



Otolith Microchemistry Applied to Environmental Effects Monitoring in the Keno Hill Mining District



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Last but not least, we wish to recognize the First Nation of Na-Cho Nyak Dun for the deep support and participation in this project, especially Josee Tremblay who made the Fish Otoliths Workshop in Mayo possible. It was a wonderful opportunity to share knowledge with the community. We also wish to acknowledge all the participants of the Mayo workshop, including the citizens who donated fish.

EXECUTIVE SUMMARY

The overall goal of this project was to assess, in three stages, the use of fish otolith microchemistry as a potentially new monitoring tool to be applied around Yukon mine sites. High quality environmental assessments are critical to sound land and water use management as part of all mining development. Improvement of the environmental assessment and prediction of potential impact of land use activities rely on the development of scientific tools and techniques. Fish otolith chemistry integrates information on contaminant exposure and life history of both individual fish and populations. This technique affords a unique opportunity: otoliths consist of a calcium carbonate structure in the inner ear of fish deposited in daily to annual increments. They have been used to determine age and life history events of fish and fish populations. As otoliths are metabolically stable, the contaminant levels within their annular structure can provide a temporal record of exposure of the fish to trace metals and can be used to get baseline data information required for environmental assessments and reconstruct historical exposure for the further protection of aquatic wildlife. As new mining projects are developing in the Yukon, it is believed that Yukon would benefit in establishing a fish otolith chemistry technique and database with the local population, which in turn gave rise to this project.

The objectives of this study were to:

- Complete the fish otolith study at the Keno Hill District site
- Engage with Yukoners and share fish otolith knowledge
- Identify gaps that remain for the development and practical use of this technique in Yukon

As a result, otoliths from grayling captured in Cristal Creek and Moose Creek and sculpins captured in Cristal Creek and Haldane Creek were collected and analysed at the University of Manitoba by Laser Ablation-Inductively Coupled Plasma-Mass Spectrometer (LA-ICP-MS). Sculpins were shown to indicate lead presence while graylings did not. However, grayling did show the potential to indicate zinc. Continued studies with periodic assessment of otoliths providing a retrospective analysis is suggested as a strategy to further define the impact of mining activities on fish otoliths.

All the water quality data, otoliths data and metal data collected have been shared amongst the project partners. In addition, a workshop was hosted in Mayo to discuss the potential application of this technology and discussions were engaged with Yukoners in various organizations.



Figure 1 Fish otolith workshop with the First Nation of Nacho Nyak Dun in Mayo, YT (June 2014)

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1. INTRODUCTION

High quality environmental assessments are critical to sound land and water management as part of all mining development. Improvement of environmental assessment and prediction of potential impacts of land use activities rely on the development of scientific tools and techniques. Fish otolith microchemistry provides both contaminant levels as well as life history information on individual fish and populations. Furthermore, it has the potential to advance our understanding of the local ecosystem and to become an additional technique in the environmental assessment toolbox. This technique also has the potential to provide First Nation peoples, other Northerners and regulators with robust data to inform land and water use decisions in Yukon.

Existing contaminant monitoring programs are based on the collection and analysis of soft tissues (e.g., liver, kidneys) of animals. Contaminant levels in muscle or visceral tissue are useful indicators of recent exposure, but changes in the metabolic condition of the animal, and tissue recompartmentalization of contaminants severely limits their use. Fish otolith microchemistry affords a unique opportunity: otoliths are small bony structures in the inner ear of fish made of aragonite (calcium carbonate, CaCO_3) that is deposited in daily to annual increments. Otoliths are used to determine age and life history events of fish and fish populations. As otoliths are metabolically stable, the contaminant levels within their annular structure can provide a temporal record of the exposure of the fish to trace metals and can be used to get baseline data information required for environmental assessments and reconstruct historical exposure for potentially further protection of aquatic wildlife. Fish otolith and contaminant research has been conducted in British Columbia (Friedrich et al 2011), Alberta (Palace et al 2007), Northwest Territories (Friedrich and Halden, 2011) and northern Manitoba (Friedrich and Halden, 2010) amongst other locations. As new mining projects are developed and old ones are re-activated fish otolith microchemistry can help answer the often asked question “will this development affect the fish?”.

The Keno Hill District (KHD), located in the traditional territory of the First Nation of Na-Cho Nyak Dun (NND FN), was identified as the most appropriate site for this study due to its long mining history, decades of wildlife and water quality monitoring and the site characterisation generated by the closure and remediation plan driven by the federal government and NND FN. As metal uptake can be species-specific, two fish species that are native to the area were selected: Arctic grayling (*Thymallus arcticus*), Figure 2 a migratory freshwater fish from the salmonidae family, and slimy sculpin (*Cottus Cognatus*), Figure 3, a freshwater nocturnal fish selected for its sedentary behaviour. Sculpins are known for their ability to spend their lifespan

within a single kilometer of river. Therefore, they have the potential of being a good indicator of local conditions.



Figure 2 Arctic grayling caught during ice fishing day with the First Nation of Nacho Nyak Dun (April 2014)



Figure 3 slimy sculpin (*Cottus Cognatus*, credits: Wikipedia)

2. METHODOLOGY

2.1. FISH SAMPLING COLLECTION

Twenty samples of Arctic grayling (*Thymallus arcticus*) were obtained from Christal Creek and a further 10 samples were collected from Moose Creek while 9 samples of slimy sculpin (*Cottus Cognatus*) were collected from each of Christal and Haldane Creeks. Table 1 summarizes the number of fish caught, species, and a preliminary assessment of their ages based on optical images and chemical scans.

Table 1 Samples used in otolith microchemistry analysis

Location	Species	No. of samples	Capture Year	Age range
Christal Creek	Grayling	20	2013	3 to 7
Moose Creek	Grayling	10	2014	3 to 9
Haldane Creek	Sculpin	9	2014	2 to 4
Christal Creek	Sculpin	9	2014	2 to 4



Figure 4 Otolith extraction from a grayling by Jody McKenzie-Grieve (April 2014). Otoliths are seen on her thumb.

2.1.1. Arctic grayling

The Arctic grayling (*Thymallus arcticus*) samples were harvested in Christal Creek (mine impacted) and Moose Creek (reference site) as indicated in the map below (Figure 5) during a field campaign conducted in the summer of 2012 to support a genomic study conducted by Dr. Caren Helbing from the University of Victoria and Jean Beckerton of Yukon Environment. Christal Creek is located within the Keno Hill Mine District and is recognized as being impacted by historical mining activity. Moose Creek, near Stewart Crossing, YT is an area which was not likely impacted by mining activity (Veldhoen et al 2014). The samples collected in Moose Creek are therefore considered as “reference” samples when compared to the “mine impacted” fish. Sample ID, fork length, total length and the mass of each fish collected is presented in Table 2 and Table 3. More information on the sampling protocol for these fish can be found in the publication issued from this genomic study (Veldhoen et al 2014).

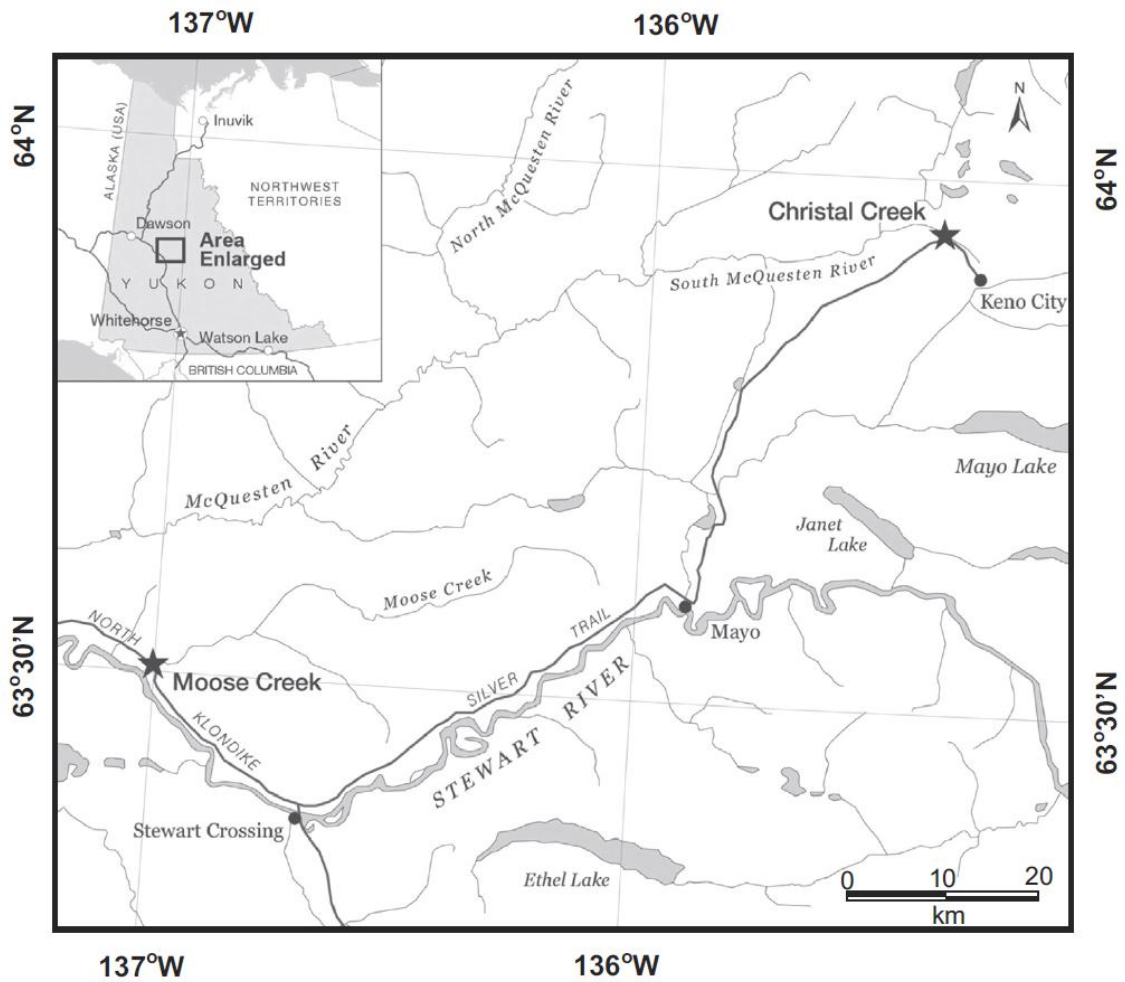


Figure 5 Moose Creek near Stewart Crossing and Christal Creek, near Keno, are indicated by stars (figure from Veldhoen et al 2014)

Table 2 Arctic Grayling (AG) sample data for Moose Creek

Fish species	Sampling location	Sampling date	Sample ID	Fish mass (g)	Fork length (mm)	Total length (mm)
AG	Moose Creek, YT	17-Aug-12	RF12	154	245	265
AG	Moose Creek, YT	17-Aug-12	RF3	363	315	340
AG	Moose Creek, YT	17-Aug-12	RF10	109	220	240
AG	Moose Creek, YT	17-Aug-12	RF8	182	265	285
AG	Moose Creek, YT	17-Aug-12	RF4	150	240	260
AG	Moose Creek, YT	17-Aug-12	RF7	>400	340	370
AG	Moose Creek, YT	17-Aug-12	RF13	82	190	210
AG	Moose Creek, YT	17-Aug-12	RF1	388	325	355
AG	Moose Creek, YT	17-Aug-12	RF14	57	175	195

Table 3 Arctic Grayling (AG) sample data for Christal Creek

Fish species	Sampling location	Sampling date	Sample ID	Fish mass (g)	Fork length (mm)	Total length (mm)
AG	Christal Creek, YT	12-Jul-12	F1/L1	154	265	280
AG	Christal Creek, YT	23-Jul-12	F2/L2	78	200	220
AG	Christal Creek, YT	23-Jul-12	F3/L3	78	195	210
AG	Christal Creek, YT	23-Jul-12	F4/L4	202	275	295
AG	Christal Creek, YT	23-Jul-12	F5/L5	400+	340	365
AG	Christal Creek, YT	23-Jul-12	F6/L6	170	260	275
AG	Christal Creek, YT	24-Jul-12	F7/L7	165	255	275
AG	Christal Creek, YT	16-Aug-12	F8/L8	70.4	199	217
AG	Christal Creek, YT	15-Aug-12	F9/L9	289	315	335
AG	Christal Creek, YT	16-Aug-12	F10/L10	161	239	379
AG	Christal Creek, YT	16-Aug-12	F11/L11	204	269	292
AG	Christal Creek, YT	16-Aug-12	F12/L12	314	321	345
AG	Christal Creek, YT	16-Aug-12	F13/L13	127	231	252
AG	Christal Creek, YT	16-Aug-12	F14/L14	296	308	334
AG	Christal Creek, YT	16-Aug-12	F15/L15	317	311	335
AG	Christal Creek, YT	16-Aug-12	F16/L16	111	215	231
AG	Christal Creek, YT	16-Aug-12	F17/L17	74.8	194	209
AG	Christal Creek, YT	16-Aug-12	F18/L18	77.7	200	217
AG	Christal Creek, YT	16-Aug-12	F19/L19	81.7	199	215
AG	Christal Creek, YT	16-Aug-12	F21/L21	44.7	163	179

2.1.2. Slimy sculpin

As element up-take can be species specific, slimy sculpin samples were harvested in October 2014 from two locations: Christal Creek (Figure 6, Figure 7), which has been impacted by mining activities in the past and from Haldane Creek, which is considered a reference site (Figure 8, Figure 9). Fish were collected using electrofisher and manual trap methods. The samples from Haldane Creek represent a background setting where no mining activities were recorded. Sampling dates, fish mass and total length (TL) are shown in the Table 4 and Table 5 below for the slimy sculpin captured in Haldane and Christal Creeks.



Figure 6 Slimy sculpin collecting in Christal Creek near Keno, YT.



Figure 7 Slimy sculpin harvesting in Christal Creek near Keno, YT.



Figure 8 Slimy sculpin harvesting in Haldane Creek near Keno, YT



Figure 9 Slimy sculpin harvesting in Haldane Creek near Keno, YT

Table 4 Slimy Sculpin (SS) sample data for Haldane Creek

Fish specie	Sampling location	Sampling date	Sample ID	Fish mass (g)	Total length (mm)
SS	Haldane Creek, YT	3-Oct-14	HC1	5.74	85
SS	Haldane Creek, YT	3-Oct-14	HC2	4.74	75
SS	Haldane Creek, YT	3-Oct-14	HC3	3.25	70
SS	Haldane Creek, YT	3-Oct-14	HC4	2.49	60
SS	Haldane Creek, YT	3-Oct-14	HC5	2.23	60
SS	Haldane Creek, YT	3-Oct-14	HC6	2.03	59
SS	Haldane Creek, YT	3-Oct-14	HC7	1.59	56
SS	Haldane Creek, YT	3-Oct-14	HC8	1.41	53
SS	Haldane Creek, YT	3-Oct-14	HC9	1.29	51
SS	Haldane Creek, YT	3-Oct-14	HC10	1.27	52

Table 5 Slimy Sculpin (SS) sample data for Christal Creek

Fish specie	Sampling location	Sampling date	Sample ID	Fish mass (g)	Total length (mm)
SS	Christal Creek, YT	3-Oct-14	CC1	6.49	89
SS	Christal Creek, YT	3-Oct-14	CC2	2.6	60
SS	Christal Creek, YT	3-Oct-14	CC3	1.77	55
SS	Christal Creek, YT	3-Oct-14	CC4	7.74	85
SS	Christal Creek, YT	3-Oct-14	CC5	11.57	95
SS	Christal Creek, YT	3-Oct-14	CC6	5.66	75
SS	Christal Creek, YT	3-Oct-14	CC7	6.58	84
SS	Christal Creek, YT	3-Oct-14	CC8	5.04	80
SS	Christal Creek, YT	3-Oct-14	CC9	5.11	73
SS	Christal Creek, YT	3-Oct-14	CC10	4.5	75

2.2. OTOLITH PREPARATION

Both right and left sagittal otoliths were removed from thawed fish in the laboratory. Otoliths were rinsed with deionized water and air-dried prior to storage. One otolith was archived and the other was sent to the University of Manitoba Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) facility (Figure 10). To prepare for LA-ICP-MS microchemical analysis, sagittal otoliths were embedded in epoxy resin. Transverse dorso-ventral sections were made across the nucleus with a precision Isomet saw to expose the core and primordial regions of the otolith. Sectioned pieces of the otoliths were mounted in 1 inch diameter polyethylene rings with epoxy resin, and polished to expose the nucleus completely (Friedrich and Halden, 2011). Prior to analysis, samples were ultra-sonic cleaned and rinsed with deionized water and allowed to air dry.

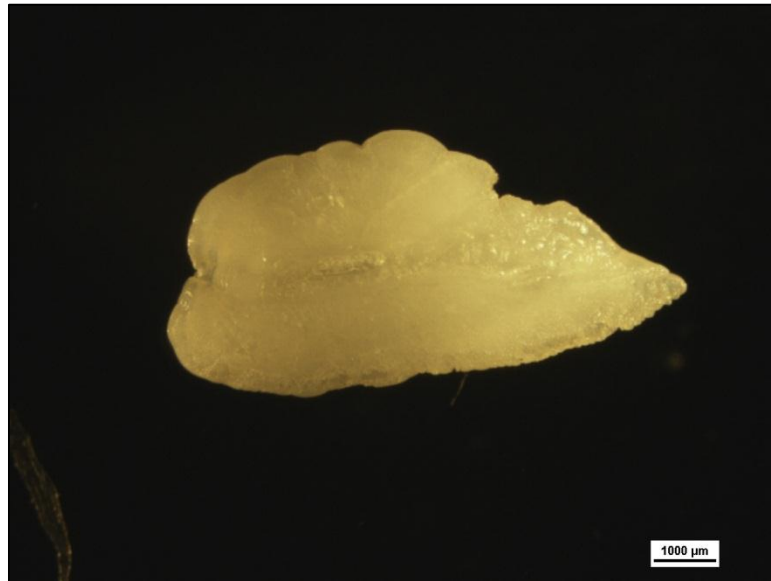


Figure 10 Arctic grayling otoliths before epoxy mounting (sample F11)

2.3. OTOLITHS ANALYSIS

LA-ICP-MS analysis was performed with a Thermo Finnigan Element2 ICP-MS coupled to Merchantek LUV 213 Nd: YAG laser. Laser scans were run through the otolith surface from edge to edge across the cores, perpendicular to annuli in time resolved mode. The path of the laser can be seen in the picture of the otoliths below (Figures 11 and 12). LA-ICP-MS analytical conditions are described in Appendix A (Tables 3 and 4). Prior to, and after analysis, the polished otoliths are imaged. The initial image can be used to age the fish and locate the line-scan for analysis and the image taken after analysis shows the exact location of the laser

analysis. Concentrations of trace elements and detection limits were processed with Iolite v2.21 Ref software and exported to commercial spreadsheet programs (Excel, Powerpoint and SigmaPlot) for final analysis and presentation. Typical detection limits are presented in Table 6 below. It should be noted that as a matter of practice and data processing, detection limits are determined for each individual element and otolith based on the actual signal compared to its background.

Table 6: Typical detection limits for trace elements in otoliths in this study in parts per million (ppm)

Element	Typical detection limit
Mn	0.1
Ba	0.04
Zn	0.3
Sr	0.9
Se	0.03
Cu	0.1
Pb	0.01
Ag	0.06
Cd	0.3

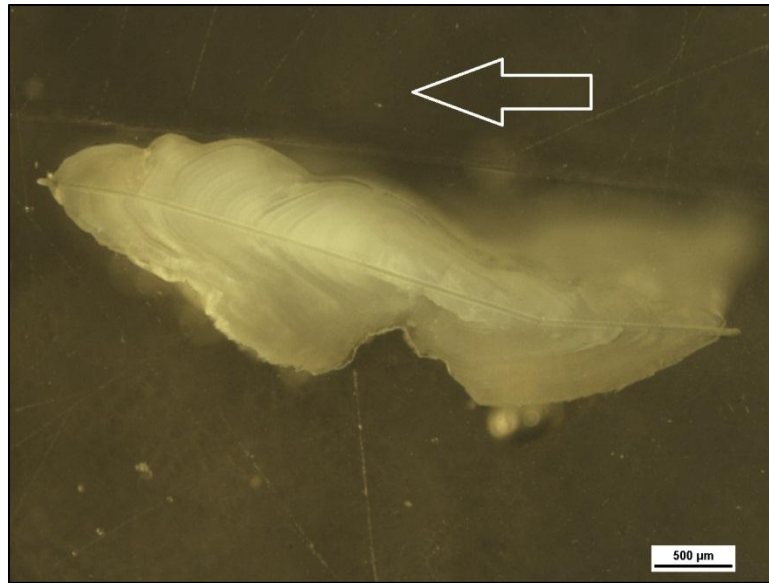


Figure 11 Arctic grayling otolith (sample F9) after LA-ICP-MS analysis (arrow indicates the direction of the laser path across the sample)



Figure 12 Slimy Sculpin otolith (sample CC7) after LA-ICP-MS analysis (arrow indicates the direction of the laser path across the sample)

2.4. WATER QUALITY MONITORING

An extensive water quality data set has been provided by Access Consulting Group for a site on Christal Creek. The site is located at the Silver Trail highway crossing just downstream of Christal Lake and provides analyses of over 90 parameters measured monthly between 2005 and 2014.

Moose Creek was sampled on August 17th 2012 during a field event when grayling were captured to support Dr. Caren Helbing's genomic study.

Haldane Creek was sampled on October 3rd 2014 during the sculpin sampling event. In addition, historical water quality data was provided by Access Consulting Group which had sampled this location on July 25th 2011. Both data sets have been utilized in this study.

3. RESULTS

3.1. ARCTIC GRAYLING OTOLITHS MICROANALYSIS

The results obtained from the grayling show Mn, Ba, Zn, Sr, and Se were present at levels greater than analytical detection limits (Table 6); in many cases Cu, Pb, Ag, Cd were not determined or were at or below detection limits. Strontium concentrations were in the range of 300 to ~1000 ppm, and variations in Sr concentrations are annual, corresponding to the annular structure of the otoliths. An example is presented in Figure 13. Zinc was present between ~10 and ~400 ppm, these concentrations variations were also annual (Figure 14). Barium fluctuated between ~5 and 40 ppm (Figure 15). Manganese also showed annual variation with typical levels varying between 10 and ~400 ppm (Figure 16). Some individuals showed short term Mn variations (i.e. < 1 year and occurring within an annulus) concentrations were up to ~400 ppm. Selenium concentrations were low (~500 parts per billion, ppb) in the core or primordial region of the otoliths with elevated concentrations ~ 2 ppm corresponding to summer regions of up to two annuli (Figure 17).

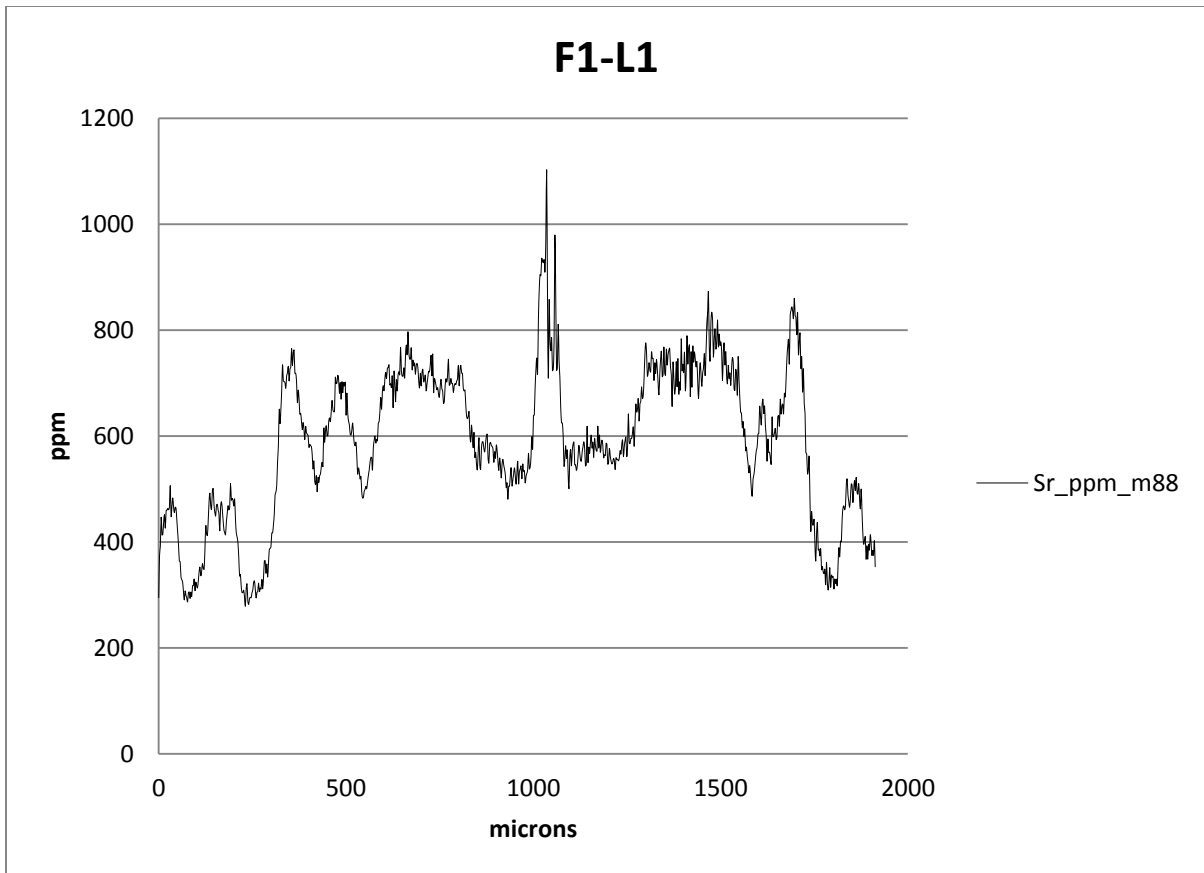


Figure 13 Strontium profile of the F1-L1 grayling individual

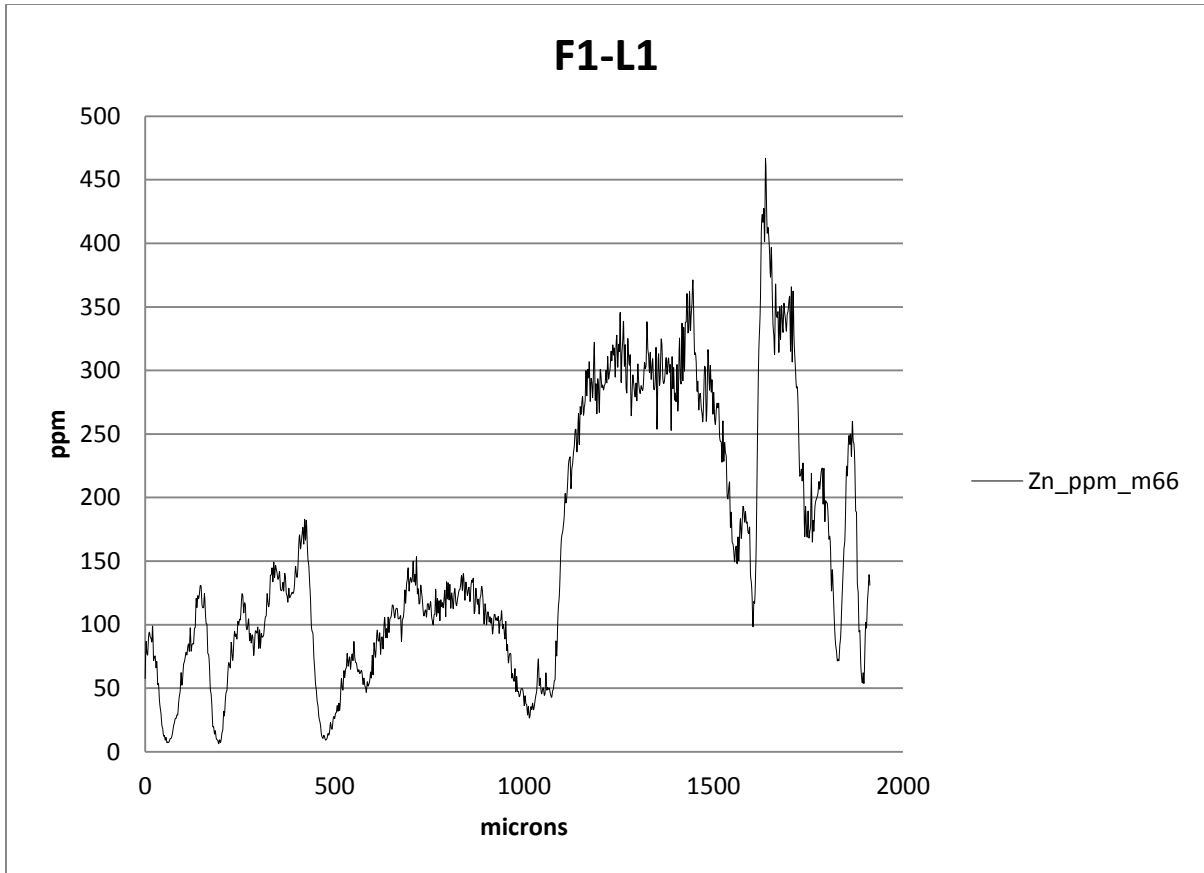


Figure 14 Zinc profile of the F1-L1 grayling individual

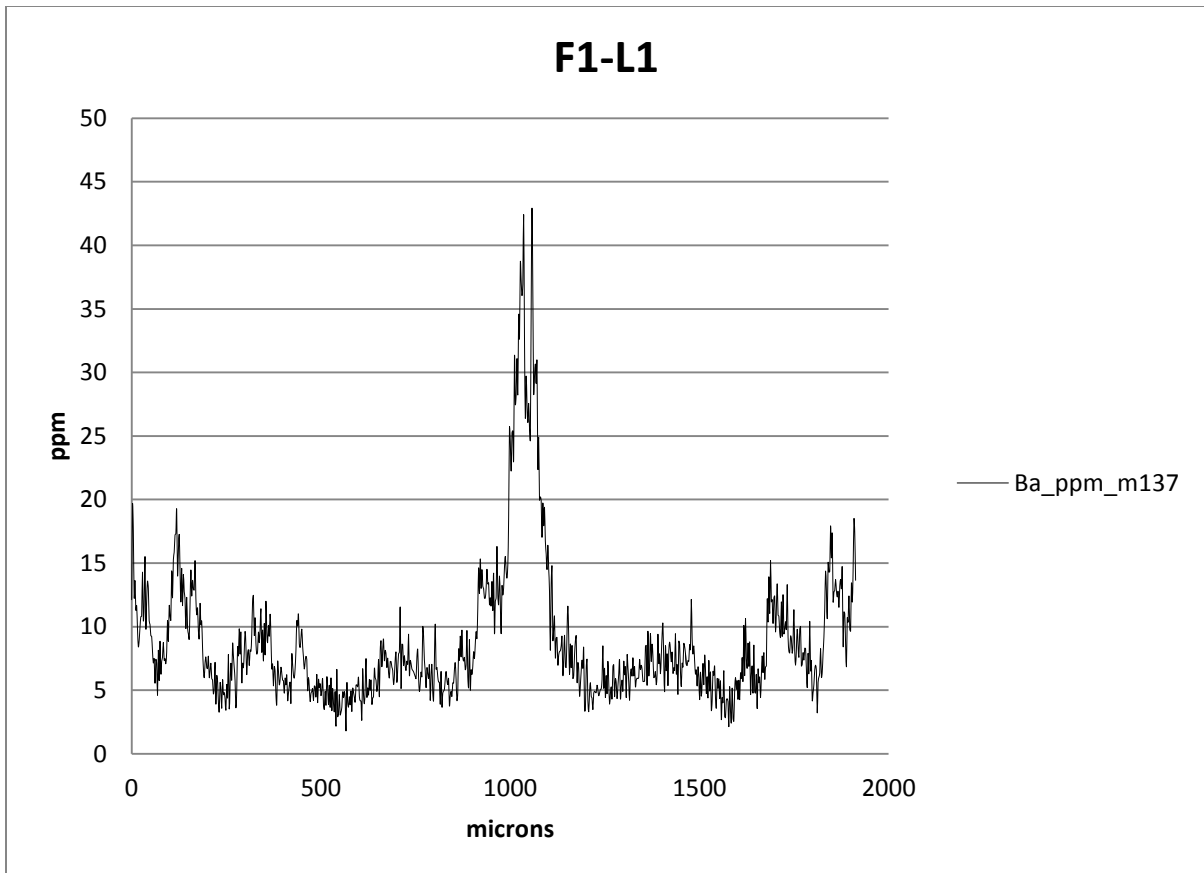


Figure 15 Barium profile of the F1-L1 grayling individual

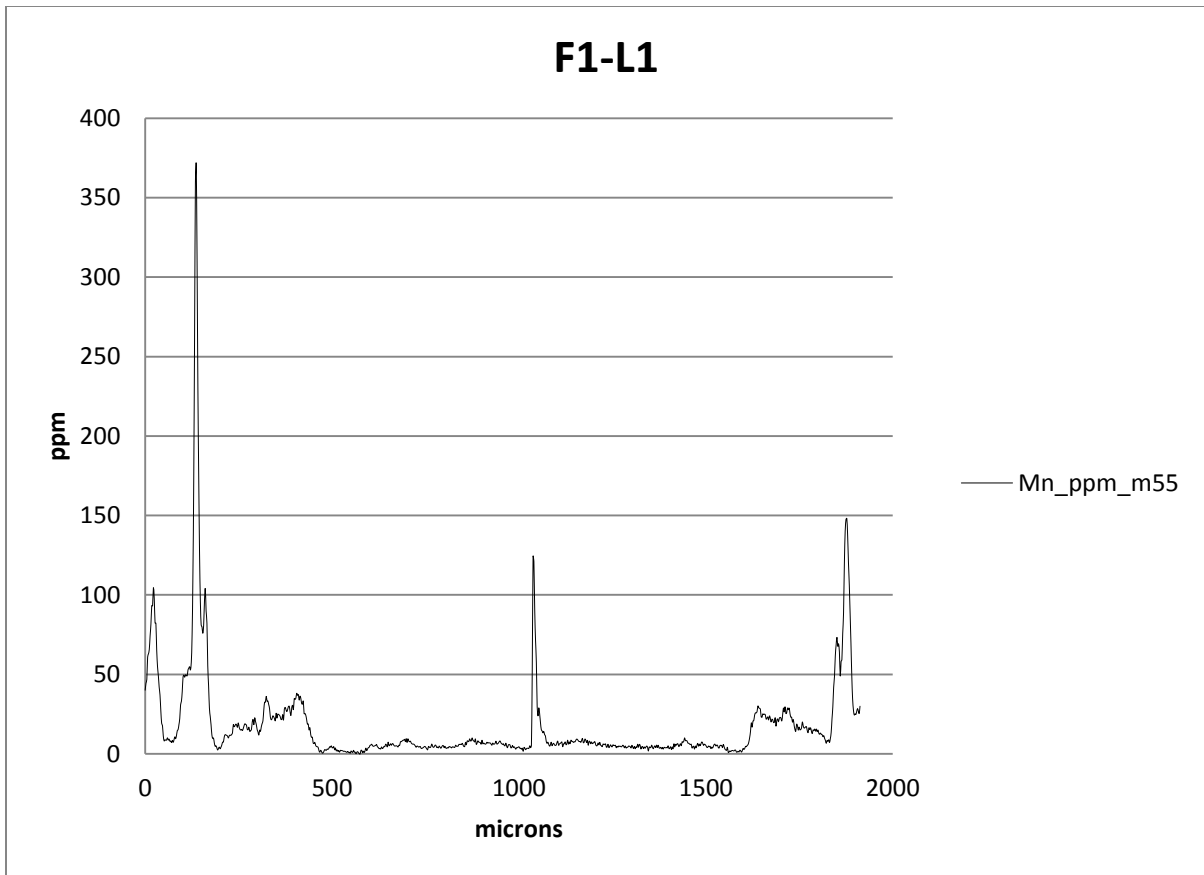


Figure 16 Manganese profile of the F1-L1 grayling individual

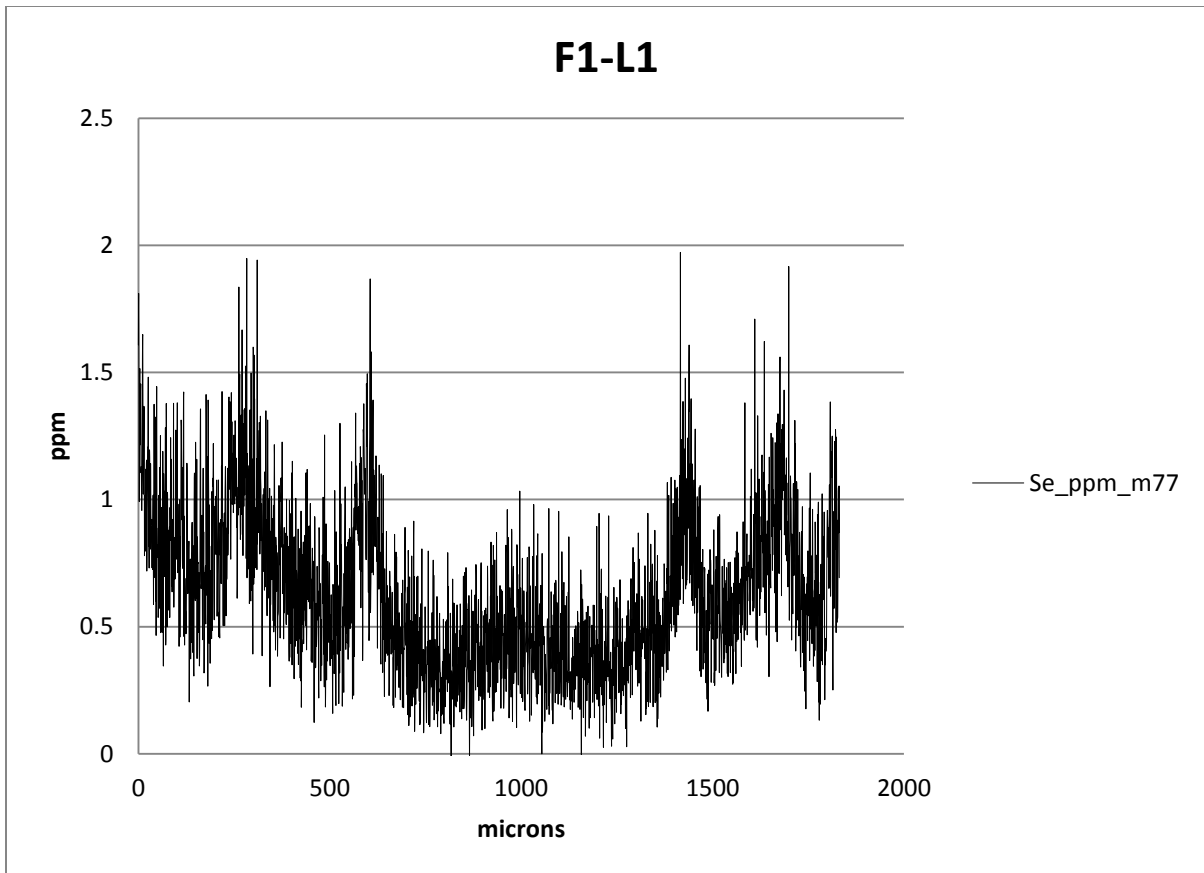


Figure 17 Selenium profile of the F1-L1 grayling individual

The reference grayling from Moose Creek ranged in age from 4 to 8 years and showed similar element variations to those from Christal Creek, but with some subtle variations. Strontium is present at concentrations around 700 ppm, and constant throughout the life of the fish. This is contrast to the annual variation seen in Christal Creek fish.. Potential reasons for this difference are discussed later. Zinc variations are annual but concentrations typically have maximum levels around 200 ppm. Mn distributions showed distinct well-resolved annual variation with maximum concentrations typically on the order of 70 ppm with one fish as high as 100 ppm; these maximum concentrations occurred in the outer (most recent) annuli. Mn concentrations in the primordial region (first year of life) were typically less than 10 ppm except for one fish that showed concentrations of 40 – 60 ppm; this fish (RF 14) also showed finely resolved Mn variations (2 to 3 peaks) within annuli. The Moose Creek fish were also different in that they showed the presence of detectable copper. Here the copper was typically higher in the core and or primordial region and was as high as 8 ppm (Figure 19).

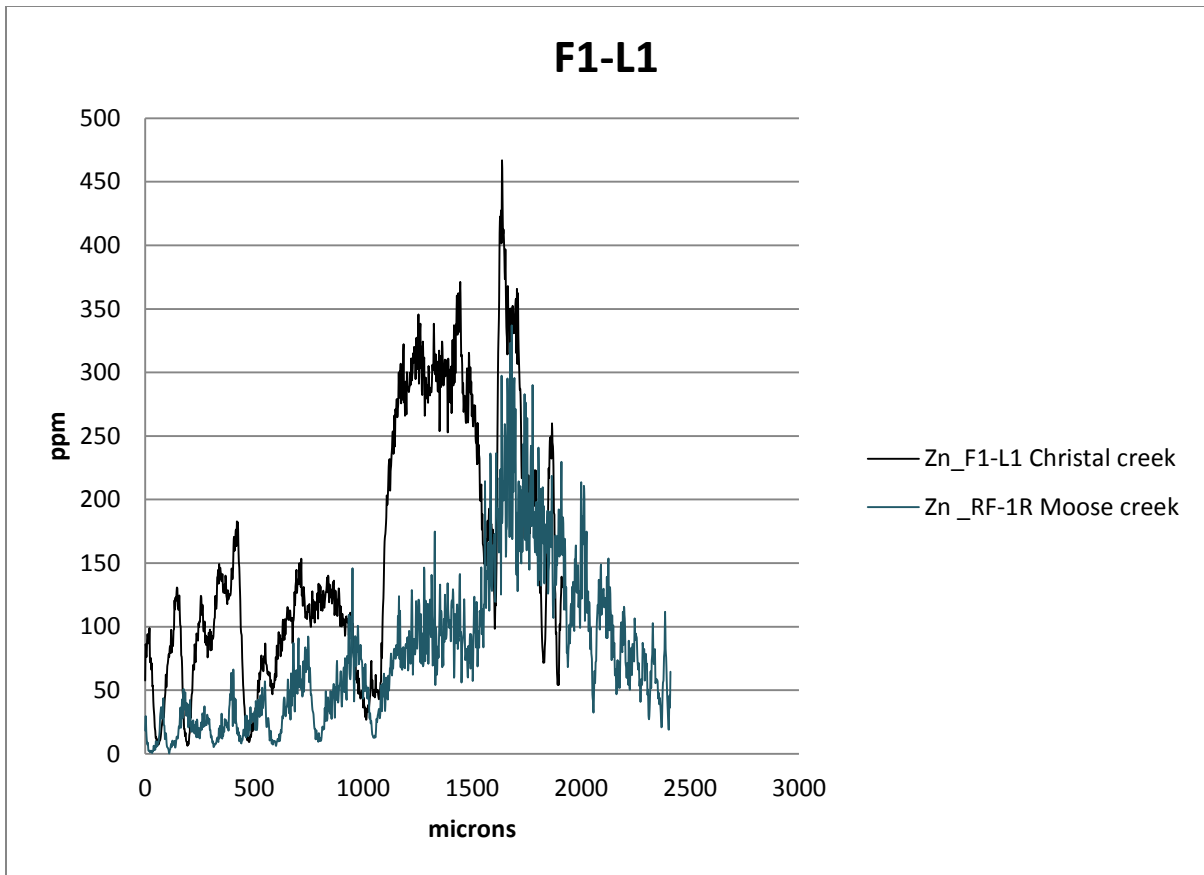


Figure 18 Zinc profiles for two individuals from Christal Creek and Moose Creek (F1-L1 and RF-1R)

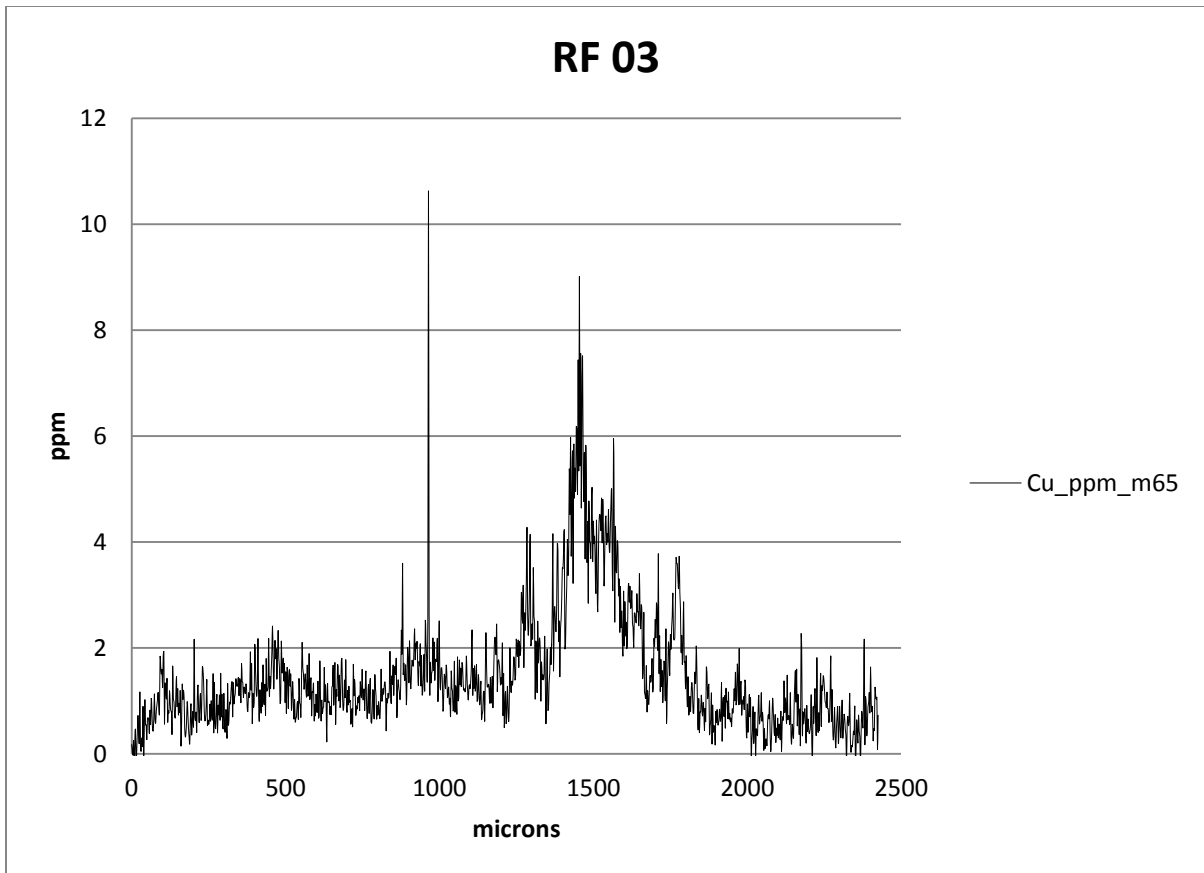


Figure 19 Copper profile of the F1-L1 grayling individual

Figure 20 shows typical element concentration data plotted initially in Excel and overlaid on an optical image of the analyzed otolith. It shows the oscillatory (changing concentration) nature of much of the data, the absolute element concentrations at any point on the line-scan, and the spatial correspondence between the element concentration and the annular structure of the otolith. This particular fish is estimated to be about 3½ years old; it would have been a juvenile fish in 2010 and caught in the summer or fall of 2013. Thinner, darker rings in the optical image typically mark winter and also periods of time when the otolith is growing slowly; it should be noted that the summer bands are brighter and wider (on the order of 200 µm.) These lighter bands correlate with higher concentrations of Zn, Sr, Mn, Ba and Se. Manganese is distinguished in that it shows double peaks signaling two periods of elevated Mn uptake (Figure 21).

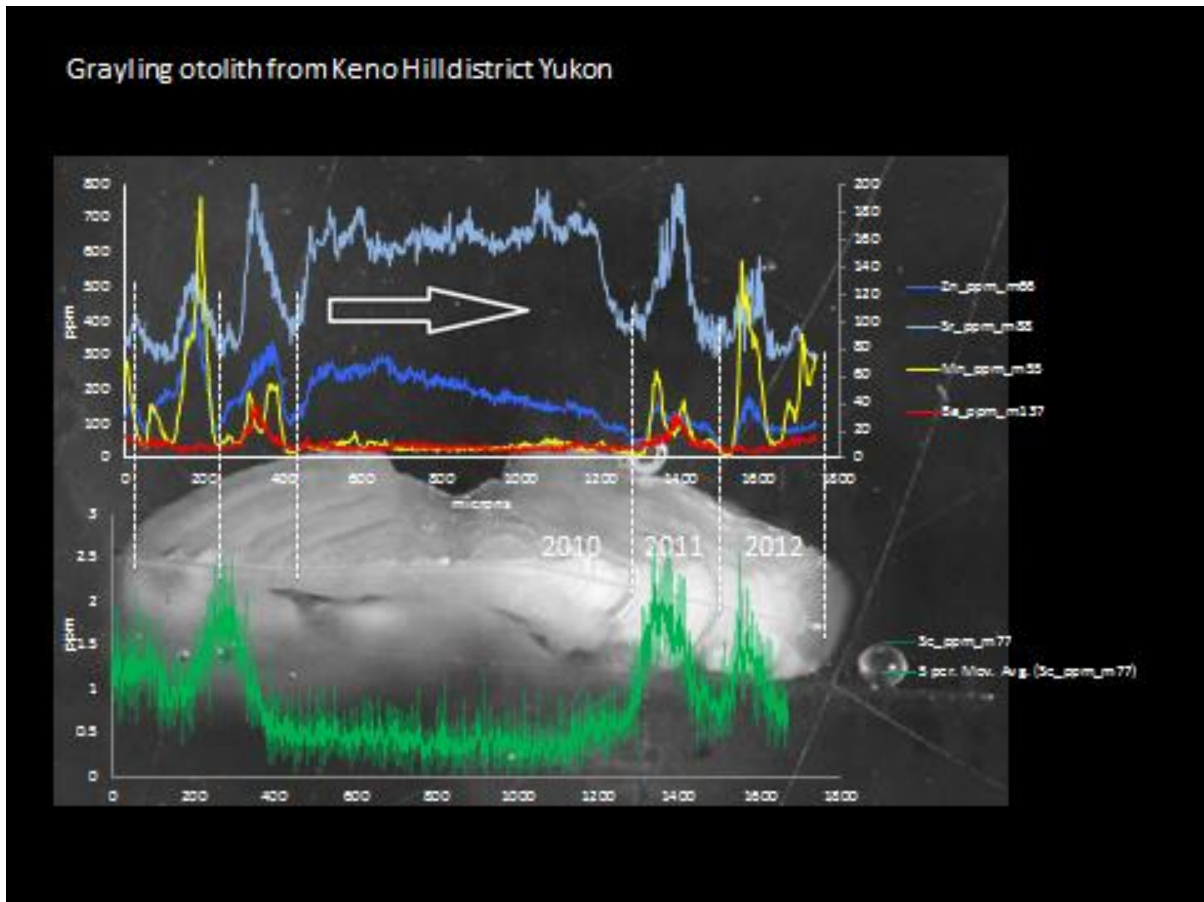


Figure 20 LA-ICP-MS linescan data for Zn (dark blue), Sr (light blue), Mn (yellow), Ba (red), and Se (green) overlaid on a reflected light optical image of a grayling otolith. The annular structure, narrow dark bands indicating slow winter growth and lighter thicker bands indicating faster summer growth, indicate the fish went through its larval stage in 2010 and was caught in 2013 (note: metal values are expressed in ppm)

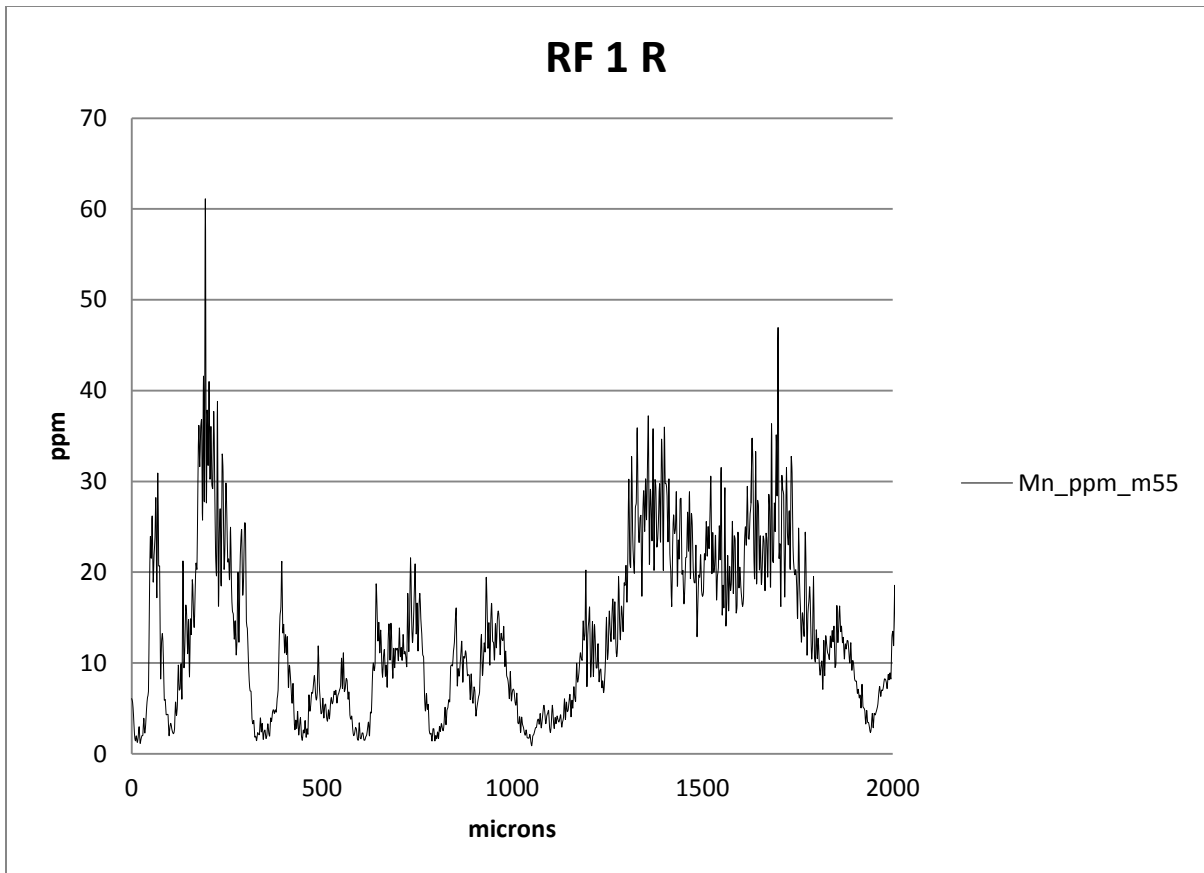


Figure 21 Manganese profile of the F1-L1 grayling individual showing double annual peaks

3.2. SLIMY SCULPIN, GRAYLING OTOLITH MICROANALYSIS

As has been suggested (Limburg and Elfman, 2010) and shown by analysis (Friedrich and Halden, 2010, 2008; Halden et al., 2000) that element uptake can be species-dependent. The ecological niche occupied by slimy sculpin is very different to that of grayling so samples of slimy sculpin from Christal Creek and Haldane Creek (reference location) were analyzed for comparison with grayling.

Strontium concentrations from Christal Creek sculpin otoliths varied between 150 to 300 ppm. They did not show distinct annual variations and were typically flat at around 200 ppm. Manganese showed distinct annual and sub-annual variation and ranged between 1-2 ppm and ~ 80 ppm. Barium showed distinct but somewhat subdued annual variation between 2 -15 ppm and 10 -35 ppm. Figure 22, Figure 23 and Figure 24 are examples of Sr, Mn and Ba profiles for 1

sculpin individual. Zn was virtually absent being at or below detection limits, which were typically around 0.3 ppm. Copper was present in several Christal Creek sculpin otoliths and ranged from 0.5 ppm (LOD 0.1 ppm) and 2 -3 ppm (Figure 25). For the fish with the higher Cu concentrations there was some correspondence between the higher concentrations and the annular structure in the otoliths, although the Cu concentrations measured were low and not nearly as well expressed or correlated as is seen in the Pb and Mn scans.

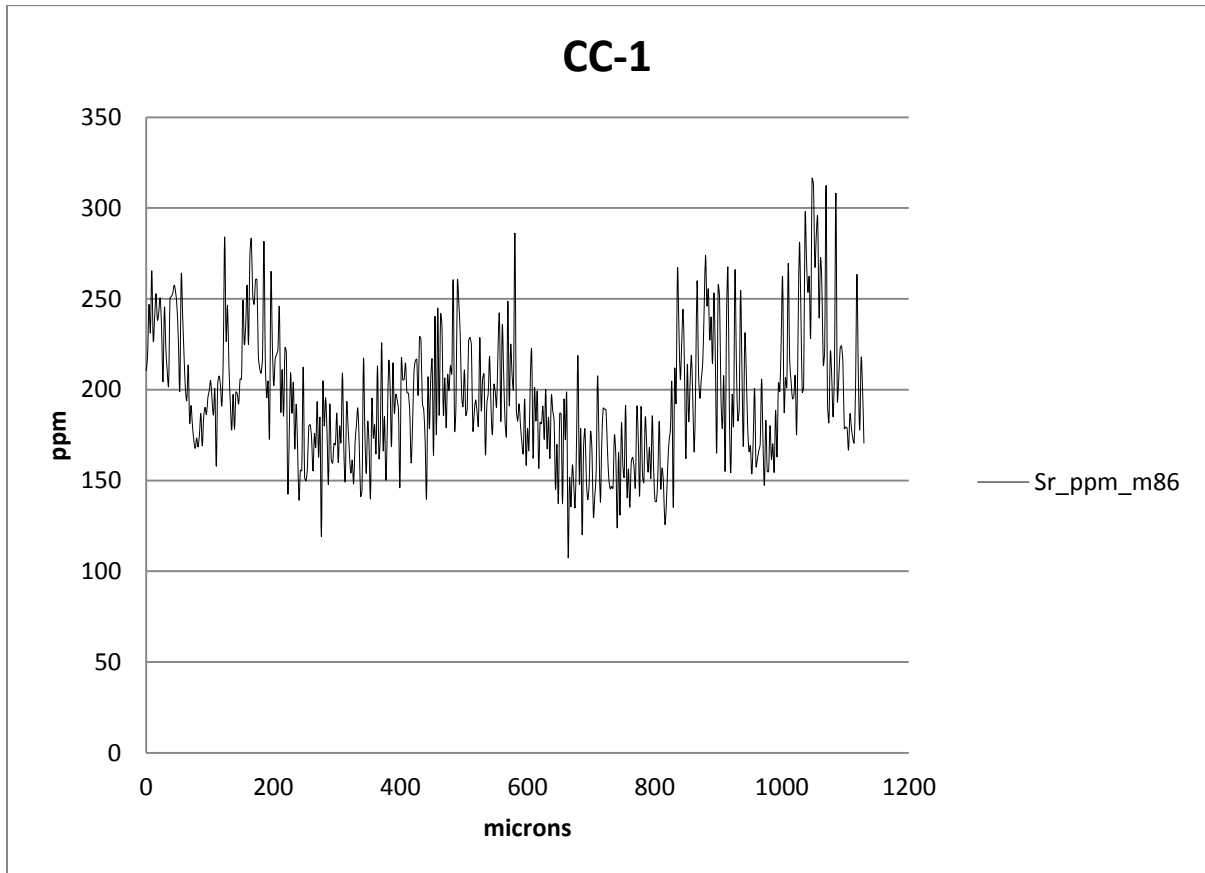


Figure 22 Strontium profile of the sculpin CC-1

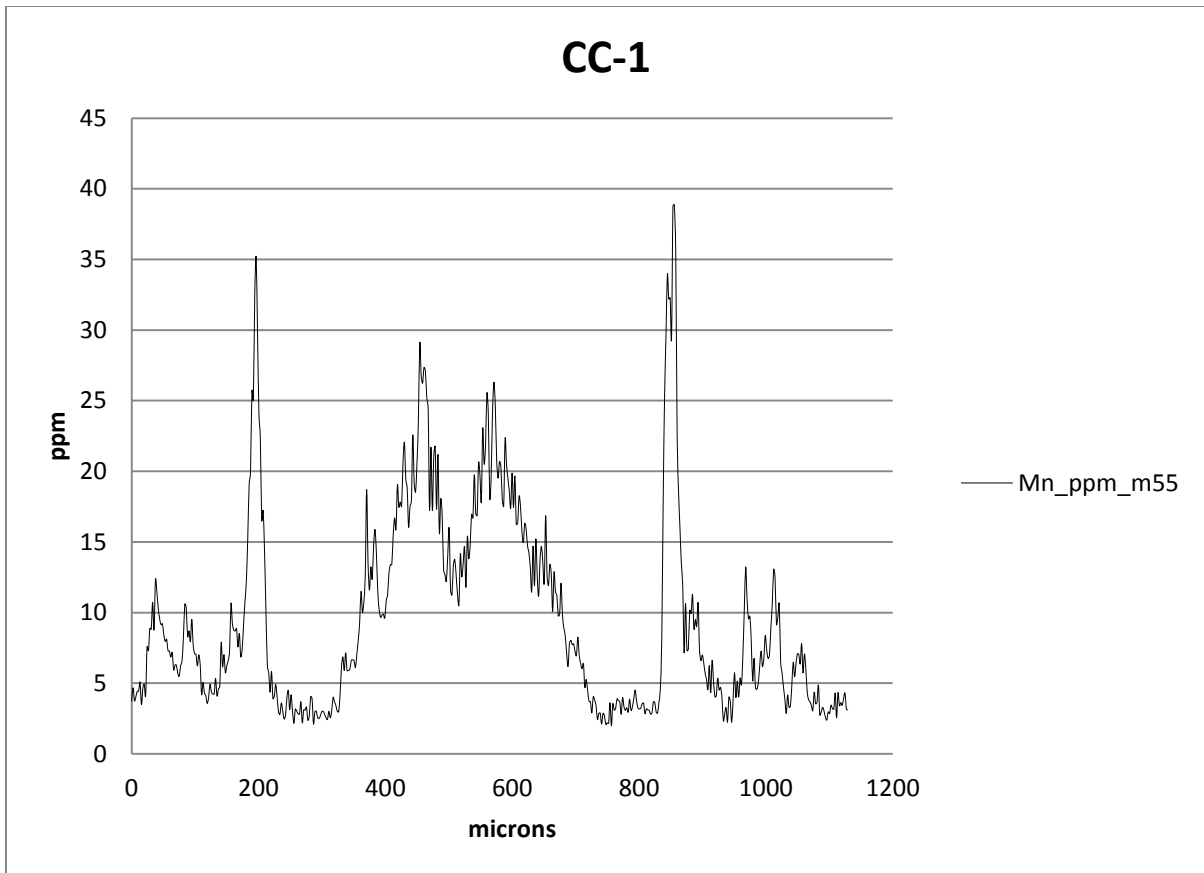


Figure 23 Manganese profile of the sculpin CC-1

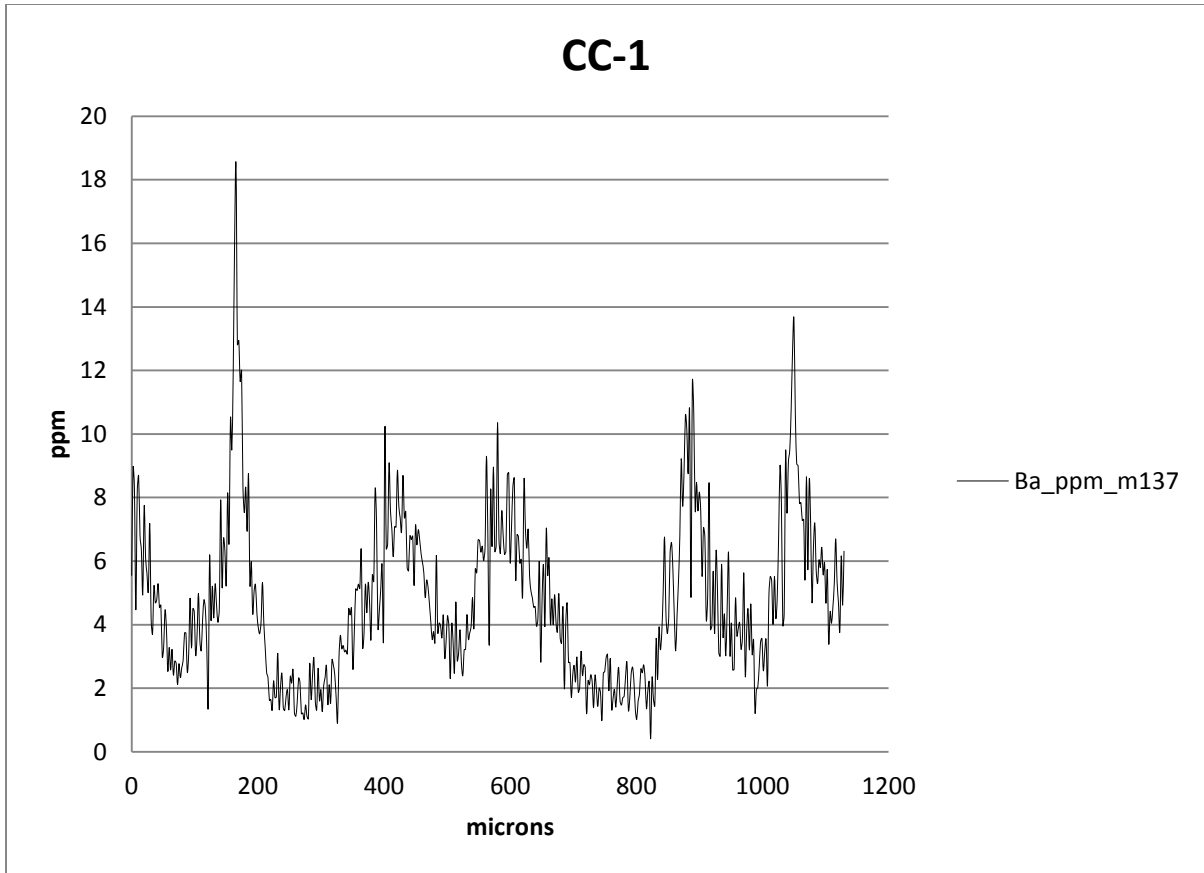


Figure 24 Barium profile of the sculpin CC-1

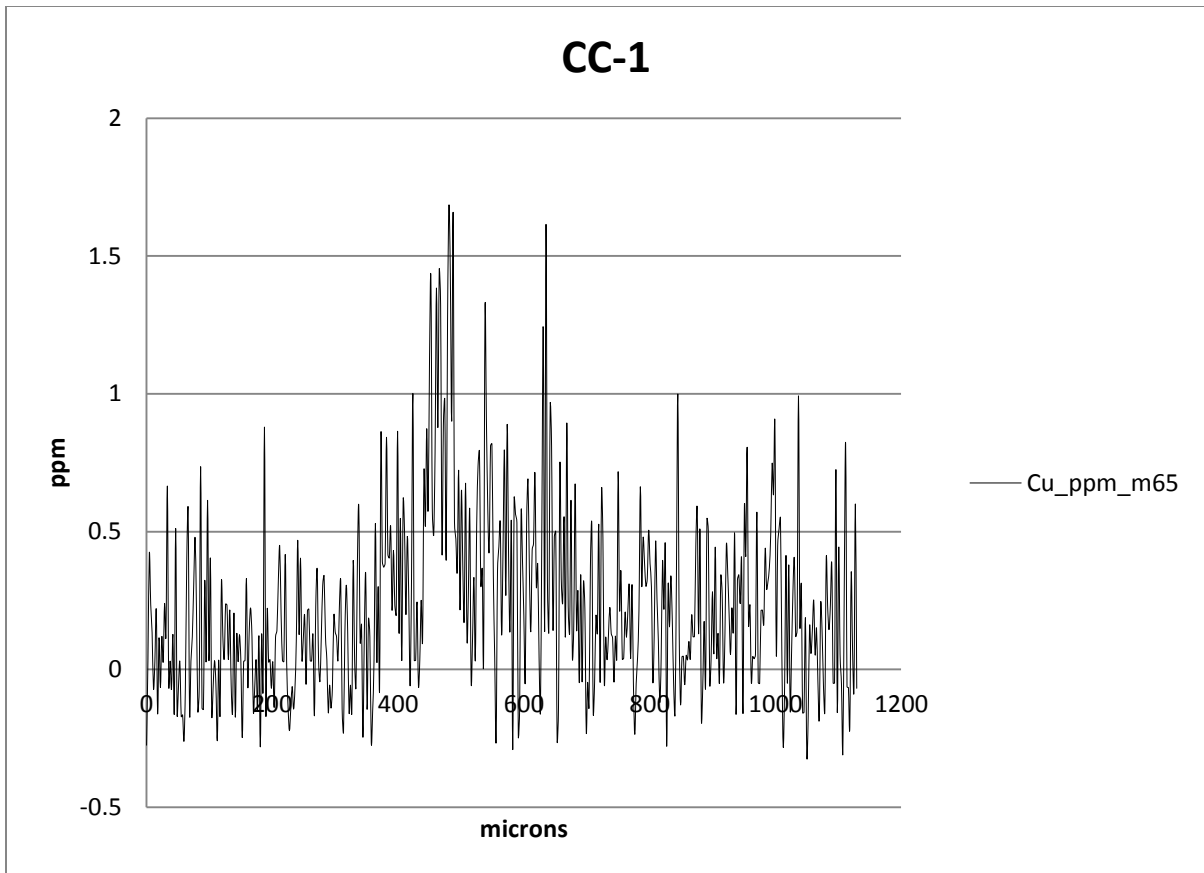


Figure 25 Copper profile of the sculpin CC-1

Lead was present in all the Christal Creek sculpin otoliths. Figure 26 shows typical element concentration data plotted initially in Excel and overlaid on an optical image of the Cristal Creek sculpin otolith. The detection limit for Pb was ~ 0.01 ppm. The Pb line scans showed prominent peaks with peak concentrations varying between 0.3 and 0.8 ppm. The Pb peaks correspond to winter growth periods in the optical images of the otoliths and the lower, or base level of Pb concentrations, were just above the detection limit. The summer bands correlate with elevated Mn, similar to what was observed in the grayling.

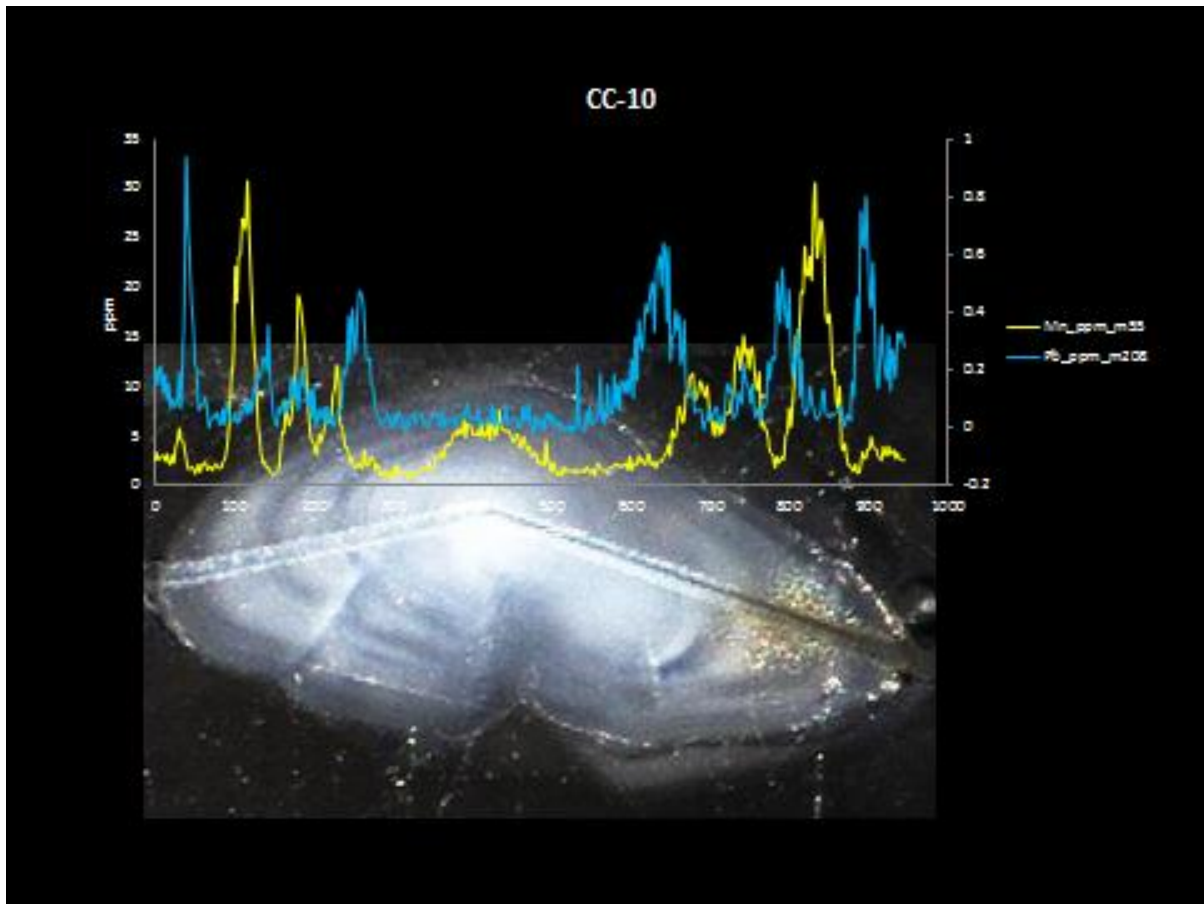


Figure 26 LA-ICP-MS linescan data for Mn (yellow), and Pb (blue) overlaid on a reflected light optical image of a 3 year old sculpin otolith from Christal Creek. The higher Pb concentrations correspond to narrow dark winter bands and the elevated Mn concentrations correlate with summer growth (note: metal values are expressed in ppm).

The sculpin otolith samples from Haldane Creek (Figure 27) have Sr profiles that showed little distinct annular variation with concentrations typically about 550 ppm. Mn concentrations showed annual variation between about 2 ppm and 80 ppm and were consistently high or had the highest concentrations in the primordial region. Annual Ba variations were between ~15 and 35 ppm, and Cu concentrations were from as high as 2 ppm (usually in the primordium) down to detection limits (LOD~ 0.1 ppm). Lead concentrations in the 10 samples were uniformly low, at or near detection limits (~ 0.2 ppm). Figure 27 shows a typical reflected light image of an otolith from a Haldane Creek (HC) sculpin with line scan overlays showing the typical distributions of Pb and Mn. The Haldane Creek otoliths are characterized by elevated

Mn concentrations in the primordium or on the margins of the primordium and low Pb concentrations throughout.

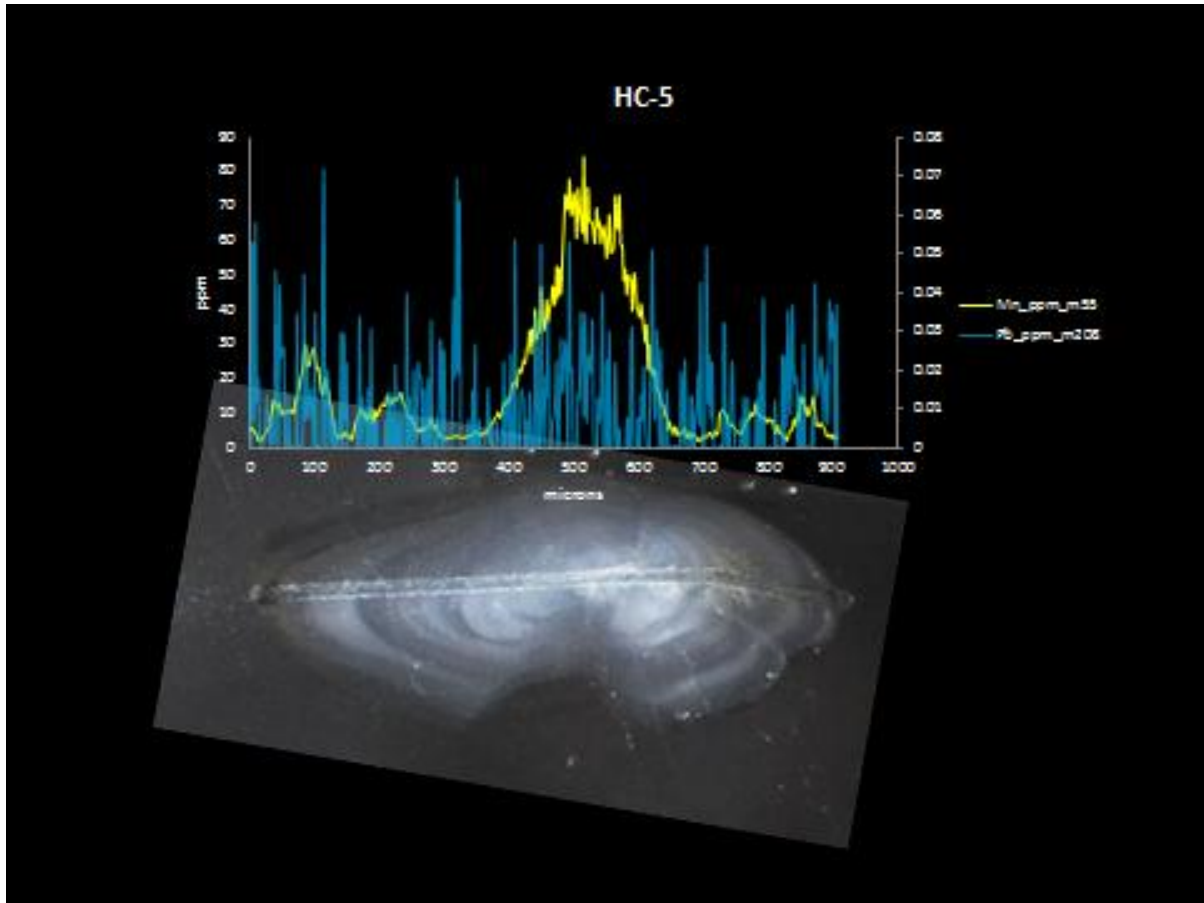


Figure 27 LA-ICP-MS linescan data for Mn (yellow), and Pb (blue) overlaid on a reflected light optical image of a 3 year old sculpin otolith from Haldane Creek. In comparison to Christal Creek the Pb concentrations are very low approaching what might be background levels. Mn is typically elevated in the primordial region for the Haldane Creeksamples with periodic increases also showing up in later annuli, but at a level lower than the primordium (note: metal values are expressed in ppm).

3.3. WATER QUALITY DATA

Water quality data for Christal Creek is extensive and made available through Access Consulting Group (D. Petkovich). Those portions that are used here are summarized briefly in Appendix A. The archival character of the information stored in otoliths now can take on additional significance with comparative water chemistry data. The first set of grayling from Christal Creek were caught in 2012. They were typically 3 – 4 years old so the “time slice” of water quality data that is relevant to their life history spans 2010 to 2013. This data was separated from the main water quality data set and plotted at a similar temporal scale (X-axis) and units to the data collected for otoliths. Figure 28 shows the line scan data (in ppm) collected from a grayling otolith and the water chemistry data for the same period in mg/L. Although mg/L and ppm are comparable, the absolute concentrations should not be compared because absolute concentrations of trace elements in otoliths reflect a combination of environmental and physiological processes. It is more appropriate to compare relative patterns over time. Nonetheless, it is interesting and useful to note: 1) Sr concentrations in the water are relatively constant and vary significantly in the otolith; 2) a significant peak in Pb concentration in the water is not recorded in the otolith; 3) variations in Zn in the water occurred in 2010 and 2011 whereas there are annual peaks of Zn in the otolith in both 2011 and 2012; and 4) double peaks in Mn concentration are observed in the otolith in both 2011 and 2012. Water chemistry data for the same period from the South McQuesten River is also shown at the same scale. The concentrations of Sr, Ba, Zn and Pb are very similar to those observed in Christal Creek, Mn is noticeably lower. The potential significance of these possibly conflicting observations will be discussed later.

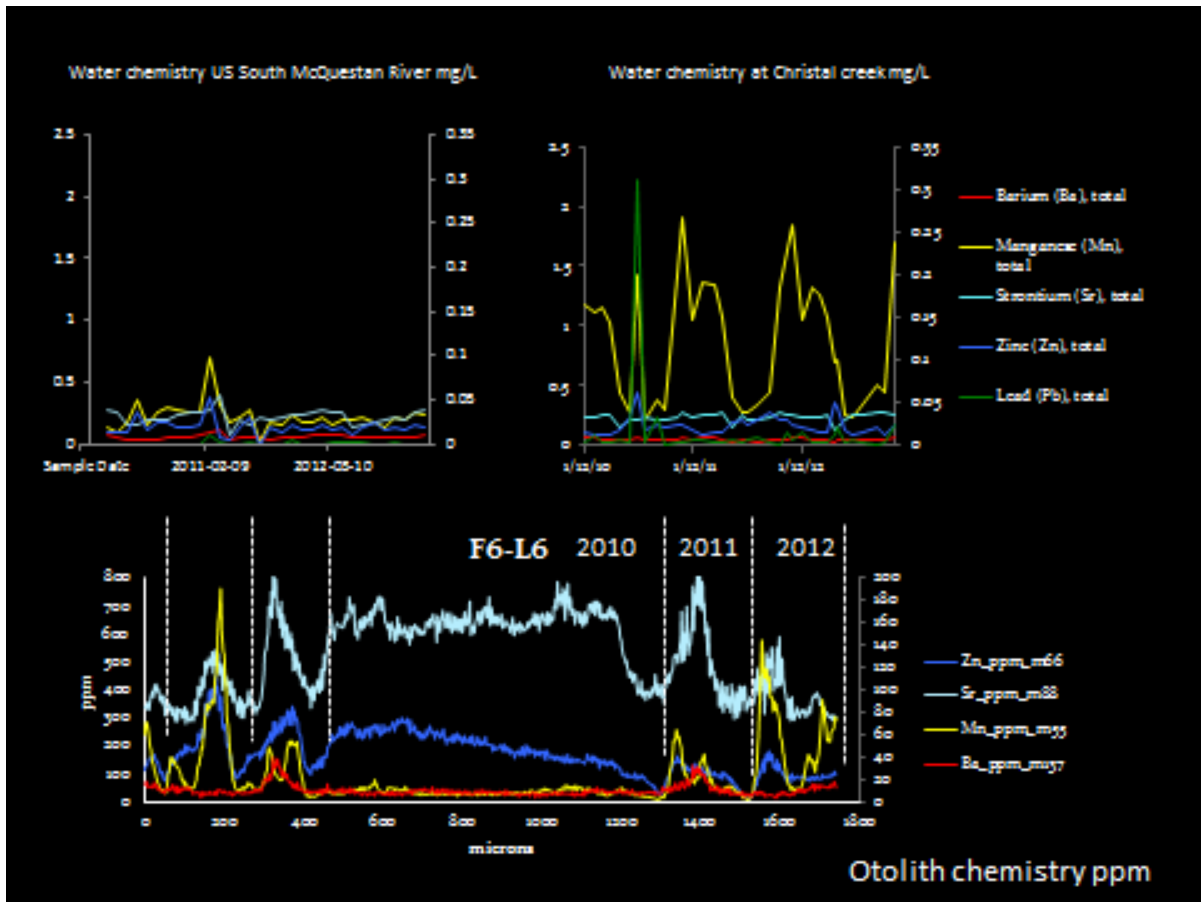


Figure 28 Line scan data for Zn, Sr, Mn and Ba in ppm from Figure 20 corresponding to the time period 2010 to 2013. Also shown are two graphs with the water quality data in mg/L from South McQuesten River and Christal Creek for the same period. A shared feature of the two data sets from the Christal Creek otolith and water quality data are the prominent double Mn peaks in 2011 and 2012.

Additional water quality is available for Haldane and Christal Creeks for 2013 addressing the sampling period for the sculpin with a comparison between Christal Creek downstream from Keno Hill and Haldane Creek as the reference system. These samples were collected in May, July, and October of 2013. Figure 29 shows water data from May to November for the creeks. Haldane Creek as the reference shows all elements as being low in concentration and flat throughout the measuring period. Christal Creek on the other hand shows significantly higher concentrations of Pb and Mn as well as variable concentrations for these two elements. The Mn data appears to show two periods of elevated concentration which might correspond with the observation made in some otoliths that there were two Mn peaks within a summer annulus.

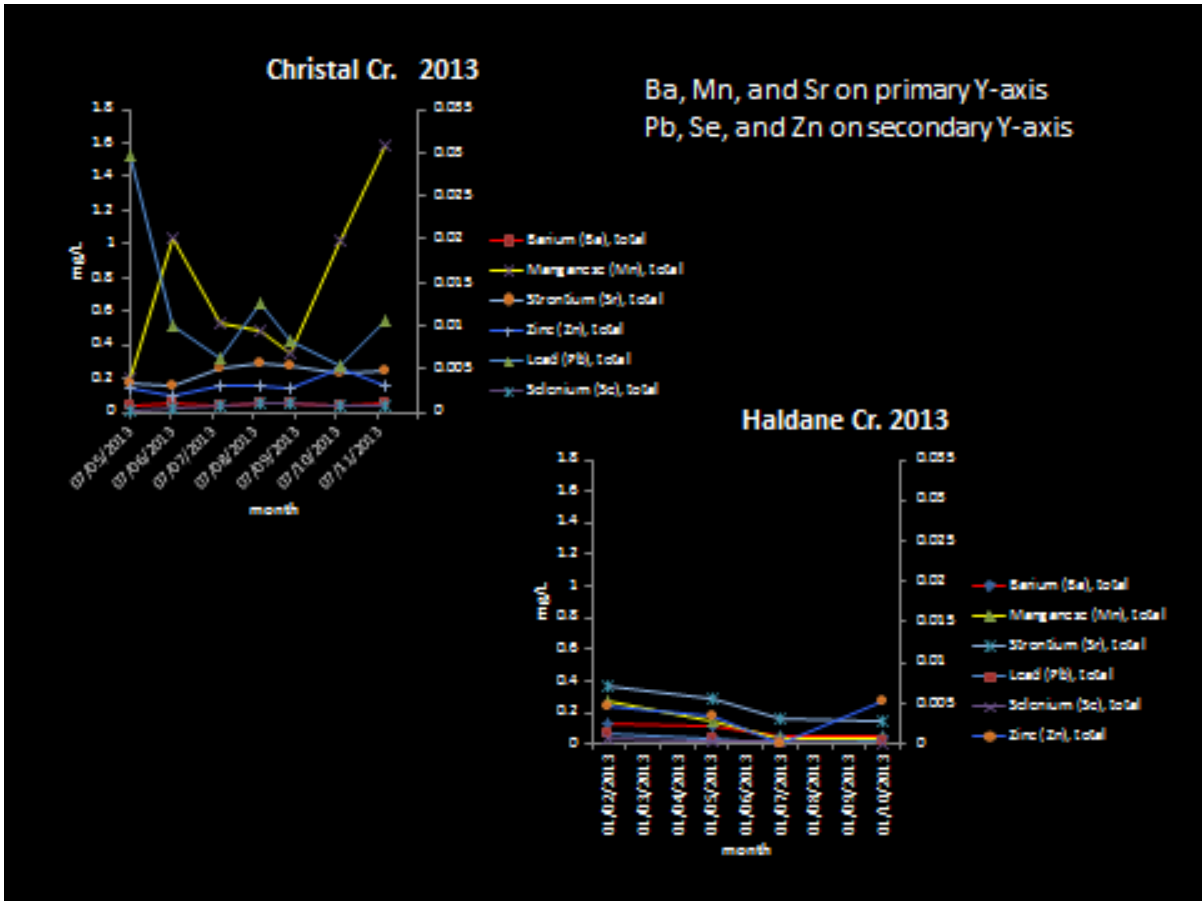


Figure 29 Comparison of water quality data from Christal and Haldane Creeks for 2013. Trace element concentrations are lower in Haldane Creek and Mn and Pb in particular are higher in Christal Creek

3.4. TRACE ELEMENT COMPARISONS BETWEEN INDIVIDUAL OTOLITHS AND LOCATIONS

Time resolved LA-ICP-MS otolith microchemistry generates a significant amount of data encompassing a range of elements, fish species and locations. Element comparison within and amongst species and locations is possible using box plots showing the range of the data and how much of it falls between certain concentration levels. Figure 31 through Figure 39 show a series of box plots (ppm v. sample) for the grayling and sculpin otoliths from Christal, Moose and Haldane Creeks; the lower boundary of the boxes in each plot represents the 25th percentile, and upper boundary the 75th percentile. Whiskers above and below the boxes indicate the 90th and 10th percentiles respectively. Within the boxes the median is shown by a thin line and the mean by a thick or dotted line. The plots capture the mean, median, and weighting of the data, along with the overall range of the data.

3.4.1. Arctic grayling

Although the overall range of Sr from grayling otoliths between Moose and Christal Creeks is similar (Figure 31 and Figure 31), the mean and median Sr values from Moose Creek are quite different; the mean, median and overall range values show much more scatter from Christal Creek. An estimated mean of the means for Moose Creek was around 703 ppm with a standard deviation of 21 ppm (2σ) and, the Christal Creek data was similar at ~591 ppm but with a standard deviation of 88 ppm (2σ). This difference may reflect the sampling location or the extent to which the fish move in and out of a particular creek. The Mn data for Christal Creek grayling (Figure 33) show distinctly greater variability than that of the Moose Creek grayling (Figure 32). At the lower end of the concentration scale they have similar means, medians and are similar up to the 75th percentile level of the data. Beyond that, very high concentrations, up to ~380 ppm, are restricted to the Christal Creek fish. In regard to Zn (Figure 34 and Figure 35), the mean Zn content in the Moose Creek grayling is about 110 ppm and, the upper range extends to ~500 ppm; the range encompassed by the 25th and 75th percentile of the data is around 100 ppm. Mean Zn contents in Christal Creek grayling is somewhat higher at about 150 ppm, the 25th to 75th percentile data is about 170-200 ppm and the extreme is around 500 ppm. Overall, Christal Creek data shows much greater variability as was the case for the Mn data.

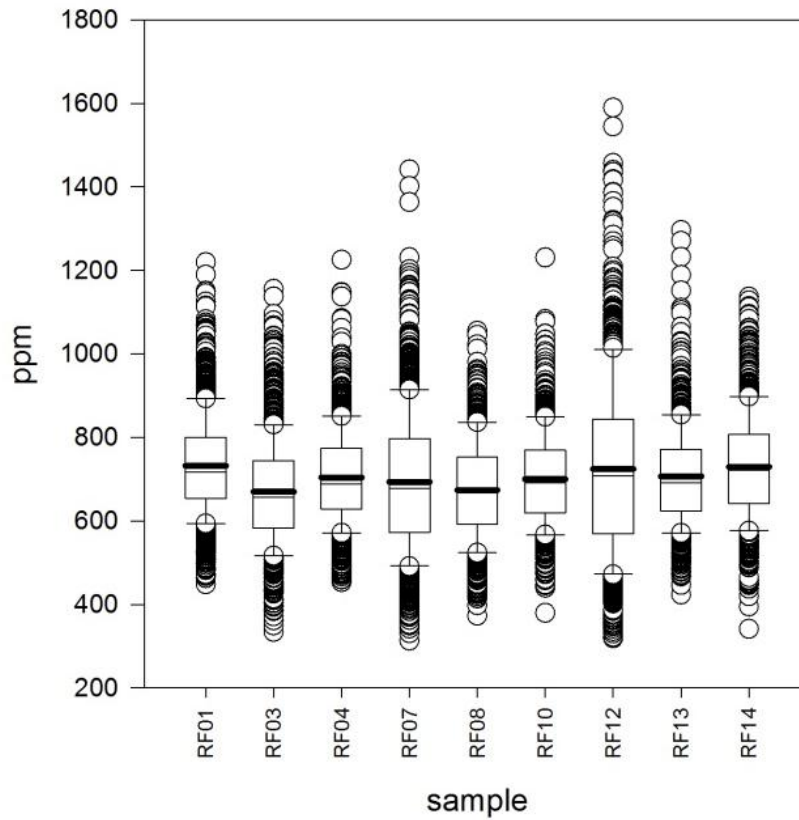


Figure 30 Plots showing Sr data for the grayling from Moose Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

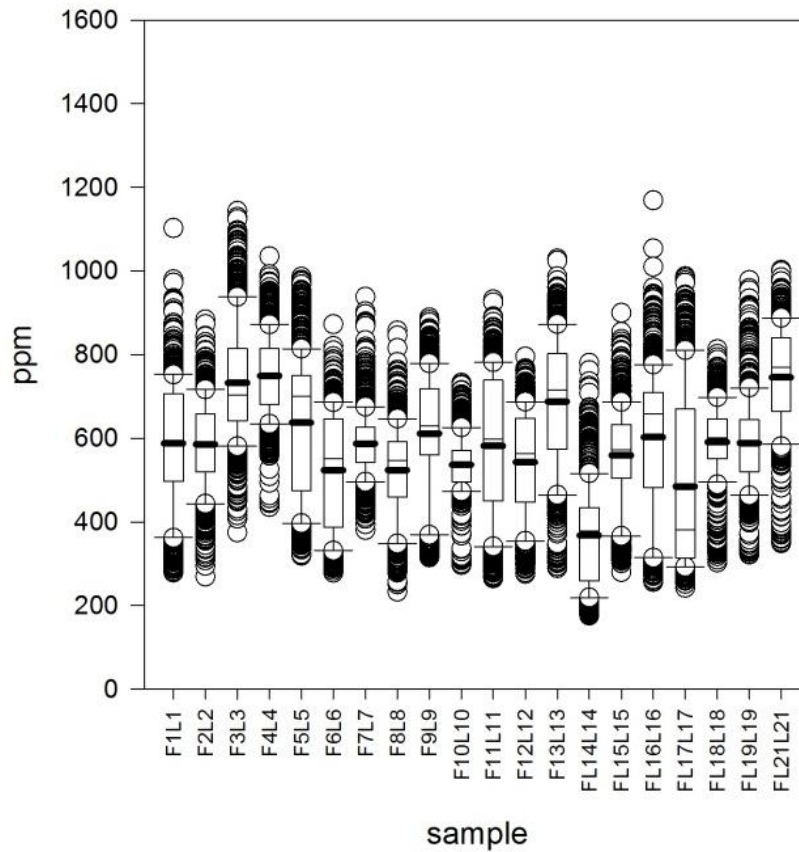


Figure 31 Plots showing Sr data for the grayling from Christal Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

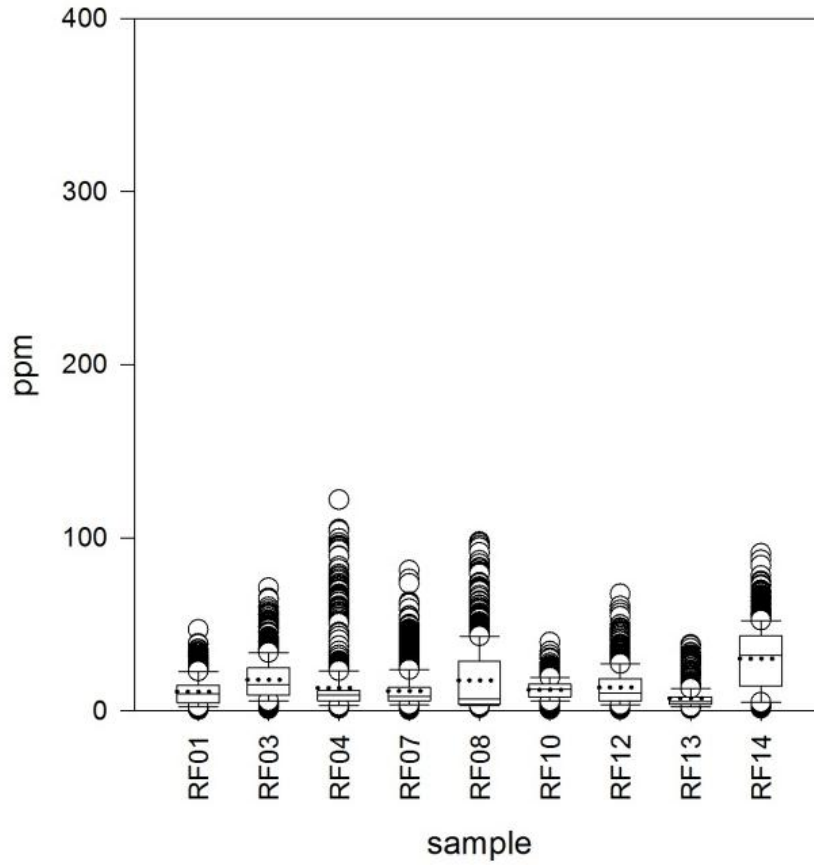


Figure 32 Plots showing Mn data for the grayling from Moose Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (dotted line).

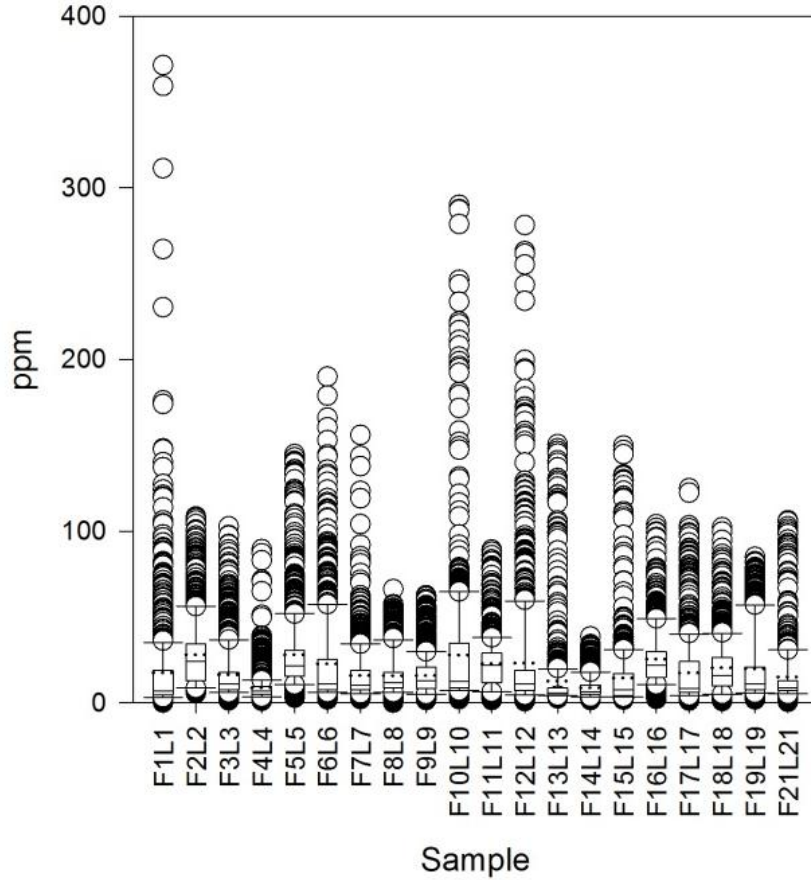


Figure 33 Plots showing Mn data for the grayling from Christal Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (dotted line).

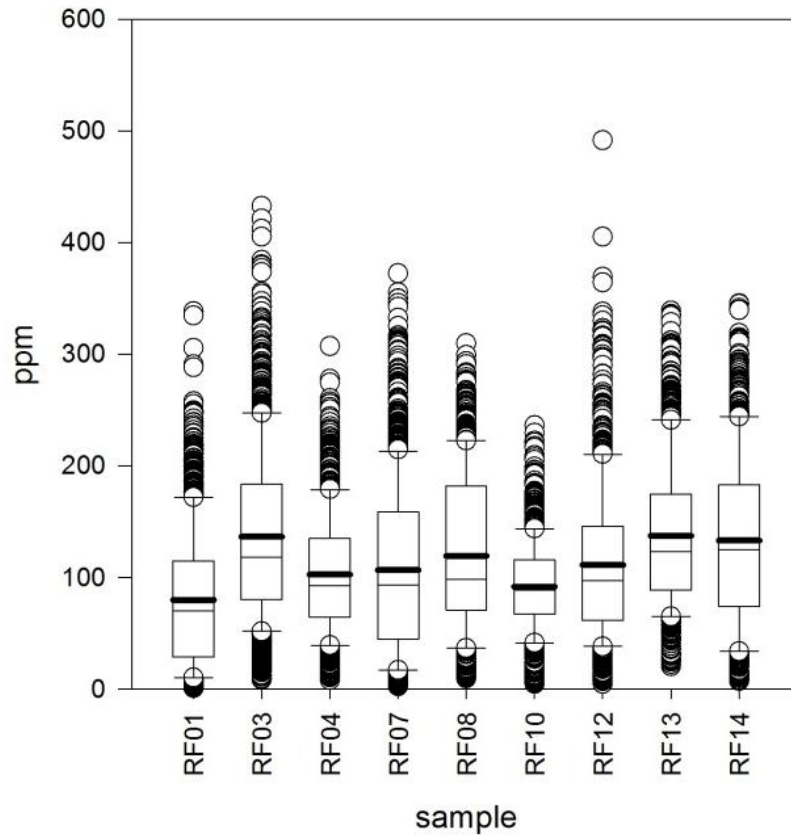


Figure 34 Plots showing Zn data for the grayling from Moose Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

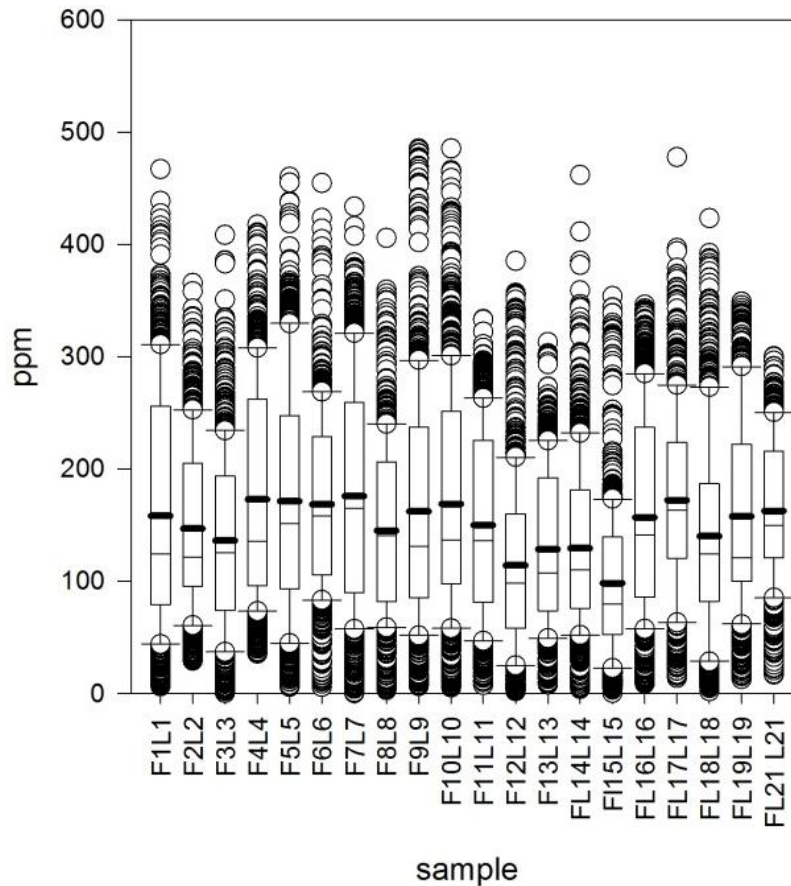


Figure 35 Plots showing Zn data for the grayling from Christal Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

3.4.2. Slimy sculpin

Figure 36 and Figure 37 show the Pb in sculpin data from Haldane and Christal Creeks. The range in Pb data is much greater from Christal Creek, with values up to almost 1 ppm. The variability of the Christal Creek data within 25th to 75th percentile range is greater than the Haldane Creek data being as high as 0.4 ppm. The data from Haldane Creek is tightly grouped around 0.05 ppm. The situation is somewhat different with regard to Mn (Figure 38 and Figure 39). In this case, other than one sample, the Mn levels, and ranges in Mn concentrations in the Christal Creek otoliths, is low in comparison to the Haldane Creek otoliths.

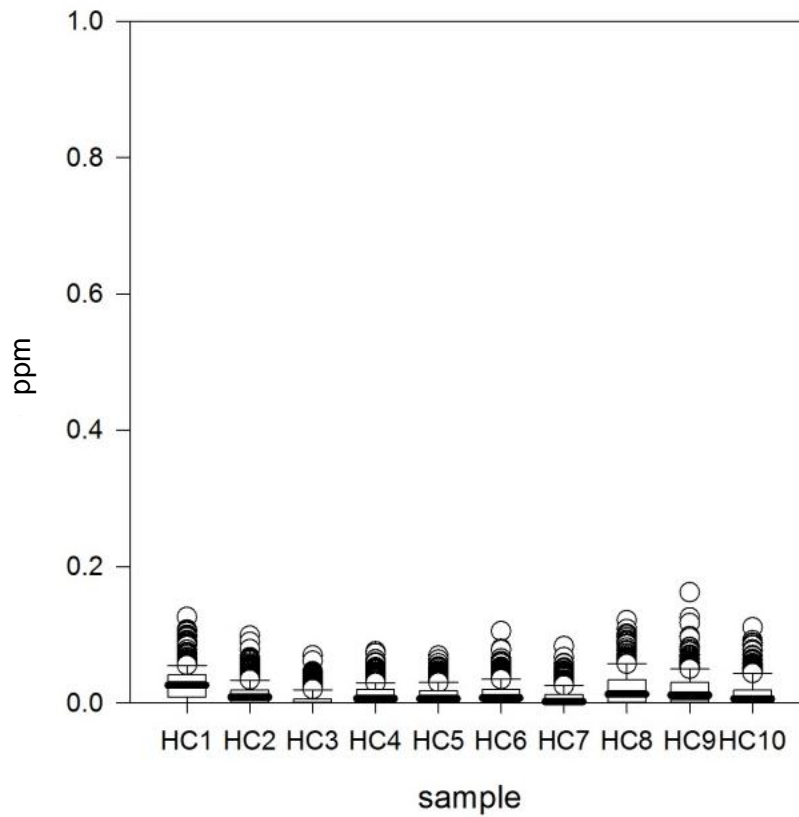


Figure 36 Plots showing Pb data for the sculpin from Haldane Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

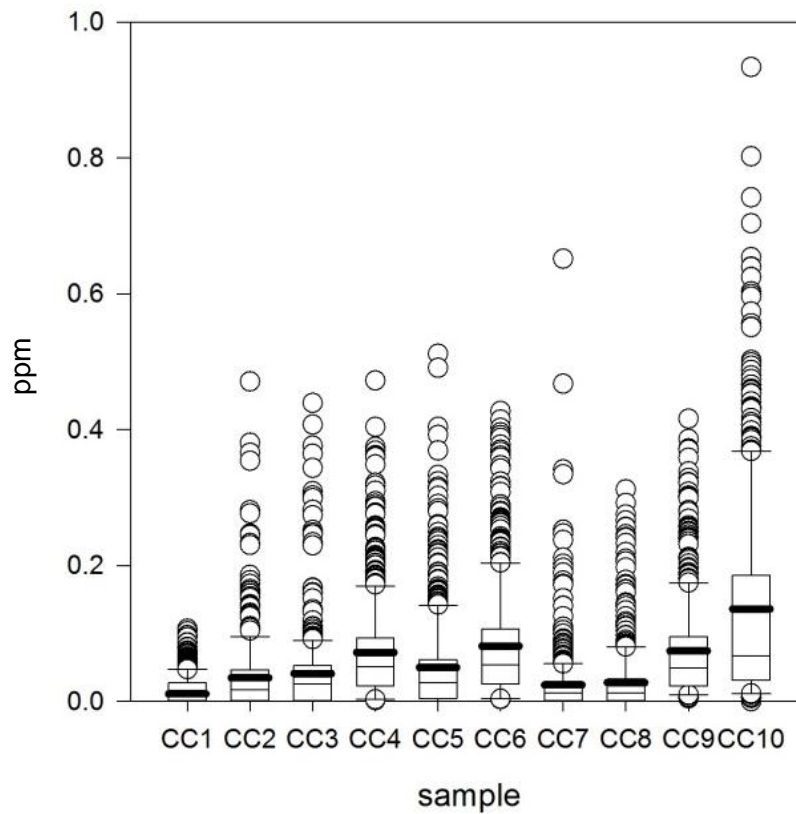


Figure 37 Plots showing Pb data for the sculpin from Christal Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

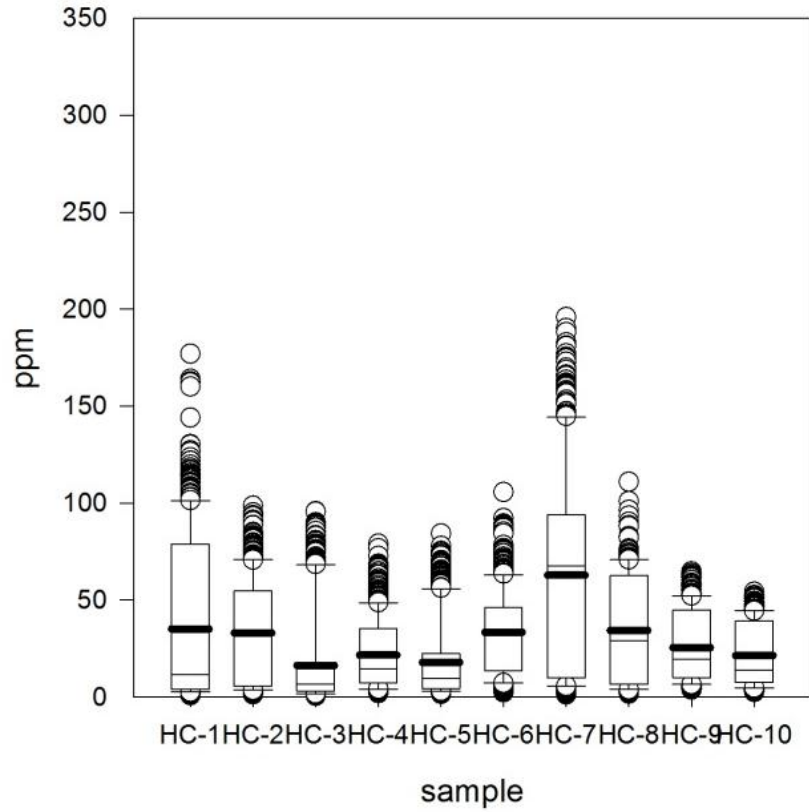


Figure 38 Plots showing Mn data for the sculpin from Haldane Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

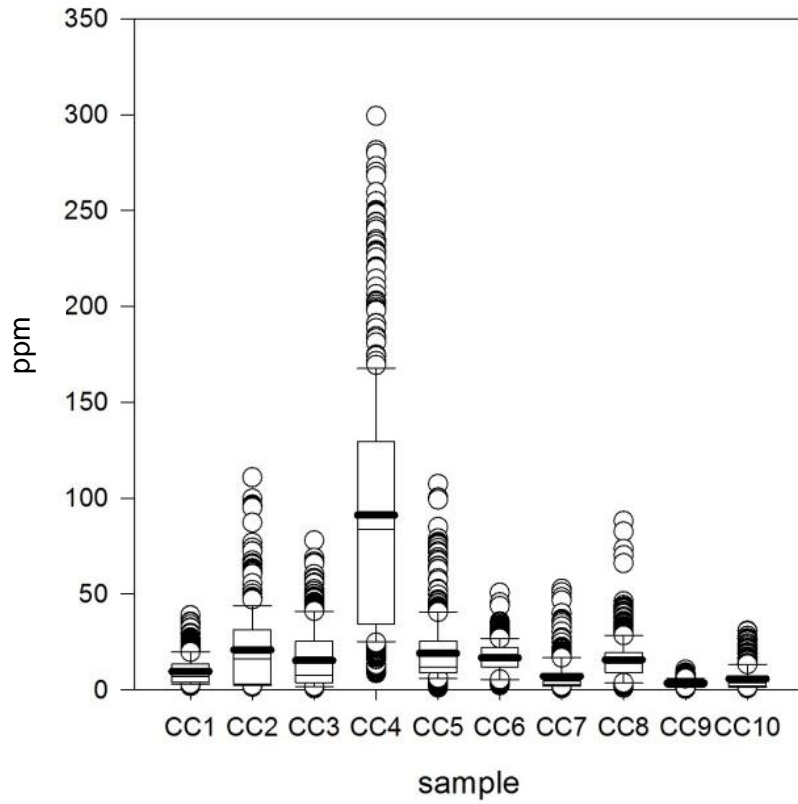


Figure 39 Plots showing Mn data for the sculpin from Christal Creek. Individual sample plots show data range, the boxes 25th and 75th percentile of the data, whiskers 10th and 90th percentile, and median (thin line) and mean (thick line).

4. DISCUSSION

Otolith microchemistry has been used extensively to track the migratory behaviour of anadromous and diadromous fish in marine environments (Walther and Limburg 2012; Babaluk et al. (in press); Loewen, et al. (in press)), and more recently it has been applied to problems of assigning spawning habitat (Mohan et al., 2015; Davoren and Halden 2014; Davoren et al., 2015). The majority of these studies have focused on the use of Sr as a marker as there is a marked difference in Sr concentrations between freshwater and marine environments. The application of “hard part” (otoliths, vertebrae, fin-rays and scales) microchemistry was discussed more recently in terms of conservation and management of freshwater fisheries by Pracheil et al. (2015). Otolith microchemistry has also been used to assess the impact of mine tailings in connection to rare element mining (Friedrich and Halden, 2008), coal mining (Palace et al., 2007) and base metal mining (Friedrich and Halden, 2010). Considering annular structure of otoliths and the archival nature of the many otolith collections that have been taken, as part of fish management and population studies, early studies were often hindered by the lack of concurrent water trace element data and limited ability to reconstruct the background environment against which an anthropogenic signal might be assessed. In this study, we have achieved good control of water chemistry that matches the time slice recorded by the otolith, and we have good control over representative background samples from a real world situation. Putting aside potential environmental and behavioural complexity that is discussed at length by Elsdon et al. (2008) and the physiological constraints imposed by the fish (e.g. Sturrock et al., 2015), very little is actually known about how the fish take up and respond to a wide range of elements. This is especially true at low concentrations and with regard to time because these types of measurements have only recently become available and are as yet by no means routine. Despite this, there is a remarkable consistency in the data and the patterns preserved in otoliths particularly in terms of location, species and trace elements. This clearly suggests the process of uptake is not random and that deconvolution of environment, physiology, sex, and species needs attention. Campana (1999) suggested a model for element uptake taking any given element through four stages from water, through gills, gut, blood and endolymph and proposed each step was governed by a partition coefficient. A reasonable assumption for a healthy fish of a specific species is that these partition coefficients are relatively constant, and underlie Limburg and Elfman’s (2010) suggestion of species-specific uptake. It would follow then that fish movement or environmental changes of water chemistry would be reordered in relative terms in the otolith.

4.1. ARCTIC GRAYLING

4.1.1. Grayling and strontium

The grayling from Christal Creek show some distinct annual variations in Sr. This is actually the first compiled Sr data for a freshwater species, clearly we cannot attribute this to any visit to an ocean. That said grayling are known to show potadromous behaviour so Sr variations would be consistent with fish moving between higher and lower Sr environments. On the face of it this might be hard to confirm alongside the water quality data, the Sr levels in the S. McQuesten River and Christal Creek are similar, although the S. McQuesten sampling site appears to show periodic elevated concentrations. In the study done by Sturrock et al. (2015), Sr was the only element to show a significant departure in element concentration between what was stored in the otolith and the ambient water chemistry. Such a lag however could still be consistent with fish moving to and from different Sr environments. It is noticeable that the Moose Creek fish do not show this difference so this may be a fundamental behavioural difference between the two populations and locations.

4.1.2. Grayling and manganese

Mn uptake between Christal Creek and Moose Creek is very different. The Moose Creek reference location otolith data set shows a more restricted concentration range than Christal Creek. Using Moose Creek as the reference, it could be argued the Christal Creek fish are moving to and from a Mn rich environment (possibly twice a year) or they are being exposed to elevated Mn concentrations, noticeably during the summer months, again possibly twice a year. It is likely that the Arctic Grayling captured in Christal Creek move into the creek to spawn in the spring, stay there for the summer, then go back into the South McQuesten River in the fall because of better overwintering habitat in the South McQuesten River. Based on otolith microchemistry alone, we cannot distinguish between movement and environmental change but a more focused analysis of the correlation between Sr and Mn might elucidate this puzzle. There may also be anecdotal evidence suggestive of spawning in Christal Creek with subsequent movement.

4.1.3. Grayling and zinc

Zinc is a biologically mediated element much more so than Mn or Sr so it is interesting to note that the overall Zn concentrations are similar between Christal and Moose Creeks. It is the variability in Zn in the Christal Creek otoliths that is much greater. Again this may be reflective

of the greater variability in Sr and Mn that could be attributed to movement or environmental variability for the Christal Creek population.

4.2. SLIMY SCULPIN

4.2.1. Sculpin and lead

There is a stark difference in both the levels of lead and periodicity in Pb content in the Haldane and Christal Creek sculpin populations. Every sample from Christal Creek (n=9) showed periodic Pb uptake and every sample from Haldane Creek (n=9) showed Pb levels at or near detection limits. The water quality data shows the Pb content of Christal Creek water is consistently higher than Haldane Creek. The Christal Creek sculpin have likely been exposed to elevated Pb from tailings in the Christal Creek watershed. Since sculpin live (and feed) in/on material in the substrate, they would likely be ingesting some sediment or ingesting material/food that would have been exposed to sediments with elevated Pb. It is worth noting that Pb uptake is annual so this behavioural and or environmental signal is reproducible. Some sculpin data is available from other studies. Sondergaard et al. (in prep) sampled sculpin in proximity to the Angel Pb-Zn mine in Greenland. Here Pb and cadmium were measured and Pb consistently correlated with winter annuli. Fish sampled from areas disconnected with mining showed no detectable Pb. In a sample set of sculpin (*Cottus aleoticus*) taken from the Squamish River system in BC, no Pb was detected at a limit of detection ~0.5 ppm (Patterson, 2012).

4.2.2. Sculpin and manganese

The sculpin Mn data does not tell us a great deal in terms of the range of concentrations, or the absolute or mean concentrations. The patterns are somewhat different over time. In Haldane Creek otoliths, the higher concentrations tend to be in the primordia. In the Christal Creek otoliths, the significant annual variations in Mn tend to be seen more in the outer annuli. Elevated primordial concentrations are not absent they just tend to be less prominent. Given that sculpin is a relatively sessile species, the two patterns suggest the variability in Mn would be an environmental feature associated with Christal Creek. Given this, at least some of the Mn variation and probably that of Sr and Zn as well in the grayling might be reasonably attributed to environmental changes in Christal Creek.

5. CONCLUSION

In conclusion, if the question was asked are the fish in Christal Creek impacted by the mine tailings, the weight of the data in comparison with the reference locations would suggest this is a likely possibility. Are the observed conditions toxic? The fish analyzed ranged in ages, but were generally 3-4 years old, indicating they are coming into contact with the variable environment of Christal Creek over several years. In addition, after 60 years of mining influence on water quality on Christal Creek, it is estimated that there has been upwards of 20 generations of sculpin produced during this period. Selection towards individuals with higher metal tolerance may have happened. If their condition factors suggested a decline in health, there would be a case for further assessment. Continued longitudinal studies with periodic assessment of otoliths providing a retrospective analysis would be a reasonable strategy to track this concern.

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APPENDIX A: WATER QUALITY DATA

Table 1 Selected water quality parameters measured for Christal Creek at the highway, in 2009

Date	1/12/2009	2/3/2009	3/10/2009	4/16/2009	5/7/2009	6/5/2009	7/7/2009	8/11/2009	9/9/2009	10/7/2009	11/13/2009	12/2/2009
Total Suspended Solids	<2	26	<5	<4	2	18	2	6	1	4	2	1
pH (field)					6.9	7.53	7.9		7.36	7.51	8.19	7.56
Hardness (from total)				251	19.4	262	379	457	448	428	449	463
Barium (Ba), total	0.093	0.02	0.053	0.0348	0.00689	0.0486	0.0608	0.0616	0.0586	0.0581	0.0591	0.0671
Cadmium (Cd), total	0.00106	0.0004	0.00092	0.00016	0.000114	0.00278	0.00137	0.00117	0.00159	0.00203	0.00112	0.00108
Calcium (Ca), total	147	128	124	87.5	5.92	83.1	117	142	141	135	140	150
Copper (Cu), total	0.002	0.002	<0.001	0.00138	0.00128	0.00132	0.00053	0.00043	0.00035	0.00067	0.00048	0.00038
Iron (Fe), total	0.07	0.1	0.26	0.066	0.076	0.358	0.095	0.195	0.134	0.378	0.271	0.266
Lead (Pb), total	0.0044	0.0005	0.0126	0.00114	0.000947	0.0449	0.0187	0.0155	0.00567	0.0472	0.0107	0.00491
Magnesium (Mg), total	26.4	14	22.5	7.94	1.12	13.2	21.5	25.1	23.3	22.2	23.9	21.8
Manganese (Mn), total	1.39	0.173	0.661	0.0765	0.0281	0.4	0.216	0.304	0.273	0.488	1.15	1.33
Molybdenum (Mo), total	<0.001	0.00106	0.00012	0.00074	0.00005	0.0002	0.00019	0.00024	0.00024	0.00052	0.00049	0.00038
Phosphorus (P), total		<0.01	0.014	0.005	0.016	0.014	0.005		0.005		0.004	0.006
Potassium (K), total	0.8	0.7	0.4	0.59	0.44	0.37	0.19	0.25	0.35	0.33	0.38	0.41
Selenium (Se), total	0.0015	<0.0006	0.0006	0.00018	<0.00004	0.00056	0.00088	0.00082	0.00081	0.00085	0.00085	0.00096
Sodium (Na), total	3.4	2.53	1.39	4.98	0.09	0.94	1.4	1.56	1.44	1.38	1.46	1.3
Strontium (Sr), total	0.286	0.205	0.205	0.173	0.0119	0.148	0.213	0.241	0.227	0.224	0.241	0.245
Sulphur (S), total	101	39.8	88.1	37	<3	62	93	116	117	111	115	124
Zinc (Zn), total	0.203	0.025	0.115	0.0107	0.0327	0.254	0.155	0.141	0.191	0.219	0.138	0.118

Table 2 Selected water quality parameters measured for Christal Creek at the highway, in 2009

Date	1/12/2009	2/3/2009	3/10/2009	4/16/2009	5/7/2009	6/5/2009	7/7/2009	8/11/2009	9/9/2009	10/7/2009	11/13/2009	12/2/2009
Barium (Ba), dissolved	0.08	0.018	0.066	0.034	0.00629	0.0484	0.0599	0.0595	0.0573	0.0553	0.0577	0.0659
Cadmium (Cd), dissolved	0.0003	0.00037	0.00036	0.000126	0.000102	0.00226	0.00127	0.000784	0.00131	0.00141	0.00087	0.000712
Calcium (Ca), dissolved	137	136	113	89.1	6.1	85.5	123	140	132	133	146	148
Copper (Cu), dissolved	0.006	0.001	<0.001	0.00086	0.00124	0.00121	0.00137	0.00049	0.00059	0.00036	0.00021	0.00018
Iron (Fe), dissolved	0.03	<0.01	0.11	0.014	0.053	0.152	0.057	0.053	0.068	0.045	0.089	0.093
Lead (Pb), dissolved	0.0011	0.0002	0.0008	0.00014	0.000639	0.00823	0.0026	0.00121	0.0017	0.000277	0.000447	0.000326
Magnesium (Mg), dissolved	26.5	15.6	20.8	7.92	1.14	13.7	22.2	24.6	22.4	22.1	23.9	21.3
Manganese (Mn), dissolved	1.28	0.186	0.757	0.0722	0.026	0.296	0.276	0.232	0.265	0.377	1.13	1.3
Molybdenum (Mo), dissolved	0.001	0.00114	0.00013	0.00076	0.00005	0.00023	0.0002	0.00027	0.00023	0.00053	0.00051	0.00037
Phosphorus (P), dissolved		<0.01	<0.01	0.002	0.009	0.008	0.005		0.004	0.004	0.003	0.005
Potassium (K), dissolved	0.8	0.6	0.4	0.58	0.44	0.42	0.28	0.26	0.33	0.33	0.4	0.41
Selenium (Se), dissolved	0.0014	<0.0006	0.0012	0.00019	<0.00004	0.00057	0.00094	0.00078	0.00077	0.00072	0.00086	0.00095
Sodium (Na), dissolved	3.7	2.5	1.3	5.07	0.1	1	1.61	1.54	1.45	1.37	1.45	1.26
Strontium (Sr), dissolved	0.239	0.211	0.266	0.176	0.0119	0.154	0.215	0.239	0.227	0.225	0.243	0.241
Sulphur (S), dissolved	94.7	47.7	82.2	38	<3	63	102	115	107	108	119	122
Zinc (Zn), dissolved	0.204	0.024	0.104	0.0071	0.0315	0.221	0.172	0.12	0.192	0.194	0.127	0.11

Otolith microchemistry applied to environmental effects monitoring in the Keno Hill mining district**Table 3 Selected water quality parameters measured for Christal Creek at the highway, in 2010**

Dates	1/12/2010	2/11/2010	3/11/2010	4/8/2010	5/5/2010	6/8/2010	7/8/2010	8/4/2010	9/14/2010	10/5/2010	11/16/2010	12/7/2010
Total Suspended Solids	2	2	2	2	2	1	3	2	6	2	1	5
pH (field)	7.94	7.68	7.91	7.77	7.74	7.62	7.74	7.85	7.05	7.88	6.81	8.04
Hardness (from total)	474	481	527	503	276	390	404	434	424	429	495	518
Barium (Ba), total	0.0635	0.0624	0.0594	0.0574	0.0429	0.0483	0.0739	0.0509	0.0494	0.0458	0.0541	0.0687
Cadmium (Cd), total	0.000934	0.0008	0.000744	0.00077	0.00102	0.00197	0.00779	0.00096	0.00151	0.000895	0.00104	0.00146
Calcium (Ca), total	149	153	168	161	87.5	118	127	133	131	133	158	162
Copper (Cu), total	0.00033	0.00033	0.00027	0.00067	0.00094	0.00055	0.00507	0.00046	0.00071	0.00026	0.00027	0.00055
Iron (Fe), total	0.482	0.496	0.481	0.543	0.564	0.139	3.7	0.147	0.741	0.181	0.196	0.112
Lead (Pb), total	0.00515	0.0136	0.00476	0.00541	0.00816	0.00543	0.312	0.00489	0.0331	0.00231	0.00506	0.00522
Magnesium (Mg), total	24.8	24.3	25.9	24.7	13.9	23.2	21.1	24.6	23.6	23.5	24.3	27.6
Manganese (Mn), total	1.17	1.11	1.15	1.03	0.45	0.275	1.42	0.216	0.386	0.293	1.42	1.91
Molybdenum (Mo), total	0.00019	0.00021	0.00021	0.00021	0.00022	0.00028	0.00028	0.00027	0.00021	0.00019	0.00019	0.00021
Phosphorus (P), total		0.006	0.009	0.009		0.007	0.071	0.007	0.014		0.005	0.015
Potassium (K), total	0.42	0.42	0.47	0.47	0.44	0.21	0.2	0.23	0.36	0.34	0.42	1.31
Selenium (Se), total	0.00099	0.00099	0.001	0.00093	0.00061	0.00077	0.0008	0.00079	0.00079	0.00083	0.00086	0.00088
Sodium (Na), total	1.53	1.53	1.67	1.57	0.91	1.42	1.21	1.47	1.48	1.41	1.53	1.64
Strontium (Sr), total	0.234	0.239	0.264	0.252	0.153	0.215	0.227	0.234	0.22	0.224	0.24	0.278
Sulphur (S), total	122	127	137	133	71	119	104	121	115	122	121	140
Zinc (Zn), total	0.107	0.101	0.0887	0.0985	0.133	0.239	0.452	0.124	0.177	0.146	0.166	0.179

Table 4 Selected water quality parameters measured for Christal Creek at the highway, in 2010

Date	1/12/2010	2/11/2010	3/11/2010	4/8/2010	5/5/2010	6/8/2010	7/8/2010	8/4/2010	9/14/2010	10/5/2010	11/16/2010	12/7/2010
Barium (Ba), dissolved	0.0679	0.0587	0.0569	0.0576	0.0441	0.0496	0.0454	0.0492	0.0449	0.0467	0.0534	0.0658
Cadmium (Cd), dissolved	0.000407	0.000291	0.000232	0.000154	0.000908	0.00184	0.00106	0.000832	0.000788	0.000764	0.000778	0.000655
Calcium (Ca), dissolved	155	159	165	167	87.4	134	127	143	138	134	162	164
Copper (Cu), dissolved	0.00117	0.00035	0.00015	0.00015	0.00216	0.0005	0.00195	0.00125	0.00056	0.00086	0.0002	0.00034
Iron (Fe), dissolved	0.318	0.191	0.153	0.176	0.521	0.045	0.085	0.076	0.059	0.064	0.06	0.025
Magnesium (Mg), dissolved	25.3	25.1	24.5	25.8	14	21.7	22.7	24.3	23.1	22.9	25.5	28.3
Manganese (Mn), dissolved	1.2	1.06	1.09	1.05	0.445	0.242	0.194	0.203	0.268	0.267	1.41	1.95
Molybdenum (Mo), dissolved	0.00026	0.0002	0.00021	0.0002	0.00023	0.00029	0.00024	0.00027	0.00019	0.0002	0.00019	0.00021
Phosphorus (P), dissolved		0.005	0.002	0.003		0.003	0.009	0.007	0.005		0.003	0.008
Potassium (K), dissolved	0.48	0.43	0.45	0.46	0.49	0.2	0.26	0.27	0.36	0.33	0.44	1.27
Selenium (Se), dissolved	0.00101	0.00087	0.00093	0.00091	0.00061	0.00078	0.00072	0.00079	0.00079	0.00082	0.00083	0.00087
Sodium (Na), dissolved	1.58	1.6	1.59	1.63	1.15	1.34	1.44	1.55	1.41	1.43	1.63	1.7
Strontium (Sr), dissolved	0.251	0.233	0.255	0.255	0.153	0.231	0.217	0.216	0.21	0.221	0.24	0.272
Zinc (Zn), dissolved	0.108	0.0905	0.078	0.0811	0.136	0.224	0.146	0.116	0.144	0.128	0.161	0.172

Otolith microchemistry applied to environmental effects monitoring in the Keno Hill mining district**Table 5 Selected water quality parameters measured for Christal Creek at the highway, in 2011**

	1/12/2011	2/8/2011	3/22/2011	4/19/2011	5/25/2011	6/22/2011	7/13/2011	8/17/2011	9/24/2011	10/29/2011	11/21/2011	12/14/2011
Total Suspended Solids	2	1	2	1	3	1	2	2	1	1	2.7	4.2
pH (field)	7.85	8.11	7.73		7.84	8.23	7.88	7.78	7.52	7.66	7.49	7.36
Hardness (from total)	481	502	573	564	303	470	407	444	447	511	497	505
Barium (Ba), total	0.0602	0.0603	0.0632	0.0578	0.0365	0.0552	0.0442	0.0382	0.0433	0.053	0.0585	0.0643
Cadmium (Cd), total	0.000802	0.00073	0.000651	0.000735	0.00189	0.00145	0.00147	0.00185	0.00215	0.00132	0.0014	0.00123
Calcium (Ca), total	150	161	183	180	99.6	146	127	138	141	159	153	159
Copper (Cu), total	0.00023	0.00014	0.00029	0.00041	0.00108	0.00037	0.00056	0.00065	0.00039	0.00026	0.00067	0.00248
Iron (Fe), total	0.249	0.4	0.489	0.398	0.362	0.143	0.176	0.249	0.544	0.297	0.366	0.492
Lead (Pb), total	0.00663	0.00795	0.00378	0.00488	0.00814	0.00441	0.00604	0.00846	0.00411	0.00243	0.0155	0.00752
Magnesium (Mg), total	25.5	24.2	28	28.1	13.2	25.5	22.1	24.3	23.1	27.7	28.2	26.1
Manganese (Mn), total	1.05	1.37	1.35	1.08	0.404	0.288	0.281	0.344	0.441	1.32	1.64	1.84
Molybdenum (Mo), total	0.00018	0.00017	0.00019	0.00017	0.00037	0.00024	0.00031	0.00024	0.00035	0.00023	0.0002	0.0002
Phosphorus (P), total	0.007	0.005	0.01	0.01	0.012	0.005	0.008	0.008	0.007	0.006	0.008	0.007
Potassium (K), total	0.42	0.43	0.48	0.51	0.51	0.21	0.17	0.32	0.41	0.43	0.47	0.61
Selenium (Se), total	0.00096	0.00082	0.00088	0.00087	0.00066	0.00078	0.00066	0.00074	0.00077	0.00082	0.00096	0.00094
Sodium (Na), total	1.61	1.48	1.69	1.7	0.95	1.57	1.43	1.54	1.41	1.63	1.77	1.87
Strontium (Sr), total	0.245	0.265	0.268	0.274	0.163	0.254	0.218	0.223	0.235	0.271	0.256	0.26
Sulphur (S), total	125	122	139	140	74	135	108	117	116	143	131	122
Zinc (Zn), total	0.123	0.101	0.103	0.104	0.19	0.207	0.183	0.226	0.275	0.218	0.219	0.175

Table 6 Selected water quality parameters measured for Christal Creek at the highway, in 2011

	1/12/2011	2/8/2011	3/22/2011	4/19/2011	5/25/2011	6/22/2011	7/13/2011	8/17/2011	9/24/2011	10/29/2011	11/21/2011	12/14/2011
Barium (Ba), dissolved	0.0574	0.0581	0.064	0.057	0.0373	0.0523	0.0455	0.0386	0.045	0.0456	0.0559	0.0625
Cadmium (Cd), dissolved	0.000101	0.000101	0.000215	0.000171	0.00176	0.00126	0.00116	0.0016	0.00166	0.00101	0.000769	0.000517
Calcium (Ca), dissolved	149	151	173	182	98.5	145	131	137	144	164	153	159
Copper (Cu), dissolved	0.00009	0.00018	0.00043	0.00032	0.00096	0.00108	0.00058	0.00068	0.00032	0.00015	0.0003	0.00066
Iron (Fe), dissolved	0.016	0.029	0.123	0.095	0.277	0.059	0.056	0.081	0.072	0.124	0.067	0.163
Lead (Pb), dissolved	0.000171	0.000306	0.000517	0.000271	0.00465	0.00216	0.00143	0.00105	0.000328	0.000755	0.000474	0.00064
Magnesium (Mg), dissolved	25.1	26.1	27.3	27.1	14.1	25.1	21.9	23.9	22.1	28.8	27.2	26.5
Manganese (Mn), dissolved	1	1.48	1.35	1.01	0.476	0.258	0.257	0.303	0.428	1.37	1.62	1.82
Molybdenum (Mo), dissolved	0.00016	0.00021	0.00018	0.00017	0.00021	0.00026	0.00029	0.00025	0.0004	0.00022	0.00018	0.00018
Phosphorus (P), dissolved	0.004	0.008	0.005	0.012	0.008	0.006	0.004	0.006	0.007	0.004	0.005	0.003
Potassium (K), dissolved	0.41	0.5	0.55	0.53	0.47	0.25	0.19	0.34	0.41	0.4	0.48	0.62
Selenium (Se), dissolved	0.00093	0.00084	0.00089	0.00085	0.0004	0.00078	0.00064	0.00069	0.0008	0.00092	0.0009	0.00096
Sodium (Na), dissolved	1.61	1.6	1.71	1.64	0.97	1.63	1.41	1.51	1.36	1.77	1.71	1.8
Strontium (Sr), dissolved	0.239	0.252	0.271	0.269	0.16	0.245	0.212	0.229	0.247	0.259	0.25	0.259
Sulphur (S), dissolved	121	134	139	137	77	122	110	117	116	141	129	129
Zinc (Zn), dissolved	0.0982	0.0831	0.0769	0.0729	0.22	0.201	0.173	0.208	0.262	0.23	0.201	0.145

Otolith microchemistry applied to environmental effects monitoring in the Keno Hill mining district**Table 7 Selected water quality parameters measured for Christal Creek at the highway, in 2012**

Date	1/15/2012	2/13/2012	3/11/2012	4/8/2012	5/1/2012	5/7/2012	6/3/2012	7/4/2012	8/1/2012	9/23/2012	10/15/2012	11/21/2012
Total Suspended Solids	2.1	1.3	1.1	1.8	2.8	3.2	<1.0	1.9	1	1.5	<1.0	2.8
pH (field)	7.3	7.21	7.1	7.69	7.67	7.93	7.92	7.93	7.82	7.75	7.56	8
Hardness (from total)	468	525	518	483	271	281	365	545	502	492	498	526
Barium (Ba), total	0.0616	0.0572	0.0585	0.0551	0.0378	0.0417	0.0465	0.0451	0.0462	0.0478	0.0495	0.0687
Cadmium (Cd), total	0.00114	0.000898	0.000818	0.000779	0.00378	0.00239	0.000729	0.000742	0.00086	0.00108	0.000769	0.00132
Calcium (Ca), total	149	167	163	151	86.7	88.4	112	171	157	155	160	165
Copper (Cu), total	0.00122	0.00019	0.00024	0.00022	0.00124	0.00143	0.00075	0.000872	0.000408	0.000277	0.000413	0.00119
Iron (Fe), total	0.535	0.571	0.48	0.514	0.991	0.872	0.148	0.21	0.187	0.314	0.202	0.441
Lead (Pb), total	0.016	0.0039	0.00362	0.00306	0.0135	0.0225	0.00336	0.00559	0.0048	0.00189	0.0027	0.0233
Magnesium (Mg), total	23.2	26.1	26.8	25.5	13.2	14.8	20.6	28.5	26.6	25.6	24	27.5
Manganese (Mn), total	1.06	1.33	1.25	1.07	0.703	0.719	0.25	0.247	0.343	0.517	0.449	1.69
Molybdenum (Mo), total	0.00019	0.00016	0.00015	0.00018	0.000189	0.000329	0.00024	0.000286	0.000335	0.000215	0.000195	0.00021
Phosphorus (P), total	0.024	0.012	0.014	0.008	0.0172	0.0156	0.0138	0.013	0.0023	0.0023	0.0044	0.0058
Potassium (K), total	0.46	0.47	0.5	0.43	0.538	0.551	0.332	0.183	0.24	0.387	0.407	0.438
Selenium (Se), total	0.00111	0.00091	0.00096	0.00089	0.000553	0.000515	0.000875	0.000758	0.000745	0.000848	0.000855	0.00101
Sodium (Na), total	2.43	1.6	1.7	1.57	0.895	1.02	1.75	1.75	1.64	1.53	1.45	1.77
Strontium (Sr), total	0.244	0.244	0.248	0.25	0.143	0.15	0.211	0.255	0.265	0.274	0.27	0.262
Sulphur (S), total	138	131	143	131	66	70	106	152	133	140	144	127
Zinc (Zn), total	0.149	0.139	0.119	0.117	0.364	0.318	0.123	0.0992	0.105	0.163	0.0988	0.173

Otolith microchemistry applied to environmental effects monitoring in the Keno Hill mining district**Table 8 Selected water quality parameters measured for Christal Creek at the highway, in 2012**

Date	1/15/2012	2/13/2012	3/11/2012	4/8/2012	5/1/2012	5/7/2012	6/3/2012	7/4/2012	8/1/2012	9/23/2012	10/15/2012	11/21/2012
Barium (Ba), dissolved	0.054	0.0568	0.0631	0.0547	0.0366	0.0406	0.0457	0.0427	0.0456	0.0457	0.0484	0.0662
Cadmium (Cd), dissolved	0.000203	0.000212	0.000104	0.000081	0.00321	0.00183	0.000608	0.000515	0.000612	0.00065	0.000684	0.000627
Calcium (Ca), dissolved	142	172	169	159	85.1	91.1	111	144	125	169	155	159
Copper (Cu), dissolved	0.0005	0.0001	0.00029	0.0001	0.00106	0.00108	0.000572	0.000232	0.000373	0.000203	0.000308	0.000392
Iron (Fe), dissolved	0.105	0.149	0.131	0.133	0.641	0.504	0.0336	0.0417	0.0518	0.0639	0.059	0.111
Lead (Pb), dissolved	0.000947	0.000139	0.000088	0.000104	0.00296	0.00409	0.000581	0.000569	0.00104	0.000189	0.000336	0.000454
Magnesium (Mg), dissolved	24.8	27.8	27.9	25.5	13.6	14.6	21	24.3	26.1	25.8	25.8	28.4
Manganese (Mn), dissolved	1.05	1.33	1.34	1.04	0.707	0.685	0.245	0.196	0.315	0.487	0.462	1.64
Molybdenum (Mo), dissolved	0.00017	0.00016	0.00018	0.00015	0.000137	0.000205	0.000238	0.000617	0.000243	0.000217	0.000159	0.000244
Phosphorus (P), dissolved	0.006	0.007	0.007	0.006	0.0125	0.0103	0.0052	0.0076	<0.0020	<0.0020	<0.0020	<0.0020
Potassium (K), dissolved	0.43	0.5	0.53	0.45	0.553	0.553	0.303	0.16	0.259	0.381	0.404	0.44
Selenium (Se), dissolved	0.00106	0.0009	0.00104	0.00094	0.000471	0.000587	0.000818	0.000771	0.000609	0.00083	0.000855	0.00105
Sodium (Na), dissolved	1.86	1.73	1.83	1.57	0.945	1.01	1.76	1.47	1.61	1.57	1.55	1.84
Strontium (Sr), dissolved	0.235	0.26	0.286	0.248	0.144	0.151	0.207	0.243	0.254	0.265	0.27	0.263
Sulphur (S), dissolved	124	136	147	134	68	75	112	128	132	136	152	133
Zinc (Zn), dissolved	0.129	0.109	0.079	0.0817	0.365	0.301	0.111	0.0835	0.0952	0.147	0.109	0.155

Table 9 Selected water quality parameters measured for Moose Creek (sampled August 17th 2012)

Parameters	Moose Creek - Values
Total Suspended Solids	<4.0
pH	8.1
Total Hardness (CaCO ₃)	140
Total Barium (Ba)	87.1
Total Cadmium (Cd)	0.0060
Total Copper (Cu)	1.40
Total Iron (Fe)	711
Total Lead (Pb)	0.087
Total Manganese (Mn)	34.7
Total Molybdenum (Mo)	0.274
Total Selenium (Se)	0.135 (1)
Total Strontium (Sr)	226
Total Zinc (Zn)	1.4 (1)
Total Calcium (Ca)	37.9
Total Magnesium (Mg)	10.8
Total Potassium (K)	0.65
Total Sodium (Na)	3.12

Table 70 Selected water quality parameters measured for Haldane Creek (sampled July 25th 2011 and October 3rd 2014)

Parameters	Haldane Creek - October 3 2014	Haldane Creek - July 25 2011
pH		7.92
Hardness (as CaCO ₃)	148	160
Total Suspended Solids (TSS)		< 5
Barium (Ba)-Total	0.0391	0.0478
Cadmium (Cd)-Total	0.000022	< 0.00001
Calcium (Ca)-Total	37.9	43
Copper (Cu)-Total	0.00187	0.00113
Iron (Fe)-Total	0.445	
Lead (Pb)-Total	0.000358	0.00068
Magnesium (Mg)-Total	11	12.7
Manganese (Mn)-Total	0.031	0.0227
Molybdenum (Mo)-Total	0.000262	0.00044
Phosphorus (P)-Total	0.077	0.012
Potassium (K)-Total	0.32	0.2
Selenium (Se)-Total	0.00018	0.0003
Sodium (Na)-Total	1.02	1.3
Strontium (Sr)-Total	0.122	0.176
Sulfur (S)-Total	10.2	N/A
Zinc (Zn)-Total	0.0032	0.005

Table 11 Selected water quality parameters measured for Haldane Creek (sampled July 25th 2011 and October 3rd 2014)

Parameters	Haldane Creek - October 3 2014	Haldane Creek - July 25 2011
Barium (Ba)-Dissolved	0.0387	N/A
Cadmium (Cd)-Dissolved	0.000011	N/A
Calcium (Ca)-Dissolved	40.2	N/A
Copper (Cu)-Dissolved	0.00161	N/A
Iron (Fe)-Dissolved	0.217	N/A
Lead (Pb)-Dissolved	0.00021	N/A
Magnesium (Mg)-Dissolved	11.4	N/A
Manganese (Mn)-Dissolved	0.0272	N/A
Molybdenum (Mo)-Dissolved	0.000235	N/A
Phosphorus (P)-Dissolved	<0.050	N/A
Potassium (K)-Dissolved	0.27	N/A
Selenium (Se)-Dissolved	0.00019	N/A
Sodium (Na)-Dissolved	1	N/A
Strontium (Sr)-Dissolved	0.132	N/A
Sulfur (S)-Dissolved	10.4	N/A
Zinc (Zn)-Dissolved	0.0019	N/A