Duncan Dam Project Water Use Plan

Upper Duncan Bull Trout Migration Monitoring

Reference: DDMMON-5

Implementation Year 5

Study Period: 2015-2016

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February 9, 2017

Upper Duncan Bull Trout Migration Monitoring (DDMMON-5)

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Citation: Okanagan Nation Alliance and Golder Associates. 2016. Upper Duncan Bull Trout Migration Monitoring – Final Report February 2017. Report prepared for BC Hydro, Castlegar, BC. 30 pages + 2 appendices.

Executive Summary

The Duncan Dam operates within an important Bull Trout (*Salvelinus confluentus*) migratory corridor of the Kootenay River watershed. To mitigate the effects of the dam on Bull Trout migrations, a Bull Trout passage program was initiated at the Duncan Dam that uses Duncan Dam gate operations and the flip bucket to facilitate passage of adults from the lower Duncan River to the Duncan Reservoir upstream. Operation of the fish passage program at Duncan Dam presents operational, safety and fish stranding risks that need to be justified, and operations should be optimized to ensure the long term success of the program. The Upper Duncan River Bull Trout Migration Monitoring Program (DDMMON-5) is intended to assess the effectiveness of the transfer program by addressing the following key management question: "Does the Bull Trout transfer program contribute to the recruitment of Kootenay Lake or Duncan Reservoir?"

The chemistry of tributary water samples and the chemistry of otoliths from juvenile Bull Trout from the same tributaries of Duncan Reservoir and Kootenay Lake were analyzed to assess differences in the chemical signature of tributaries upstream and downstream of Duncan Dam. The ratios of strontium (^{86}Sr) to calcium (^{43}Ca; Sr:Ca) and barium (^{138}Ba) to calcium (Ba:Ca) were used to classify juvenile Bull Trout to their capture locations using linear discriminant analysis (LDA). The predictive model from juvenile Bull Trout otoliths captured in their natal tributaries was used to predict the natal origin of adult Bull Trout captured in Kootenay Lake, Duncan Reservoir, and in the flip bucket transfer program. These data were used to estimate the proportion of adults sampled that were spawned and reared in areas upstream of Duncan Dam. The analyses included data for juvenile and adult Bull Trout otoliths analyzed by a laboratory at the University of Victoria ("Victoria lab") in 2008/2009 and data from juvenile otoliths analyzed at the University of Manitoba ("Winnipeg lab") in 2016.

Analyses of Sr:Ca and Ba:Ca ratios in water samples indicated distinct chemical signatures in most of the Duncan and Kootenay watershed tributaries assessed. Linear regressions indicated a significant relationship between elemental ratios in juvenile otoliths and water samples, which supported the idea that tributaries have distinct water chemistry that result in distinct elemental ratios in the otoliths of Bull Trout.

Thirteen juvenile Bull Trout otoliths previously analyzed in the Victoria lab were re-analyzed in the Winnipeg lab in 2016 to assess the comparability of elemental ratios. Sr:Ca was not different between labs but Ba:Ca was consistently greater at the Victoria lab than the Winnipeg lab. Values of Ba:Ca measured in the Victoria lab were adjusted using the linear relationship between Ba:Ca at the two labs and adjusted Ba:Ca values were used in all subsequent analyses.

LDA models using otolith chemistry data measured by the Winnipeg lab correctly classified 75% of juvenile Bull Trout to their capture location. This was lower than the percentage correctly classified using data from the Victoria lab, which was 84%. The models using data from the Winnipeg lab had poor predictive ability for tributaries of the Duncan watershed, as none of the juveniles from Houston Creek or Westfall River were correctly classified to those tributaries, and only 50% of Upper Duncan River juveniles were correctly classified. Poor ability to correctly identify Duncan watershed tributaries was related to small sample sizes for two of three tributaries, high within-stream variability

in both Sr:Ca and Ba:Ca, and very similar chemistry between some of the Duncan and Kootenay watershed tributaries. All of the Kootenay watershed tributaries, except for Hamill Creek, were predicted accurately by both Victoria and Winnipeg data-sets.

The poor classification rate of juvenile otoliths from Duncan watershed tributaries using data from the Winnipeg lab means that predictions of the proportion of Duncan-origin adults are not reliable. Few adults of unknown origin were predicted to be from the Duncan watershed using data analyzed at the Winnipeg lab but this could have been because the model was unable to correctly classify Duncan watershed otolith chemistry based on the juvenile results. This was in contrast to results using data from the Victoria lab, which predicted that a majority of adult Bull Trout from the flip bucket (78%) and Kootenay Lake (69%) samples were from Duncan watershed tributaries. These conflicting results make it difficult to draw conclusions regarding the contribution of Duncan watershed tributaries to Bull Trout recruitment in Kootenay Lake. However, one result that was consistent between both data-sets was the finding that 26% of adult Bull Trout from the flip bucket and 23% of those from Kootenay Lake were predicted to have been reared in the Upper Duncan River proper. This suggests that the Upper Duncan River is important for the recruitment of Bull Trout in Kootenay Lake. There remains uncertainty in the proportion of adults originating from the other Duncan watershed tributaries, Houston Creek and Westfall River, because these tributaries could not reliably be classified using the data analyzed by the Winnipeg lab.

Overall, data analyzed by the Winnipeg lab mostly agreed with previous results from the Victoria lab, except for a few of the tributaries (Hamill Creek, Houston Creek and Westfall River), which had much greater within-stream variability and worse predictive ability using Winnipeg data than Victoria data. Unfortunately, these differences had a significant impact on the prediction of adult Bull Trout of unknown origins and related conclusions regarding inter-basin recruitment of Bull Trout in the Kootenay and Duncan watersheds. Recommendations intended to reduce uncertainties in future years of the study include:

- For streams in which there was significant overlap in element ratios, we recommend investigating differences in the ratio of ^{88}Sr:^{86}Sr to determine if these isotopes may help discriminate among streams.

- If uncertainty in the classification of natal origins cannot be adequately resolved using the isotope ratios, an additional 10 juvenile samples previously analyzed by the Victoria lab should be analyzed at the Winnipeg lab to confirm the relationship between the data sets.

Acknowledgements

BC Hydro
Jason Watson
Brenda Thomas
Kris Wiens
Len Wiens
Alf Leake

Okanagan Nation Alliance Fisheries Department
Amy Duncan
Michael Zimmer

University of Winnipeg
Norman Halden
Panseok Yang

Head Collection Locations
Gill & Gift - Balfour
Woodbury Resort
Cooper Creek Store
Meadow Creek Store

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1. INTRODUCTION

The Duncan Dam operates within an important Bull Trout (*Salvelinus confluentus*) migratory corridor of the Kootenay River watershed. To mitigate the effects of the dam on Bull Trout migrations, a Bull Trout passage program was initiated at the Duncan Dam that uses Duncan Dam gate operations to facilitate passage of adults from the lower Duncan River to the Duncan Reservoir upstream. The passage program involves the installation of a weir to facilitate Bull Trout access into the flip bucket, which is an energy dissipating structure at the end of the discharge tunnel. After Bull Trout accumulate in the flip bucket, the fish transfer procedure uses gate operations to alter water levels in the tunnel and allow access into the reservoir (O'Brien 1999). BC Hydro has undertaken the Upper Duncan River Bull Trout Migration Monitoring Program (DDMMON-5) with the consultation of the Duncan Dam Water Use Planning Consultative Committee, to assess the effectiveness of the transfer program in benefiting recruitment above and below Duncan Dam.

The DDMMON-5 program's primary objective is to determine natal origin of Bull Trout captured in Kootenay Lake and Duncan Reservoir by using otolith microchemistry. The microchemistry of tributary water samples and microchemistry of otoliths from juvenile Bull Trout from the same tributaries of Duncan Reservoir and Kootenay Lake were analyzed to assess differences in the chemical signature of tributaries upstream and downstream of Duncan Dam. Data from juvenile Bull Trout captured in their natal tributaries were used to predict the natal origin of adult Bull Trout captured in Kootenay Lake and Duncan Reservoir. These data were used to determine the proportion of adults sampled that spawn and rear in areas upstream of Duncan Dam.

1.1 Project Background

The Duncan River is located within south eastern British Columbia and flows out of the Selkirk and Purcell mountains to the north before entering the northern arm of Kootenay Lake, northeast of the town of Nelson, BC (Figure 1). The Duncan Dam was constructed in 1967 as a part of the Columbia River Treaty process between the US and Canada and is situated on the Duncan River, 12 km upstream of the inflow to Kootenay Lake. Duncan Dam is an earth fill and concrete structure 39.7 m high and 792 m long, with two low level outlets and a single spillway (DVH Consulting 2001). The dam provides storage to improve hydroelectric generation and flood control downstream in the Kootenay River basin. There are no power generation facilities at the Duncan Dam. Duncan Reservoir stores up to 1.73 billion m^3 of water (Anon 1986).

The Duncan River is an important migration corridor for Bull Trout and other fish species moving between Kootenay Lake and the tributaries of the Duncan River. After the completion of Duncan Dam in 1967, this migration route was thought to be blocked (Peterson and Withler 1965), as Bull Trout were observed at the base of the dam. In 1968, the first senior dam operator ("Dutchie" Wageningen) altered flows in the spring and summer to facilitate Bull Trout passage into the upstream Duncan Reservoir (O'Brien 1999). Subsequently, fish transfers have occurred annually since 1968 and currently occur from late spring (early May) to early fall (mid-September) (BC Hydro 2008).

Previous years of this monitoring study provide background information about Bull Trout migration behaviour in the Upper and Lower Duncan systems. Annual spawning movement of adfluvial Bull Trout has been shown to occur from Kootenay Lake to the Upper Duncan River, tributaries of the lower Duncan River, the Lardeau River and Trout Lake (O'Brien 1999). Mark-recapture studies have been conducted during Bull Trout transfers and indicated that spawning adults may pass upstream in later years (Ord et al. 2000). This confirms that some adults may migrate through Duncan Dam, go back downstream, and migrate back up through the dam to the Duncan Reservoir in later years.

The operation of the fish passage program at Duncan Dam presents operational, safety and fish stranding risks that need to be justified and operations should be optimized to ensure the long term success of the program. The weir installation poses safety risks for dam operators due to the need for working in water and operations need to be managed during installation. Furthermore, passage operations require up to 24 hours of no flow from the dam, which can result in fish stranding particularly when Lardeau River flows are too low to backwater the tailrace and downstream Duncan River habitats. Further study regarding the optimization of fish passage operations is being conducted by the Duncan Dam Bull Trout Passage Monitoring Program (DDMMON-6).

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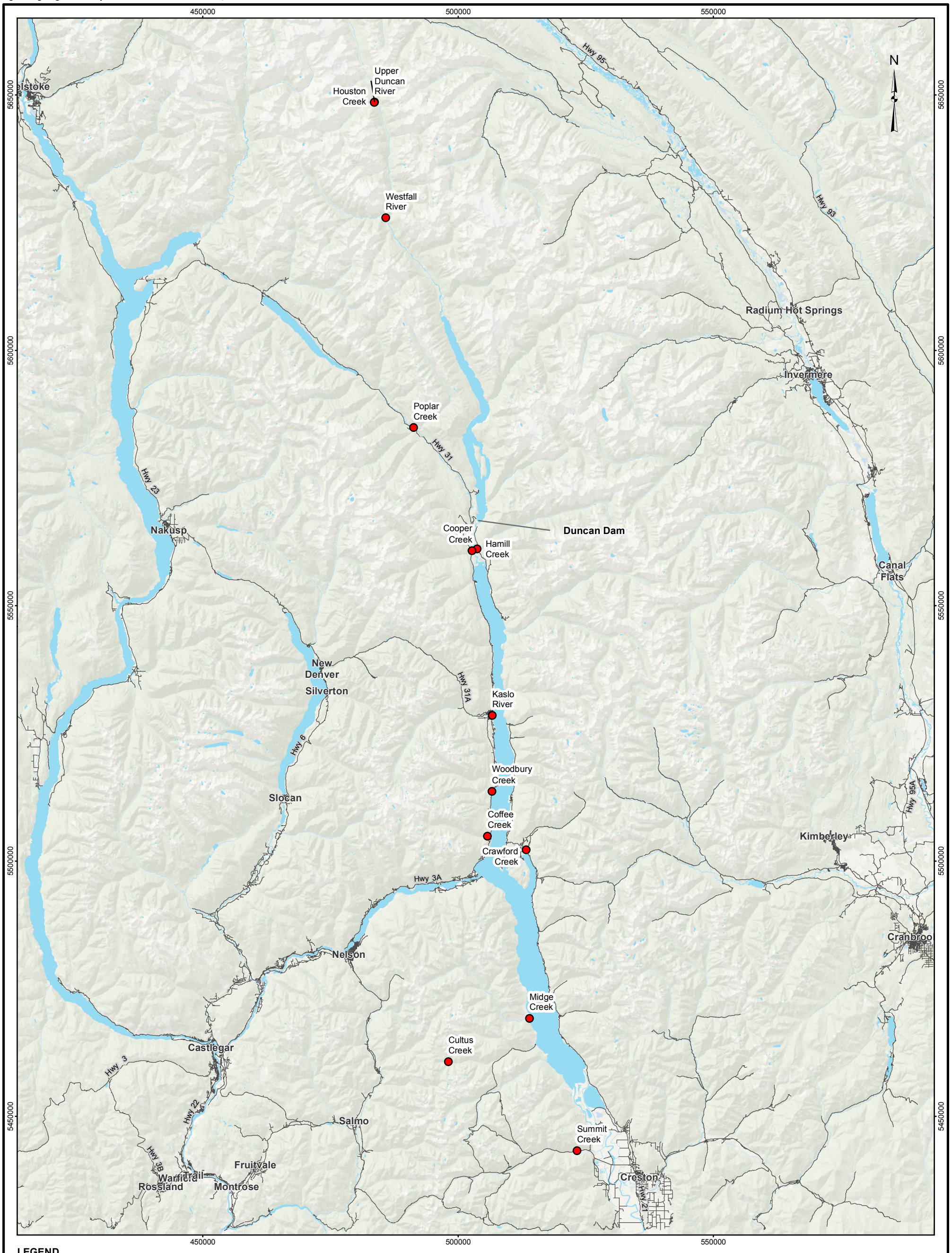

LEGEND

SAMPLE LOCATION

- WATER CHEMISTRY AND JUVENILE BULL TROUT COLLECTION LOCATION
- ROAD SEGMENT

15 0 15

SCALE 1:700,000 KILOMETRES

REFERENCE

SERVICE LAYER CREDITS: ESRI, DELORME, GEBCO, NOAA NGDC, AND OTHER CONTRIBUTORS.
TRIM MAPPING PROVIDED BY BC HYDRO.

DATUM: NAD83 PROJECTION: UTM ZONE 11

PROJECT

UPPER DUNCAN RIVER
BULL TROUT MIGRATION MONITORING (DDMMON#5)

TITLE

STUDY AREA OVERVIEW

Golder Associates

PROJECT 1524544 / 1000 / 7			FILE No.	
DESIGN	DR	20 APR. 2016	SCALE AS SHOWN	REV. 0
GIS	JG	20 APR. 2016		
CHECK	DR	21 APR. 2016		
REVIEW	DS	21 APR. 2016		

FIGURE: 1

1.2 Project Objectives

The Duncan Dam operates a fish transfer program to allow for the passage of adult Bull Trout from the lower Duncan River into the Duncan Reservoir. However, it is unknown if recruitment from those spawners is contributing to populations above or below the dam. The main objective of the study is to determine whether the Bull Trout transfer program facilitates the recruitment of Bull Trout above and/or below Duncan Dam (BC Hydro 2008). The objectives outlined in the terms of reference are to:

1) Estimate the proportion of Bull Trout entering the Duncan Reservoir that originate from the Duncan Reservoir system;
2) Document the life histories of Bull Trout sampled from the Kootenay and Duncan systems; and
3) Identify differences in life histories between systems that may be associated with migration between systems.

These objectives have been identified to address the overall management question in the terms of reference:

"Does the Bull Trout transfer program contribute to the recruitment of Bull Trout to Kootenay Lake or Duncan Reservoir?"

This management question will be answered by addressing the following questions:

1) What are the origins of Bull Trout individuals sampled in Duncan Reservoir and Kootenay Lake watersheds?
2) Do the distribution and analyzed life histories of the sampled fish denote a bottleneck to recruitment at Duncan Dam?

Once these questions have been answered, the final management question can be investigated:

"What changes to the Bull Trout transfer program are recommended to improve Bull Trout in the Duncan Reservoir and Kootenay Lake?"

The program has been designed to test two hypotheses based on the water and otolith microchemistry methodology used in the study. The hypotheses in the RFP terms of reference are as follows:

> ***H01:*** *Stream chemistry is not sufficiently different between tributaries of the Kootenay and Duncan watersheds to determine the natal origins of Bull Trout sampled in the area.*
>
> ***H02:*** *The proportion of natal to non-natal Bull Trout is not statistically different between the Kootenay and Duncan watersheds.*

The first hypothesis assesses whether or not the otolith microchemistry methodology is effective for addressing the management questions. The second hypothesis assesses whether spawning and rearing areas in the Duncan watershed contribute to recruitment in

Kootenay Lake. Differences in the proportion of Bull Trout of non-natal origin between watersheds would suggest that the fish passage program at Duncan Dam is important for Bull Trout populations in the study area.

Results from the 2008 and 2009 study years of this program indicated that the otolith microchemistry methodology was effective for discriminating the natal origin of most of the tributaries sampled (Golder 2010). These 2008/2009 results suggested that a large percentage of adult Bull Trout captured at Duncan Dam during fish transfer operations and in Kootenay Lake likely originated from tributaries in the Upper Duncan River watershed. In 2013, a different laboratory was used to analyze otolith microchemistry of Bull Trout captured from 2008 to 2013. The data analyzed in 2013 indicated much greater variability in otolith chemistry within each stream, which resulted in overlapping chemistries among streams, and poor ability to discriminate the natal origins of Bull Trout (ONA and Golder 2013). For this reason, conclusions to-date regarding the effectiveness of the fish transfer program are mostly based on 2008/2009 results, whereas the data analyzed in 2013 provided little utility for addressing the management questions. A third laboratory was used for otolith microchemistry analyses in 2016, because the laboratory used in 2008/2009 was not available. The main objectives of this report are:

1) Analyze data for a subsample of juvenile Bull Trout otoliths analyzed at both the 2008/2009 and 2016 laboratories to corroborate previous results and confirm that results from the new laboratory will be useable for discriminating and predicting the natal origin of Bull Trout; and

2) If data analyzed in 2016 are comparable to 2008/2009 results, then additional juvenile otoliths will be analyzed to improve the model predicting the natal origin of Bull Trout. Otoliths collected and analyzed for this report include additional samples for streams previously analyzed and samples from new streams, both of which could help improve ability to discriminate and predict natal origins.

1.3 Study Area

The study area covers an approximate distance of 150 km from the northern end of the Upper Duncan River to southern Kootenay Lake (Figure 1). Three tributaries (Houston Creek, Upper Duncan River, and Westfall River) were upstream of Duncan Dam and were used to represent the Upper Duncan watershed. All other streams were downstream of Duncan Dam and were chosen to represent different portions of the Kootenay Lake watershed. Poplar Creek was chosen to represent the Lardeau River, which is a tributary that joins the Duncan River ~800 m downstream of the Duncan Dam. There were two sites in the lower Duncan River (Hamill Creek and Cooper Creek), three in the north arm of Kootenay Lake (Kaslo River, Woodbury Creek and Coffee Creek), and two sites in central/south Kootenay Lake (Crawford Creek and Midge Creek). In 2015, Cultus Creek and Summit Creek were sampled for the first time to represent the southern end of Kootenay Lake. Water samples were collected from each location where juvenile Bull Trout were collected. Site locations and description are provided in Table A1, Appendix A.

2. METHODS

2.1 Field Methods

Field sampling in 2015 included collection of juvenile Bull Trout from Cultus and Summit creeks, collection of adult Bull Trout heads from Duncan Reservoir and Kootenay Lake, and water samples from Cultus and Summit creeks at locations of juvenile capture, as described in detail below. Water samples and juvenile Bull Trout otoliths were also previously collected for this program in 2008, 2009, 2012, and 2013. Adult Bull Trout otoliths were also previously collected in 2008, 2012, and 2013 from Duncan Reservoir, Kootenay Lake and the Duncan Dam flip bucket.

2.1.1 Water Sampling

All samples were collected in sterilized 125 mL high-density polyethylene narrow mouth bottles and labelled with the following:

- Company name
- Date and time sampled
- Sample Number
- Site
- Replication #
- Preserved or Not
- Temperature

Samples were collected with 60 mL filtering syringes equipped with 0.45 µm filters. Filtering syringes were conditioned by triple rinsing with stream water prior to sample collection. Sample bottles were conditioned by triple rinsing with filtered stream water prior to filling the bottle to the neck with the sampled stream water. Two samples were taken from each tributary and preserved with 0.5 mL HNO_3 (diluted to 0.4% HNO_3 once applied to stream water sample) for trace metals analysis. All samples were collected upstream of the field technician to prevent contamination. One preserved and one unpreserved field blank of deionized water were collected at each sampling location. All samples were stored in a cooler with ice packs for transport. Samples were refrigerated until ready to be shipped in a cooler with ice packs to the lab. Samples were shipped in coolers with ice packs to keep the samples as cool as possible during transport.

2.1.2 Juvenile Fish Collection

Juvenile fish were collected using a backpack electrofisher. All collection activities were carried out as per the Resource Inventory Standards Committee standards and methods for fish collection (RISC 1997). After collection, fish were euthanized using diluted clove oil and measured for length (mm) and weight (g). All fish were labelled according to tributary and sample date, and frozen for transport to the lab where otoliths were extracted.

2.1.3 Otolith Collection - Adult Fish

Transfer Station Sampling

The weekly visits that coincided with the transfer schedule provided by BC Hydro enabled the collection of adult Bull Trout from the flip bucket. The collection permit allowed for a maximum of 30 adult Bull Trout to be collected each year (up to a maximum of 10% of the fish present at each transfer). An estimate of the total number of Bull Trout present during each transfer was established prior to sampling.

Staff randomly selected the allotted number of fish during each transfer, attempting to distinguish gender externally with a preference for sacrificing males at the request of the Ministry of Forests, Land, and Natural Resource Operations. The fish collected had meristic data recorded (weight, fork length, gender and other information as requested) and the heads were collected. Each head was stored in a plastic bag in a cooler and then frozen until the otoliths were removed. Otolith removals were conducted in the lab after sufficient thawing. Adult Bull Trout were collected from the flip bucket in 2009, 2012 and 2013.

Recreational Bull Trout Fishery Collection Program

An opportunistic fish-head collection program was established for the Duncan Reservoir and Kootenay Lake targeting the collection of adult Bull Trout from recreational fisheries at each reservoir. The program included design and display of signage, communication of the program objectives at major marinas and guide offices along Kootenay Lake, and establishment of designated drop off locations for each area to facilitate the collection and storage of samples. Where possible, fish data (length, age, sex and scale sample) from fish heads was obtained. Samples were collected and frozen until the otoliths were removed. This collection program was used in 2009, 2012, 2013, and 2015.

2.2 Laboratory Methods

2.2.1 Water Chemistry Analysis

Water samples were analyzed for trace metals by Caro Analytical Services using a Thermo X Series II X7 quadruple Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Calibration was done by analysis of synthetic multi-element standards. Drift and matrix correction were done by addition of Rh, In, Re, Bi (internal standards). Accuracy and precision were determined by replicate analyses of the standard reference material in *APHA Standard Methods for the Examination of Water and Wastewater, American Public Health Association*, and all data was reported in ug/L (ppb).

2.2.2 Otolith Microchemistry Analysis

Sagittal otoliths were collected from juvenile and adult Bull Trout in 2008, 2009, 2012, 2013 and 2015. Individual otoliths were stored in microcentrifuge tubes until processing. Three different laboratories have been used for otolith microchemistry analyses during the monitoring program because laboratories that had previously conducted the analyses were unavailable in subsequent years. In 2008 and 2009, otolith samples were analyzed at the School of Earth and Ocean Sciences, University of Victoria (hereafter “Victoria lab”). In 2013, otoliths were analyzed at the University of Adelaide in Australia. In 2016, otoliths were analyzed at the University of Manitoba in Winnipeg (hereafter “Winnipeg lab”).

All three labs used laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to measure the concentrations of elements in the Bull Trout otoliths. The methodology used by the Victoria lab was provided to the other two labs, and they attempted to follow these methods as closely as possible. Details of the laboratory methodology for otolith analysis by the Victoria lab and University of Adelaide lab are provided in Golder (2010) and ONA and Golder (2013). Details of the otolith analysis conducted in 2016 by the Winnipeg lab are provided below.

The number of otoliths analyzed by capture location and laboratory is provided in Table 1. In 2016, only juvenile Bull Trout otoliths were analyzed whereas adult otoliths were not. New adult otoliths were not analyzed in 2016 because the priorities were to assess whether data from the Winnipeg lab would be directly comparable to those from the Victoria lab, and corroborate the earlier results and predictive model. Juvenile otoliths analyzed by the Winnipeg lab in 2016 included: a) 13 otoliths that were previously analyzed by the Victoria lab (1-2 juveniles from each of eight tributaries); b) 106 juvenile otoliths that were re-analyzed from the Australian lab; and c) 31 juvenile otoliths that had not been previously analyzed. Statistical analyses in 2016 used all juvenile and adult otoliths analyzed in Victoria and juvenile otoliths analyzed in Winnipeg. Otolith chemistry data analyzed by the Australian lab was not used in 2016 because large within-stream variability resulted in poor ability to classify juveniles to their known stream of capture, and therefore was not useful for predicting the natal tributary of adults of unknown origin.

Table 1. Numbers of Bull Trout otoliths analyzed by year, capture location, and laboratory.

Collection Year	Capture Location	Life Stage	Total Number Analyzed	Number Analyzed by Laboratory		
				Victoria	Australia	Winnipeg
2009	Flipbucket	Adult	23	23	0	0
2012/2013	Flipbucket	Adult	44	0	44	0
2015	Flipbucket	Adult	0	0	0	0
2009	Kootenay Lake	Adult	13	13	0	0
2012/2013	Kootenay Lake	Adult	33	0	33	0
2015	Kootenay Lake	Adult	0	0	0	0
2009	Duncan Reservoir	Adult	12	12	0	0
2013	Duncan Reservoir	Adult	0	0	0	0
2015	Duncan Reservoir	Adult	0	0	0	0
Total Adults			**125**	**48**	**77**	**0**
Duncan Watershed						
2008/2009/2013	Houston Creek	Juvenile	26	7	12	9
2008/2009/2013	Upper Duncan River	Juvenile	33	7	13	13
2008/2009/2013	Westfall River	Juvenile	31	7	11	13
Kootenay Watershed						
2008/2009/2013	Coffee Creek	Juvenile	24	7	10	8
2008/2009/2013	Cooper Creek	Juvenile	27	7	9	11
2008/2009/2013	Crawford Creek	Juvenile	59	7	27	25
2015	Cultus Creek	Juvenile	30	0	0	30
2008/2009/2013	Hamill Creek	Juvenile	18	7	6	5
2008/2009/2013	Kaslo River	Juvenile	32	7	15	11
2013	Midge Creek	Juvenile	48	0	26	22
2008/2009/2013	Poplar Creek	Juvenile	16	7	9	1
2015	Summit Creek	Juvenile	1	0	0	1
2008/2009/2013	Woodbury Creek	Juvenile	9	7	1	1
Total Juveniles			**354**	**70**	**139**	**150**

University of Manitoba Otolith Microchemistry Laboratory Methods

The otoliths were extracted and provided to the laboratory. Otoliths were embedded in epoxy (Buehler Epoxy-Cure Resin), scored with a scalpel, and sectioned using a Buehler isomet saw. Secondary epoxy embedding was accompanied by placing 13 to 16 sectioned otoliths into a one inch diameter acrylic tubing where more epoxy was added to secure the otoliths. The otolith core was exposed by polishing with sand paper in 320, 600, and 1200 grit sizes (Buehler Carbimet). To achieve a highly polished surface, otoliths were further polished with 3 µm diamond paste (Buehler mfg.) and then with 0.1 µm aluminum oxide paste. Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) was accomplished at the Winnipeg lab using the UP-213 Laser Ablation System (New Wave Research) attached to a ThermoFinigan Element 2 high resolution ICP-MS (Thermo Electron Corporation). Operating parameters of the LA-ICP-MAS are provided in Tables 2 and 3.

Transects ran from the outer edge of the otolith through the core to the opposite edge in a straight line. All ablations were preceded by pre-ablation in order to remove surface contaminants. Pre-ablation laser settings were: 55 µm laser beam diameter, at a pulse repetition rate of 5 Hz and a scan speed of 100 µm/s. For laser ablation, laser settings were reconfigured to 30 µm laser beam diameter, at a pulse repetition rate of 5 Hz and a scan speed of 2-3 µm/s depending on the size of otoliths. Prior to each ablation, gas blank was collected for 50 seconds to correct for background.

The isotopes chosen for analysis were: lithium (^{7}Li), magnesium (^{25}Mg), zinc (^{66}Zn), strontium (^{86}Sr), and barium (^{138}Ba). Calcium (^{43}Ca) was used as an internal standard. All isotopic counts were ratioed to ^{43}Ca. A reference standard (National Institute of Standards and Technology: NIST SRM 610) was analyzed at the interval of one hour to correct for machine drift. Program Iolite (version 2.3.1) was used for data reduction.

Table 2. Merchantek New Wave UP-213 laser ablation conditions.

Laser	Nd:YAG
Wavelength (nm)	213
Pulse Width (nsec)	4
Repetition rate (Hz)	5
Beam shape	Flat top
Fluence (J/cm^2)	~6
Beam size (µm)	30
Ablation mode	Line
Background time (sec)	50

Table 3. ThermoFinnigan Element2 high resolution ICP-MS conditions.

Plasma power (W)	1285
Cool gas (L/min)	15.8
Auxiliary gas (L/min)	1.0
Sample gas (L/min)	0.91
He carrier gas (L/min)	0.68
ThO/Th (%)	0.20
Analytical Method	
Mass window (%)	10
Sample time (ms)	10
Sample/peak	100
Scanning type	EScan
Detection mode	Counting and analogue
Integration type	Average
Data Reduction	
Standard reference material	NIST SRM 610
Internal standard	Ca
Software	Iolite (v. 2.3.1)

2.2.3 Otolith Data Processing

LA-ICP-MS transects included the entire cross-section of the otoliths. The core of otoliths are chemically distinct from the layers deposited after hatching and represent the chemical influence of the mother and the environment that the mother lived in (Elsdon et al. 2008). The portion of the otolith of primary interest for this study was immediately outside the core, which reflects the water chemistry during the first summer of rearing in the natal stream. For age-0 juveniles sampled in their natal stream, the entire otolith outside the core represents the chemistry of the natal stream. For adults, the portion representing the natal tributary was immediately outside the core but inside the region corresponding to downstream migration to larger tributaries, lakes or reservoirs during the juvenile life stage.

Profile data of the LA-ICP-MS scans were graphed and provided by the laboratories, showing the element concentrations on the vertical axis and elapsed time or distance during the scan on the horizontal axis. The core was identified by an abrupt change in element concentrations (^{86}Sr, ^{138}Ba, and ^{66}Zn) on each side of the core. For adults, the outmigration of juveniles from the natal stream was identified as an abrupt change in elemental concentrations. The values corresponding to the natal rearing area were extracted from the profile scans in spreadsheet software and averaged. Portions of the scan with the relatively stable values of ^{86}Sr and ^{138}Ba were selected to represent the natal area. Areas corresponding to large spikes or changes in ^{25}Mg were avoided because this may indicate changes in the crystalline form of the otolith (see below) which affects the concentrations of ^{86}Sr and ^{138}Ba. Mean values of elements for the natal area were the unit of analysis for both juveniles and adults.

A portion of juvenile and adult Bull Trout samples showed sudden changes to extremely low concentrations of ^{86}Sr and ^{138}Ba in parts of the otolith. Review of the literature concerning otolith chemistry suggested that these sections likely reflect layers deposited as a different crystalline form of calcium carbonate. Teleost fish otoliths are typically composed primarily of aragonite but aragonite can sometimes be partially or fully replaced

by the vaterite form of calcium carbonate. As rates of incorporation of strontium and other elements are greatly reduced in the vaterite form compared to aragonite, elemental signatures in these two forms are not comparable (Gauldie 1996). Otoliths that had anomalous chemistry signatures due to vaterite deposition in the area of interest (natal origin outside of core) were not included in the analyses.

2.3 Statistical Analysis

All analyses used ^{86}Sr (hereafter "Sr") and ^{138}Ba (hereafter "Ba") to describe and compare the chemistry of water and otoliths samples. Other metals (lithium and manganese) were considered in previous study years but exploratory analyses indicated that these elements did not significantly improve discrimination among streams so they were not used in subsequent analyses.

Raw data from the LA-ICP-MS metals analyses were in units of parts per million (ppm). Values of ^{43}Ca (hereafter "Ca") for all Bull Trout were assumed to be 388,000 ppm because previous studies showed that fish otoliths were composed of 38.8% Ca (Yoshinaga et al. 2000). Values of Sr, Ba, and Ca in ppm were converted to moles by dividing by their molar mass. The Sr:Ca ratio is presented in mmol/mol and the Ba:Ca is presented in µmol/mol.

Multivariate analysis of variance (MANOVA) was used to test for differences in water chemistry among years (2008, 2009, 2013, and 2015). The two response variables were Sr:Ca ratio and Ba:Ca ratio, year was the fixed effect of interest, and tributary was a block effect. Differences in the individual chemical ratios (Sr:Ca and Ba:Ca) were assessed using mixed effect analysis of variance (ANOVA) with year as a fixed effect and tributary as a random block effect. If there were no significant differences among years, then mean values for all available years combined were used for each tributary.

The relationship between elemental ratios of water samples and juvenile otoliths was described using linear regression. Mean values representing the natal area for each juvenile Bull Trout analyzed in the Winnipeg lab were regressed against mean values (all years combined, if applicable) for each tributary. Juvenile otolith chemistries analyzed by the Victoria lab in 2008/2009 were not re-analyzed because a relationship with water chemistry at the capture location was already established in previous years (Golder 2010).

Otolith chemistry analyzed at the Winnipeg lab included otoliths of juvenile Bull Trout captured in 2008, 2009, 2013, 2015, although the majority of samples were from 2008 and 2013. Mixed effect ANOVA was used to assess differences in Sr:Ca and Ba:Ca (the response variables) between years (fixed effect) within tributary (random block effect). If elemental ratios did not differ by year, then year was not used as an explanatory factor in subsequent analyses.

Values of Sr:Ca and Ba:Ca were compared between labs for the 13 juvenile otolith samples analyzed in both Victoria and Winnipeg labs. For each elemental ratio, values from one lab were plotted against the other, and a 1:1 line was used to assess if values were consistently biased. A mixed effect ANOVA was used to assess if differences between labs were significant. The response variable was either Sr:Ca or Ba:Ca, laboratory was a fixed effect, and random effects were tributary and individual fish within tributary. If there was a significant difference between lab values, then linear regression was used to describe the relationship between lab values. The estimated slope and

intercept from the regression were used to correct the Victoria lab values for bias, so they were directly comparable to Winnipeg values. Values of Sr:Ca were not different between labs and were not adjusted for subsequent analyses. Values of Ba:Ca were significantly different between labs (see Results). All values of Ba:Ca measured at the Victoria lab, including values from juveniles and adults captured in 2008/2009, were adjusted using the regression equation described above. Adjusted Ba:Ca values were used in all subsequent analyses.

MANOVA was used to test for differences in water chemistry among capture locations, with the Sr:Ca and Ba:Ca ratios as the two response variables. Linear discriminant analysis (LDA) was used to describe differences in otolith chemistry among capture locations and develop a predictive model of the natal area of Bull Trout. Leave-one-out classification was used to cross-validate models. Split-sample LDA was used to predict the natal origins of adult Bull Trout based on the LDA model of juvenile Bull Trout of known origin. Three separate LDA models were assessed, using data analyzed by the Victoria lab, the Winnipeg lab, and a combined Victoria/Winnipeg data-set. In all three cases, the LDA models were used to predict the natal tributary of adults analyzed in Victoria. Adult otoliths were not analyzed by the Winnipeg lab.

For the combined Victoria/Winnipeg juvenile otolith data-set, MANOVA was used to test for differences in water chemistry between labs with capture location as a block effect. Differences in the individual chemical ratios (Sr:Ca and Ba:Ca) were assessed using mixed effect ANOVA with year as a fixed effect and tributary as a random block effect. All LDA and MANOVA analyses that used the combined data-set included the 13 juvenile otoliths that were analyzed in Victoria and Winnipeg, and both lab values were used in the analyses. Although these paired samples violate the assumption of independent observations, they needed to be included for adequate sample sizes, and the remaining majority of otoliths (n=185) were independent, so the effect of this violation is expected to be minor.

The natural logarithms of Ba:Ca and Sr:Ca were used in MANOVA and LDA to better meet assumptions of normality. For all analyses, effects were considered significant if the p-value was less than 0.05. All analyses were conducted using R version 3.2.2 (R Core Team 2015) and LDA was conducted using the MASS package (Venables and Ripley 2002).

All of the 70 juvenile otoliths analyzed by the Victoria lab and included in previous reports were used in the analysis. Of the 150 juvenile otoliths analyzed in the Winnipeg lab (Table 1), 16 were not used because of questionable and highly variable element concentrations in the scans, which could be related to phase shifts from aragonite to vaterite crystal structure, migrations to and from capture locations, or other unknown reasons. In addition, six juvenile otolith samples were considered outliers that had Sr and Ba values much greater or lower than other samples from the same tributary (FishIDs 95, 403, 418, 434, 435, and 440). These outliers were included in scatterplots of elemental ratios by capture location, and regressions between water and juvenile otolith chemistry but were not included in comparisons between groups (ANOVA/MANOVA) or predictive models of natal origin (LDA) because they were considered unreliable and not representative of chemistry of the tributary where they were captured. Possible reasons for outliers with otolith chemistry much different than other fish from the same stream are discussed further in Section 4.2.

3. RESULTS

3.1 Water Chemistry

Ratios of Ba:Ca and Sr:Ca varied among tributaries, especially for Poplar and Cooper creeks, which had much higher values of Sr:Ca and Ba:Ca than the other tributaries in all three years of sampling (Figure 2). Water chemistry was similar for Hamill Creek, Houston Creek, Midge Creek, Upper Duncan River, and Westfall River. Samples from Woodbury, Kaslo, and Coffee creeks consistently had the lowest Ba:Ca ratios. Water chemistry did not differ significantly among sampling years (MANOVA; P=0.08). Neither Sr:Ca (P=0.1) nor Ba:Ca (P=0.1) differed among years with tributary as a block effect. Therefore, mean values from all available years for each tributary were used for comparisons with Bull Trout otolith chemistry.

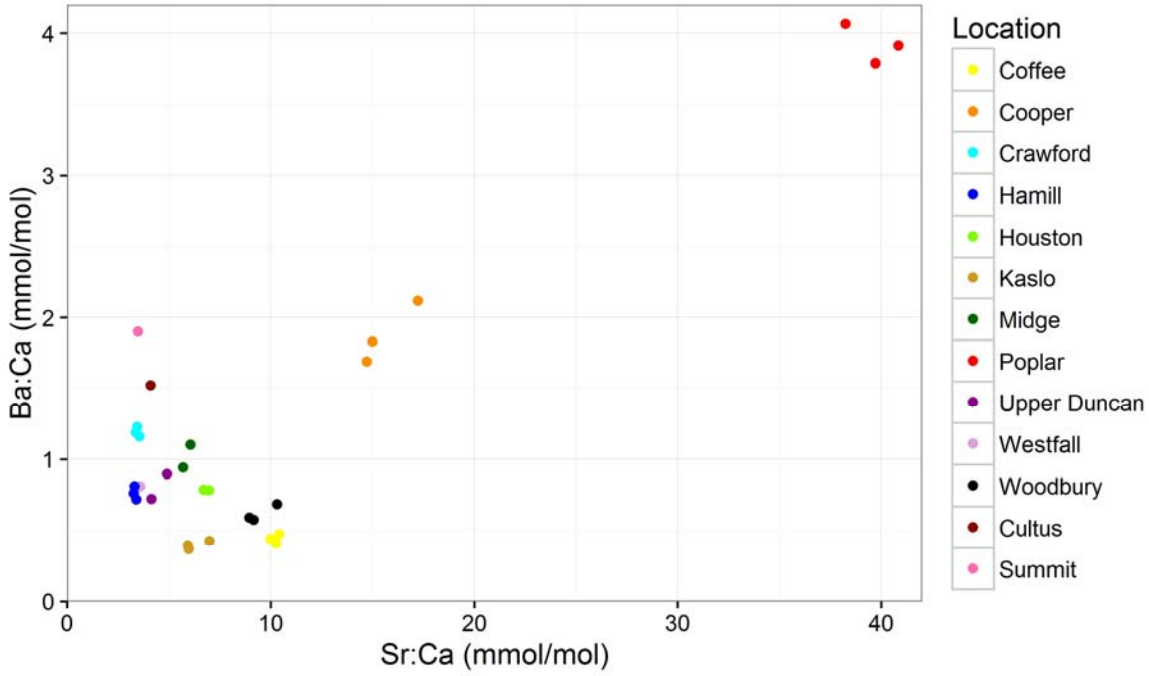

Figure 2. Water chemistry ratios by tributary. Multiple points for each tributary represent different sampling years.

3.2 Juvenile Otolith Chemistry

There was a strong relationship between the Sr:Ca ratio in juvenile Bull Trout otoliths analyzed in the Winnipeg lab and water samples from the tributaries where fish were captured (y = 0.11x + 0.15; P<0.0001; r^2=0.73; Figure 3). Sr:Ca ratios in otoliths from Houston Creek were lower than expected based on water chemistry. The two obvious outliers in Cooper Creek and the Westfall River (Figure 3) were included in the regressions but were removed from LDA to classify and predict natal origins.

There was a significant relationship between the Ba:Ca ratio in juvenile Bull Trout otoliths analyzed in the Winnipeg lab and water samples from the tributaries where fish were captured (y = 6.88x + 2.54; P<0.0001; r^2=0.32; Figure 3). Water chemistry explained less of the variation in Ba:Ca in otoliths (32%) than in Sr:Ca in otoliths (73%). Ba:Ca in juvenile

otoliths was greater than predicted by water chemistry in Crawford Creek and lower than predicted for Coffee Creek. Most of the otolith Ba:Ca ratios for other tributaries were centered around the regression line, although some had high variability within the same tributary (Figure 3).

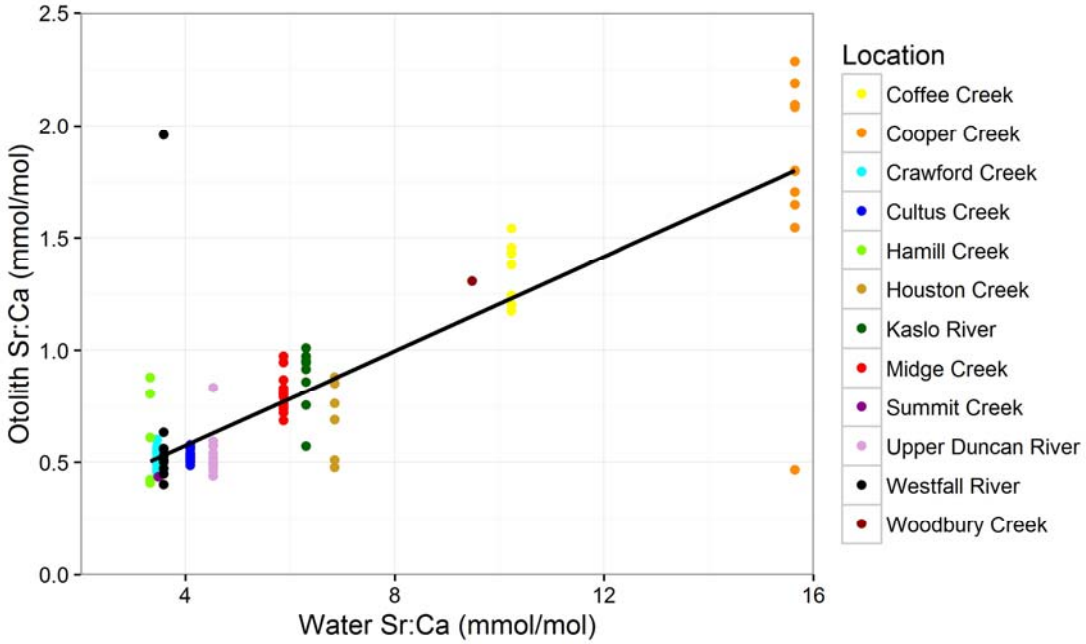

Figure 3. Relationship between the Sr:Ca ratio in water samples and juvenile Bull Trout otoliths analyzed in 2016.

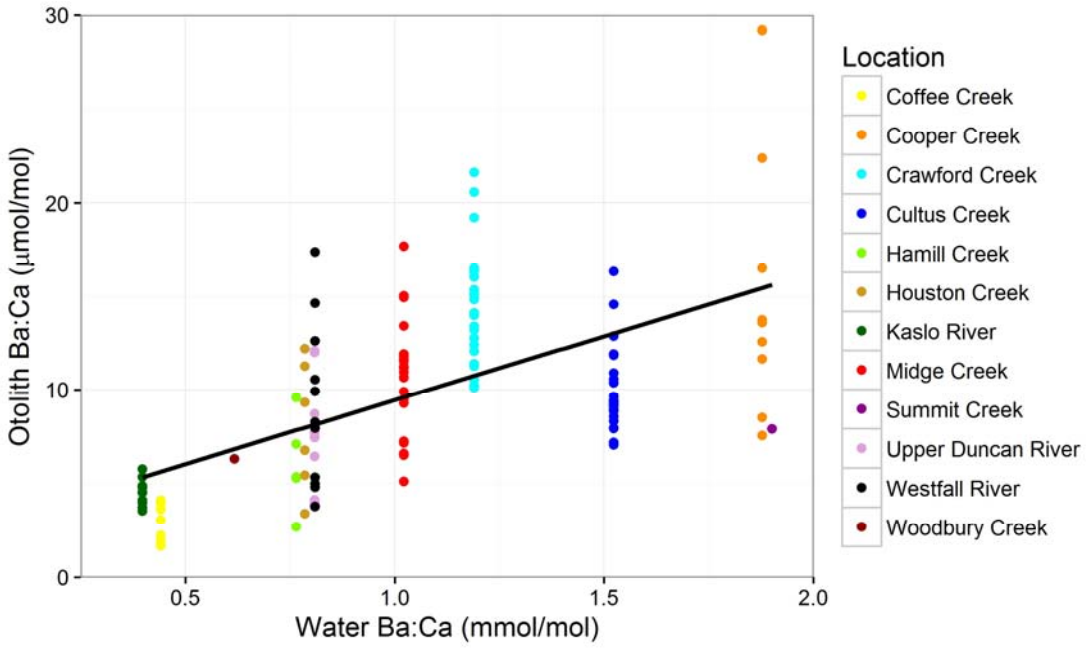

Figure 4. Relationship between the Ba:Ca ratio in water samples and juvenile Bull Trout otoliths analyzed in 2016.

Thirteen juvenile Bull Trout otoliths previously analyzed in the Victoria lab were re-analyzed in the Winnipeg lab in 2016 to assess the comparability of elemental ratios. Sr:Ca was not significantly different between labs for paired samples of juvenile otoliths from eight of the tributaries (mixed effect ANOVA; *P*=0.6). This is also supported by the plot of Sr:Ca ratios from Winnipeg versus Victoria labs, which shows similar values and relatively equal spread of points above and below the 1:1 line, indicating lack of consistent bias (Figure 5).

Ba:Ca values were significantly greater at the Victoria lab than the Winnipeg lab (mixed effect ANOVA; *P*=0.0001). Nearly all the points on the plot fall below the 1:1 line, suggesting a consistent positive bias for Victoria lab values relative to Winnipeg lab values (Figure 6). Linear regression was used to describe the relationship between Winnipeg and Victoria lab Ba:Ca values (Figure 7; y = 0.82x + -0.9; *P*<0.0001; r^2=0.97). The estimated slope and intercept from the regression equation were used to adjust the Ba:Ca values for all juvenile and adult otoliths previously analyzed in the Victoria lab so that they were directly comparable to the Winnipeg lab Ba:Ca values. These adjusted Ba:Ca values were used for all subsequent analyses, including LDA to classify and predict natal origins based on otolith chemistry.

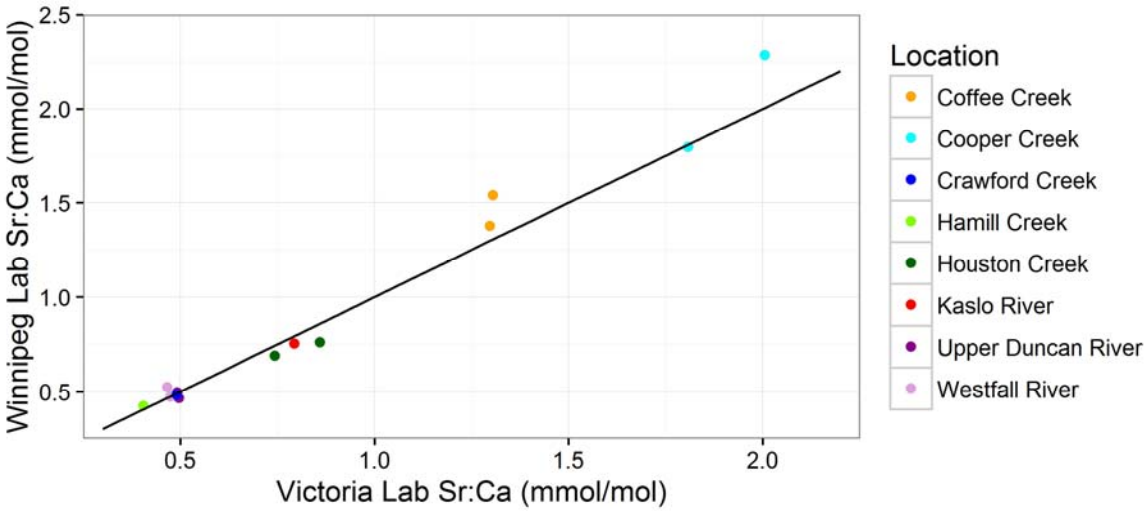

Figure 5. Sr:Ca ratios for thirteen juvenile Bull Trout otoliths analyzed at two laboratories. Each point represents the mean value corresponding to the natal rearing area for an individual fish. The line represents a 1:1 relationship.

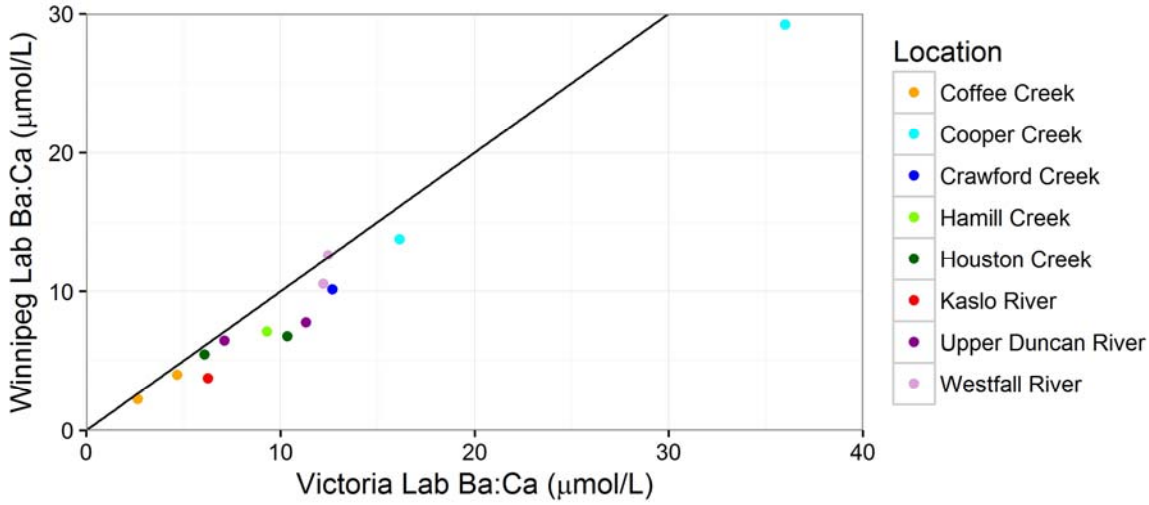

Figure 6. Ba:Ca ratios for thirteen juvenile Bull Trout otoliths analyzed at two laboratories. Each point represents the mean value corresponding to the natal rearing area for an individual fish. The line represents a 1:1 relationship.

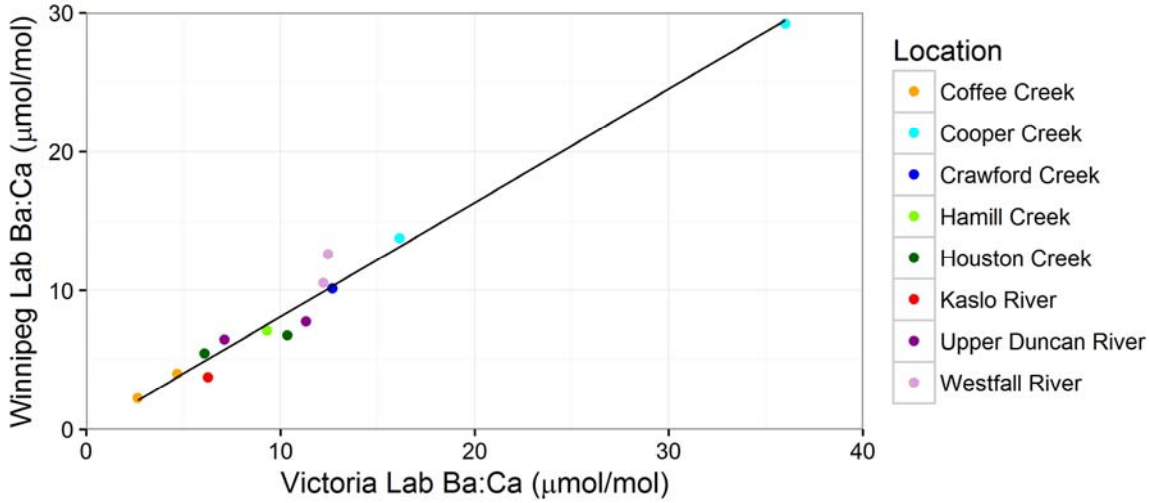

Figure 7. Linear regression between Ba:Ca ratios for thirteen juvenile Bull Trout otoliths analyzed at two laboratories. Each point represents the mean value corresponding to the natal rearing area for an individual fish.

For juvenile otoliths captured in various years (2008-2015) and analyzed by the Winnipeg lab, neither Sr:Ca nor Ba:Ca differed among years (P=0.2 and P=0.3 respectively; Figure B1, Appendix B). Therefore, year was not considered as an explanatory factor in subsequent analyses. The Sr:Ca and Ba:Ca ratios of juvenile otoliths analyzed by the Winnipeg lab were fairly well grouped by capture location for most of the streams (Figure 8). However, some of the streams, such as Hamill Creek, Houston Creek, Westfall River, and Upper Duncan River had much greater within-stream variability in both Sr:Ca and Ba:Ca than the other tributaries. There were some outliers from Cooper Creek, Hamill Creek, Houston Creek, and Westfall River that were much greater or lower in Sr:Ca than all other samples from that tributary. These outliers, listed in Section 2.3, were included in Figure 8 but removed from the data set before linear discriminant analyses to predict natal origins.

There were three streams, Cultus, Midge, and Summit creeks, that were not sampled or analyzed in previous study years. Otolith chemistry data from Cultus Creek was tightly grouped, especially in terms of Sr:Ca, but overlapped with several other streams, including Crawford Creek, Hamill Creek, Westfall River, and Upper Duncan River (Figure 8). Midge Creek had greater within-stream variability in Sr:Ca but had less overlap in chemistry with other streams than Cultus Creek. Only one juvenile Bull Trout was captured in Summit Creek but Sr:Ca and Ba:Ca values were similar to several other streams .

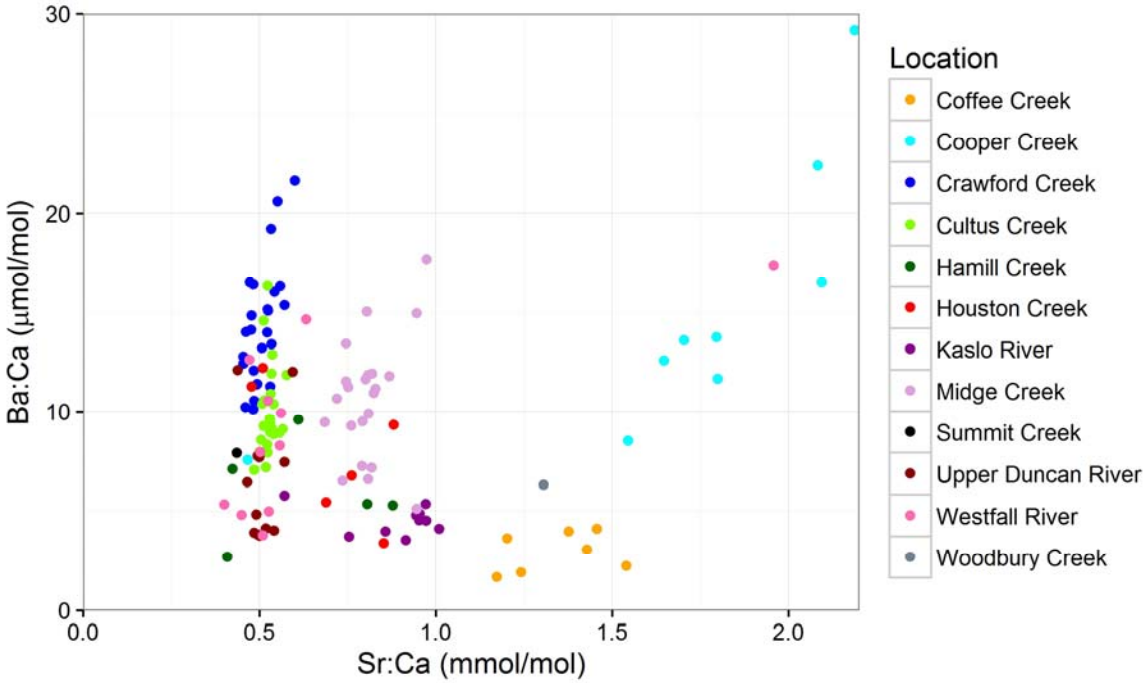

Figure 8. Natal otolith chemistry of juvenile Bull Trout analyzed at the Winnipeg lab in 2016 by capture location.

Juvenile Bull Trout otolith chemistry data analyzed in the Victoria lab were well grouped by capture location, with Cooper and Poplar creeks having the most distinct chemistry in terms of Sr:Ca and Ba:Ca (Figure 9). There was overlap between some of the tributaries, especially for Hamill Creek, Houston Creek, Westfall River, and Upper Duncan River, which was also observed in the Winnipeg lab data. However, these streams with overlapping chemistry were more tightly grouped, with less within-stream variability in the Victoria data than the Winnipeg data. There was greater within-stream variability in Ba:Ca than Sr:Ca in data analyzed at Victoria and Winnipeg labs (Figures 8 and 9).

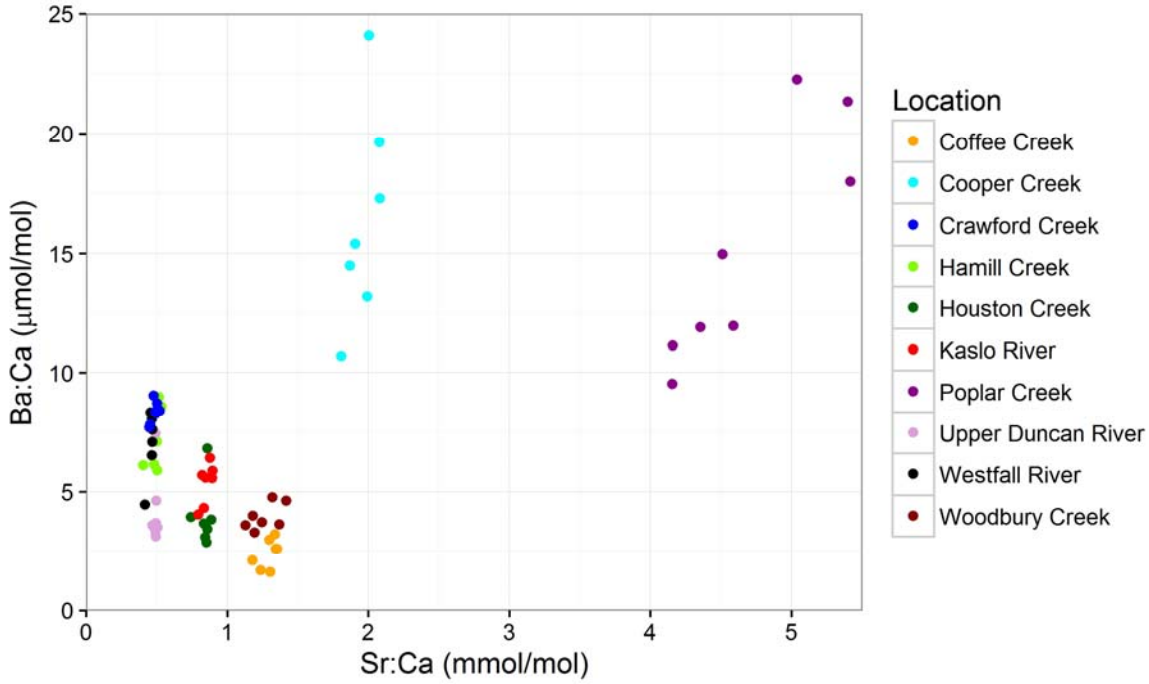

Figure 9. Natal otolith chemistry of juvenile Bull Trout analyzed at the Victoria lab in 2016 by capture location.

For the juvenile otolith data analyzed by the Winnipeg lab, Sr:Ca and Ba:Ca ratios differed significantly among capture locations (MANOVA; $P<0.0001$). LDA correctly classified the capture locations for 76% of individual fish, based on the unvalidated model using Winnipeg lab data (Table 4; Figure 10). Using the leave-one-out cross-validated LDA, juvenile capture locations were correctly classified in 75% of cases. The coefficients of discriminants indicated that the first discriminating function (LD1) was most related to Sr:Ca and the second discriminating function (LD2) was more related to Ba:Ca. Most of the between-group variance was explained by LD1 (91%) with a smaller percentage explained by LD2 (9%). The percentage of correct classification was greater than 80% for seven of the streams but none of juvenile otoliths from Hamill Creek, Houston Creek, Westfall River were correctly classified based on their Sr:Ca and Ba:Ca ratios (Table 4). Only 50% of juvenile otoliths from the Upper Duncan River were correctly classified.

For the juvenile otolith data analyzed by the Victoria lab, Sr:Ca and Ba:Ca ratios differed significantly among capture locations (MANOVA; $P<0.0001$). LDA using the Victoria lab data correctly classified the capture locations for 84% of individual fish based on the unvalidated model, and 79% of fish using leave-one-out cross-validation (Table 4; Figure 11). The coefficients of discriminants indicated that LD1 was most related to Sr:Ca and LD2 was more related to Ba:Ca. Most of the between-group variance was explained by LD1 (97%) with a small percentage explained by LD2 (3%). The percent of fish whose capture location was correctly classified by cross-validated models based on Sr:Ca and Ba:Ca ratios ranged from 50% to 100% depending on the stream (Table 4).

LDA using the combined data-set from Victoria and Winnipeg labs correctly classified 71% of juveniles to their capture locations (Table 4; Figure 12). The tributaries that were poorly predicted using the Winnipeg data (Hamill, Houston, and Westfall) were also poorly predicted by the combined data-set. Crawford Creek was the only tributary where the combined data-set had a better classification rate than the Victoria-only and Winnipeg-only data-sets.

The largest difference between LDA models using data from different laboratories was that the Duncan watershed tributaries (Houston Creek, Westfall River, and Upper Duncan River) were predicted reasonably well using data from the Victoria lab, and very poorly using data from the Winnipeg lab (Table 4). This difference was related to greater within-stream variability in Sr:Ca and Ba:Ca for Duncan watershed tributaries in the Winnipeg data (Figure 10) than the Victoria data (Figure 11). All of the Kootenay watershed tributaries, except for Hamill Creek, were predicted accurately by both Victoria and Winnipeg data-sets.

Table 4. Percentage of correct classification of capture location of juvenile Bull Trout based on linear discriminant analysis for samples analyzed by Winnipeg and Victoria labs.

	Winnipeg		Victoria		Combined	
Capture Location	**Un-validated Model**	**Cross-Validated Model**	**Un-validated Model**	**Cross-Validated Model**	**Un-validated Model**	**Cross-Validated Model**
Duncan Watershed						
Houston Creek	0%	0%	71%	71%	9%	9%
Upper Duncan River	50%	50%	86%	86%	63%	63%
Westfall River	0%	0%	86%	57%	24%	18%
Kootenay Watershed						
Coffee Creek	100%	100%	100%	100%	93%	93%
Cooper Creek	100%	89%	100%	100%	94%	94%
Crawford Creek	92%	92%	57%	57%	94%	94%
Cultus Creek	88%	88%	-	-	75%	75%
Hamill Creek	0%	0%	67%	50%	0%	0%
Kaslo River	90%	90%	71%	71%	76%	71%
Midge Creek	95%	95%	-	-	91%	91%
Poplar Creek	-	-	100%	100%	100%	100%
Summit Creek	0%	n/a	-	-	0%	n/a
Woodbury Creek	100%	n/a	100%	86%	88%	88%
Total	**76%**	**75%**	**84%**	**79%**	**71%**	**71%**

- A hyphen indicates that otoliths were not analyzed for a particular site.
- “n/a” indicates that the classification rate could not be cross-validated because there was only one sample at that site.

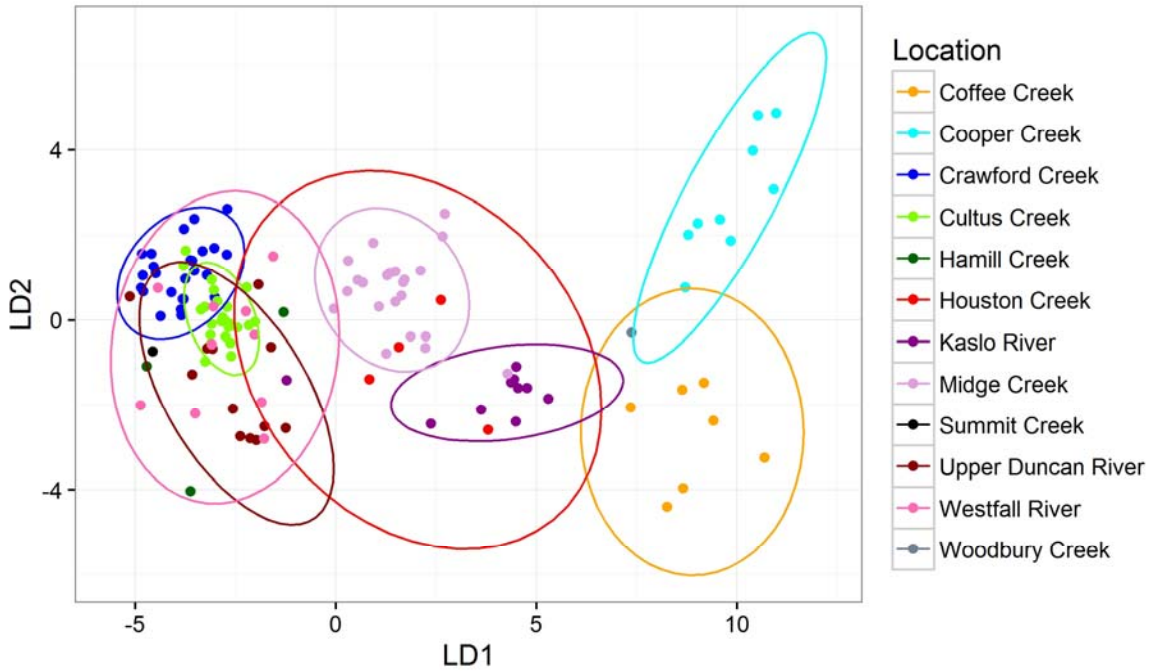

Figure 10. Discriminant scores for natal otolith chemistry of juvenile Bull Trout analyzed by the Winnipeg laboratory. Ellipses show 95% confidence intervals of the discriminant scores. Ellipses are not shown for Hamill, Summit, and Woodbury creeks because sample sizes were too small to calculate confidence intervals.

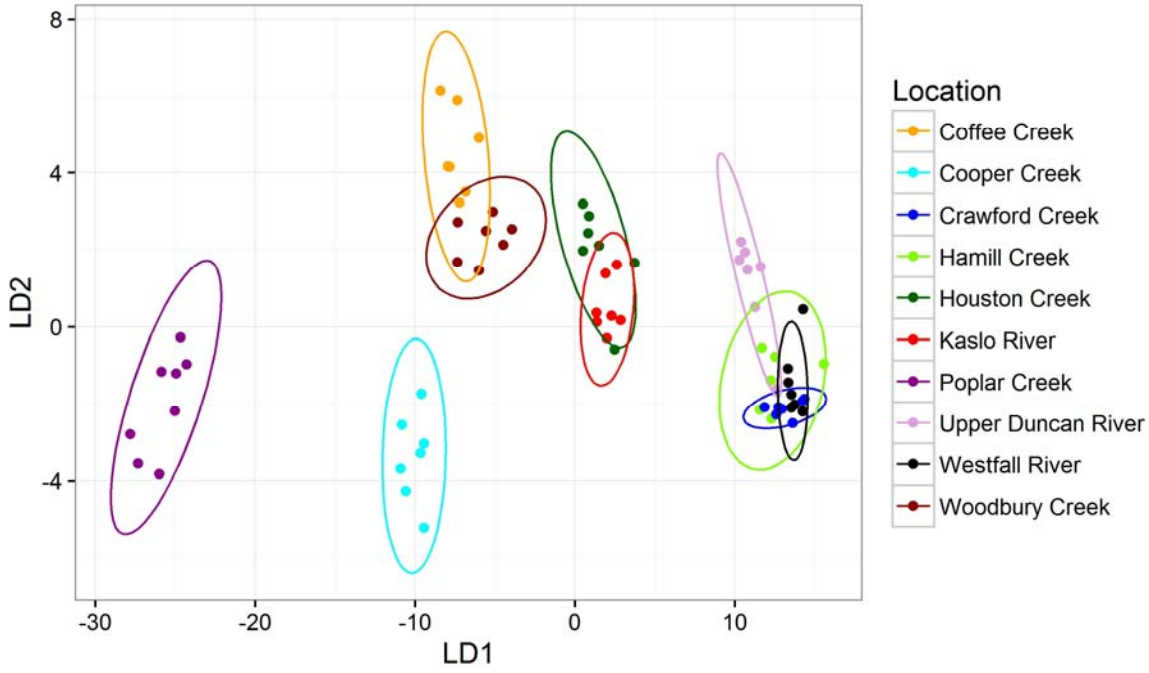

Figure 11. Discriminant scores for natal otolith chemistry of juvenile Bull Trout analyzed by the Victoria laboratory. Ellipses show 95% confidence intervals of the discriminant scores.

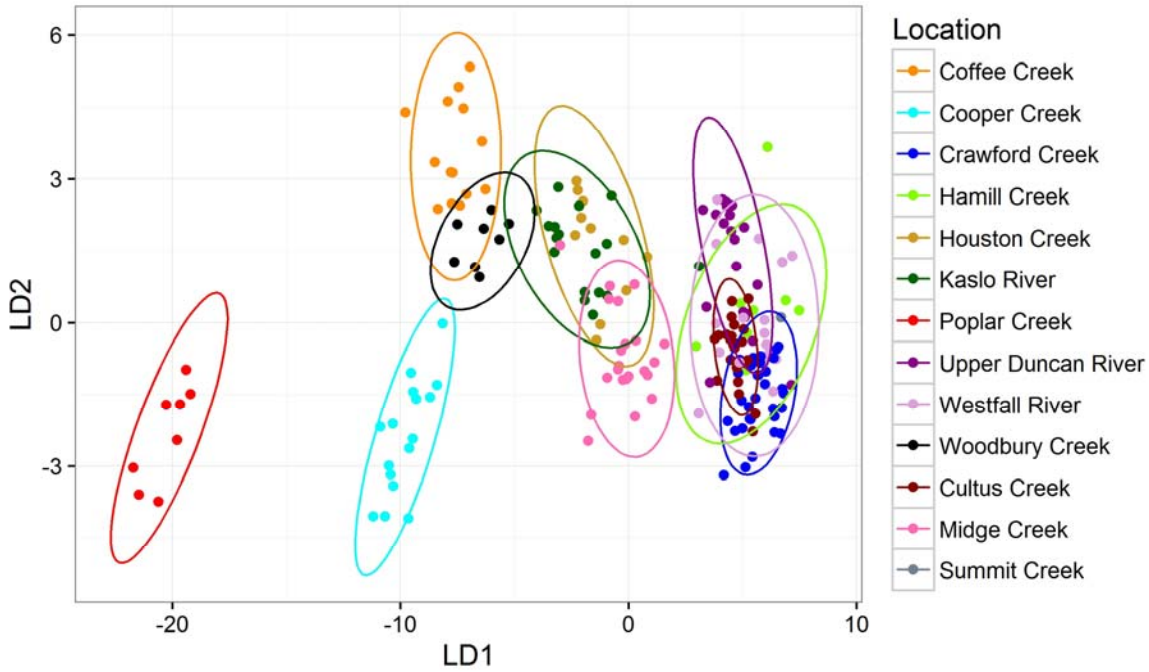

Figure 12. Discriminant scores for natal otolith chemistry of juvenile Bull Trout using combined data analyzed by the Victoria and Winnipeg labs. Ellipses show 95% confidence intervals of the discriminant scores. Ellipse is not shown for Summit Creek because the sample size was too small to calculate confidence intervals.

3.3 Predicting Natal Area of Adults

The Ba:Ca and Sr:Ca ratios corresponding to the natal portions of adult Bull Trout otoliths did not differ among the Duncan Lake, Kootenay Lake, and the flip bucket capture locations (MANOVA; *P*=0.6; Figure 13). Based on the split-sample LDA that used otolith chemistry of juveniles analyzed by the Winnipeg lab to predict adult natal origin, 23% of adults captured in Kootenay Lake were classified to one of the three natal tributaries in the Duncan Watershed (Table 5). Eight percent of the adults captured in Duncan Reservoir and 39% of those captured in the flip bucket were predicted to be from the Duncan Watershed tributaries. In contrast, the LDA using juvenile data analyzed by the Victoria lab classified a majority of adults to Duncan Watershed tributaries (Table 6). This model using Victoria lab data predicted that 50% of adults captured in Duncan Reservoir, 78% of those from the flip bucket, and 69% of those from Kootenay Lakewere from one of the Duncan watershed tributaries. The LDA model using the combined Winnipeg/Victoria data-set resulted in predictions intermediate to the Winnipeg-only and Victoria-only analyses. In the combined data-set analysis, 33% of adults captured in Duncan Reservoir, 52% from the flip bucket, and 46% from Kootenay Lake were predicted to be from Duncan watershed tributaries (Table 7). Discriminant scores from the Sr:Ca and Ba:Ca ratios of adult Bull Trout otoliths are plotted with 95% confidence intervals from the juvenile otolith LDA models are shown in Appendix B, Figures B2-B4.

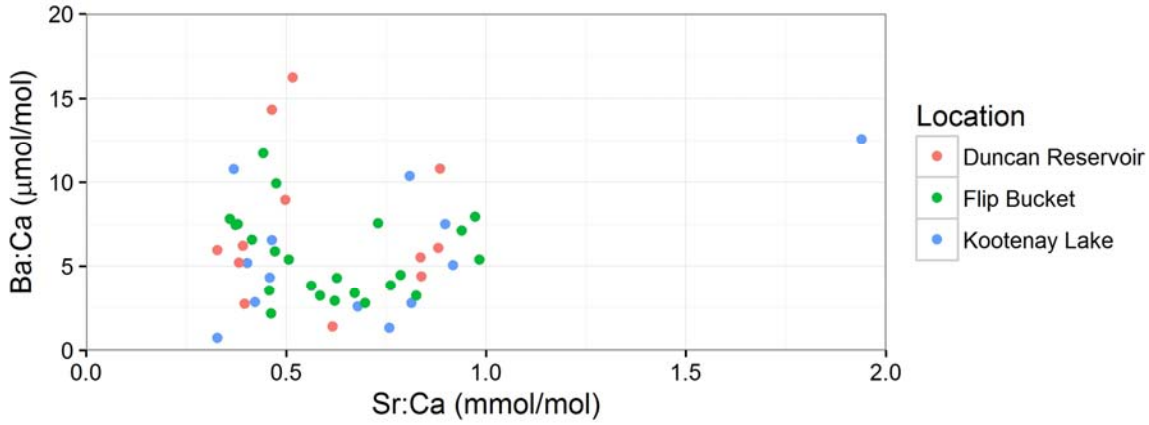

Figure 13. Natal otolith chemistry of adult Bull Trout analyzed by the Victoria lab.

Table 5. Classification of the natal origin of adults captured in 2008 using predictive model of juveniles captured in 2008-2015 and analyzed in Winnipeg laboratory.

	Adult Capture Location					
	Duncan Reservoir		Flip Bucket		Kootenay Lake	
Predicted Natal Tributary	**#**	**%**	**#**	**%**	**#**	**%**
Houston Creek	0	0	3	13	0	0
Upper Duncan River	1	8	6	26	3	23
Westfall River	0	0	0	0	0	0
Total Duncan Watershed	**1**	**8**	**9**	**39**	**3**	**23**
Coffee Creek	0	0	0	0	0	0
Cooper Creek	0	0	0	0	1	8
Crawford Creek	2	17	2	9	1	8
Cultus Creek	1	8	1	4	1	8
Hamill Creek	1	8	0	0	1	8
Kaslo River	4	33	7	30	4	31
Midge Creek	1	8	1	4	2	15
Summit Creek	2	17	3	13	0	0
Woodbury Creek	0	0	0	0	0	0
Total Kootenay Watershed	**11**	**92**	**14**	**61**	**10**	**77**
Grand Total	**12**	**100**	**23**	**100**	**13**	**100**

Table 6. Classification of the natal origin of adults captured in 2008 using predictive model of juveniles captured in 2008-2009 and analyzed in Victoria laboratory.

	Adult Capture Location					
	Duncan Reservoir		Flip Bucket		Kootenay Lake	
Predicted Natal Tributary	#	%	#	%	#	%
Houston Creek	2	17	7	30	4	31
Upper Duncan River	1	8	6	26	3	23
Westfall River	3	25	5	22	2	15
Total Duncan Watershed	**6**	**50**	**18**	**78**	**9**	**69**
Coffee Creek	0	0	0	0	0	0
Cooper Creek	0	0	0	0	1	8
Crawford Creek	2	17	1	4	0	0
Hamill Creek	1	8	0	0	1	8
Kaslo River	3	25	4	17	2	15
Poplar Creek	0	0	0	0	0	0
Woodbury Creek	0	0	0	0	0	0
Total Kootenay Watershed	**6**	**50**	**5**	**22**	**4**	**31**
Grand Total	**12**	**100**	**23**	**100**	**13**	**100**

Table 7. Classification of the natal origin of adults captured in 2008 using predictive model of juveniles analyzed in Winnipeg and Victoria labs combined.

	Adult Capture Location					
	Duncan Reservoir		Flip Bucket		Kootenay Lake	
Predicted Natal Tributary	#	%	#	%	#	%
Houston Creek	1	8	4	17	1	8
Upper Duncan River	1	8	7	30	3	23
Westfall River	2	17	1	4	2	15
Total Duncan Watershed	**4**	**33**	**12**	**52**	**6**	**46**
Coffee Creek	0	0	0	0	0	0
Cooper Creek	0	0	0	0	1	8
Crawford Creek	2	17	2	9	1	8
Cultus Creek	1	8	0	0	0	0
Hamill Creek	0	0	0	0	0	0
Kaslo River	3	25	5	22	4	31
Midge Creek	1	8	1	4	1	8
Poplar Creek	0	0	0	0	0	0
Summit Creek	1	8	3	13	0	0
Woodbury Creek	0	0	0	0	0	0
Total Kootenay Watershed	**8**	**67**	**11**	**48**	**7**	**54**
Grand Total	**12**	**100**	**23**	**100**	**13**	**100**

4. DISCUSSION

4.1 Stream Chemistry

Analyses of Sr:Ca and Ba:Ca ratios in water samples suggested distinct chemical signatures in most of the Duncan and Kootenay watershed tributaries assessed in this study. Hamill Creek, Upper Duncan River, and Westfall Creek had similar chemistry but all the other tributaries were well separated by Sr:Ca and Ba:Ca ratios. Regressions suggested significant relationships between Ba:Ca and Sr:Ca ratios in water samples and juvenile Bull Trout otoliths. These results support the idea that tributaries in the Kootenay and Duncan watersheds have distinct water chemistry that result in distinct elemental ratios in the otoliths of Bull Trout.

In regressions of elemental ratios in juvenile otoliths versus water samples, the otolith ratios from a few tributaries were consistently above or below the regression line, suggesting that the rate of incorporation of Sr or Ba may not be consistent for these tributaries (e.g. Coffee and Crawford creeks; Figure 4). However, as ratios of Sr:Ca and Ba:Ca in otoliths for these tributaries were distinct from other tributaries, the deviation from the water-otolith regression line does not affect use of otolith data for classifying and predicting natal origin using elemental ratios. A more problematic issue was the large within-stream variability in Ba:Ca and, for some tributaries, Sr:Ca in otoliths (Figures 3 and 4). There was very little variability in Sr:Ca and Ba:Ca ratios among years within stream (Figure 2), which suggests that variability in elemental ratios in otoliths was not related to inter-annual fluctuations in water chemistry.

4.2 Otolith Analysis

The objectives of analyses in 2016 were to assess whether otolith chemistry data analyzed at the Winnipeg lab were comparable to data previously analyzed at the Victoria lab, and if so, corroborate the earlier results based on the Victoria data. Comparison of juvenile otolith samples analyzed at both labs suggested no differences in Sr:Ca ratios between labs. Ba:Ca was consistently greater in the Victoria data than the Winnipeg data. Linear regression was used to adjust the Ba:Ca values measured by the Victoria lab so they were directly comparable to values measured by the Winnipeg lab. Because there was a consistent relationship and the regression used to adjust Victoria lab values had good fit (r^2=0.97), this adjustment likely did not affect conclusions regarding classifying natal origins based on otoliths chemistry. The reason for the systematic difference in Ba concentrations measured by the two labs was likely a difference in the standard reference materials used for LA-ICP-MS. The standard reference used by the Winnipeg lab (National Institute of Standards and Technology [NIST] 610) has greater uncertainty associated with the reported Ba values (435 ± 23) than the reference material used by the Victoria lab (NIST 611; 452 ± 9) which may explain the systematic bias in measured otolith values (P. Yang, Univ. of Manitoba, pers. comm.).

Analysis of otolith chemistry data measured by the Winnipeg lab correctly classified 75% of juveniles to their capture location. This was lower than the percentage of juveniles correctly classified to their natal tributary using data from the Victoria lab (84%) and greater than models using than data from the Australian lab (44%; ONA and Golder 2013). However, the models using data from the Winnipeg lab had poor predictive ability for Duncan watershed tributaries, as 0% of the juveniles from Houston Creek and Westfall River, and 50% from Upper Duncan River were correctly classified to their natal tributaries. Poor ability to correctly identify Duncan watershed tributaries was related to small sample

sizes for one of three tributaries (Houston Creek), high within-stream variability in both Sr:Ca and Ba:Ca, and very similar chemistry between the some of the Duncan and Kootenay watershed tributaries. For instance, Westfall River (Duncan watershed) had similar Sr:Ca ratios as several Kootenay watershed streams (Cultus and Hamill creeks; Figure 2) and high variability in Ba:Ca in otoliths that did not allow the model to discriminate Westfall Creek samples. Instead, many Westfall River and Houston Creek samples were assigned to Cultus or Midge creeks (Appendix B, Table B1), which are Kootenay tributaries with similar chemistry, larger sample sizes or lower within-stream variability in Sr:Ca and Ba:Ca.

The poor classification rate of juvenile otoliths from Duncan watershed tributaries using data from the Winnipeg lab means that predictions of the proportion of Duncan-origin adults are not reliable. Few adults of unknown origin were predicted to be from the Duncan watershed using data analyzed at the Winnipeg lab but this could have been because the model was unable to correctly classify Duncan watershed otolith chemistry based on the juvenile results. This was in contrast to results using data from the Victoria lab, which predicted that a majority of adult Bull Trout from the flip bucket (78%) and Kootenay Lake (69%) samples were from Duncan watershed tributaries, based on a model with classification rates of 71-86% (Table 4). These conflicting results make it difficult to draw conclusions regarding the contribution of Duncan watershed tributaries to Bull Trout recruitment in Kootenay Lake. However, one result that was consistent between both data-sets was the finding that approximately one quarter of adult Bull Trout from the flip bucket and one quarter of those from Kootenay Lake were predicted to have been reared in the Upper Duncan River (Tables 5 and 6). This suggests that the Upper Duncan River is important for the recruitment of Bull Trout in Kootenay Lake. There remains uncertainty in the proportion of adults originating from the other Duncan watershed tributaries, Houston Creek and Westfall River, because of differing results between data from the two different laboratories.

The lower rates of correct classification for some tributaries (especially Hamill Creek, Houston Creek, and Westfall River) based on the Winnipeg data compared to the Victoria data, was related to greater within-stream variability in either Sr:Ca, Ba:Ca, or both depending on the stream. Reasons for this greater variability could be related to natural phenomena, or analytical and laboratory differences. The Winnipeg lab attempted to follow Victoria lab methodology as closely as possible. Other than the positive bias in Ba values due to the standard reference material used, there are no other analytical differences reported by the Winnipeg lab that could explain the greater variability. Some tributaries (e.g., Cultus Creek) had relatively low levels of within-stream variability in juvenile otolith chemistry, which suggests that high variability in other streams cannot be attributed to poor precision of laboratory methods at the Winnipeg lab. During data analysis in 2008/2009, the Victoria lab provided data extracted from the raw data corresponding to the natal portion of the otolith for juvenile and adult Bull Trout. The Winnipeg lab provided raw data and we extracted the data corresponding to the natal portions of the scan following the approach reported by the Victoria lab (Section 2.2.3). However, because we did not conduct the data extraction for the Victoria data, it is possible that differences in the methods for selecting the natal area of the otolith could have contributed to the greater precision in the Victoria data.

Some juvenile Bull Trout otoliths had very different ratios of Sr:Ca and Ba:Ca than other juveniles from the same stream, with raw data showing little variability and stable values of elemental concentrations for each fish. This suggests that there was real variation (i.e., not a product of laboratory analysis) in elemental ratios of otoliths of juvenile Bull Trout captured in the same tributary. Other studies in the literature (Kennedy et al. 2000; Wells et al. 2003) and data presented here both support the contention that elemental ratios in freshwater and rates of incorporation of elements into otoliths are likely consistent over long periods of time. Therefore, large differences in Sr:Ca and Ba:Ca ratios among juveniles captured in the same tributary could be because some of these juveniles were spawned and reared elsewhere but migrated to the capture location before sampling. When selecting data from the portion of the otolith that represents the natal rearing area, any regions that had large changes in element concentrations were avoided, as these regions could represent changing water chemistry due to migrations, especially for age-1 and older juveniles that could have migrated downstream to lake environments and back to the location of capture. For age-0 Bull Trout, we hypothesized that individuals that had substantially different otolith chemistry than other juveniles from the same capture location may have hatched and initially reared in upstream tributaries with different water chemistry but moved downstream prior to sampling. Outliers were removed from the analysis but it is possible that some of remaining within-stream variability in otolith chemistry was related to immigration of age-0 fish from upstream tributaries.

In summary, the model using data from the Winnipeg lab correctly classified 75% of juveniles to their capture location based on otolith chemistry. Unfortunately, correct classification of juveniles to their capture locations was 0% for two of the three Duncan watershed tributaries due to high, within-stream chemistry variability and small sample sizes. Poor classification rates of Duncan-origin otoliths using Winnipeg lab data may explain the small proportion of adults predicted to be from Duncan watershed tributaries, which conflicted with results based on data analyzed by the Victoria lab. The uncertainty in the percentage of adults that were spawned and reared in the Duncan watershed makes it difficult to draw conclusions regarding the contribution of Duncan watershed to Bull Trout recruitment in Kootenay Lake and the importance of fish transfer program at Duncan Dam. Nonetheless, both Winnipeg and Victoria data-sets predicted that 25% of adult Bull Trout captured in the flip bucket and Kootenay Lake were spawned and reared in the Upper Duncan River, suggesting that this tributary is important to recruitment in Kootenay Lake.

5. CONCLUSIONS

The overall management question of this monitoring program is:

"Does the Bull Trout transfer program contribute to the recruitment of Kootenay Lake or Duncan Reservoir?"

Although there is uncertainty regarding the proportion of adfluvial Bull Trout from Kootenay Lake that migrate past Duncan Dam to spawn in upstream tributaries, the otolith chemistry data suggest that a considerable portion of adults caught in the flip bucket and in Kootenay Lake were reared in tributaries upstream of Duncan Dam. Based on data from the Winnipeg lab, ~25% of adults caught in the flip bucket and in Kootenay Lake were predicted to be from the Upper Duncan River, whereas the percentage of fish originating from the other Duncan watershed tributaries (Houston Creek and Westfall River) could not

reliably be predicted. The Victoria lab data predicted a larger percentage of adult Bull Trout captured in the flip bucket (78%) and Kootenay Lake (69%) were from natal rearing areas in the Duncan watershed including the Upper Duncan River, Houston Creek and Westfall River. Because of these conflicting results, we can only conclude that 25% of adults classified to the Upper Duncan River is the estimated minimum contribution of the Duncan watershed rearing areas to adult populations captured in the flip bucket and Kootenay Lake. This interpretation of the available data suggests that the transfer program is important for the recruitment of Bull Trout in Kootenay Lake and Duncan Reservoir.

The first management hypotheses related to the management question above was:

> ***H01:*** *Stream chemistry is not sufficiently different between tributaries of the Kootenay and Duncan watersheds to determine the natal origins of Bull Trout sampled in the area.*

The distinct water chemistries among most of the sampled tributaries and their association with the otolith chemistry of juvenile Bull Trout provided support for rejecting null hypothesis H01. Similar water chemistry between some of the sampled tributaries, especially Hamill Creek, Westfall River, and the Upper Duncan River, resulted in some uncertainty in predicted natal tributaries, which was estimated using classification rates of juveniles of known origin.

The second management hypotheses related to the management question above was:

> ***H02:*** *The proportion of natal to non-natal Bull Trout is not statistically different between the Kootenay and Duncan watersheds.*

Analyses using data from Victoria and Winnipeg labs both predicted that a portion of adults from Kootenay Lake and the flip bucket originated from the Duncan watershed, and that a portion of adults from Duncan Reservoir originated from the Kootenay Watershed. The results show that rearing areas in the Duncan watershed contribute to recruitment in the Kootenay watershed. Because of the conflicting results and larger within-stream variability in Winnipeg lab chemistry data, statistical tests of the differential proportion of non-natal fish were not possible and H02 cannot be addressed at this time.

The final management question identified in the terms of reference is:

"What changes to the Bull Trout transfer program are recommended to improve Bull Trout in the Duncan Reservoir and Kootenay Lake?"

The current monitoring program is not designed to address this question. The results highlight the importance of continuing operation of the transfer program for Bull Trout in the study area, as individuals from both watersheds use the flip bucket during migrations. Specific recommendations regarding the operation of the transfer program, including issues regarding the timing of migrations and flip bucket operation, are beyond the scope of the results presented in this report. One method to address this hypothesis would be to assess temporal composition of the flip bucket captures to determine if changes in stock structure occur during the migration of Bull Trout. Sample sizes would need to be sufficient for each time period of interest to provide sufficient statistical power to discern changes.

6. RECOMMENDATIONS

The results suggest that the management questions regarding inter-basin recruitment of Bull Trout can be addressed using otolith microchemistry but that additional samples and refinements are needed to reduce uncertainty in predicted natal origins. Recommendations for future study include the following:

1) For streams in which there was significant overlap in element ratios, investigating differences in the ratio of ^{88}Sr:^{86}Sr is recommended to determine if these isotopes may help discriminate among streams. Sr isotope ratios are recommended as the highest priority for analysis in future years of this program. Samples to be analyzed for Sr isotope ratios should be all of the juvenile otoliths analyzed at the Winnipeg lab and the 23 adult otoliths collected from Bull Trout captured in the flip bucket, contingent on budget and laboratory availability. The laboratory recommended for Sr isotope ratio analyses is the WM Keck Collaboratory for Plasma Spectrometry at Oregon State University because the University of Winnipeg lab that was used for previous otolith microchemistry is not equipped to measure Sr isotope ratios. In other studies, the Winnipeg lab relied on the Oregon State University laboratory for Sr ratio analyses and the results were integrated into their otolith stock origination investigations.

2) If uncertainty in the classification of natal origins cannot be adequately resolved using the isotope ratios, then the following additional otolith microchemistry analyses are recommended to reduce uncertainty.

    - Otolith chemistry data analyzed at the Winnipeg lab showed reasonably distinct groupings by tributary and were comparable to the previous data from the Victoria lab. It is recommended that future otolith chemistry analyses should be conducted at the Winnipeg lab.

    - The 13 juvenile otolith samples analyzed at both the Victoria and Winnipeg labs suggested no differences in concentrations of Sr and a systematic bias in Ba that was corrected using linear regression. A re-analysis of an additional 10 samples previously analyzed by the Victoria lab is recommended to confirm these relationships.

    - If additional juvenile otoliths are analyzed, samples should include the streams that had small sample sizes and high within-stream variability, especially Hamill Creek and Houston Creek. Increased sample sizes for these streams will likely improve the classification rates and help discern whether the large within-stream variability was related to natural variability, such as seasonal changes or immigration of juveniles, or the precision of laboratory analyses. Increased sample sizes for these streams will also provide a more balanced data-set, which results in more accurate classifications in linear discriminant analyses than data with unbalanced sample sizes among classes, which tend to underestimate the proportions of groups with smaller sample sizes (Xue and Hall 2015).

- Data analysis for both juveniles and adults involved selecting the portion of the raw data from LA-ICP-MS scans that corresponds to the natal rearing period for each fish. Mean values representing the natal portion were provided by the Victoria lab but this data selection was performed during our data analysis for samples analyzed by the Winnipeg lab. Although the method for selection of the natal portion was thought be consistent between data-sets, and the 13 samples analyzed by both labs suggested consistent values, we cannot be sure that differences in how the data were selected contributed to tighter within-stream groupings in Victoria lab data than Winnipeg lab data. It recommended that the natal portion be re-selected from the original raw data for a sub-sample of otoliths analyzed by the Victoria lab to confirm that similar values are obtained.

3) If the classification rate of tributaries with high within-stream variability and chemistry overlap are not improved with the addition of Sr ratio testing, we recommend that a power analysis be conducted, if feasible, to determine the effect of increased sample sizes on the discriminatory power of the best model based on all available data to date. These results would be available to guide decisions by BC Hydro and stakeholders to either increase sampling to improve the model or address the management questions using data collected to date.

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Appendix A – Site Locations and UTM Coordinates

Table A1. Descriptions of samples sites for water chemistry and juvenile Bull Trout sampling, 2008-2015.

Duncan Watershed Tributaries

Upper Duncan River (UTM 11U 0483681E 5648552N)

This site was approximately 400 m upstream of the confluence with Houston Creek and isolated from the upper reaches of the river. The site is accessed by a small spur road off of the Duncan Forest Service Road. Water sampling took place immediately upstream of a small bridge crossing the river on the left upstream bank. Juvenile sampling took place starting at the water sample site and moving downstream on both banks of the stream, until a suitable number of fish were collected.

Houston Creek (UTM 11U 0483579E 5648542N)

This sample location was located at a tributary in the study area which Bull Trout spawning had been confirmed. The water sample site was taken approximately 20 m upstream of a bridge on the Duncan Forest Service Road that crosses Houston Creek on the left upstream bank. All juvenile collection occurred within 120 m upstream of the water sample site.

Westfall River (UTM 11U 0485870E 5625830N)

This site was identified as a Bull Trout spawning location. The sample site is approximately 500 m upstream of the confluence with the Upper Duncan River on the right upstream bank at a bridge that crosses the river. The bridge is located approximately 1 km west on a spur road off of the Duncan Forest Service Road. Juvenile were sampled on the right upstream bank and extended from the water sample site upstream for approximately 150 m.

Kootenay Watershed Tributaries (Excluding Those Above Duncan Dam)

Poplar Creek (UTM 11U 0491337E 5584815N)

This location was on the Lardeau River system. The water sample site is on the right upstream bank immediately upstream of a bridge on the Highway 31 (Trout Lake Highway). This water sample was collected approximately 100 m upstream of the confluence of Poplar Creek with the Lardeau River. Juvenile collection occurred starting at the water sample location proceeding upstream approximately 150 m along the right upstream bank.

Hamill Creek (UTM 11U 0503757E 5561121N)

This water sample was collected on the left upstream bank downstream from the bridge on the Duncan Forest Service Road, and is approximately 500 m upstream of the confluence with the lower Duncan River. Juveniles were sampled from the water sample location proceeding upstream for approximately 400 m.

Cooper Creek (UTM 11U 0502705E 5560725N)

This water sampling site is located on the left upstream bank above a bridge on Highway 31. The water sample site is approximately 700 m upstream from the confluence with the lower Duncan River. Juvenile samples were collected upstream of the bridge on the left upstream bank approximately 300 m.

Kaslo River (UTM 11U 0506725E 5528471N)

This water sample location is on the right upstream bank just above the bridge on Highway 31, approximately 600 m upstream of Kootenay Lake. Juvenile Bull Trout were collected from the water sampling location, approximately 500 m upstream of the bridge on Highway 31 on the right upstream bank.

Woodbury Creek (UTM 11U 0506675E 5513621N)

This water sample location is on the left upstream bank just above the bridge on Highway 31, and is approximately 300 m upstream from Kootenay Lake. Juvenile sampling was conducted by proceeding approximately 100 m upstream and 200 m downstream from the water sample site.

Coffee Creek (UTM 11U 0505803E 5504863N)

This water sample location is on the left upstream bank approximately 30 m upstream from the bridge on Highway 31 and approximately 1 km upstream from Kootenay Lake. Juveniles were sampled on the left upstream bank of the creek and extended approximately 250 m upstream from the water sample site.

Crawford Creek (UTM 11U 0513338E 5502181N)

The water sample location is on the left upstream bank just upstream from the bridge on Highway 3A, approximately 900 m upstream from Kootenay Lake (Crawford Bay). Juveniles were sampled on both banks of the creek and extended upstream from the water sample site approximately 500 m.

Midge Creek (UTM 11U 0514003E 5469193N)

The water sample location is on the right upstream bank just downstream from the rail bridge, approximately 300 m upstream from Kootenay Lake. Juveniles were sampled on both banks of the creek and extended upstream from the water sample site 300 m.

Cultus Creek (UTM 11 U 498100E 5460704N / 501684E 5460904N)

There were two juvenile Bull Trout and water sampling sites on Cultus Creek. The sites were located ~18 km from Kootenay Lake.

Summit Creek (UTM 11U 0523343E 5443291N / 0514874E 5442496N)

There were two juvenile Bull Trout and water sampling sites on Summit Creek. The sites were accessed from Highway 3 and were ~8 km and ~16 km upstream from the Kootenay River.

Appendix B – Supplementary Results

Table B1. Classification of juvenile Bull Trout otoliths analyzed by the Winnipeg lab based on linear discriminant analysis (unvalidated model).

Known Capture Location	Predicted Capture Location											
	Coffee Creek	Cooper Creek	Crawford Creek	Cultus Creek	Hamill Creek	Houston Creek	Kaslo River	Midge Creek	Summit Creek	Upper Duncan River	Westfall River	Woodbury Creek
Coffee Creek	7	0	0	0	0	0	0	0	0	0	0	0
Cooper Creek	0	9	0	0	0	0	0	0	0	0	0	0
Crawford Creek	0	0	23	2	0	0	0	0	0	0	0	0
Cultus Creek	0	0	3	21	0	0	0	0	0	0	0	0
Hamill Creek	0	0	0	2	0	0	0	0	0	1	0	0
Houston Creek	0	0	0	0	0	0	1	3	0	0	0	0
Kaslo River	0	0	0	0	0	0	9	0	0	1	0	0
Midge Creek	0	0	0	0	0	0	1	21	0	0	0	0
Summit Creek	0	0	0	1	0	0	0	0	0	0	0	0
Upper Duncan River	0	0	1	5	0	0	0	0	0	6	0	0
Westfall River	0	0	1	5	1	0	0	0	0	3	0	0
Woodbury Creek	0	0	0	0	0	0	0	0	0	0	0	1

Table B2. Classification of juvenile Bull Trout otoliths analyzed by the Victoria lab based on linear discriminant analysis (unvalidated model).

Known Capture Location	Predicted Capture Location									
	Coffee Creek	Cooper Creek	Crawford Creek	Hamill Creek	Houston Creek	Kaslo River	Poplar Creek	Upper Duncan River	Westfall River	Woodbury Creek
Coffee Creek	7	0	0	0	0	0	0	0	0	0
Cooper Creek	0	7	0	0	0	0	0	0	0	0
Crawford Creek	0	0	4	1	0	0	0	0	2	0
Hamill Creek	0	0	1	4	0	0	0	0	1	0
Houston Creek	0	0	0	0	5	2	0	0	0	0
Kaslo River	0	0	0	0	2	5	0	0	0	0
Poplar Creek	0	0	0	0	0	0	8	0	0	0
Upper Duncan River	0	0	0	1	0	0	0	6	0	0
Westfall River	0	0	1	0	0	0	0	0	6	0
Woodbury Creek	0	0	0	0	0	0	0	0	0	7

Table B3. Classification of juvenile Bull Trout otoliths analyzed by labs in Victoria and Winnipeg lab based on linear discriminant analysis (unvalidated model).

Known Capture Location	Predicted Capture Location												
	Coffee Creek	Cooper Creek	Crawford Creek	Cultus Creek	Hamill Creek	Houston Creek	Kaslo River	Midge Creek	Poplar Creek	Summit Creek	Upper Duncan River	Westfall River	Woodbury Creek
Coffee Creek	13	0	0	0	0	0	0	0	0	0	0	0	1
Cooper Creek	0	16	0	0	0	0	0	0	0	0	0	0	0
Crawford Creek	0	0	25	5	0	0	0	0	0	0	0	2	0
Cultus Creek	0	0	6	17	0	0	0	0	0	0	0	1	0
Hamill Creek	0	0	0	3	0	0	0	0	0	0	2	4	0
Houston Creek	0	0	0	0	0	1	7	3	0	0	0	0	0
Kaslo River	0	0	0	0	0	1	15	0	0	0	1	0	0
Midge Creek	0	0	0	0	0	0	1	21	0	0	0	0	0
Poplar Creek	0	0	0	0	0	0	0	0	8	0	0	0	0
Summit Creek	0	0	1	0	0	0	0	0	0	0	0	0	0
Upper Duncan River	0	0	1	3	0	0	0	0	0	0	12	3	0
Westfall River	0	0	2	5	0	0	0	0	0	0	4	6	0
Woodbury Creek	1	0	0	0	0	0	0	0	0	0	0	0	7

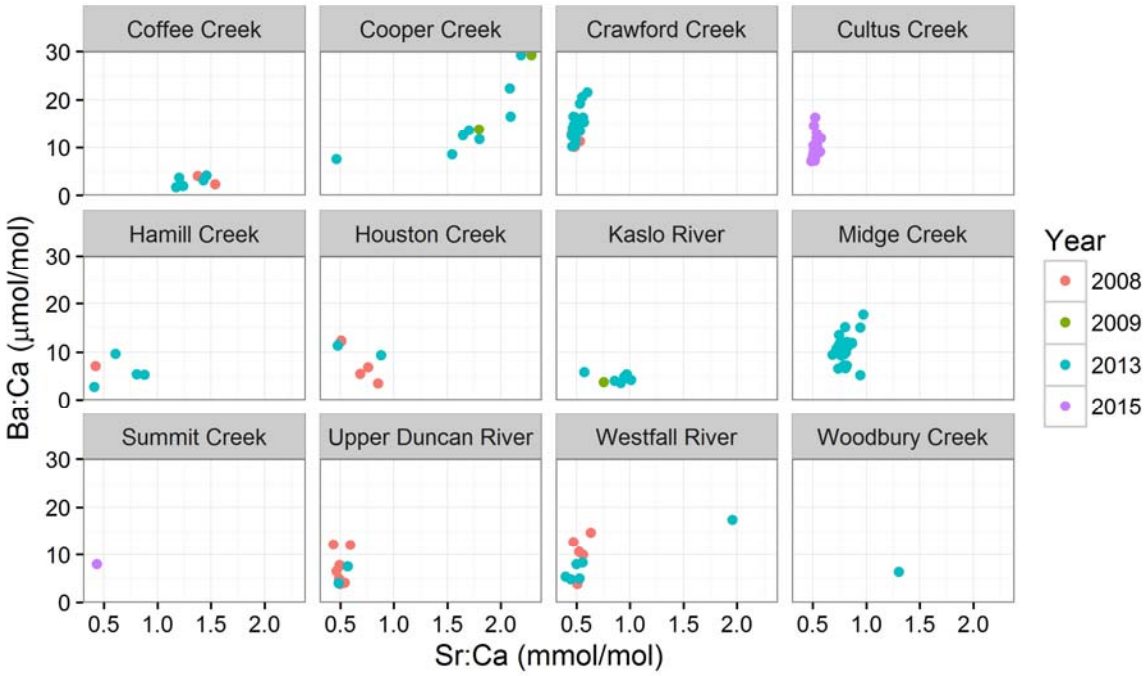

Figure B1. Otolith chemistry ratios from juvenile Bull Trout analyzed in the Winnipeg lab by capture location and year.

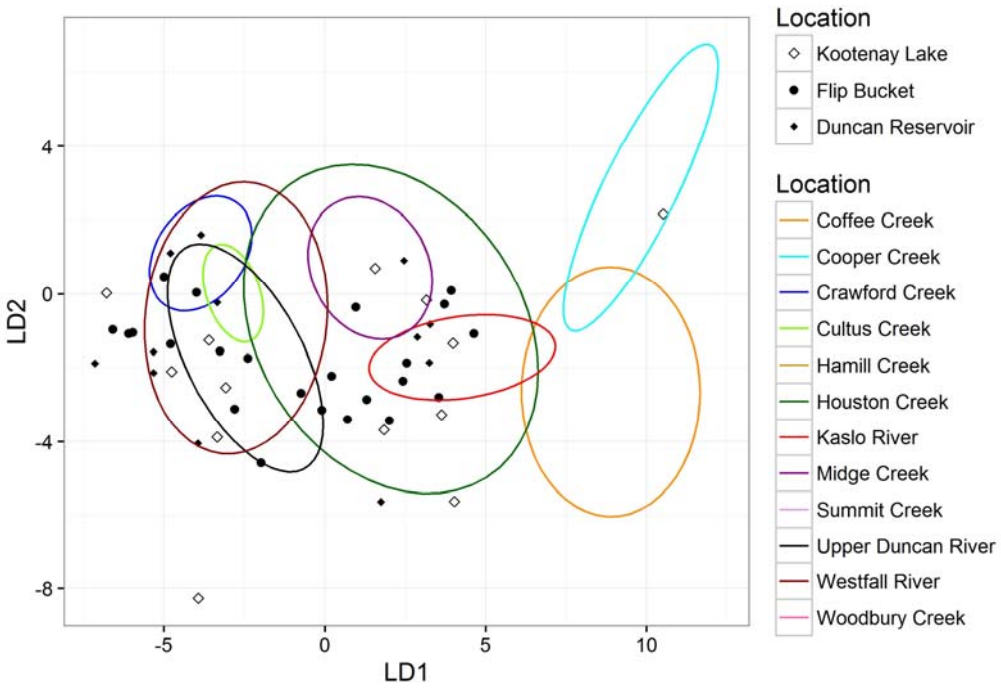

Figure B2. Discriminant scores for natal otolith chemistry of adult Bull Trout by capture locations (points) shown with 95% confidence intervals of discriminant scores from juvenile Bull Trout captured in their natal tributaries and analyzed in Victoria (ellipses).

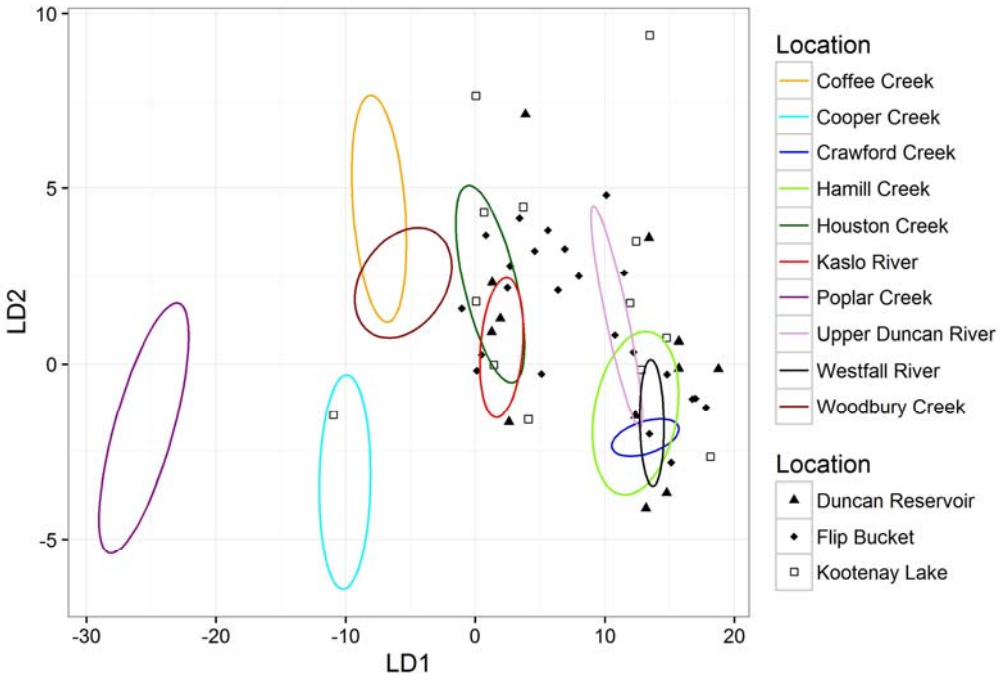

Figure B3. Discriminant scores for natal otolith chemistry of adult Bull Trout by capture locations (points) shown with 95% confidence intervals of discriminant scores from juvenile Bull Trout captured in their natal tributaries and analyzed in Winnipeg (ellipses).

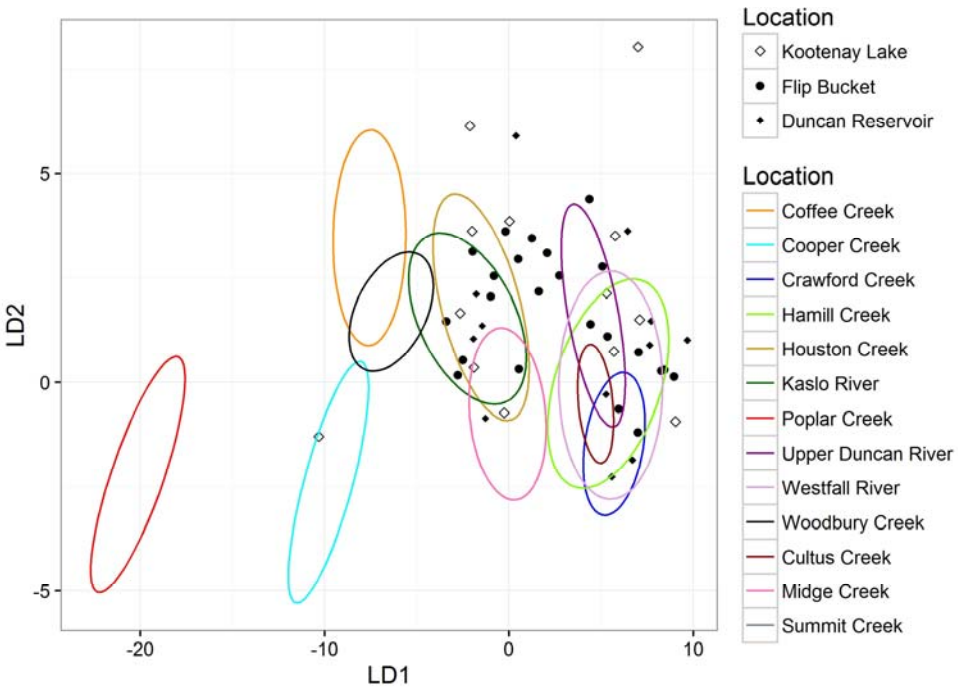

Figure B4. Discriminant scores for natal otolith chemistry of adult Bull Trout by capture locations (points) shown with 95% confidence intervals of discriminant scores from juvenile Bull Trout captured in their natal tributaries and analyzed in Winnipeg and Victoria labs (ellipses).