal signals on Co, La and Th with low oxide production rates ( $\text{ThO}/\text{Th} < 0.3\%$ ). Calibration was performed using NIST SRM 612 in conjunction with internal standardization using <sup>43</sup>Ca. All data were processed offline using Iolite v3 (Paton et al., 2010, <http://www.iolite-software.com>). Analyses of the secondary standards (e.g., MACS-3) were typically within 5–10% of reference values at the 95% confidence level. LODs, at the 3-sigma level, were calculated using the Longerich method (Longerich et al., 1997) with the exception of some samples with very low counts per second background, where the Pettke method (Pettke et al., 2012) was used.

## 2.6. Trace element analysis by SO-ICP-MS

Solution based inductively coupled plasma mass spectrometry (SO-ICP-MS) was used on a subsample of whole otoliths (63 otoliths from 15 sites, Table 1) primarily as a quality assurance step for the LA-ICP-MS data, since SO-ICP-MS has superior capability for measuring low limits of detection (Ludsin et al., 2006). SO-ICP-MS methods were conducted at the Soil, Water, Air, Manure and Plants Lab (SWAMP Lab) at the University of Alberta. The performance of the analytical method was monitored using carbonate reference materials (IGGE/GSR-6 Limestone, ST SEV 5365–85 Aragonite). Each otolith was dissolved with 1 mL ultrapure HNO<sub>3</sub>, kept at room temperature for 30 min, diluted with ultrapure Milli-Q water (MilliporeSigma, 18 MΩ·cm at 25 °C) to a concentration of 1 mg/mL, and refrigerated until analysis. The concentrations of trace metals in the acid-digested otolith samples and reference materials were determined using ICP-MS (iCAP-Q, Thermo-Finnigan, Bremen, Germany). The instrument was equipped with nickel cones (sample and skimmer) and a quartz cyclonic spray chamber coupled to a Teflon (PFA) microflow nebulizer. Sample introduction was performed in self-aspirating mode at the rate of 400 µL/min (1 L/min Ar) using an autosampler (SC-2 DX, Elemental Scientific, CO) housed in a class 100 all-polypropylene laminar flow clean air cabinet. Daily tuning of the instrument was performed using a 2% HNO<sub>3</sub> multi-element solution containing 1 µg/L Indium (In) to keep the instrument at an optimal compromise between <sup>115</sup>In intensity and the relative abundance of oxides (<sup>140</sup>Ce<sup>16</sup>O/<sup>140</sup>Ce < 0.5%, RSD < 2%). Multi-element calibration curves in 2% HNO<sub>3</sub> ranged from 1 to 10,000 pg/mL (range varied per element), with In (500 pg/mL) as an internal standard (IS). Digestion blanks, consisting only of the digestion acid medium (HNO<sub>3</sub>), were included in every batch and analysed alongside the samples. Carbonate reference materials for trace elements (IGGE/GSR-6 Limestone, ST SEV 5365–85 Aragonite) were included in every digestion batch and analysed to monitor

analytical performance. The ICP-MS acquisitions started with five replicates of method blanks (2% HNO<sub>3</sub> + IS) and a seven-point calibration curve, followed by the study samples, punctuated with a set of five primary standard quality control samples (QCs) at the following concentrations: 10, 50, 100, 500 and 1000 pg/mL. Recoveries of reference samples and QC samples were considered acceptable when concentrations were within  $\pm 20\%$  of the certified value.

## 2.7. Data quality control

### 2.7.1. LA-ICP-MS

Elements were excluded from statistical analyses when the measured concentrations of >50% of samples were below the LOD. For the remaining elements, individual samples that were <LOD were replaced with the measured LOD value estimated for each sample during analysis. Owing to a bias observed in the estimated LOD resulting from instrument drift, the LODs were replaced with imputed values randomly drawn from the respective distribution of measured LODs for each element. The distribution was generated using the Epanechnikov kernel function (Liebenberg, 2014). Outliers were identified separately for each element using median absolute deviation methods (Leys et al., 2013).

### 2.7.2. SO-ICP-MS

Similarly to LA-ICP-MS data quality control, elements where >50% of measured values were < LOD were discarded from further consideration.

In the remaining data, samples with <LOD values were replaced with the measured LOD. The SO-ICP-MS results were primarily used to independently assess the quality of the LA-ICP-MS data. However, the SO-ICP-MS data were included in statistical analysis for some elements that were not quantifiable using LA-ICP-MS. These elements included Al, Bi, Li, Re, Sc, Th, U, and Y. Outliers were identified in the multi-element dataset using Mahalanobis distance, and samples with a value >6 were removed (Mahalanobis, 1936). However, additional outliers were removed on a per element basis if observed in the residual plot assessment for the linear mixed-effects models (see below).

## 2.8. Statistical analyses

Linear mixed-effects models (LMM) were used to assess whether element concentration differed significantly among four locations in the LAR: 1. upstream-Clearwater River, 2. upstream-Athabasca River, 3. midstream and 4. downstream (Table 2, Pinheiro and Bates, 2000). Sites between A18 and Bitumount, inclusive, were categorized as midstream based on their proximity to the majority of industrial activity in the LAR (Fig. 1). Year was included as a fixed effect to account for variations between 2014 and 2015 samples and site and fork length were included as random effects. A second set of LMMs were used to compare element concentrations between LAR samples and Batchawana River (Ontario) samples. Site and fork length were included as a random effects. Year was not included in this analysis because we did not expect differences between

**Table 2**

Mean (ppm), standard deviation (SD), percent of samples below limit of detection (LOD), and LOD (ppm) measured for otoliths analysed using LA-ICP-MS (left) and SO-ICP-MS (right) methods. For SO-ICP-MS data ppm is equivalent to  $\mu\text{g/g}$  dry weight. Only elements with <50% of samples below LOD were used in statistical analyses, and presented in black. n for LA-ICP-MS = 251, n for SO-ICP-MS = 63. The number of outliers removed from the LA-ICP-MS data set is indicated in the table, and 5 outliers were removed from the SO-ICP-MS data set.

	LA-ICP-MS				SO-ICP-MS		
	Mean (ppm) ( $\pm$ SD)	% < LOD	Outlier n	LOD (ppm) ( $\pm$ SD)	Mean (ppm) ( $\pm$ SD)	% < LOD	LOD (ppm)
Ag	Below LOD	92		0.015 (0.010)	Below LOD	98	0.001
Al	Discarded	34		0.180 (0.134)	4.277 (2.918)	10	0.478
As	Discarded	6		0.020 (0.008)	Below LOD	100	0.003
Ba	20.21 (4.332)	0	4	0.008 (0.005)	29.36 (12.26)	0	0.004
Be	Not Run	-		-	Below LOD	100	0.003
Bi	Not Run	-		-	0.004 (0.001)	0	3.73E-4
Cd	Below LOD	79		0.007 (0.005)	Below LOD	59	2.97E-4
Co	0.054 (0.033)	41	4	0.042 (0.025)	0.007 (0.002)	0	0.001
Cr	0.534 (0.190)	0	9	0.152 (0.063)	0.010 (0.011)	17	0.002
Cu	0.075 (0.027)	2	15	0.019 (0.009)	0.090 (0.139)	32	0.015
Fe	13.58 (3.68)	0	1	1.143 (0.393)	27.02 (1.36)	0	0.231
Ga	Not Run	-		-	Below LOD	95	0.001
Li	Not Run	-		-	0.231 (0.063)	0	0.002
Mg	22.77 (7.71)	0	4	0.198 (0.123)	Not Run	-	-
Mn	4.503 (2.656)	0	8	0.019 (0.009)	8.853 (5.299)	0	0.005
Mo	Below LOD	82		0.010 (0.003)	Below LOD	81	0.002
Na	3131.1 (294.3)	0	1	0.330 (0.203)	Not Run	-	-
Ni	0.150 (0.080)	15	6	0.070 (0.059)	0.310 (1.039)	2	0.024
Pb	0.007 (0.012)	31	1	0.001 (0.001)	0.006 (0.009)	0	2.56E-4
Rb	0.209 (0.127)	0	2	0.004 (0.003)	0.268 (0.127)	0	0.002
Re	Below LOD	83		4.42E-04 (2.72E-04)	1.57E-03 (2.71E-4)	0	6.01E-5
Sb	Below LOD	64		0.009 (0.004)	Below LOD	78	0.003
Sc	Below LOD	100		0.015 (0.011)	0.021 (0.002)	0	0.008
Se	Discarded	31		0.175 (0.058)	Below LOD	100	0.031
Sn	Below LOD	54		0.004 (0.002)	Not Run	-	-
Sr	599.0 (137.6)	0	3	0.016 (0.008)	636.9 (160.1)	0	0.011
Th	Below LOD	76		4.36E-4 (4.93E-4)	0.010 (0.002)	0	7.76E-5
Tl	0.019 (0.008)	0	4	0.002 (0.001)	0.019 (0.007)	0	0.001
U	Below LOD	57		1.25E-4 (1.64E-4)	1.18E-03 (2.06E-4)	0	3.13E-4
V	Below LOD	73		0.004 (0.002)	Below LOD	98	0.003
W	Below LOD	73		0.001 (0.001)	Not Run	-	-
Y	Below LOD	52		0.001 (0.001)	0.002 (0.001)	0	4.25E-4
Zn	1.304 (0.575)	0	6	0.127 (0.064)	1.018 (0.382)	0	0.207

Lines in grey reflect elements that were not run using one of the methods, analyses where >50% of data were below LOD or elements that were discarded because of suspect data.

Ontario and Alberta data to be the result of different sampling years. LMMs were conducted using the lme4 package in R (Bates et al., 2015; R Core Team, 2016). Multiple comparison tests were used to assess differences between locations on the river using the multcomp package (Hothorn et al., 2008). Visual assessments were conducted on residual plots to assess normality and heterogeneity, and data were transformed using log transformations when required (Zuur, 2009). A principal components analysis (PCA) was conducted on LA-ICP-MS data including elements where >50% of otolith samples had values above LOD from Alberta and Ontario sites. The PCA was conducted in R using the prcomp function. Kaiser's criterion and scree plots were used to determine the number of factors to retain (Cattell, 1966; Kaiser, 1960). In the following, the term significant is reserved for statistical significance at the 95% confidence level (i.e.  $p < 0.05$ ).

### 3. Results

#### 3.1. Data quality

Within the otoliths 17 of 29 elements analysed using LA-ICP-MS methods met the LOD threshold for data quality (i.e. >50% of samples >LOD), but of the four elements typically associated with bitumen only Ni was included (Table 2). Of the 17 quantifiable elements, Al was discarded as a precaution because aluminum oxide was used in the polishing step of otolith preparation. As well, As and Se were discarded because the results were not compatible with results from the SO-ICP-MS data which indicated both elements were <LOD. The discrepancy between methods may be explained by the use of low resolution mode and interference from other elements/molecules (Halden and Friedrich, 2008). The remaining 14 elements were included in further statistical analyses.

Twenty of 29 elements analysed using SO-ICP-MS methods met the LOD threshold for data quality, including bitumen-associated elements Ni and Re (Table 2). Of these 20 elements, only eight elements were included in statistical analysis, including two that were not analysed using LA-ICP-MS (Bi, Li), five that were < LOD using LA-ICP-MS analysis (Re, Sc, Th, U, Y) and one of the three elements discarded from the LA-ICP-MS data (Al).

#### 3.2. Linear mixed-effects models

Comparisons among locations along the Clearwater and Athabasca Rivers (upstream-Clearwater River, upstream-Athabasca River, midstream, downstream) for individual elements found no significant differences in 19 of 22 comparisons (Table 3, Fig. 2). The three elements which did show significant differences in concentrations were Li, Cu, and Pb which were all more abundant upstream of industry. Specifically, Cu concentrations were significantly lower in otoliths from fish collected from the downstream location compared to those from the upstream-Athabasca River location, Pb was significantly higher in the upstream-Athabasca River location compared each of the three other location in the LAR, and Li was significantly lower in otoliths from the downstream location compared to those from the upstream-Athabasca River and midstream locations (Table 3, Fig. 2).

Comparison of Ontario and Alberta locations yielded no significant differences in 9 of 22 elements. For 10 elements (Ba, Bi, Li, Mg, Na, Re, Sc, Sr, Th, Y), otoliths from Alberta had significantly greater concentrations than those found in Ontario. In contrast, Cr, Rb, and Tl were significantly more abundant in the otoliths from Ontario (Table 3, Fig. 3).

#### 3.3. Principal component analysis

The first five principal components were retained from the principal components analysis and accounted for 21.2, 15.4, 13.3, 10.3 and 8.0% of the variation in the data, respectively. Variation along PC1 was driven mainly by differences in Sr, Rb, Tl, Na, Ba and Mg and PC1 differentiated strongly between Ontario samples and Alberta samples (Fig. 4A). Variation along PC2 was driven mainly by differences in Zn, Ni, Cr, Fe and Cu, but these differences were not useful in differentiating among locations

**Table 3**

Summary of post-hoc multiple comparison results comparing locations along the Lower Athabasca Region (LAR) main stems of the Clearwater and Athabasca Rivers (upstream-Athabasca River, upstream-Clearwater River, midstream and downstream) and comparing Ontario to Alberta study locations.

Analysis	Element	Among LAR Locations	Ontario vs Alberta
LA-ICP-MS	Ba	>0.05	ON<AB
	Co	>0.05 (log)	>0.05 (log)
	Cr	>0.05	ON>AB (log)
	Cu	Downstream < Upstream-Athabasca River	>0.05
	Fe	>0.05	>0.05 (log)
	Mg	>0.05	ON<AB
	Mn	>0.05 (log)	>0.05 (log)
	Na	>0.05	ON<AB
	Ni	>0.05	>0.05
	Pb	Upstream-Athabasca River > Upstream-Clearwater River, Midstream & Downstream (log)	>0.05 (log)
	Rb	>0.05	ON>AB (log)
	Sr	>0.05	ON<AB
	Tl	>0.05	ON>AB
	Zn	>0.05 (log)	>0.05 (log)
SO-ICP-MS	Al	>0.05	>0.05
	Bi	>0.05	ON<AB
	Li	Downstream < Upstream-Athabasca River & Midstream	ON<AB
	Re	>0.05	ON<AB
	Sc	>0.05	ON<AB
	Th	>0.05	ON<AB
	U	>0.05	>0.05
	Y	>0.05	ON<AB

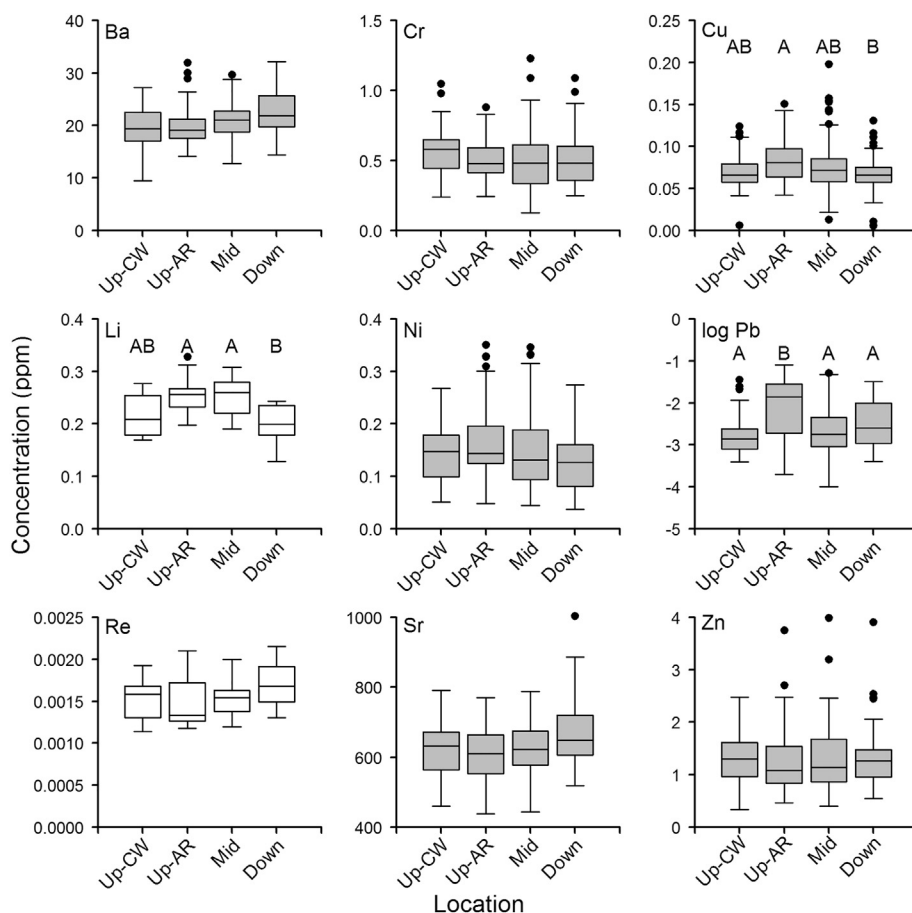
or provinces. Similarly, variations in PC3, PC4 and PC5 were driven by differences in Co, Mn, Na, Pb, and Mg (PC3), Pb, Cu, Tl, Ba and Fe (PC4), and Ba, Co, Mn, Zn and Mg (PC5) but those principal components were also not effective in differentiating between Alberta locations along the Athabasca and Clearwater Rivers, or between provinces (Fig. 4B).

### 4. Discussion

Concentrations of approximately one third of the elements analysed in this study were below quantifiable levels which illustrates the need for ultraclean lab facilities and procedures (Shotyk et al., 2017). Of those that could be measured with confidence, few differences were found among Trout-perch otoliths from locations upstream (in the Athabasca and Clearwater Rivers), midstream and downstream of the majority of industrial activity in the LAR. Of the four elements known to be enriched in bitumen (V, Ni, Mo and Re), only Ni and Re were quantifiable in fish otoliths. However, no significant differences in Ni and Re concentrations were found among locations in the LAR study area. The only elements which showed significant differences in concentrations were Li, Cu and Pb, and these were more abundant upstream of industry, compared to downstream. Overall, spatial patterns in otolith chemical concentrations in Trout-perch in the Athabasca and Clearwater main stems failed to reveal a detectable impact from industrial activity. Significant differences in element concentrations were found between otoliths from Ontario versus Alberta, however, these differences seemed to be driven largely by their respective geological settings.

#### 4.1. Impacts of oil exposure on otolith microchemistry

Only a small number of studies have applied otolith microchemistry techniques to examine impacts to fish from exposure to oil, and none, to our knowledge have examined exposure to bitumen. A lab study assessed the impacts of oil ingestion on fish and otolith growth and otolith composition (Morales-Nin et al., 2007) and three studies used the presence of oil marker elements in otoliths to evaluate whether fish were using off-shore oil platforms and rigs as habitat (Fowler et al., 2015; Love et al., 2009; Nowling et al., 2011). Otolith microchemistry



**Fig. 2.** Boxplots of selected element concentrations of Trout-perch otoliths from Lower Athabasca Region sites conducted on LA-ICP-MS data (grey) or SO-ICP-MS data (white). Locations reflected in the boxplots include sites upstream (Up-CW, Up-AR), proximal (Mid) and downstream (Down) of the majority of industrial activity. The upstream sites are separated into those on the Athabasca main stem (Up-AR) and those on the Clearwater River (Up-CW). Where significant differences exist, letters above the boxplots indicate similarities and differences.

techniques were also applied to assess whether the Deep Horizons oil spill in the Gulf of Mexico in 2010 could be detected in fish otoliths (Granneman et al., 2017; Lopez-Duarte et al., 2016; Nelson et al., 2015). Nelson et al. (2015) found that while they could discriminate among sites primarily using concentrations of Mn, Sr, and Ba, they could not discriminate between oiled and non-oiled sites. In fact, the authors found that many of the elements typically associated with oil were either below the LOD (i.e. V, Cr, Ni, Se) or did not differ among sites (i.e. Mg, Al, Cu, Zn, Sn, Pb). Granneman et al. (2017), however, noted that the fish analysed by Nelson et al. (2015) were spawned after the oil spill and comparisons were conducted by presuming oil and non-oiled sites. Further, the LODs reported by Nelson et al. (2015) are considerably higher than concentrations reported in subsequent studies, including the current study. Lopez-Duarte et al. (2016) were able to detect Ni and V, but found no evidence that either element increased following the 2010 oil spill. The authors did find an increase in Ba, Pb and Cu associated with oil impacted sites after the spill, however, they speculated that the increases may reflect increased freshwater inputs from the Mississippi River which were intentionally released in response to the spill to limit upstream movement of the oil. Granneman et al. (2017) were also able to detect the elements of interest in their study (including V, Ni), but similar to Lopez-Duarte et al. (2016) they found no significant differences in metal concentrations before, during and after the oil spill. The lack of detectable change in oil marker elements associated with the oil spill was suggested to result from lower concentrations of these elements in Macondo oil, or that the effects of the spill were obscured by the presence of metals from other sources in the Gulf of Mexico (Granneman et al., 2017; Lopez-Duarte et al., 2016; Nelson et al., 2015). In at least one study, however, V concentrations in otoliths

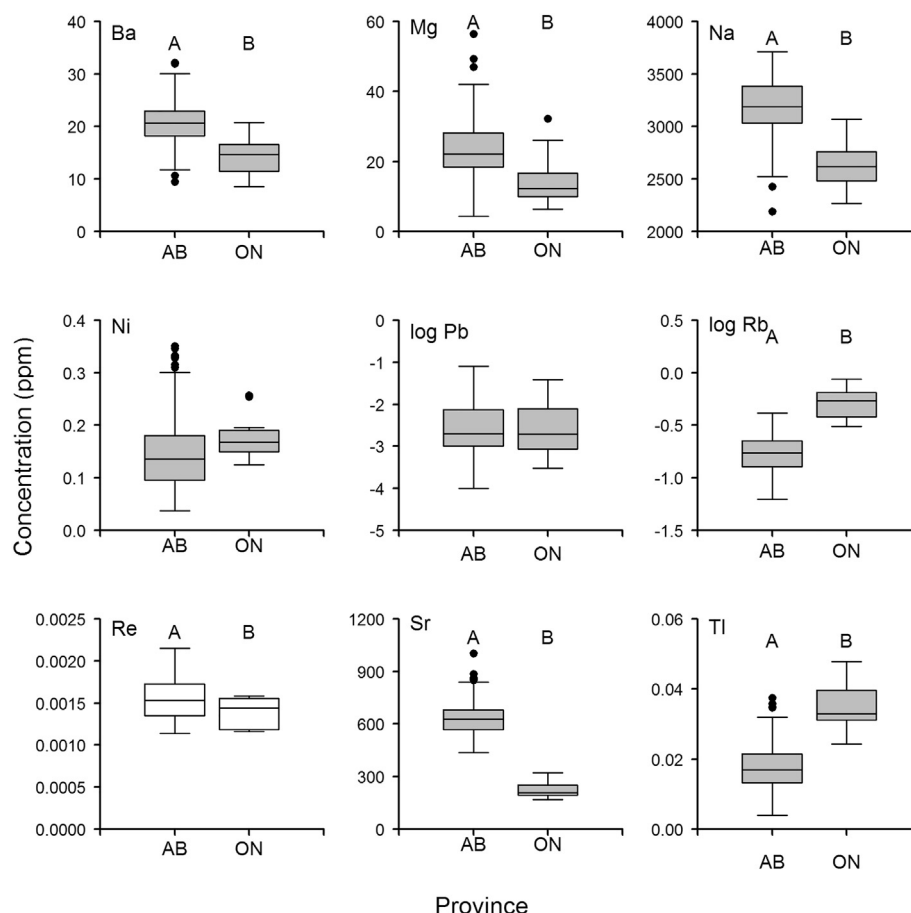
of marine fishes collected near oil and gas structures were found to be significantly more abundant compared to fish from control locations (Nowling et al., 2011).

#### 4.2. Bitumen-associated elements: Mo, Ni, Re and V

Despite the limited success in detecting impacts from oil exposure on fish otolith microchemistry in these previous studies, application of these methods to fish in the LAR was nevertheless considered promising, given the enrichment of V, Ni, Re, and Mo in bitumen relative to the earth's crust. Further, the expectation of detecting elevated concentrations of these elements in otoliths due to bitumen exposure was supported by the proximity of tailings ponds and open pit bitumen mines to the river (RAMP, 2012), evidence of tailings pond seepage (Golder Associates, 2009), and the presence of at least 35 natural outcrops of bitumen along the river banks. The most abundant metal in the bitumen fraction of the Athabasca bituminous sands is V (Bicalho et al., 2017), and previous studies indicate that V will be readily incorporated in the otolith lattice when present in sufficient concentrations (Granneman et al., 2017; Lopez-Duarte et al., 2016). For example, Granneman et al. (2017) reported mean V concentrations up to 28 ppb. However, despite the low LODs obtained here (4 ppb using LA-ICP-MS), we were unable to detect V in 73% of our samples. The low concentrations of V in fish otoliths from the Athabasca River (this study) are consistent with the low concentrations of V in the river reported for the dissolved fraction which are well within the range reported for other major river systems (Shotyk et al., 2017).

Of the four elements typically enriched in bitumen, only Ni and Re were detectable in LAR otoliths. However, no differences were observed in either Ni or Re concentrations among the locations sampled, despite the profound





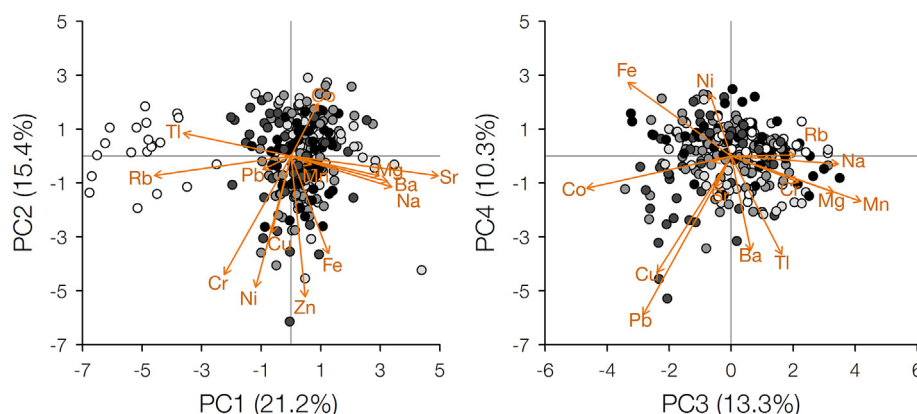
**Fig. 3.** Boxplots of selected element concentrations of Trout-perch otoliths from Lower Athabasca Region, Alberta (AB) and Ontario (ON) sites conducted on LA-ICP-MS data (grey) or SO-ICP-MS data (white). Where significant differences exist, letters above the boxplots indicate similarities and differences.

(approximately 3-fold) increase in dissolved Re concentrations with distance downstream (Shotyk et al., 2017). Mean concentrations of Ni measured in LAR Trout-perch otoliths were within the range reported by Granneman et al. (2017) and below the LOD reported by Nelson et al. (2015). Comparison with Ontario otoliths showed no difference in Ni, but Re concentrations were lower in fish from Ontario. Taken together, and despite the slight increase in dissolved V and Ni, and profound increase in dissolved Re with distance downstream (Shotyk et al., 2017), there was no

significant increase in concentrations of bitumen-related elements (V, Ni, Mo, Re) in the LAR otoliths. At this time, there is no evidence of industrial impacts of bitumen-derived elements in fish otoliths.

#### 4.3. Other elements: Ag, Be, Cd, Cr, Cu, Fe, Li, Pb, Sb, Tl and Zn

Concern has been expressed regarding a long list of potentially toxic elements that apparently exceeded Canada's or Alberta's guidelines for



**Fig. 4.** (A) Biplot of the first two principal components describing element concentrations from LA-ICP-MS analyses of Trout-perch otoliths. Points indicate individual fish from the Clearwater River upstream of the confluence with the Athabasca River and industrial activity (black), and on the Athabasca River upstream of industrial activity (dark grey), within the industrial zone on the Athabasca River (medium grey), downstream of the main industrial zone (light grey), and the Batchawana River, Ontario (white). The arrows indicate which elements are responsible for driving the variation in the data where a longer arrow indicates a larger driver and a smaller angle between the arrow and the axis indicates on which principal component the element is more influential. (B) Biplot of the third and fourth principal components. All combinations of the first five principal components were visually assessed using biplots, however, only these two are presented as examples.



the protection of aquatic life in melted snow or water samples near or downstream of development in the LAR (Kelly et al., 2010). For the most part, we found that these elements were either below our LODs (i.e. Ag, Be, Cd, Sb), or that no significant differences were observed among locations in the LAR (i.e. Cr, Tl, Zn). The lack of change in concentration of Cd and Tl in otoliths downstream versus upstream was consistent with the extremely low concentrations of these elements in the dissolved fraction of the Athabasca River (at natural, background values), and their lack of change in concentration with distance downstream of industry (Shotyk et al., 2017). Some elements were identified by the second principle component describing variation among individual fish otoliths. Individuals with higher Zn, Ni, Cr, Fe and Cu were differentiated along this axis, however, there were no known characteristics unifying those individuals. Individuals came from all locations within the LAR, and were not remarkable based on length or weight characteristics. As such, the observed multivariate patterns may reflect individual variation related to physiology or some other biological factor that was not measured rather than proximity to natural or anthropogenic inputs. Where differences were observed (i.e. Cu, Pb), the higher concentrations were not associated with locations near or downstream of industrial development, but rather were upstream of such activities on the Athabasca River. In the case of Cu and Pb, it is suspected that these anomalous data may reflect upstream inputs from tributaries. The water chemistry of a number of tributaries and their role in the Athabasca River is currently being investigated by an independent, ongoing study on spatiotemporal variation in trace element speciation. Lithium was also instrumental in differentiating locations in the LAR, however, it should be noted that these patterns were observed only in the smaller SO-ICP-MS data set. Otolith Li concentrations were lower at the downstream location compared to upstream-Athabasca River and midstream locations despite the increasing importance of inputs of saline groundwaters with distance downstream (Shotyk et al., 2017).

#### 4.4. The role of geology

Differences in element concentrations of otoliths from the LAR versus the Batchawana River in Ontario are most likely driven by differences in geology. Ontario samples were characterized largely by higher concentrations of Rb and Tl which probably reflects the abundance of potassium-bearing silicates such as potassium feldspar and mica in Precambrian rocks of the Canadian Shield (Goff et al., 1995), whereas LAR otoliths were characterized by higher concentrations of elements (e.g. Sr, Na, Mg) typically associated with the soluble salts (e.g. calcite, dolomite, halite) of the Western Canada Sedimentary Basin. The comparison between regions was performed to provide context to the element concentrations using otoliths from fish taken in a region outside of the LAR. However, the findings should be interpreted with caution since the influence of geology was not studied in any detail, and the potential contributions to the Batchawana River from industrial activity in northern Ontario were ignored. The Batchawana River area has known mineral occurrences (Siragusa, 1986) and the region has seen some historical mining activity (Northwatch, 2001).

#### 4.5. Element uptake and incorporation into the otolith

The differences found both among the LAR study locations, and between the LAR and Batchawana River highlight the importance of understanding the complex mechanisms involved in otolith element uptake. Early application of otolith microchemistry focused largely on interpreting environmental concentrations of elements to act as environmental tracers (e.g. Campana, 1999; Campana and Thorrold, 2001); however, the rate of element incorporation can vary based on a number of factors, such as, how environmental Ca concentration impacts Ca uptake to maintain homeostasis within the fish, the properties of the element (e.g. valence, size), and physiology (e.g. whether an element is essential or non-essential to the biological function of the

organism) (Geffen et al., 1998; Izzo et al., 2018; Loewen et al., 2016). Before elements are incorporated into the otolith, they encounter three barriers that can each impact how accurately the final otolith concentration reflects the initial environmental concentration: 1. Entry into the blood plasma, 2. Entry into the endolymph and 3. Incorporation into the otolith (Campana, 1999). Entry into the blood plasma can occur through branchial uptake at the gills or through ingestion to varying degrees (Campana, 1999; Ranaldi and Gagnon, 2008, 2009). Elements with no known biological function (non-essential), particularly those that are similar in size and valence to Ca (e.g. Sr, Ba, Pb), tend to compete with Ca at uptake sites in the gills. These elements are typically incorporated into the otolith at higher concentrations and are thought to enter the otolith with fewer modifications relative to environmental concentrations (Wood et al., 2011). However, a recent review on physiology and biomineralization highlighted the role of environmental Ca concentrations on determining the uptake rate of those elements (Loewen et al., 2016). The differences in environmental Ca among locations and between regions in this study, however, are small relative to those reviewed by Loewen et al. (2016). As such, environmental concentrations of the elements competing with Ca (e.g. Sr, Ba) are likely to play a large role in the patterns observed in this study.

Essential elements (e.g. Na, Mg), which have vital biological functions, are thought to be under tight physiological control and less likely to reflect environmental concentrations. Nevertheless, the differences in otolith concentrations observed here are consistent with the differences in environmental concentrations observed between the LAR (Mg:  $9.6 \pm 0.9$  ppm, Na:  $12.4 \pm 1.9$  ppm, Shotyk et al., 2017) and the Batchawana River (Mg:  $1.2 \pm$  ppm, Na:  $0.9 \pm 0.1$  ppm, DFO, unpublished data). Interestingly, mean Na concentrations from the LAR otoliths were more typical of marine fish otoliths (LAR mean =  $3175 \pm 258$  ppm, reported by Friedrich and Halden, 2008 = 3000 ppm) as opposed to the Ontario otoliths that were more similar to those of freshwater fish (ON mean = 2628 ppm, Friedrich and Halden, 2008 = 2500–2600 ppm).

The potential effects of sex on otolith element concentrations were not evaluated in this study since sex was not recorded for all fish. Sex has been shown to play a role in otolith element concentrations for some elements (Izzo et al., 2018; Kalish, 1989; Sturrock et al., 2015). Sturrock et al. (2015) demonstrated that the large sex-specific differences in blood plasma concentrations are generally not reflected in the otolith, however, sex-specific differences were observed in Sr, Zn, Li and K in the otoliths of marine fish. While we cannot say definitively whether sex played a role in our results, the key findings of our study, in particular the low concentrations of elements enriched in bitumen and other potentially toxic elements and the large differences between regions, are likely to be robust to the potential confounding influence of sex. However, the influence of sex and other physiological differences may limit the ability to detect small differences (Izzo et al., 2018).

## 5. Conclusion

We assessed a wide range of elements including several trace metals known to be enriched in bitumen (Hitchon and Filby, 1983; Selby and Creaser, 2005), and other elements of possible toxicological concern (Kelly et al., 2010) in otoliths from Trout-perch obtained throughout the Lower Athabasca Region. Element concentrations were generally low: of the elements enriched in bitumen (V, Ni, Mo and Re), only two could be measured (Ni, Re) and neither was more abundant downstream of industry, compared to upstream. Of the potentially toxic elements, only Cu and Pb showed significant differences in spatial variation, but they were more abundant upstream of industry compared to downstream. The study failed to yield evidence of anthropogenic contamination for any of the elements. Differences in element abundance between otoliths from Alberta and Ontario were seen, but these appear to be mainly due to differences in geology, with elements such as Mg and Sr more abundant in Alberta (Western Canada Sedimentary

Basin) while Rb and Tl were more abundant in Ontario (Precambrian Shield). Trout-perch are abundant in the Athabasca River, non-migratory, and considered a sentinel species in respect to environmental monitoring. The data presented here can be viewed as baseline information, against which possible changes in future may be compared.

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