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## Alkali Element Uptake in Otoliths: A Link Between the Environment and Otolith Microchemistry

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Otoliths taken from fish in the vicinity of rare element pegmatites in eastern Manitoba, Canada, were analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and found to contain lithium, sodium, rubidium, cesium, and strontium at levels ranging from a few ppm to a few 10s of ppm. In some otoliths, the concentrations varied in correspondence to the annular structure of the otoliths, suggesting a periodicity to their incorporation. This is the first reported detection and resolution of periodic signals of Rb and Cs in otoliths. The correspondence between the elements in the otoliths and surrounding rocks suggests there can be a strong link between the microchemistry of a fish's otoliths and its environment, particularly where there are distinctive rock types. These elements may serve as natural markers in certain environments and may be used to characterize and monitor lacustrine environments over a period of time.

### Introduction

Otoliths are hard calcareous structures in the inner ear of teleost fish that grow throughout the life of the fish and form part of the organs that sense position and motion (1). They are composed of layers of calcium carbonate, typically aragonite, deposited annually in a noncollagenous organic matrix (2). Observed in reflected light, each layer is composed of a bright and a dark band, the bright band representing a period of summer growth, and the dark band corresponding to winter growth, when the nutrient supply is diminished (1). Annuli also act as "information storage structures" that record the life-history of fish: annually deposited aragonite layers indicate the age of the fish, the elemental composition of otoliths can provide information as to the past environmental conditions experienced by the fish, and quantitative trace element otolith composition can be used to deduce population structure and movement (3–5).

In recent years, research on biominerals such as otoliths, teeth, and bone has increased with improvement of resolution and sensitivity of instrumental techniques, which has allowed for the analysis of a broader suite of elements and isotopes. Otolith trace element composition has been the focus of many studies, and strontium in particular has been well studied as a proxy for water temperature variations and as a method of establishing migratory behavior of fish (6–8). However, the aragonite structure ( $\text{CaCO}_3$ ) has the capacity

to incorporate a wide range of other trace elements, including barium, transition elements, rare earth elements, and, as shown in this study, alkali elements. The incorporation of such elements into otoliths has the potential to record information about fish behavior and diet, as well as on the local environment, including the surrounding geology. New insights are emerging into the role of biominerals as records of environmental change, as well as how the trace element chemistry of the environment can impact an organism (9, 10). The objectives of this study are to (1) analyze a suite of trace elements (Li, Na, Rb, Sr, Cs) across the annular structure of otoliths (i.e., life history of the fish); (2) compare and contrast trace element content with respect to time, species, and local geology; and (3) establish what constitutes a characteristic trace element pattern in otoliths for the region.

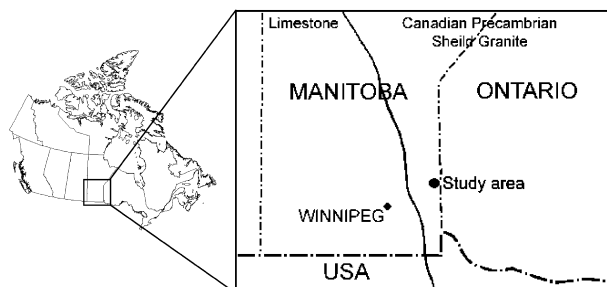
### Study Area and Geologic Setting

Samples were collected from lakes in the Lac du Bonnet region of southeastern Manitoba, Canada, close to the Manitoba–Ontario border, in the Canadian Precambrian Shield (Figure 1). The area is approximately 2.6 billion years old and lies within the Bird River greenstone belt (11). An east–west trending suite of metamorphosed sedimentary and volcanic rocks underlying the belt have been intruded by mafic to ultramafic rocks (12). Large plutonic masses of granitic rocks lie north and south of the belt, and there are many pegmatites hosting minerals of rare elements, of which the Tanco pegmatite is particularly distinct (11).

The Tanco pegmatite is an igneous body believed to be the product of very advanced differentiation of granitic magma that was emplaced approximately 2.55 billion years ago (13). It is classified as a relatively high-temperature, low-pressure lithium pegmatite and is famous for its concentrations of tantalum, lithium, and cesium minerals (14). Tantalum primarily occurs in the minerals wodginite  $\text{Mn}^{2+}$ -(Sn, Ta)(Ta, Nb) $_2\text{O}_8$ , tantalite (Fe, Mn)(Ta, Nb) $_2\text{O}_6$ , and micro-lite (Ca, Na) $_2\text{Ta}_2\text{O}_6(\text{O}, \text{OH}, \text{F})$ . Cesium is mined from pollucite (Cs, Na)(AlSi $_2\text{O}_6$ ) $\cdot n\text{H}_2\text{O}$ , and this pegmatite contains over 80% of the world's known Cs/pollucite reserves. Lithium is found in spodumene, LiAlSi $_2\text{O}_6$ , which is used in the manufacturing of glassware and ceramics. The locality was chosen because a great deal is known about the unique mineralogy and geochemistry of the rocks.

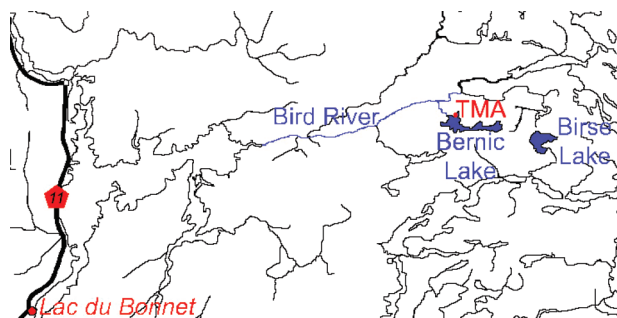
### Materials and Methods

Otoliths from northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*), white suckers (*Catostomus commersoni*), and



**FIGURE 1.** Map of southeastern Manitoba showing the location of the study area in the English River gneissic belt of the western Superior Province of the Canadian Shield (12). Reproduced with permission of the Canadian Institute of Mining, Metallurgy and Petroleum (Figure originally printed in *CIM Bulletin*, Vol. 72, No. 802, p 142).

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**FIGURE 2.** Map of the study area near the town of Lac du Bonnet in southeastern Manitoba showing the sample locations: Bernic Lake, TMA, Birse Lake, and up- and downstream Bird River (26). Scale 1:500 000. Reproduced with the permission of the Government of Manitoba (figure originally printed in Davies et al. (26) as Figure 10).

**TABLE 1.** LA-ICPMS Operating Conditions and Data Acquisition Parameters for Otolith Analysis

	ICPMS
forward power	1303 W
gas flows	
plasma (Ar)	16 L/min
auxiliary (Ar)	0.80 L/min
sample (Ar/He)	0.89/0.335 L/min
shield electrode	used for analysis
	LA
repetition rate	20 Hz
preablation warm-up	~120 s
pulse duration	5 ns
spot size	30 $\mu\text{m}$
power	80%
incident pulse energy	~0.020 mJ
energy density on sample	~6.22 Jcm <sup>-2</sup>
laser scan speed	2 $\mu\text{ms}^{-1}$
	data acquisition
data acquisition protocol	time resolved analysis
scanning mode	BScan and EScan
detector mode	analog and counting
isotopes determined	<sup>7</sup> Li, <sup>23</sup> Na, <sup>44</sup> Ca, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>133</sup> Cs
dwel time (segment duration)	0.3 ms
magnet settling time	0.001–0.3 ms
time/pass	1.7 s
runs/pass <sup>a</sup>	1000–1 (17m 09s)

<sup>a</sup> Pass is a measurement cycle through the mass spectrum; 1 pass collected 1000 times producing 1000 blocks of data per sample.

ciscos (*Coregonus artedii*) were collected and archived in 1998 as part of a preliminary environmental study of the distribution of trace elements. This collection forms the basis of an ongoing survey. Five locations were sampled: Bernic Lake (white sucker  $n = 11$ , cisco  $n = 10$ ), Birse Lake (northern pike  $n = 8$ , walleye  $n = 4$ ), the Tanco mine tailings management area (TMA; white sucker  $n = 8$ ), and Bird River up- (northern pike  $n = 3$ , walleye  $n = 3$ ) and downstream (northern pike  $n = 3$ , walleye  $n = 3$ ) from Bernic Lake (Figure 2). Birse Lake is outside the Bernic Lake watershed and is not in contact with the pegmatite and was therefore sampled to act as a control site. Sagittal otoliths were removed from the fish, embedded in epoxy resin, and cut transverse to create a dorso–ventral cross section through the core of the otolith and expose all annuli. The posterior half of each cut otolith was re-embedded in a 25-mm lucite microprobe mount, ground, and polished. Prior to analysis, samples were rinsed with DDW and allowed to air dry.

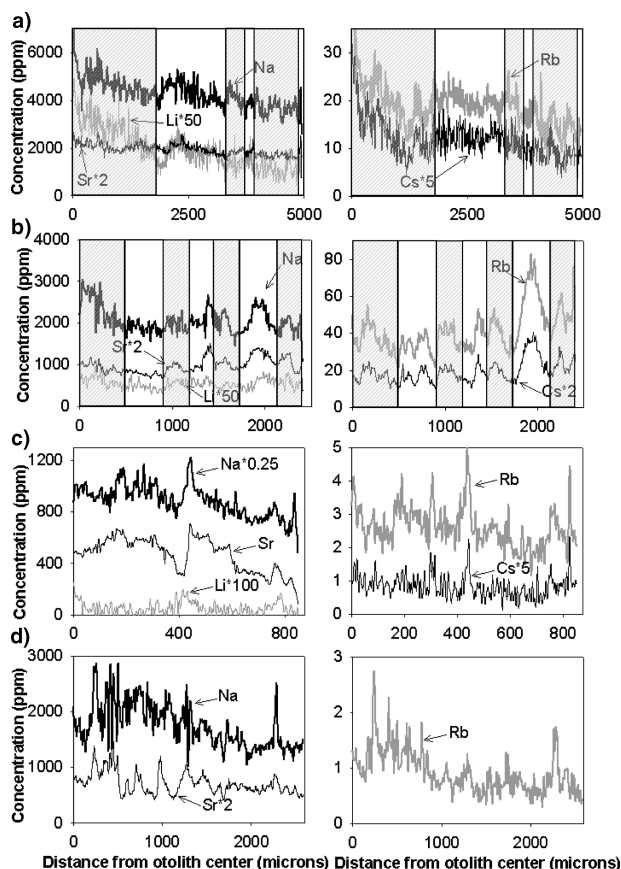
While X-ray emission analysis of trace elements in otoliths has been done using proton-induced X-ray emission (PIXE) and electron microprobe analysis (EMPA), laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) can detect a broader suite of elements with better sensitivity and can detect Li. LA-ICP-MS analyses were done using a Thermo Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd:YAG laser. Table 1 summarizes the instrument parameters used during analysis. Running conditions used to optimize sensitivity and resolution of the annular growth features in these samples included a 30- $\mu\text{m}$ -diameter beam traveling 2  $\mu\text{ms}^{-1}$ . Calcium as 56 wt% CaO was used as an internal standard, and the external calibration was done using NIST glass 610 with the nominal values reported by Pearce et al. (15). Line scans were run across the otolith surface from core to edge, perpendicular to annuli in time-resolved or scanning mode. Standard analyses were collected prior to each analytical run. Measured trace element concentrations, standard deviations, and detection limits were processed using GLITTER software (16) and exported to EXCEL for final presentation.

## Results

Table 2 summarizes the trace element contents from otoliths analyzed in this study. Sodium, Rb, and Sr are found in substantial concentrations in all otoliths, the concentrations varying among sample sites as well as among species. The presence or absence of Li and Cs depends on location, and elevated levels of Li, Cs, and Rb were detected in areas proximal to the pegmatite. In fish taken from water bodies outside the Bernic Lake watershed, Li and Cs are below detection limits, approximately 2.9 and 0.1 ppm, respectively. Representative chemical profiles from four of the sites are

**TABLE 2.** Summary of Otolith Trace Element Content in Parts Per Million from the Study Area

	Li	Na	Rb	Sr	Cs
Bernic Lake					
white sucker	0.2–28	953–3838	7–34	202–773	0.4–4.8
cisco	3–98	1234–8880	8–50	627–1528	0.4–6.4
TMA					
white sucker	2.9–38	459–4614	8–83	212–839	1.7–26.3
Bird River – downstream					
northern pike	2.9–8	2254–4700	1–4	325–661	0.1–0.5
Bird River – upstream					
white sucker	<2.9	1057–3355	0.2–2	192–636	<0.1
northern pike	<2.9	980–2867	0.15–3	203–746	<0.1
Birse Lake					
walleye	<2.9	807–2808	0.15–2	94–218	<0.1
typical detection limits	2.9	5.2	0.15	0.25	0.1
typical 1 $\sigma$ Error	2	200	1	30	0.1



**FIGURE 3.** Representative line scans from the LA-ICP-MS showing trace element concentrations in parts per million (ppm) versus distance in microns ( $\mu\text{m}$ ) across otoliths from rim to core. Shading represents the best estimate of annuli based principally on reflected light microscopy. (a) Cisco otolith from Bernic Lake. (b) White sucker otolith from Tailings Management Area. (c) Northern pike otolith from downstream Bird River. (d) Northern Pike otolith from upstream Bird River.

shown in Figure 3 and may be used to establish what constitutes a characteristic chemical pattern for each location, as described below. In cases where annular structures were visible on the otolith section, a link was made between such structures and the periodic nature of the chemical profiles (Figures 3 and 4). Some otolith sections did not have clear annular structures, but periodic variations were still present

in the chemical profiles (Figure 3). Indeed, periodic patterns of all elements are evident in many samples, but there are differences in element concentration and correlations, also described below. Uptake of elements into the otolith structure varied with sample location as well as species.

**Bernic Lake.** All otoliths from Bernic Lake contain the entire suite of elements analyzed and fall within the following typical ranges: Li 0.2–90 ppm, Na 900–4300 ppm, Rb 7–50 ppm, Sr 200–1500 ppm, and Cs 0.4–6 ppm. Four out of seven cisco samples from this site contain Na peaks that reach up to 8500 ppm. Rubidium and Cs concentrations are relatively consistent across species, whereas concentrations of Li, Na, and Sr are higher in cisco otoliths than in otoliths from white suckers.

**Tailings Management Area (TMA).** As in Bernic Lake, all otoliths from the TMA contain all five elements. Typical ranges for the elements in otoliths from TMA are as follows: Li 3–35 ppm, Na 450–4500 ppm, Rb 8–80 ppm, Sr 200–825 ppm, and Cs 2–26 ppm. Otoliths were only obtained from white suckers in this location, as no other species were caught.

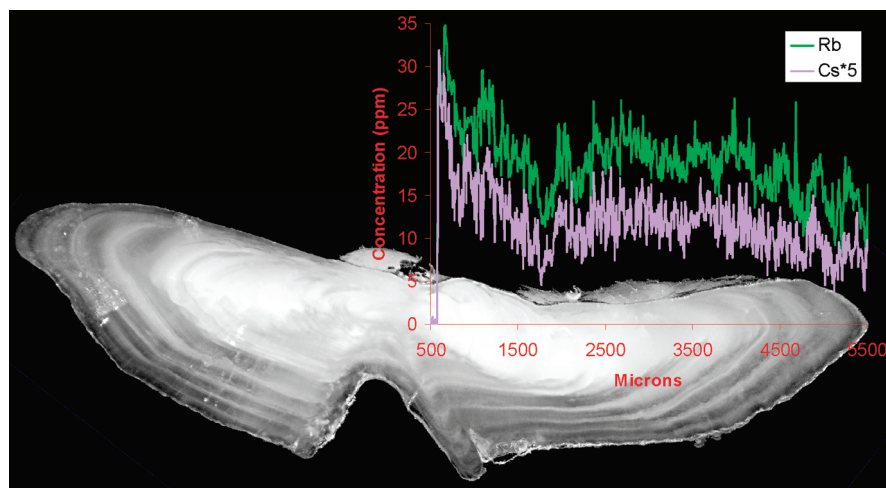
**Bird River Downstream.** The chemical signature of samples from Bird River downstream from the Bernic Lake outlet is characterized by the presence of all five elements, in abundances that are less than those in Bernic Lake and the TMA. Typical concentration ranges in this sample location are the following: Li 3–8 ppm, Na 2200–4500 ppm, Rb 0.8–4 ppm, Sr 300–650 ppm, and Cs 0.1–0.5 ppm. Northern pike was the only species captured from this site.

**Bird River Upstream.** Sodium, Rb, and Sr are ubiquitous in samples from upstream Bird River in concentrations in the ranges of 950–2500 ppm, 0.2–2.5 ppm, and 200–750 ppm, respectively. Concentrations from northern pike and white sucker otoliths are of similar magnitude. Lithium and Cs were below detection limits in all samples from this location.

**Birse Lake.** Birse Lake was sampled as a control lake, being in a different watershed as well as distant from the pegmatites and mining activity. Chemical signatures from the walleye captured in this location are characterized by Na in the range of 800–2800 ppm, Rb in the range of 0.2–2 ppm, and Sr in the range of 90–210 ppm. Similar to what was observed in samples from upstream Bird River, Li and Cs were below detection limits.

## Discussion

In this and other studies (e.g., refs 17 and 18) we have shown that the trace element signature in otoliths can be connected



**FIGURE 4.** Overlay of LA-ICP-MS analyses on image of otolith from Bernic Lake. The laser scan that produced the element traces shown lies directly under the x axis of the plot.



**TABLE 3. Comparison of Otolith Microchemistry (ppm) Among Locations and Species**

	Li	Na	Rb	Sr	Cs
Upstream Bird River white sucker	<2.9	1057–3355	0.2–2	192–636	<0.08
TMA white sucker	2.9–38	459–4614	8–38	013–839	1.7–26.3
Bernic Lake white sucker	2.9–28	953–3838	7–34	202–773	0.4–4.8
Bernic Lake cisco	3–98	1234–8880	8–50	627–1528	0.4–6.4

to the geological character of the sample location. Otoliths from the Lac du Bonnet region have also shown variations between different species of the same location.

**Alkali Element Concentrations.** Sodium levels average about 3000 ppm for marine fish (19), and freshwater otoliths typically range from 2500 to 2600 ppm (20). Values detected in the Lac du Bonnet region fall within the range of 450–4500 ppm. However, some samples in this study show concentrations up to 8500 ppm. The incorporation of so much sodium raises several questions from a crystal chemical standpoint. If the Na<sup>+</sup> is substituting for Ca<sup>2+</sup> in the aragonite structure, it must be coupled with the incorporation of another monovalent cation to maintain a neutral charge balance of the crystal, a phenomenon that is not uncommon in minerals. However, the incorporation of such high amounts of Na would clearly have an adverse effect on the structure of the crystal. It is possible that at least some of the Na is being incorporated into the protein matrix of the otolith. Resolution of this issue by means of protein analysis is part of this ongoing study.

Detection of Rb, Cs, and Li indicates that these relatively rare elements are present and mobile in this particular environment. Lithium-, Cs-, and Rb-bearing minerals, including carbonates, are common in the surrounding rocks; moreover these minerals are easily weathered and, as such, would release elements to the aqueous environment. The presence of Li has previously been detected in cod otoliths from the Gulf of St. Lawrence area, in amounts averaging from 0.6 to 1.0 ppm (21). In this study however, we have detected Li in amounts >90 ppm. In addition to this, the incorporation of Li, as well as Rb and Cs, into the otolith structure is periodic, suggesting a seasonal or dietary control. It is apparent from the site-specific uptake of Li, Cs, and increased amounts of Rb that the most likely source is the Tanco pegmatite based on spatial distribution of elements and element association.

The pathway of these elements into the otolith structure is not well understood. Early studies stated that calcium deposited in otoliths comes predominantly from the surrounding water, via the gills, to the endolymphatic sac (22), with only a small proportion attributed to dietary sources (23). These two major pathways have also been proposed as the mechanism of incorporation for trace elements (24). It seems likely that the alkali elements detected in otoliths of this study have followed similar pathways. Indeed the data presented in Table 3 suggest that there is more than one process involved in trace element uptake into otoliths, although their relative roles as sources of such elements remains to be assessed.

**Variations with Sample Location.** In a comparison of white sucker otoliths from three samples sites (Bernic Lake, TMA, upstream Bird River), several observations can be made (Table 3). Concentrations of Na and Sr are about equal for all three locations, and although Rb is detectable in all three sites, the concentration of this element is an order of magnitude higher in the otoliths from Bernic Lake and Tanco Mine Area than in the otoliths from upstream Bird River. Lithium and Cs are below detection limits at upstream Bird River but are present in significant amounts at the other two sites. The strong correlation between elements present in the otolith and proximity to the Tanco pegmatite indicate

that otoliths are incorporating a chemical signature that reflects the geology of the local environment, either directly from the water or through food.

**Variations with Species.** Table 3 shows a comparison of otoliths from two different species from Bernic Lake; a cisco, which is a pelagic species feeding mainly on plankton, and a white sucker, which feeds on a variety of benthic organisms, such as insect larvae and small crustaceans. Both types of fish contain the same suite of elements, but these data show that the cisco otoliths contain higher concentrations of all five elements than do the otoliths from the white suckers. This suggests that otoliths may also incorporate a chemical signature related to the fish's diet.

In a recent study by Friedrich et al. (25) trout otoliths from an area near mining operations were found to contain periodic signatures of Cu, Pb, and Zn. Anomalous high concentrations in individual annuli were believed to represent the fish moving into areas where levels of these elements are high, such as near the tailings. Similarly, Palace et al. (17) concluded that annular concentrations of selenium in otoliths of rainbow trout indicated the fish had come in contact with selenium-bearing effluent from a coal mining operation. In this study, we have shown that otoliths collected from five sample locations have distinct trace element signatures that are highly correlated to the local geology, indicating that the chemical signatures of otoliths vary on a local scale. In areas where mining is important to the local economy, specific suites of elements may be present in the environment, and otoliths may provide a method of determining and monitoring the impact on fish by acting as natural markers of the trace element chemistry of the environment.

A mechanism for understanding the concentration and periodic uptake of alkali element signals in otoliths remains to be established. However, by expanding the suite of elements analyzed in otoliths, there is the potential for a more detailed assessment of environmental changes experienced by the fish in this area. It should be possible to use these records to study the life histories of fishes and the environment during their lives. Areas with unique geology and geochemistry that contain a distinctive suite of elements, such as the Tanco pegmatite, are ideal for such studies.

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