Duncan Dam Project Water Use Plan

Upper Duncan River Bull Trout Migration Monitoring

Reference: DDMMON-5

Year 10 Data Report

Study Period: 2017-2018

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DDMMON-5: UPPER DUNCAN RIVER BULL TROUT MIGRATION MONITORING

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Photo: Duncan Dam discharge units, low level operation gates 1 and 2, with Lower Duncan River at high levels (fish weir under water).

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EXECUTIVE SUMMARY

The Duncan Dam operates within an important Bull Trout (*Salvelinus confluentus*) migratory corridor between the Kootenay Lake and Upper Duncan River watersheds. To mitigate the effects of the dam on Bull Trout migrations, a Bull Trout passage program was initiated at the Duncan Dam that uses gate operations and the flip bucket structure to facilitate passage of adults from the Lower Duncan River to the Duncan Reservoir upstream. The Upper Duncan River Bull Trout Migration Monitoring Program (DDMMON-5) is intended to assess the effectiveness of the transfer program and address the following key management questions (MQs):

MQ1: Does the Duncan Dam Bull Trout transfer program contribute to the recruitment of Bull Trout to Kootenay Lake or Duncan Reservoir?"

MQ2: What are the origins of Bull Trout individuals sampled in Duncan Reservoir and Kootenay Lake watersheds?

MQ3: Do the distribution and analyzed life histories of the sampled fish denote a bottleneck to recruitment at Duncan Dam?

MQ4: What changes to the Bull Trout transfer program are recommended to improve Bull Trout in the Duncan Reservoir and Kootenay Lake?

This monitoring program used differences in the chemistry of otoliths (ear bones) of Bull Trout to predict the tributaries where they were spawned and reared. Otolith chemistry data from juvenile Bull Trout captured in known spawning tributaries were used to develop a predictive model, which was used to predict the natal spawning tributary of adults captured in Duncan Reservoir, the Duncan Dam flip bucket, or Kootenay Lake. The three predictor variables used to predict natal tributaries were the strontium (^{86}Sr) to calcium ratio (Sr:Ca), the barium (^{138}Ba) to calcium ratio (Ba:Ca), and the strontium isotope ratio ($^{87}Sr/^{86}Sr$). Two statistical methods were used to classify juveniles and adults: linear discriminant analysis (LDA) and random forest analysis.

$^{87}Sr/^{86}Sr$ was the most useful variable for discriminating between tributaries, followed by Sr:Ca, and Ba:Ca. LDA and random forest analysis had similar overall classification accuracy (~80%) but differed slightly in the classification success for some tributaries. Random forest analysis was preferred over LDA because it had better classification success for the three tributaries of the Upper Duncan watershed. In addition, random forest analysis does not require assumptions about data distribution, unlike LDA, which can be biased if assumptions of equal variance and multivariate normality are not met. Therefore, random forest analysis was primarily used for interpretation and to address the management questions in this report.

Using random forest analysis, most of the Kootenay watershed tributaries were well-classified, with classification rates ranging from 78 to 100%. The exceptions were Summit and Woodbury creeks, which both had a sample size of one, and Hamill Creek, which had three juvenile samples and highly variable chemistry among individuals. Classification rates for Duncan watershed tributaries were 60% for Houston Creek, 58% for Upper Duncan River, and 10% for Westfall River. Relatively poor classification success for some tributaries was attributed to overlapping chemistry, small and unbalanced sample sizes, and high within-group variability for some tributaries. Classification tables suggested that juveniles from tributaries in the Duncan watershed may be misclassified to the Kootenay watershed, whereas misclassification of Kootenay watershed Bull Trout to the Duncan watershed were less likely. This suggests that

predictions of the proportions of adult Bull Trout that were reared in the Duncan watershed may be underestimates.

The random forest analysis predicted that 73% of adults captured in Duncan Reservoir, 60% of adults from the flip bucket, and 13% of adults captured in Kootenay Lake were from tributaries in the Duncan watershed. The estimate that 60% of Bull Trout captured in the flip bucket were spawned in the Duncan watershed supports the idea that the fish transfer program contributes to recruitment by allowing Bull Trout to migrate between Kootenay Lake and natal spawning areas in the Upper Duncan River watershed. Because of poor classification rates of Duncan tributaries, there remains uncertainty in the proportion of Bull Trout in Kootenay Lake and Duncan Reservoir that can be attributed to spawning areas upstream of Duncan Dam. The importance of the transfer program to recruitment is also supported by previous tagging and recaptures at the flip bucket (Ord et al. 2000), and telemetry work (O'Brien 1999), both of which demonstrated adult Bull Trout making migrations between Kootenay Lake and spawning areas in the Upper Duncan watershed.

With regard to the management questions, current results and previous research support the idea that the fish transfer program contributes to recruitment in Kootenay Lake and the Duncan Reservoir (MQ1). There remains uncertainty regarding MQ2, because of limitations of the current microchemistry data-set, but the best estimates are that a majority (88%) of adults captured in Kootenay Lake were spawned from tributaries below the dam, the majority of adults from Duncan Reservoir were spawned above the dam, and 60% of adults migrating through the Duncan Dam were spawned upstream of the dam. There is evidence that adfluvial Bull Trout migrate between Kootenay Lake and the Upper Duncan River (this study), and that passage is at least partially limited at Duncan Dam (O'Brien 1999), but it is unknown whether this results in a "bottleneck" to recruitment (MQ3). This program was not designed to address MQ4 but continued operation of fish transfer using the weir to reduce the required jump by Bull Trout is recommended, along with improved passage monitoring, as recommended by Thorley (2009).

If future otolith microchemistry studies are undertaken to reduce uncertainty in predicted natal origins, the following recommendations are provided:

- Part of the uncertainty in juvenile classifications was related to very small sample sizes and high variability for some tributaries. Analysis of additional juvenile samples from these tributaries would likely improve classification rates, and help discern whether within stream variability was related to natural variability, such as immigration of juveniles, or the precision of laboratory analyses. The highest priority tributary is Hamill Creek, which had a small useable sample size (n=3) and highly variable chemistry that partly overlapped Duncan watershed tributaries.
- In this analysis, elemental ratios (Sr:Ca and Ba:Ca) were measured at a laboratory in Victoria for adults but at a different laboratory in Winnipeg for juveniles. It is recommended that additional adult otoliths are analyzed at the Winnipeg lab to compare results to data from Victoria and whether conclusions regarding Bull Trout recruitment are the same. Additional adult Bull Trout heads and otoliths have already been collected from the three adult capture locations and are being stored for analysis. Future analysis of adult otoliths at the Winnipeg lab should include at least 15 samples previously analyzed by the Victoria lab for comparison, as well as new, previously un-measured samples.

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1 Introduction

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 Kaslo, BC (Figure 1). The Duncan Dam was finished construction by 1967 as a part of the Columbia River Treaty deal between the US and Canada. Duncan Dam is situated on the Duncan River, 12 km upstream of its confluence with Kootenay Lake. Duncan Dam is an earth fill and concrete structure 39.7 m high and 792 m long, with two low level outlet gates (LLOG) and a single spillway (DVH Consulting 2001). The dam's reservoir provides storage to improve hydroelectric generation and flood control to downstream areas in the Kootenay and Columbia river basins. There are no power generation facilities and no structures purposely built for fish passage at the Duncan Dam (Anon 1986).

The Duncan River is an important spawning migration corridor for Bull Trout (*Salvelinus confluentus*) and other fish species moving between Kootenay Lake and the tributaries to the Upper Duncan River. Bull Trout populations are generally thought to be in decline in many areas of the province due to over-exploitation and habitat loss, and have been classified as 'blue-listed' by the British Columbia Conservation Data Centre (BC CDC 2018). In 1968, the first senior operator at Duncan Dam altered flows in the spring and summer to facilitate Bull Trout passage through the low level gates and tunnel into the upstream Duncan Reservoir (O'Brien 1999). Subsequently, fish transfers have occurred annually since 1968 and have occurred periodically between early May and mid-September (BC Hydro 2008), but cannot be conducted during periods of high flows (Ord et al. 2000).

The transfer procedure utilizes two gates, the second lower level operating gate (LLOG#2) and the Lower Level Maintenance Gate (LLMG) to create a lock-like mechanism to allow fish passage through a discharge tunnel into the reservoir upstream (BC Hydro 2010). To gain access to the discharge tunnel, Bull Trout must first leap up into the flip bucket, which is a structure designed to direct water away from the base of the dam. In 1994, a removable weir was constructed at the toe of LLOG#2 to reduce the height that fish must jump over the cement lip of the flip bucket structure, which can be up to 3.3 m above the surface of the river. The transfer process takes approximately 3 days to complete. Some years the fish weir was not installed for various reasons (BC Hydro 2010).

During some transfers, all Bull Trout within the flip bucket were enumerated before the transfer process, while during other transfers, a visual estimate of numbers of fish was recorded. Typically, the volume of water in the flip bucket was reduced to allow crews on the catwalk above to make a more accurate visual estimate. Bull Trout transfers at the Duncan Dam have been monitored annually since 1995, although the number of transfers and the dates of first and last transfer have been inconsistent over the years. Thorley (2009) completed an assessment on whether the fish weir was necessary for Bull Trout passage and recommended that the fish weir be installed in all years, regardless of the height of the jump required to access the flip bucket. The existing fish weir was damaged in 2011 and has not been put into service since, potentially limiting the size of fish which are able to migrate upstream of Duncan Dam (BC Hydro 2010). LLOG#2 flows have been manipulated to allow some adult migration during the last 6 years (BC Hydro 2015).

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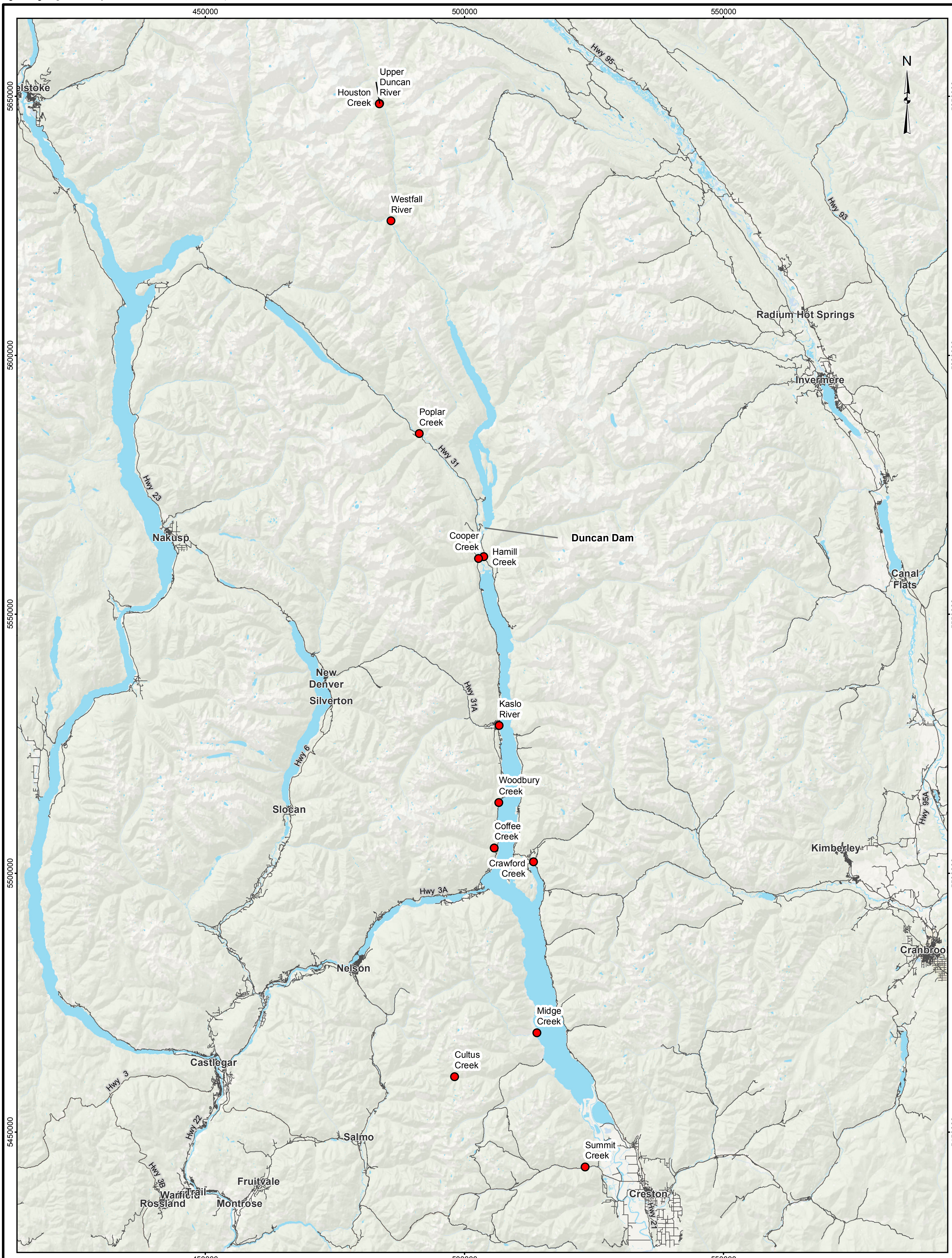

LEGEND

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	**FIGURE: 1**	
CHECK	DR	21 APR. 2016		
REVIEW	DS	21 APR. 2016		

Annual migration movements of adfluvial Bull Trout have been shown to occur from Kootenay Lake to the Upper Duncan River, with Bull Trout observed at the dam in peak numbers in early July to mid-August (O’Brien 1999; Ord et al. 2000). Bull Trout passing through the dam are known to spawn throughout Upper Duncan River above the reservoir, including Westfall River, Houston Creek, Geigerich Creek, Stevens Creek, Hatteras Creek, and Marsh Adams Creek (O’Brien 1999). Bull Trout were also tracked to the Upper Duncan River mainstem (from Giegerich Creek confluence upstream past Houston Creek confluence). O’Brien (2001) assumed that Bull Trout migrating to mainstem destinations in the Upper Duncan River did not spawn in that location, based on the tagged Bull Trouts’ smaller body size, no visual detection of spawning redds, and general lack of suitable spawning habitat at mainstem destinations compared to literature description (Baxter and McPhail, 1996). After the upstream migration period, non-resident Bull Trout are thought to return to Duncan Reservoir. Overwinter telemetry studies have indicated that between 74% and 86% of the tagged fish emigrate through the Duncan Dam discharge structure to continue downstream to Kootenay Lake (O’Brien 1999). During the telemetry study, O’Brien (1999) found that six Bull Trout returned to the flip bucket in the following year to immigrate upstream again. These Bull Trout, followed over multiple years, appeared to be returning annually to the Upper Duncan watershed but did not show a precise homing to individual sites or tributaries, and fine-scale site selection was related more to body size. The population of Bull Trout intermixes significantly with fish in Kootenay Lake and are provincially managed as one population.

For BC Hydro staff, the operation of the fish passage program at Duncan Dam presents operational, safety, and downstream fish stranding risks that need to be justified. BC Hydro would like to optimize operations to ensure the long term success of the program. The temporary stop-log weir installation procedure has posed potential safety risks for dam operators due to facility operations changes that are needed during weir installation. Fish passage operations require up to 24 hours of no flow from the dam, which can potentially result in fish stranding issues in the discharge channel, when Lardeau River flows are also low. Duncan Dam Bull Trout Passage Monitoring Program (DDMMON-6) suggested that to reduce the safety concerns, an improved fish weir should be designed and implemented (BC Hydro 2010; 2015).

1.1 Project Objectives

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.

1.2 Key Management Questions

The objectives have been developed to address the overall management question in the terms of reference:

MQ1: Does the Duncan Dam 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:

MQ2: What are the origins of Bull Trout individuals sampled in Duncan Reservoir and Kootenay Lake watersheds?

MQ3: 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:

MQ4: What changes to the Bull Trout transfer program are recommended to improve Bull Trout in the Duncan Reservoir and Kootenay Lake?

1.2.1 Management Hypotheses

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:

$H0_1$: 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.

$H0_2$: 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 the degree to which the fish passage program at Duncan Dam is important for Bull Trout populations in the study area.

Previous years of this monitoring program established that water chemistry in the sampled tributaries differed among tributaries, and that these differences corresponded with chemistry of Bull Trout otoliths from the same tributaries (Golder 2010, ONA and Golder 2013, 2016). These previous studies found that Bull Trout otoliths from many of the tributaries could be classified successfully, but there was overlapping chemistry between some tributaries that resulted in poor prediction and uncertainty in the origin of adult Bull Trout. Furthermore, otolith chemistry was measured at three different laboratories during different study years, and data-sets from the different laboratories resulted in conflicting results in terms of classification success of juveniles of known origin, and predicted origin of adults (see Section 2.2.1 and ONA and Golder 2013 for additional details). The approach of this study year of the program was to use the existing juvenile Bull Trout data from ONA and Golder (2016), combined with adult Bull Trout data collected from Golder (2010) for analysis. The main objective of this year of the project was to improve accuracy of tributary classification by measuring an additional chemical variable, the strontium isotope ratio ($^{87}Sr/^{86}Sr$), for all of the juvenile and adult Bull Trout otoliths in the data-set chosen for analysis.

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 in the study area (Houston Creek, Upper Duncan River, and Westfall River) are upstream of Duncan Dam and were used to represent the Upper Duncan watershed. All other tributaries are downstream of Duncan Dam and were chosen to represent different portions of the Kootenay Lake watershed. In this report, the term Kootenay Lake watershed includes all tributaries flowing into Kootenay Lake except those upstream of Duncan Dam. Tributaries in the Kootenay Lake watershed sampled in this study included 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 in central/south Kootenay Lake (Crawford Creek and Midge Creek). Cultus Creek and Summit Creek were sampled to represent the southern end of Kootenay Lake. Poplar Creek is a tributary to the Lardeau River. The Lardeau River joins the Duncan River ~800 m downstream of Duncan Dam. Poplar Creek was included in the Golder (2010) report but samples from Poplar Creek were not included in the current data-set because additional juvenile otolith samples were not available for analysis at the laboratory used for this report. 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

No field sampling for juvenile Bull Trout, adult Bull Trout, or water samples was conducted during the current study period (2017-2018). Otoliths from juvenile Bull Trout and water samples were collected at various locations in 2008, 2009, 2012, 2013, and 2015. Adult Bull Trout otoliths were collected from Duncan Reservoir, Kootenay Lake and the Duncan Dam flip bucket in 2008, 2012, 2013, and 2015. A summary of fish collection for the previous sampling is provided below. Details of water sampling are presented in previous reports (Golder 2010; ONA and Golder 2013, 2016).

2.1.1 Juvenile Fish Collection

Juvenile fish were collected using a Smith-Root battery powered 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 transportation. Sagittal otoliths were extracted and sent to the laboratory for all juvenile and adult samples.

2.1.2 Adult Fish Collection

Transfer Station Sampling

Sampling 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) 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 Duncan Reservoir and Kootenay Lake targeting the collection of adult Bull Trout from recreational fisheries. 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, data (length, age, sex and scale sample) from fish heads were 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 Otolith Microchemistry – Elemental Ratios

Three different laboratories were used during previous years of the otolith microchemistry analysis because laboratories that had previously conducted the analyses were unavailable in subsequent years. All three laboratories measured a suite of elemental concentrations using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The elemental ratios from these data that were used for statistical analysis were strontium to calcium (^{86}Sr:^{43}Ca), and barium to calcium (^{138}Ba:^{43}Ca) ratio. 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”).
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.

All analyses in this report used juvenile microchemistry data from the Winnipeg lab, and adult microchemistry data from the Victoria lab. Adult otoliths were not analyzed at the Winnipeg lab. Microchemistry data analyzed at the University of Australia were highly variable and considered not useful for addressing the management questions (ONA and Golder 2013, 2016). Juvenile data from the Victoria lab were not used in the current analysis to classify juveniles and adults to tributaries. However, 13 samples analyzed at both Victoria and Winnipeg were used to compare, and correct elemental concentrations, which was necessary to use juvenile data from Winnipeg to predict adult origin using data from the Victoria lab (Section 2.3.1).

University of Manitoba Otolith Microchemistry Laboratory Methods

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 1 and 2.

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, a 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 intervals of one hour to correct for machine drift. Program Iolite (version 2.3.1) was used for data reduction.

Table 1. 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 2. 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)

2.2.2 Otolith Microchemistry – Strontium Isotope Ratio

A new laboratory analysis was conducted during the 2017-2018 study period to measure the strontium isotope ratio ($^{87}Sr/^{86}Sr$), which had not previously been measured for any of the otoliths. The analysis was conducted at the W. M. Keck Collaboratory for Plasma Spectrometry at Oregon State University because the labs used in previous years were not equipped to measure Sr isotope ratios. Samples analyzed included the juvenile otoliths previously analyzed for elemental ratios at the Winnipeg lab, with the exception of the 13 analyzed at both Victoria and Winnipeg labs. These 13 samples were mis-located by the Winnipeg lab and were therefore not available for analysis of strontium isotopes. All of the adults previously analyzed at the Victoria lab that had useable data for ^{86}Sr:^{43}Ca and ^{138}Ba:^{43}Ca (i.e. no vaterite or otherwise unreliable data) were analyzed for strontium isotope ratio. Technical details of the strontium isotope ratio analysis are presented below.

Otoliths had previously been mounted during the elemental analysis. Juvenile otoliths were mounted on epoxy rounds and finely polished as described in Section 2.2.1. Adult otoliths were mounted on epoxy rounds and finely polished as described in Golder (2010). Epoxy rounds were loaded into a Photon Machines Analyte G2 ArF Excimer laser system along with quality control standard glasses NIST 610 and NIST 612, primary standard Gastropod AP-1 and secondary standard BB-1 clinopyroxene. The laser ablation introduction system was coupled to a Nu Instruments Plasma 1 multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) using ultrapure helium (0.4 L/min) as a carrier gas. Ablated sample material was mixed with argon nebulizer gas just prior to the ICP-MS torch using an in-house assembled mixing “T”.

Full system checks were conducted following collector gain cross-calibrations at the beginning of each analytical session using NIST 610 glass to verify adequate system sensitivity, peak shape and peak coincidence. The general method of Woodhead et al. (2005) was used to correct for potential Kr and Rb interferences and to monitor for

Ca argide/dimer formation. Background interferences by Kr isotopes and contributions from any other gas species present within the plasma and carrier gas supplies were corrected by measuring an on-peak baseline prior to ablation. Measured backgrounds were subtracted from measured intensities during analysis. Mass biases were corrected by reference to the ratio of ^{86}Sr to ^{88}Sr of 0.1194 and isobaric interference of ^{87}Rb on ^{87}Sr was corrected by measuring beam intensity for ^{85}Rb and calculating the contribution of ^{87}Rb. Calcium argide/dimer was measured at mass 82.

Bull Trout otoliths were analyzed in batch experiments, each batch comprising four otolith samples. All analytical runs began and ended with a gastropod→ cpx → gastropod standard block and all otolith analyses were bracketed by an additional gastropod analysis. Adequate signal intensity for the otoliths samples was found to require 85 μm spots and adequate special resolution was determined to be achieved through the use of transects across the samples progressing at 10 μm/s. Similar signal intensity and ample analytical duration for the primary gastropod standard and secondary clinopyroxene standards were achieved using a 65 μm spot progressing at 5 μm/s. All analyses were performed at a laser repetition rate of 15 Hz and a laser energy of 4.8 J/cm^2.

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 or age-1 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 for both elemental concentrations and $^{87}Sr/^{86}Sr$ were graphed and provided by the laboratories, showing the element concentrations or ratio 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 often visible 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 other elements including strontium and barium. 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. The same portions of some otoliths corresponded to erratic and highly variable patterns in $^{87}Sr/^{86}Sr$. 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

The overall approach was to use otolith chemistry data from juveniles to develop a predictive model of natal tributaries and evaluate its classification accuracy, and use this model to predict the natal tributary of adult Bull Trout of unknown origin. Two alternative methods were used to classify juvenile and adult Bull Trout based on otolith chemistry: linear discriminant analysis and random forest analysis. Details of these two methods and data processing prior to analysis are presented in the following sections.

2.3.1 Data Processing

The three variables used to classify otolith chemistry by tributary were the ratio of ^{86}Sr to ^{43}Ca (hereafter "Sr:Ca"), the ratio of ^{138}Ba to ^{43}Ca (hereafter "Ba:Ca"), and the ratio of ^{87}Sr to ^{86}Sr (hereafter "^{87}Sr/^{86}Sr"). Raw data for ^{86}Sr and ^{138}Ba were in units of parts per million (ppm). Values of ^{43}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 ^{86}Sr, ^{138}Ba, and ^{43}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. ^{87}Sr/^{86}Sr data provided by the laboratory were already in ratio format.

For juvenile Bull Trout, there were two juvenile otoliths with missing values for Sr:Ca and Ba:Ca and 13 otoliths with missing values for ^{87}Sr/^{86}Sr. For all statistical analyses in this report, observations with missing values are not useable and need to be excluded from models, which is not desirable because of small samples sizes for some tributaries. Therefore, missing values were filled with the mean value from the tributary of origin for each sample. To assess the sensitivity of the results to using tributary-specific mean values for missing data, we examined two alternative approaches to handling missing data. The first was to exclude all observations with missing data. The second was to use an alternative data-filling technique called Multiple Imputation by Chained Equations. (MICE). The predictive mean matching method in the package "mice" (Buuren and Groothuis-Oudshoorn 2011) in R version 3.4.1 (R Core Team 2017) was used to create plausible values for the missing data. The MICE method used capture location, Sr:Ca, Ba:Ca, and ^{87}Sr/^{86}Sr as predictors in the model and produced values within the observed range of data for each variable that reflect the within-group variability for each tributary. Five iterations of the model were conducted to produce five alternative data-sets, each with a different combination of values used to fill the missing data. The linear discriminant analysis (LDA) described below for juvenile Bull Trout was run for each of the five data-sets from the MICE method, as well as the data-set where observations were deleted if they had a missing value, and the data-set with missing data filled with tributary-specific mean values. LDA results from these seven data-sets were compared to assess sensitivity of results to the method of handling missing data. The results of these sensitivity analyses are presented in Appendix B. The data-set using mean values to fill missing data was used for all subsequent analyses that predicted the origin of adult Bull Trout.

Of the 150 juvenile otoliths analyzed in the Winnipeg lab, 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, seven juvenile otolith samples were considered outliers that had Sr:Ca, Ba:Ca, or $^{87}Sr/^{86}Sr$ values much greater or lower than other samples from the same tributary (FishIDs 95, 403, 418, 434, 435, 440, and 452). Outliers can have a large influence on the classification and prediction methods used and therefore these seven outliers were not used because they were considered unreliable and not representative of the 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 4.4. One adult Bull Trout (FishID 232) was removed from the data-set because of an asymmetrical and highly variable pattern in Sr:Ca and Ba:Ca that made it impossible to determine the values associated with the natal portion of the otolith. After removing unuseable data and outliers, and filling missing data, the samples sizes used in the analysis in this report were 131 juveniles and 39 adults.

Sr:Ca and Ba:Ca values were measured at the Winnipeg lab for juveniles and the Victoria lab for adults. Previous analysis of 13 juveniles analyzed at both labs for comparison showed that Sr:Ca was not different between labs, but values of Ba:Ca were consistently lower at the Winnipeg lab than the Victoria lab (ONA and Golder 2016). Therefore, Ba:Ca values measured at the Victoria lab needed to be corrected for bias before being used in subsequent analyses. Linear regression was used to describe the relationship between lab values for Ba:Ca. 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. Instead of using mean Ba:Ca values from the natal portion of the otolith for each juvenile as in ONA and Golder (2016), means of all the values from the entire scan of each otolith were used in the linear regression. This was done to avoid any potential differences between values due to picking different portions of the otolith to represent the natal area, because the Victoria lab selected the natal area in their data, and we selected the natal area for the samples analyzed in Winnipeg.

2.3.2 Linear Discriminant Analysis

Linear discriminant analysis (LDA) was used to describe differences in otolith chemistry among capture locations and develop a predictive model of the natal area using data from juvenile 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. The three predictor variables in the model were Sr:Ca, Ba:Ca, and $^{87}Sr/^{86}Sr$. Sr:Ca and Ba:Ca were transformed using the natural logarithm to better meet model assumptions of normality. $^{87}Sr/^{86}Sr$ did not require transformation prior to analysis. All three predictor variables were standardized by subtracting the mean and dividing by the standard deviation prior to LDA. Standardization of variables was needed to interpret the factor loadings, which represent the correlation between predictor variables and the linear discriminant axes, and are used to understand the relative importance of the predictor variables in discriminating between tributaries. LDA was performed using the “MASS” package in R (Venables and Ripley 2002).

The LDA predicting adult natal tributaries was run twice using two alternatives for the prior probabilities for each natal tributary. The first used equal prior probabilities between all 12 tributaries. The second used prior probabilities based on the proportions of fish by tributary in the model training (juvenile) data-set, which is the default method in the software and was used in previous years’ analyses. Equal prior probabilities are likely more suitable because the relative abundance of juveniles sampled and analyzed likely does not reflect abundance in the adult capture locations. However, both methods are presented for comparison.

The LDA to predict the natal tributary of adults used un-standardized variables for both juveniles and adults. Standardized variables could not be used to predict adult origin using the juvenile LDA because standardization changed the relationship between the adult and juvenile data. As the means and standard deviations of the juvenile and adult data-sets were different, standardizing the variables changed the relationship between juveniles and adults, which biased the predictions. The juvenile LDA is equivalent regardless of whether standardized or un-standardized data are used; therefore, the classification rates from the juvenile LDA using standardized data also apply to the LDA using un-standardized data.

2.3.3 Random Forest Analysis

Random forest analysis is a method used to predict group membership using classification trees (Breiman 2001). A classification tree recursively splits the data into binary groups based on a criterion for one or more of the predictor variables (e.g., Ba:Ca >0.5). At each split, a criterion is selected to maximize within-group homogeneity, and the splitting continues until there is no additional homogeneity gained. Each tree is constructed using a random subset of data selected using bootstrap resampling. The selected samples (“in-bag samples”) are run down the tree to predict group membership whereas the non-selected samples (“out-of-bag samples”) are used to calculate prediction ability. Random forest analysis consists of a large number of classification trees (i.e., a forest) and the final model prediction is based on the majority of “votes” from each classification tree.

Random forests are becoming increasingly popular for classifying fish stocks using otolith chemistry (Tournois et al. 2013; Loewen et al. 2015). Unlike linear discriminant analysis, which assumes homogenous within-group dispersion and multivariate normality, random forests do not require any assumptions about data distribution. Random forests can perform better than linear discriminant analysis and are the recommended option for otolith microchemistry if distributional assumptions are not met (Mercier et al. 2011).

For the random forest analysis, the juvenile otolith data (n=131) were used as the training data-set and the adult otolith data (n=39) were used as the test data-set. Transformation and standardization of the data was not required for the random forest analysis. The software’s default setting was used to decide the number of predictors considered at each split, which resulted in one predictor at each split in the trees. The analysis used 5000 trees to ensure that predictive ability had stabilized, which was selected after examining plots of misclassification rate versus the number of trees. Separate cross-validation of the random forest was not required because the "out-of-bag" estimates of misclassification are already based a random subset of the data, similar to splitting the data for cross-validation, and these estimates are considered unbiased (Breiman 2001). The random forest analysis was conducted using the "randomForest" package in R.

3 Results

Previous reports established that water chemistry differed among tributaries, and that these differences were reflected by elemental ratios of otoliths of Bull Trout rearing in these tributaries. The current analysis attempts to improve classification of natal area for juveniles of known origin by adding a third predictor variable, $^{87}Sr/^{86}Sr$, in addition to the Sr:Ca and Ba:Ca ratios used in previous analyses. Models using these three variables were used to predict adults of unknown origin captured in Duncan Reservoir, Kootenay Lake, and the Duncan Dam flip bucket.

3.1 Juvenile Otolith Chemistry

3.1.1 Trends in Juvenile Otolith Chemistry

The data-set for juvenile Bull Trout included 131 fish, of which 27 were from tributaries of the Duncan watershed and 104 were from the Kootenay watershed. For all juveniles, Sr:Ca and Ba:Ca were measured at the Winnipeg lab and $^{87}Sr/^{86}Sr$ was measured at the Oregon lab. As described in Section 2.3.1, seven juveniles were excluded from all analyses because they were outliers that differed drastically from other samples from the same tributary. In addition, juveniles that were missing data for one of the variables were included in the analysis but missing values were filled with the mean value from other fish from the same tributary. This included two fish that had missing values for Sr:Ca and Ba:Ca, and 13 fish that had missing values for $^{87}Sr/^{86}Sr$.

As in previous years of the study, Sr:Ca and Ba:Ca differed among streams but there was overlap in values between several of the tributaries (Figure 2). Houston Creek, Kaslo River, and Midge Creek overlapped in Sr:Ca and Ba:Ca. Crawford, Cultus, and Summit Creek and Upper Duncan and Westfall rivers all overlapped in Sr:Ca and were only partially separated by Ba:Ca (Figure 2; top panel). $^{87}Sr/^{86}Sr$ differed among streams with low within-group variability for some tributaries (e.g., Houston Creek, Kaslo River) but greater variability for Cultus Creek, Hamill Creek, Upper Duncan River and Westfall River (Figure 2; middle panel). Streams that overlapped in chemistry based on Sr:Ca and Ba:Ca but were differentiated by $^{87}Sr/^{86}Sr$ included Crawford Creek, Houston Creek, Kaslo River, and Midge Creek. Note that the plots in Figure 2 show the data used in the linear discriminant analyses and therefore include mean-imputed values but do not includes the seven outliers that were removed from the data (see Section 2.3.1).

All of the values discussed above are mean values from the portion of the otolith corresponding to juvenile rearing in the natal stream. For juveniles, this region was the outer edges of the otolith. For adults, this was the region directly outside of the core of the otolith, but inside of the region corresponding to downstream migration to lake or reservoir habitat. Examples of the raw elemental concentration data from the full LA-ICP-MS scans across the otolith are shown for a juvenile and an adult Bull Trout in

Appendix B (Figures B1 and B2). In both juveniles and adults, the concentration of strontium appeared to be fairly uniform throughout the seasons while Bull Trout were in presumed to be in the same stream. On the other hand, barium showed seasonal fluctuations for many but not all Bull Trout, with the largest fluctuation typically occurring outside the core during the first summer of growth (Appendix B, Figure B1). When possible, an area of stable Ba and Sr was selected to represent natal areas, although for some fish, only areas with peaks or variation in concentrations were available to calculate mean values.

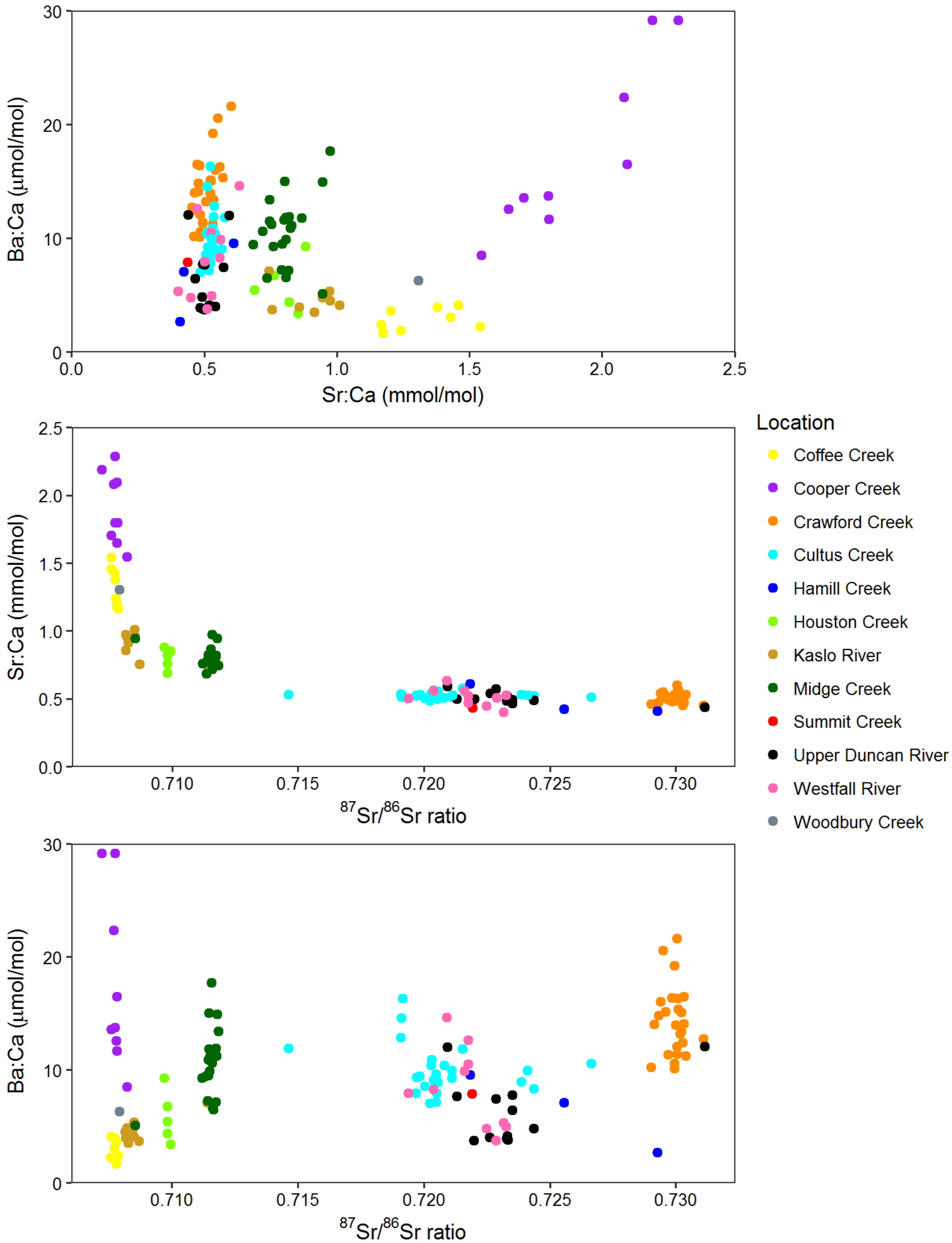

Figure 2. Natal otolith chemistry of juvenile Bull Trout by capture location. Panels are scatterplots showing bivariate relationships between each of the three chemistry variables.

3.1.2 Linear Discriminant Analysis

The LDA used for interpretation in this report used tributary-specific mean values to fill missing data for 15 of the juvenile otolith samples. In the LDA, the first, second and third discriminant axes (LD1, LD2, and LD3), explained 85%, 11%, and 4% of the variance between tributaries, respectively. Factor loadings, which represent the correlation between the discriminant axes and the predictor variables, indicated that LD1 was most related to $^{87}Sr/^{86}Sr$ but also negatively correlated with Sr:Ca (Table 3). LD2 was most related to Sr:Ca but also positively related to $^{87}Sr/^{86}Sr$. LD3 mostly represented Ba:Ca. Together, these results indicate that $^{87}Sr/^{86}Sr$ and Sr:Ca provide the majority of the power to discriminate between tributaries but that Ba:Ca is also provided a small amount of discriminatory power. Separation of tributaries by the discriminant axes is shown visually in Figure 3.

The overall classification success rate of the LDA was 81% for the un-validated model. Two of the three tributaries in the Duncan watershed were classified poorly (Table 4) with a classification rate of 20% for Houston Creek and 10% for Westfall River. The Upper Duncan River was classified correctly for 75% of the juveniles. Classification of Kootenay watershed tributaries was relatively strong with rates between 75 and 100% for most tributaries (Table 4). Exceptions were Summit Creek (0%), which only had one sample, and Hamill Creek (67%), which had three samples with highly variable values of all three predictor variables (Figure 2).

In LDA, un-validated classification rates can be overly optimistic because the same data are used to train and test the model. Therefore, cross-validation of classification rates is recommended, such as the leave-one-out procedure used in this analysis. In the cross-validated model, the overall classification rate was 78%. However, the classification rates for Houston Creek, Westfall River, and Hamill Creek were all reduced to 0% (Table 4). This indicates substantial uncertainty in classifying these tributaries. All of the other tributaries had the same classification rate in the cross-validated model as the un-validated model.

Classification tables can be used to assess which tributaries were assigned in the cases where juveniles were misclassified (Appendix B, Tables B1 and B2). For this program's objectives, tributaries misclassified to a tributary within the same watershed (Kootenay or Duncan) are less problematic, but tributaries classified to a tributary in the wrong watershed are of concern. Hamill Creek was assigned to either Cultus or Hamill Creek (both in the Kootenay watershed) in the un-validated model (Table B1), but two of three samples were assigned to Upper Duncan River in the cross-validated model (Table B2). Juveniles from Cultus Creek were mostly classified correctly (85%) but 11% were incorrectly assigned to the Upper Duncan River. All of the Houston Creek juveniles were incorrectly assigned to Kootenay watershed tributaries (Kaslo and Midge creeks) in the cross-validated model. Two thirds of juveniles from Westfall River (Duncan watershed) were classified to a tributary in the Kootenay watershed tributaries, and the remaining third were classified to the Upper Duncan River. Overall, two of three Duncan watershed tributaries (Houston and Westfall) were mostly classified incorrectly to the Kootenay watershed, which suggests that the proportion of Bull Trout from the Duncan watershed could be underestimated when using this data-set and LDA. Only one of the nine Kootenay tributaries (Hamill Creek) had a substantial portion of samples misclassified to a tributary in the Duncan watershed.

Table 3. Factor loadings representing the correlation between the variables and the discriminant functions.

Variable	LD1	LD2	LD3
log(Sr:Ca)	-2.49	4.35	-0.84
log(Ba:Ca)	0.64	-0.17	1.79
$^{87}Sr:^{86}Sr$	3.89	4.13	-1.41

Table 4. Percentage of correct classification of capture location of juvenile Bull Trout based on linear discriminant analysis.

Capture Location	Un-validated Model	Cross-Validated Model	Sample Size
Duncan Watershed			**27**
Houston Creek	20%	0%	5
Upper Duncan River	75%	75%	12
Westfall River	10%	0%	10
Kootenay Watershed			**104**
Coffee Creek	100%	100%	8
Cooper Creek	100%	100%	9
Crawford Creek	100%	100%	25
Cultus Creek	85%	85%	26
Hamill Creek	67%	0%	3
Kaslo River	78%	78%	9
Midge Creek	95%	95%	22
Summit Creek	0%	-	1
Woodbury Creek	100%	-	1
Total	**81%**	**78%**	**131**

- A hyphen.indicates that the classification rate could not be cross-validated because there was only one sample at that site.

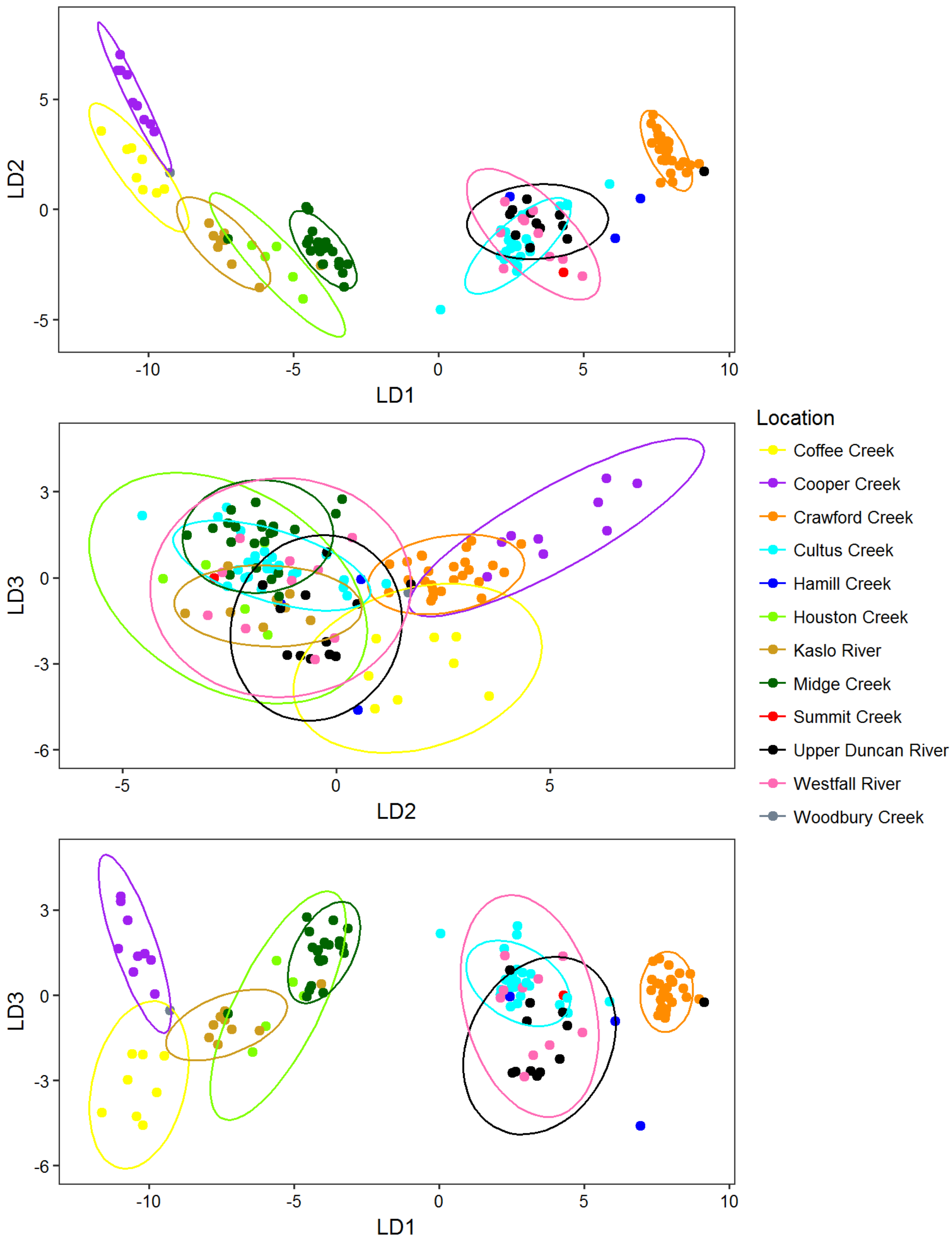

Figure 3. Discriminant scores for natal otolith chemistry of juvenile Bull Trout. Ellipses show 95% confidence intervals of the discriminant scores. Ellipses are not shown for Hamill Creek, Summit Creek, and Woodbury Creek because the sample sizes were too small to calculate confidence intervals.

3.1.3 Sensitivity Analysis for Linear Discriminant Analysis

The LDA presented in Section 3.1.2 and used to predict adult origin (Section 3.3.1) used tributary-specific mean values to fill missing data for juveniles. To assess sensitivity of the results to filling missing data with mean values, the juvenile LDA was repeated using alternative data-sets: 1) a data-set where observations (juveniles) were deleted if they had a missing value for one or two of the predictor variables; 2) five alternative data-sets with missing values filled using MICE, which is a method that produces random, plausible values reflecting within-tributary variability (Appendix B, Figures B3 and B4).

In the LDA where observations with missing data were deleted, the overall classification rate was 84% for the un-validated model and 83% for the cross validated model (Appendix B, Table B3). These values were similar to, but slightly better than the LDA where missing values were filled with tributary-specific means (81% and 78%; Table 4). Tributaries that had better classification rates with the deleted-observation data-set than in the analysis with mean values included Westfall River (0% vs. 13%) and Kaslo River (78% vs. 88%). However, this is somewhat misleading, because the greater classification rate was likely because deleted observations were at the edges of the range of data for those tributaries, which reduced within-group variability in the analysis with deleted observations. Because filling missing data with mean values preserves the variability in the predictors that were not missing for that observation, the mean-filled data likely better represents true variability in the data-set. The classification tables for the analysis with missing data deleted (Appendix B, Tables B4 and B5) indicated that similar types of misclassifications were made as with the mean-filled analysis. Half of Hamill Creek juveniles (1 of 2) were assigned to the Upper Duncan River, many Westfall River juveniles were assigned to Cultus Creek, and Houston Creek juveniles were assigned to Midge Creek and Kaslo River.

The LDA using five alternative data-sets with values added using the MICE method also had similar results the mean-filled data-set. All of the Kootenay watershed tributaries had the same classification rates (Appendix B, Table B6) as the mean-filled analysis (Table 4). There were some small differences in classification rates for Duncan tributaries. For instance, rates for the Upper Duncan River ranged from 58 to 75% (Table B6), compared to 75% in the mean-filled analysis. The classification tables show small differences (± 1 fish per tributary) between MICE data-sets in the number of fish classified to different tributaries, which indicates a small amount of model instability with respect to the value chosen to fill the missing data. Cases where classifications differed among the five MICE data-sets are highlighted bold and yellow in Tables B7 and B8 (Appendix B).

Overall, the sensitivity analysis indicates only minor differences in overall classification rate (≤5%) between the data-sets filled with mean values, filled with the MICE method, or with observations deleted. Classification rates for individual tributaries differed by 0 to 17% between data-sets, which reflects the uncertainty in classifications due to overlapping chemistry for Upper Duncan River and some other tributaries.
The sensitivity analysis supports the use of mean values to fill missing data, as it gave similar predictions and classification rates as the other alternatives assessed. One implication of using mean-values is that it would reduce within-group variance slightly for the predictor variable and tributaries that had had missing data. Since 13 of the 15 observations with missing data were missing $^{87}Sr/^{86}Sr$, the within-group variance for $^{87}Sr/^{86}Sr$ is likely underestimated in this analysis.

3.1.4 Random Forest Analysis

The random forest analysis was conducted as an alternative method to assign juvenile and adult Bull Trout to tributaries using the three predictor variables. The overall successful classification rate ("out-of-bag" estimate) was 80%. Classification success rate of Kootenay watershed tributaries was high, ranging from 78 to 100% for most tributaries, but was zero for two tributaries with only one sample, and for Hamill Creek, which only had three samples. Classification rates for Duncan watershed tributaries were 60% for Houston Creek, 58% for Upper Duncan River, and 10% for Westfall River. Some of the classification errors from Duncan watershed tributaries were assigned to the wrong tributary but the correct watershed. These included 30% (3 of 10) of juveniles from the Westfall River, and 17% (2 of 12) of juveniles from the Upper Duncan River.

In comparison to LDA, the classification rate of Kootenay watershed tributaries using random forest analysis was nearly the same, with the exception of Cultus Creek, which had a slightly higher success rate using random forest (92%) than LDA (85%). Classification of the Upper Duncan River was slightly worse using random forest analysis (58%) than LDA (75%). The largest difference between the methods was for Houston Creek, which was never classified correctly using LDA (cross-validated model) but was classified correctly in 60% of cases (3 of 5) using random forest analysis.

An example of a representative classification tree from the random forest is provided in Apppendix B (Appendix B, Figure B5). The final classifications of tributary were based the majority of votes from the 5000 trees in the analysis, each of which used a different random subset of the data and different criterion at each split. More than 2000 trees appeared to be required before misclassification error rates stabilized (Appendix B, Figure B6).

Table 5. Percentage of correct classification of capture location of juvenile Bull Trout using random forest analysis.

Known Capture Location	Predicted Capture Location												Sample Size	Classification Success Rate (%)
	Houston Creek	Upper Duncan River	Westfall River	Coffee Creek	Cooper Creek	Crawford Creek	Cultus Creek	Hamill Creek	Kaslo River	Midge Creek	Summit Creek	Woodbury Creek		
Duncan Watershed														
Houston Creek	3	0	0	0	0	0	0	0	1	1	0	0	5	60
Upper Duncan River	0	7	2	0	0	1	2	0	0	0	0	0	12	58
Westfall River	0	3	1	0	0	0	6	0	0	0	0	0	10	10
Kootenay Watershed														
Coffee Creek	0	0	0	8	0	0	0	0	0	0	0	0	8	100
Cooper Creek	0	0	0	0	9	0	0	0	0	0	0	0	9	100
Crawford Creek	0	0	0	0	0	25	0	0	0	0	0	0	25	100
Cultus Creek	0	1	1	0	0	0	24	0	0	0	0	0	26	92
Hamill Creek	0	1	1	0	0	1	0	0	0	0	0	0	3	0
Kaslo River	1	0	0	0	0	0	0	0	7	1	0	0	9	78
Midge Creek	0	0	0	0	0	0	0	0	1	21	0	0	22	95
Summit Creek	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Woodbury Creek	0	0	0	1	0	0	0	0	0	0	0	0	1	0

3.2 Comparison of Elemental Ratios Between Laboratories

The model to predict natal origin of adults used juvenile Sr:Ca and Ba:Ca values measured at the Winnipeg lab but adult Sr:Ca and Ba:Ca values measured at the Victoria lab (Section 3.3). Therefore, 13 juvenile otoliths that were analyzed at both labs were used to check the comparability of Sr:Ca and Ba:Ca between laboratories. Previous comparison of elemental ratios analyzed at both Victoria and Winnipeg labs showed that Sr:Ca values were not significantly different (Figure 4) but Ba:Ca values were consistently lower at the Winnipeg lab than the Victoria lab (Figure 5; ONA and Golder 2016). Linear regression was used to describe the relationship between Winnipeg and Victoria lab Ba:Ca values (Figure 6; $y = 0.92x + -0.46$; $P<0.0001$; $r^2=0.99$). The estimated slope and intercept from the regression equation were used to adjust the Ba:Ca values for all adult otoliths previously analyzed in the Victoria lab so that they were directly comparable to the juvenile Winnipeg lab Ba:Ca values. These adjusted Ba:Ca values for adult Bull Trout were used in the LDA and random forest analysis to classify and predict natal origins based on otolith chemistry.

The regression used in this report used mean values from the entire otolith for each fish, whereas the previous report (ONA and Golder 2016) used mean values for the natal portion of the otolith. The regression in this report had marginally better fit ($r^2=0.99$ vs. $r^2=0.97$). Using means from the entire scan was considered more reliable because it removed potential variability associated with choosing different portions of the otolith when calculating the value for the natal region.

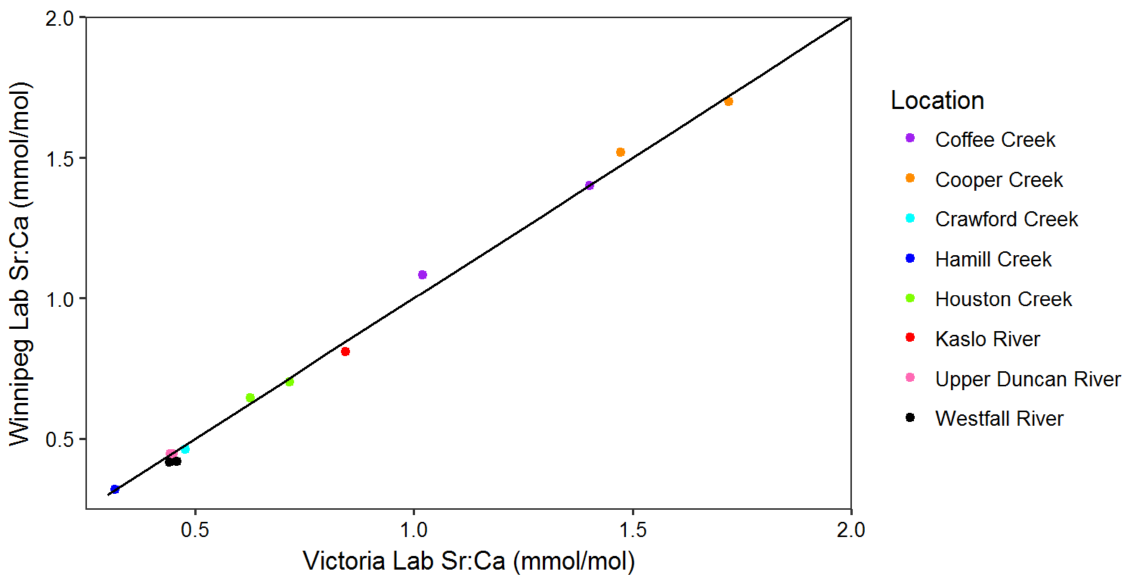

Figure 4. Sr:Ca ratios for thirteen juvenile Bull Trout otoliths analyzed at two laboratories. Each point represents the mean value from the entire otolith scan for each fish. The line represents a 1:1 relationship.

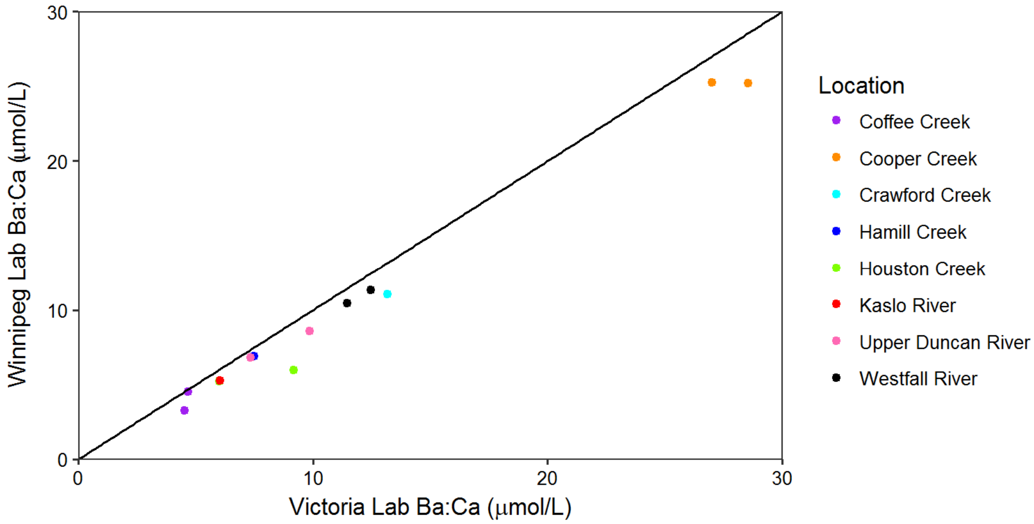

Figure 5. Ba:Ca ratios for thirteen juvenile Bull Trout otoliths analyzed at two laboratories. Each point represents the mean value from the entire otolith scan for each fish. The line represents a 1:1 relationship.

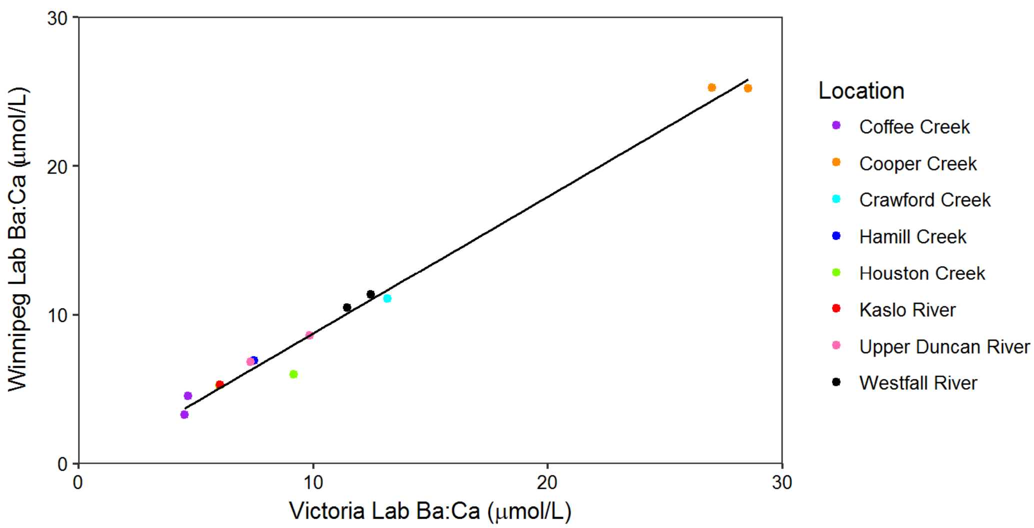

Figure 6. Linear regression between Ba:Ca ratios for thirteen juvenile Bull Trout otoliths analyzed at two laboratories. Each point represents the mean value from the entire otolith scan for each fish.

3.3 Predicting Natal Area of Adults

There were 39 adult Bull Trout with useable data for all three predictor variables, including 20 captured from the flip bucket, 11 from Duncan Reservoir, and 8 from Kootenay Lake (Figure 7). The range of values of Sr:Ca, Ba:Ca, and $^{87}Sr/^{86}Sr$ was similar between the three capture locations (Appendix B, Figure B7). These three variables were used to predict the natal tributary of each adult using the juvenile data and two types of classification model: LDA and random forest analysis.

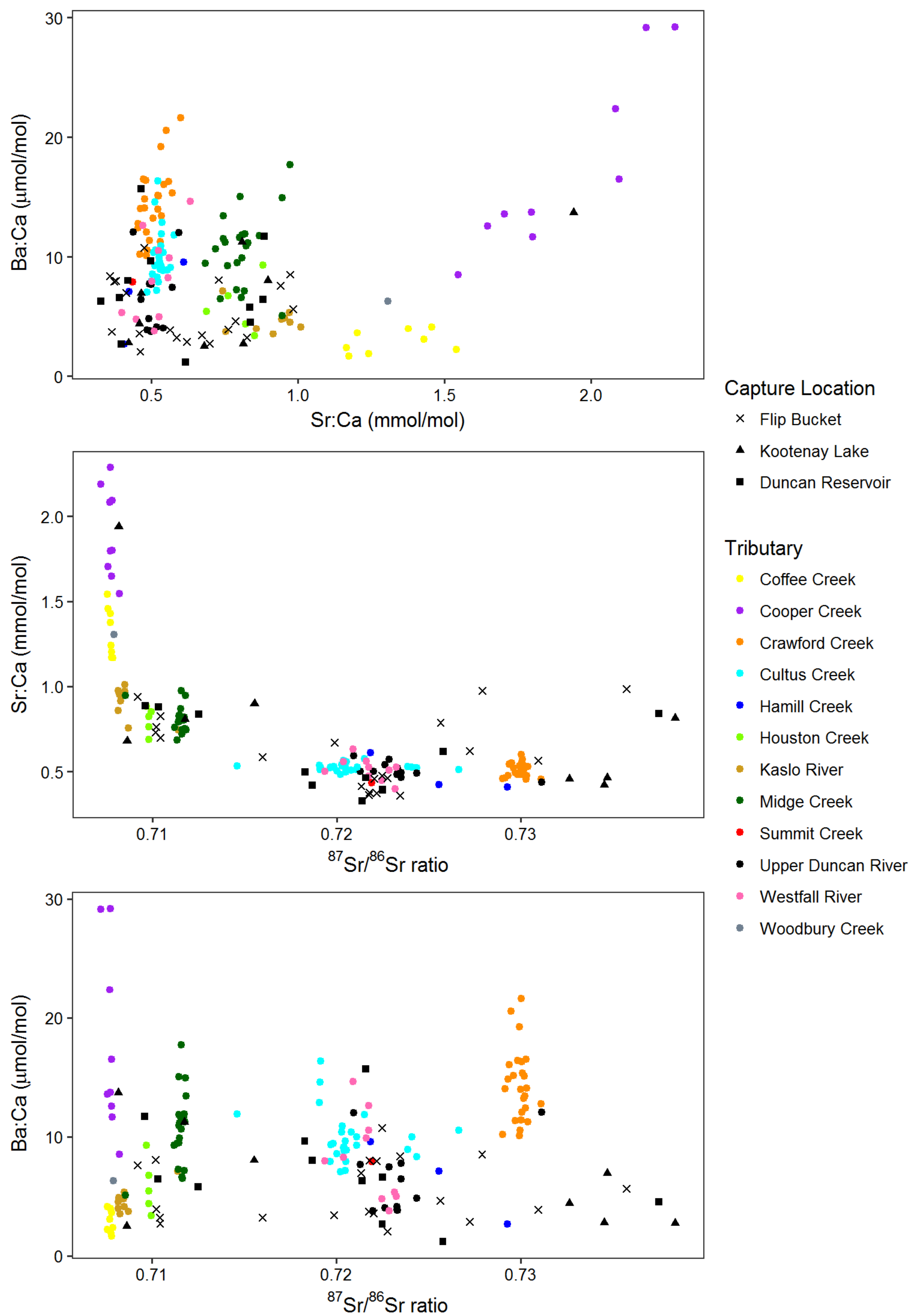

Figure 7. Mean values Ba:Ca, Sr:Ca, and $^{87}Sr/^{86}Sr$ from the natal portion of otoliths of juvenile and adult Bull Trout by capture location. Values for juveniles captured in tributaries are shown by coloured circles and adults are shown by black shapes by capture location.

3.3.1 Linear Discriminant Analysis

The natal tributary of adults was predicted using two LDA models, one using equal prior probabilities for the tributaries and one using prior probabilities based on the sample sizes in the juvenile data. The model using equal priors is considered more appropriate but both are presented for reference (Table 6). As expected, the model with the priors from the juvenile model tended to predict more adults from the tributaries with large sample sizes (e.g., Cultus Creek) and fewer adults from those with small juvenile sample sizes (e.g., Summit Creek), compared to the equal prior model.

Using the equal-prior LDA, 36% of adults captured in Duncan Reservoir, 35% of adults captured in the flip bucket, and 13% of adults captured in Kootenay Lake were classified to one of the Duncan watershed tributaries (Table 6). The low rates of classification to tributaries in the Duncan watershed, including for adults captured in Duncan Reservoir, are not surprising, because cross-validation of the juvenile LDA suggested successful classification rates of 0% for Houston Creek and Westfall River for juveniles known to be from those tributaries. The majority of adults from Kootenay Lake were classified to Crawford and Midge creeks, and these tributaries were also predicted for appreciable proportions of the adults from Duncan Reservoir and the flip bucket. Adults that had discriminant scores (Figure 8; top right of figure) and $^{87}Sr/^{86}Sr$ values (Figure 7) much different than any of the juveniles suggests that some adults may have originated from tributaries with chemistry different than any of those sampled for juveniles during this program.

One unexpected result was that 36% of adults from Duncan Reservoir and 30% from the flip bucket were classified to Summit Creek, but none of the adults from Kootenay Lake were classified to Summit Creek. Summit Creek is located at the far south end of Kootenay Lake, the furthest away from Duncan Dam. This result may be because the single juvenile captured in Summit Creek had chemistry very similar to the Upper Duncan River and Westfall River, and the sample size of one caused variance to be underestimated and the frequency of membership to this group to be overestimated. Another possibility is that these adults were from an un-sampled tributary, and the single Summit Creek juvenile had the chemistry the most similar to these individuals.
The adults assigned to Summit Creek had low scores on the second discriminant axis (LD2) and fairly high scores on the first axis (LD1; Figure 8), which reflects high values of Sr:Ca and moderately high values of $^{87}Sr/^{86}Sr$.

Table 6. Classification of the natal origin of adults using linear discriminant analysis.

Predicted Tributary	Equal Prior Probabilities						Juvenile Prior Probabilities					
	Duncan Reservoir		Flip Bucket		Kootenay Lake		Duncan Reservoir		Flip Bucket		Kootenay Lake	
	#	%	#	%	#	%	#	%	#	%	#	%
Duncan Watershed	4	36	7	35	1	13	2	18	7	35	1	13
Houston Creek	2	18	3	15	1	13	0	0	2	10	1	13
Upper Duncan River	2	18	4	20	0	0	2	18	5	25	0	0
Westfall River	0	0	0	0	0	0	0	0	0	0	0	0
Kootenay Watershed	7	64	13	65	7	88	9	82	13	65	7	88
Coffee Creek	0	0	0	0	0	0	0	0	0	0	0	0
Cooper Creek	0	0	0	0	1	13	0	0	0	0	1	13
Crawford Creek	1	9	3	15	4	50	1	9	3	15	4	50
Cultus Creek	1	9	0	0	0	0	3	27	2	10	0	0
Hamill Creek	0	0	1	5	0	0	0	0	0	0	0	0
Kaslo River	0	0	2	10	0	0	1	9	2	10	0	0
Midge Creek	1	9	1	5	2	25	2	18	2	10	2	25
Summit Creek	4	36	6	30	0	0	2	18	4	20	0	0
Woodbury Creek	0	0	0	0	0	0	0	0	0	0	0	0
Grand Total	11	100	20	100	8	100	11	100	20	100	8	100

Note: percentages by tributary or watershed may not add up to 100% because they are rounded to the nearest percent.

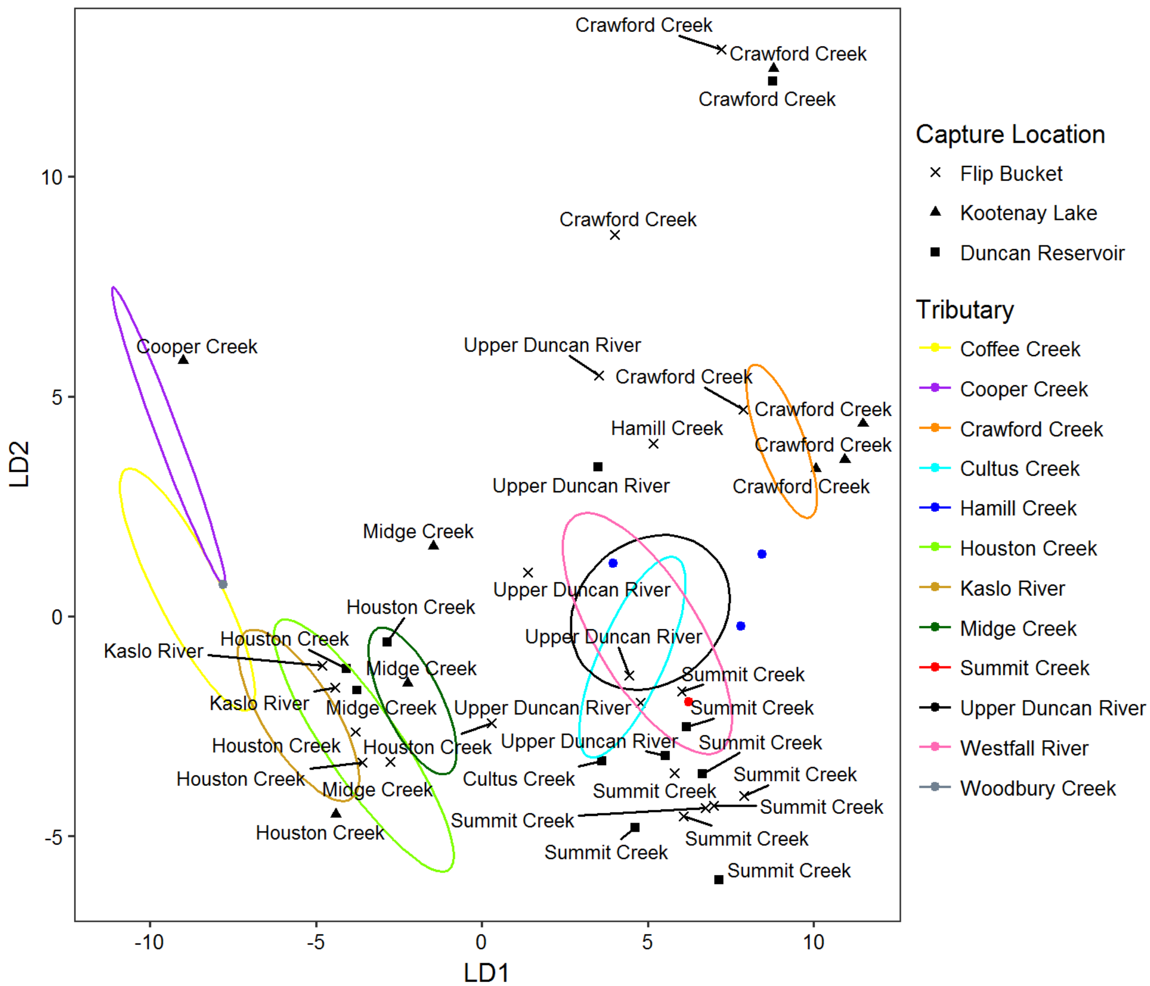

Figure 8. Predictions of natal tributary of adults by capture location from linear discriminant analysis using equal priors. Coloured ellipses are 95% confidence regions by from the juveniles of known origin. For Hamill, Summit, and Woodbury creeks, 95% confidence ellipses could not be calculated due to small sample size so the discriminant of the individual juveniles are shown instead.

3.3.1 Random Forest Analysis

The random forest analysis predicted that 73% of adults captured in Duncan Reservoir, 60% captured in the flip bucket, and 13% captured in Kootenay Lake were from tributaries in the Duncan watershed (Table 6). The greatest proportion of adults from Duncan Reservoir was classified to the Westfall River (45%). Adults captured in the flip bucket were classified to Houston Creek (30%), Upper Duncan River (20%), and Westfall River (10%), with the remaining 40% from various Kootenay watershed tributaries. Only 1 of the 8 adults (13%) captured in Kootenay Lake were classified to the Duncan watershed. Adults from Kootenay Lake were classified to five of the tributaries in that watershed, with the largest proportion classified to Midge Creek (38%).

Five of the adults classified to Midge Creek were beyond the range of values in the juvenile data, with very high values of $^{87}Sr/^{86}Sr$ (Figure 9). These individuals were mostly classified to Crawford Creek by the LDA. The distinct otolith chemistry in the natal region of the otoliths for these adults suggests they could be from a tributary not sampled during this monitoring program.

Table 7. Classification of the natal origin of adults using random forest analysis.

Predicted Tributary	Adult Capture Location					
	Duncan Reservoir		Flip Bucket		Kootenay Lake	
	#	%	#	%	#	%
Duncan Watershed	**8**	**73**	**12**	**60**	**1**	**13**
Houston Creek	2	18	6	30	0	0
Upper Duncan River	1	9	4	20	1	13
Westfall River	5	45	2	10	0	0
Kootenay Watershed	**3**	**27**	**8**	**40**	**7**	**88**
Coffee Creek	0	0	0	0	0	0
Cooper Creek	0	0	0	0	1	13
Crawford Creek	0	0	1	5	1	13
Cultus Creek	1	9	1	5	0	0
Hamill Creek	0	0	1	5	1	13
Kaslo River	0	0	0	0	1	13
Midge Creek	2	18	3	15	3	38
Summit Creek	0	0	2	10	0	0
Woodbury Creek	0	0	0	0	0	0
Total	**11**	**100**	**20**	**100**	**8**	**100**

Note: percentages by tributary or watershed may not add up to 100% because they are rounded to the nearest percent.

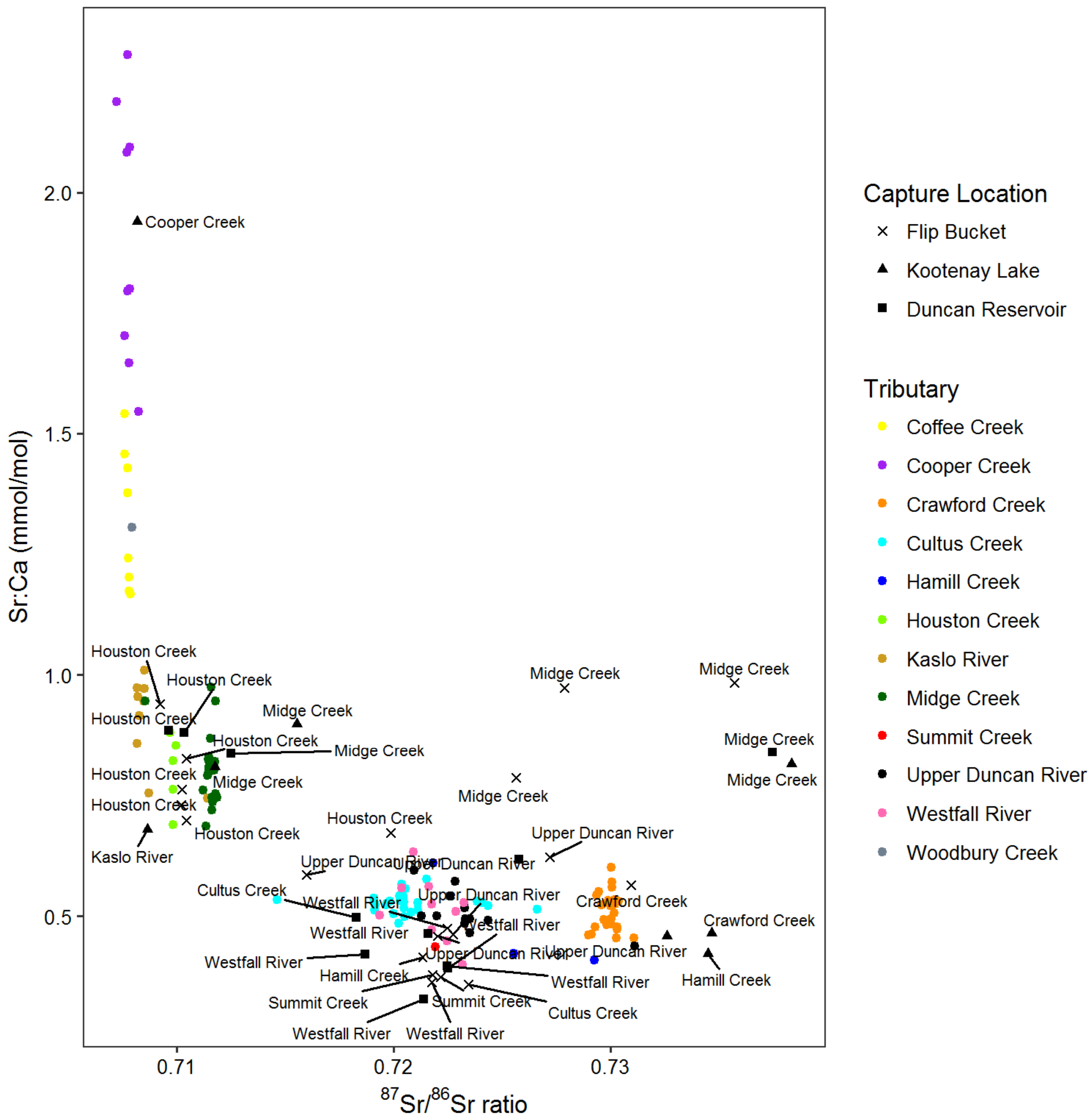

Figure 9. Predictions of natal tributary of adults by capture location from random forest analysis. Juvenile data are shown by coloured circles. Adult data are shown by black shapes, and each fish is labeled with the natal tributary predicted by the random forest analysis.

4 Discussion

4.1 Chemistry Differences Between Streams

Previous reports demonstrated that water chemistry was distinct in terms of Sr:Ca and Ba:Ca for most of the Duncan and Kootenay watershed tributaries assessed in this study (Golder 2010; ONA and Golder 2013, 2016). 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. Previous results also demonstrated that differences in water chemistry were also reflected by differences in the Sr:Ca and Ba:Ca ratios in the otoliths of Bull Trout from these tributaries. Chemical analysis of water samples did not include measurement of $^{87}Sr/^{86}Sr$, but the juvenile otolith data show that $^{87}Sr/^{86}Sr$ varies between tributaries, with distinct values for most of the streams (Figure 2). Previous analyses also indicated no significant differences in water or otolith chemistry between years within the study tributaries (ONA and Golder 2016), which suggests that Bull Trout otoliths collected in different years can be combined for analysis. Plots of the data (Figure 2), as well as the LDA and random forest analysis, indicated that $^{87}Sr/^{86}Sr$ was the most useful variable for discriminating between tributaries, followed by Sr:Ca, and Ba:Ca.

4.2 Classification of Juveniles of Known Natal Tributaries

Two statistical methods were used to develop of predictive model to classify juveniles to their natal tributary: LDA and random forest analysis. The methods had very similar overall classification accuracy (~80%) but differed slightly in the classification success for some tributaries. Random forest analysis had better classification success than LDA for Houston Creek (60% vs 0%) and Westfall River (10% vs. 0%), and worse classification success than LDA for the Upper Duncan River (58% vs. 75%). For both LDA and random forest analysis, some Westfall River juveniles were assigned to the Upper Duncan River (both in the Duncan watershed). In the random forest model, some of the Upper Duncan juveniles were assigned to Westfall River. Therefore, if classifications to the wrong tributary but the correct watershed are considered successful, then the classification success of Duncan watershed tributaries is slightly better than reported. Genetic studies demonstrated a lack of genetic differentiation between Bull Trout from different spawning areas in the Duncan watershed (O'Brien 1999). In addition, radio tagged spawners returning to the Duncan watershed did not show precise homing to specific locations, and often migrated to different tributaries in the Upper Duncan watershed in subsequent years (O'Brien 1999). These findings support the idea that classifications to the wrong tributary but within the correct watershed are good enough for the objectives of this report, given the biology of adfluvial Bull Trout in the study area. Regardless of which model and classification rates are used, the fairly low classification success of Houston Creek and the Upper Duncan and Westfall rivers (0-75%) suggests that the proportion of Bull Trout classified to the Duncan watershed would be underestimated using this data-set.

Most of the Kootenay watershed tributaries were well-classified by both LDA and random forest analysis, with classification rates ranging from 78 to 100%. The exceptions were Summit and Woodbury creeks, which both had a sample size of one, and Hamill Creek, which had three juvenile samples and highly variable chemistry among individuals. Of these three tributaries, there is potential for Bull Trout from Hamill Creek and Summit Creek to be misclassified to the Duncan watershed tributaries, based on similar chemistry (Figure 2) and the classification tables for both LDA (Appendix B, Table B2) and random forest analysis (Table 7). Bull Trout from Woodbury Creek and all six tributaries with good classification rates were unlikely to be misclassified to the Duncan watershed.

4.3 Predicting Adult Natal Tributary

The two predictive models, LDA and random forest analysis, did not agree on the proportion of adults classified to tributaries of the Duncan and Kootenay watersheds. The LDA predicted that a majority of adults from Duncan Reservoir (64%), the flip bucket (65%), and Kootenay Lake (88%) were reared in tributaries of Kootenay Lake. The random forest analysis predicted that 73% of adults captured in Duncan Reservoir, 60% of adults from the flip bucket, and 12% of adults captured in Kootenay Lake were from tributaries of the Duncan watershed. The predictions of the random forest analysis make intuitive sense, in that the majority of Kootenay Lake Bull Trout were spawned in that watershed, the majority of Duncan Reservoir Bull Trout were spawned in that watershed, and Bull Trout captured in the flip bucket were a mixture of fish reared in one of the two watersheds.

The random forest analysis is considered more reliable than LDA because the juvenile data had uneven variance between groups (tributaries) and LDA can be biased when assumptions about data distribution are violated (Mercier et al. 2011). In addition, classification success for juveniles from the Duncan watershed tributaries was better using random forest analysis than LDA. For these reasons, the predictions of adult origin from the random forest analysis are considered more appropriate for addressing this project's management questions. However, the conflicting results from the LDA reflect uncertainty in the predictions, which was due to overlapping chemistry between some tributaries of the Duncan and Kootenay watersheds, and small and unbalanced sample sizes.

Previous research, including floy-tagging and telemetry studies, are available to compare the results of the predictions from the current otolith microchemistry assessment. Ord et al. (2000) found that of Bull Trout tagged at the flip bucket and later recovered by anglers, 77% were captured in Kootenay Lake, 10% were captured in Duncan Reservoir, and 5% were captured in the Lardeau River. Of the Bull Trout that moved into Kootenay Lake, the majority were located in the north arm of Kootenay Lake. A telemetry study found that the majority of adult Bull Trout captured at Duncan Dam, in the Upper Duncan River, and the Lower Duncan River subsequently migrated to Houston Creek, Westfall River, or the Upper Duncan River above Houston Creek (O'Brien 1999). The majority of these radio-tagged Bull Trout (74 to 86% per year) that migrated into the Upper Duncan watershed subsequently migrated down through Duncan Dam and into Kootenay Lake. All of these tagging and telemetry observations indicate repeated migrations between Kootenay Lake and spawning grounds in the Upper Duncan watershed by adfluvial Bull Trout. In comparison, the random forest analysis predicted that 60% of Bull Trout captured in the flip bucket had been spawned in tributaries in the Duncan watershed. Therefore, the random forest analysis agrees with tagging and telemetry studies and support the idea that a majority of fish captured in the flip bucket were likely spawned in the Upper Duncan, use habitat in Kootenay Lake as adults, and return to spawning areas in the Upper Duncan to reproduce.

Other locations where Bull Trout were re-located by O'Brien (1999) and thought to be important spawning tributaries were Poplar Creek and Hamill Creek. Poplar Creek was not included in the current analysis but had much greater Sr:Ca (> 4 mmol/mol) than any other tributary (ONA and Golder 2016) and as all adults in the analysis had Sr:Ca less than 2 mmol/mol, it is unlikely any of the adults in this analysis were from Poplar Creek. Hamill Creek had overlapping Sr:Ca and Ba:Ca with Westfall and Upper Duncan rivers but was partially separated from tributaries by $^{87}Sr/^{86}Sr$ (Figure 2).

Six adult Bull Trout that had greater $^{87}Sr/^{86}Sr$ than any of the tributaries sampled for juveniles suggested that these adults could be from tributaries not included in this analysis. Four of these Bull Trout were captured in Kootenay Lake, one was captured in the flip bucket and one was captured in Duncan Reservoir. The tributaries selected for this study aimed to cover the most important Bull Trout spawning tributaries in Kootenay Lake, the Lower Duncan River, and the Upper Duncan River. One tributary not included in this study but recently identified as an important Bull Trout spawning area is Meadow Creek, which is a tributary to the Lower Duncan River (Andrusak 2014; Masse Environmental Consultants Ltd. 2015). Houston Creek and Westfall River are likely the most important Bull Trout spawning tributaries in the Duncan watershed, as half of radio tagged Bull Trout migrated to these streams, and redd counts were greatest in these tributaries (O'Brien 1999). However, other tributaries of the Upper Duncan watershed also likely provide spawning habitat, including Giegerich and Stevens creeks (O'Brien 1999), and it is possible that some of the sampled adults were from these streams. In our analysis, five of the six Bull Trout with distinct $^{87}Sr/^{86}Sr$ values were assigned to Kootenay watershed tributaries (Crawford, Midge, or Hamill creeks) and one was assigned to the Upper Duncan River.

4.4 Limitations of Data and Modelling Approach

Several aspects of the data-set contribute to uncertainty in interpretation and conclusions. One of these limitations is the consistency between laboratories in measurements of the elemental ratios (Sr:Ca and Ba:Ca). The analysis uses juvenile data from the Winnipeg lab and adult data from the Victoria lab, and because there was a consistent difference in values of Ba:Ca between the labs, linear regression was used to correct adult values of Ba:Ca. Although the regression had good fit (r^2=0.99), using corrected values from the Victoria lab for adults adds some uncertainty to the analysis and its interpretation.

Another limitation of the current data-set is missing values for some of the predictor variables for some of the juveniles. The majority (13 of 15) of juveniles with missing data were missing $^{87}Sr/^{86}Sr$ but had values of Sr:Ca and Ba:Ca. Missing values were filled using tributary-specific means in the LDA and random forests analysis. Deleting observations was undesirable because of small sample sizes, and is likely to lead to bias. Filling missing data with mean values before statistical analysis can perform as well or better than more sophisticated methods in some cases (Mundfrom and Whitcomb 1998). Our sensitivity analysis suggested similar classification rates by tributary when using means as when using randomly selected values using the MICE method. Therefore, we think that the approach to handling missing data was appropriate and had relatively little impact on the results and conclusions.

Some juvenile Bull Trout otoliths had very different chemistry than other juveniles from the same stream. Seven outliers that were distinct from all other samples from the same tributary were removed from the analysis. However, some tributaries had variable chemistry across many of the juvenile samples, and needed to be included in the analysis (e.g. Hamill Creek and Cultus Creek for $^{87}Sr/^{86}Sr$; Figure 2). The raw data for these samples showed 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 the chemistry of otoliths of juvenile Bull Trout captured in the same tributary. Other studies in the literature (Kennedy et al. 2000; Wells et al. 2003) support the contention that elemental and isotope 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, Ba:Ca, and $^{87}Sr/^{86}Sr$ 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. Many of the tributaries have upstream sub-tributaries where spawning is known to occur (Andrusak and Andrusak 2012). We hypothesize that some of the variability in chemistry between juveniles from the same capture location may be because some juveniles were hatched and initially reared in upstream tributaries with different water chemistry but moved downstream prior to sampling.

4.5 Summary

The addition of $^{87}Sr/^{86}Sr$, which was not measured in previous years of the program, improved classification success, including better separation of juveniles from Duncan watershed tributaries from those from Kootenay Lake tributaries. The random forest analysis used in this analysis also seems to be an improvement over the LDA used in previous reports, likely because of less strict assumptions about data distribution. However, there remains uncertainty due to misclassification errors for juveniles of known origin, especially for the Duncan watershed tributaries, which had classification rates ranging from 10 to 58%. Relatively poor classification success was attributed to overlapping chemistry between some of the tributaries, small and unbalanced sample sizes, and high within-group variability for some tributaries. Classification tables suggested that juveniles from tributaries in the Duncan watershed may be misclassified to the Kootenay watershed, whereas misclassifications of Kootenay watershed Bull Trout to the Duncan watershed were less likely. This suggests that predictions of the proportions of adult Bull Trout that were reared in the Duncan watershed may be underestimates. The predicted proportion of adult Bull Trout from Duncan watershed tributaries was 60% of adults from the flip bucket and 73% of those captured in Duncan Reservoir, and 12% of those captured in Kootenay Lake.

5 Management Questions Summary

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

The results indicate that 60% of adult Bull Trout captured in the flip bucket were from one of the three tributaries of the Duncan watershed. This suggests that a large proportion of adults transferred upstream by the flip bucket at Duncan Dam were spawned and reared in the Duncan watershed, migrated downstream to Kootenay Lake, and later returned to the Duncan watershed, possibly to spawn. Because of the uncertainties in this analysis discussed above, a precise estimate of the contribution of spawning areas upstream of Duncan Dam to the Bull Trout population in Kootenay Lake and Duncan Reservoir is not possible. However, the results support the idea that the transfer program contributes to recruitment by allowing access to spawning and rearing habitats in the Upper Duncan watershed to Bull Trout residing downstream of the Duncan Dam as adults. The importance of the transfer program to recruitment is also supported by previous tagging and recapture at the flip bucket (Ord et al. 2000), and telemetry work (O'Brien 1999), both of which demonstrated adult Bull Trout making return migrations to the Upper Duncan watershed using the flip bucket in subsequent years. O'Brien (1999) reported that nearly all (74-86% per year) radio-tagged adults that migrated to spawning tributaries in the Upper Duncan watershed later migrated downstream through the dam and into Kootenay Lake, which demonstrates the contribution of the transfer program to recruitment in the study area.

5.1.1 $H0_1$: 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 $H0_1$. Similar chemistry in water and juvenile otoliths between some of the sampled tributaries, especially Summit Creek, Cultus 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.

5.1.2 $H0_2$: The proportion of natal to non-natal Bull Trout is not statistically different between the Kootenay and Duncan watersheds.

The current analysis indicated that 73% of adult Bull Trout from Duncan Reservoir were spawned in tributaries of that watershed, and 88% of adults captured in Kootenay Lake were spawned downstream of Duncan Dam. This suggests similar proportions of natal vs. non-natal Bull Trout in the adults sampled from recreational fisheries in Duncan Reservoir and Kootenay Lake. However, as previously discussed, the analysis suggested that estimates of the contribution of spawning tributaries in the Upper Duncan watershed may be underestimates, based on classification tables for juveniles of known origin. Of the adults collected in flip bucket, 60% were predicted to be from tributaries in the Duncan watershed, which suggests Bull Trout spawned in the Duncan watershed contribute to the adult population in Kootenay Lake. Because of uncertainties in the current data-set and analysis, a statistical test of natal and non-natal proportions was considered inappropriate and was not conducted. Instead of a statistical test, a weight-of-evidence approach considering all analyses and sources of uncertainty is recommended to address this hypothesis. Using this approach, we fail to reject $H0_2$ at this time because the preferred statistical model (random forest analysis) suggested similar proportion of natal Bull Trout between watersheds. However, the conflicting results from the LDA, and poor classification rates of Duncan watershed tributaries limit the strength of this conclusion.

5.2 MQ2: What are the origins of Bull Trout individuals sampled in Duncan Reservoir and Kootenay Lake watersheds?

The current best estimates of the origins of Bull Trout sampled in this program, which are based on otolith microchemistry and random forest analysis, are provided in Table 7. These results suggested that 73% of adult Bull Trout sampled in Duncan Reservoir, 60% of those sampled in the flip bucket, and 12% of those sampled in Kootenay Lake had origins in known spawning tributaries in the Upper Duncan watershed, and the remainder had origins in spawning tributaries of the Kootenay Lake watershed.

5.3 MQ3: Does the distribution and analyzed life histories of the sampled fish denote a bottleneck to recruitment at Duncan Dam?

The Duncan Dam could be considered a “bottleneck” to recruitment if three conditions were met: 1) Bull Trout were migrating between the Duncan Reservoir and Kootenay Lake watersheds for different parts of their life history; 2) availability of spawning and rearing habitat was limiting recruitment of Bull Trout in Duncan Reservoir or Kootenay Lake; and 3) the transfer program at Duncan Dam was limiting the number of adult Bull Trout migrating between watersheds.

This monitoring program provides evidence that Bull Trout migrate between the watersheds (condition #1), as 60% of adults from the flip bucket were predicted to have origins in the Duncan watershed, and smaller proportions of adults from Kootenay Lake (13%) and Duncan Reservoir (27%) were not spawned in the watershed where they were caught. Migrations between the Kootenay and Duncan watersheds are also well-documented by previous tagging and telemetry studies (O’Brien 1999; Ord et al. 2000). This monitoring program was not designed to assess condition #2 and spawning and rearing habitat are just one of many possible factors that could be limiting recruitment. This program also cannot assess passage limitations at Duncan Dam (condition #3), but other studies provide some relevant information. O’Brien (1999) reported that of radio tagged Bull Trout detected at the base of the dam, the proportion that successfully passed the dam via flip bucket transfer ranged from zero to one-third between years, although not all of these fish were necessarily attempting to move upstream. In addition, 47.0 to 52.7% of Bull Trout counted in the flip bucket were successfully passed upstream during fish transfers, with the remainder still in the flip bucket after transfer, or moving downstream into the tailrace. These results suggest that passage success of the fish transfer program is limited, although the precise passage efficiency is unknown. Thorley (2009) reported that in years when the weir was not installed to reduce the jump height required for fish to enter the flip bucket, the body size distribution of Bull Trout was larger, which suggests smaller Bull Trout were not able to pass the dam. Therefore, previous studies indicate that even with the fish transfer program, the dam remains an impediment to a proportion of Bull Trout attempting to migrate upstream. With regard to MQ3, it is possible that migration at the dam could be a bottleneck to recruitment but sufficient evidence to support this contention is lacking.

5.4 MQ4: 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 (number, body size, and natal origin) occur over the migration period of Bull Trout. Sample sizes would need to be sufficient for each time period of interest to provide sufficient statistical power to discern changes. O'Brien (1999) provides information on the preferred timing of gate movements during fish transfer based on his observations. Thorley (2009) recommended installing the weir in all years, which would reduce the height of the required jump to access the flip bucket and reduce size selectivity of the transfer program. Thorley (2009) also recommended installing water level logger at the base of the lower level gates to more accurately measure the jump height required to enter the flip bucket, to improve future assessments of Bull Trout passage. Inability to pass Bull Trout upstream using the fish transfer program during high flows, as reported by Ord et al. (2000), could also be problematic if significant numbers of Bull Trout are attempting to migrate upstream during these conditions, which should be considered during any future modifications to the transfer program.

6 RECOMMENDATIONS

Current otolith microchemistry results, combined with previous tagging and telemetry studies, provide sufficient evidence to demonstrate the importance of the fish transfer program for allowing inter-basin migrations of adfluvial Bull Trout, which contribute to recruitment in Kootenay and Duncan watersheds. Based on the otolith microchemistry results, there remains uncertainty in the proportion of adults that were spawned and reared in the Duncan and Kootenay watersheds. If future studies are undertaken to reduce uncertainty in predicted natal origins, the following recommendations are provided:

- To ensure consistency with the current data-set, any future otolith microchemistry analysis for elemental ratios should be conducted at the Winnipeg lab, and analysis of isotope ratio analysis should be conducted at the Oregon lab.
- In this report, only adults captured in 2008/2009 and analyzed at the Victoria lab were used in the statistical analysis. It is recommended that additional adult otoliths are analyzed at the Winnipeg lab to compare results to data from Victoria and whether conclusions regarding Bull Trout recruitment are the same. Additional adult Bull Trout heads and otoliths have already been collected from the three adult capture locations and are being stored for analysis.
  Future analysis of adult otoliths at the Winnipeg lab should include at least 15 samples previously analyzed by the Victoria lab for comparison, as well as new, previously un-measured samples.

- Part of the uncertainty in juvenile classifications was related to very small sample sizes and high variability for some tributaries. Analysis of additional juvenile samples from these tributaries would likely improve classification rates, and help discern whether within-stream variability was related to natural variability, such as immigration of juveniles, or the precision of laboratory analyses. More balanced sample sizes between tributaries is also likely to provide more accurate classification for both LDA and random forest analysis (Boulesteix et al. 2012; Xue and Hall 2015). The highest priority tributary for analyzing additional juvenile samples is Hamill Creek, because there were only five samples, of which two were excluded because they were outliers, and the remaining three samples had highly variable chemistry that overlapped Duncan watershed tributaries. Additional juvenile otolith samples from many of the tributaries were previously analyzed by other laboratories (Victoria or Australia) but not used in this report. Some of these otoliths could be re-analyzed to increase sample sizes of the current data-set without conducting additional field data collection.

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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 using linear discriminant analysis (un-validated model). These results are from the data-set with missing data filled with mean values for each capture location.

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	8	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	25	0	0	0	0	0	0	0	0	0
Cultus Creek	0	0	1	22	0	0	0	0	0	3	0	0
Hamill Creek	0	0	0	1	2	0	0	0	0	0	0	0
Houston Creek	0	0	0	0	0	1	2	2	0	0	0	0
Kaslo River	0	0	0	0	0	1	7	1	0	0	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	2	0	0	0	0	0	9	0	0
Westfall River	0	0	0	6	0	0	0	0	0	3	1	0
Woodbury Creek	0	0	0	0	0	0	0	0	0	0	0	1

Table B2. Classification of juvenile Bull Trout otoliths using linear discriminant analysis (cross-validated model). These results are from the data-set with missing data filled with mean values for each capture location.

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	8	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	25	0	0	0	0	0	0	0	0	0
Cultus Creek	0	0	1	22	0	0	0	0	0	3	0	0
Hamill Creek	0	0	0	1	0	0	0	0	0	2	0	0
Houston Creek	0	0	0	0	0	0	2	3	0	0	0	0
Kaslo River	0	0	0	0	0	1	7	1	0	0	0	0
Midge Creek	0	0	0	0	0	0	1	21	0	0	0	0
Summit Creek	0	0	0	0	0	0	0	0	0	0	0	0
Upper Duncan River	0	0	1	2	0	0	0	0	0	9	0	0
Westfall River	0	0	0	6	0	0	0	0	1	3	0	0
Woodbury Creek	0	0	0	0	0	0	0	0	0	0	0	0

Table B3. Percentage of correct classification of capture location of juvenile Bull Trout based on linear discriminant analysis using data-set with fish with missing values for a variable deleted.

Capture Location	Un-validated Model	Cross-Validated Model	Sample Size
Duncan Watershed			**20**
Houston Creek	0%	0%	2
Upper Duncan River	70%	70%	10
Westfall River	13%	13%	8
Kootenay Watershed			**96**
Coffee Creek	100%	100%	7
Cooper Creek	100%	100%	7
Crawford Creek	100%	100%	24
Cultus Creek	88%	88%	24
Hamill Creek	50%	0%	2
Kaslo River	88%	88%	8
Midge Creek	95%	95%	22
Summit Creek	0%	-	1
Woodbury Creek	100%	-	1
Total	**84%**	**83%**	**116**

Note: "-" hyphen indicates that percentage could not be calculated in leave-one-out cross-validated model because there was only one sample for that location.

Table B4. Classification of juvenile Bull Trout otoliths using linear discriminant analysis (un-validated model). These results are from analysis with observations with missing data deleted from the data-set.

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	7	0	0	0	0	0	0	0	0	0	0
Crawford Creek	0	0	24	0	0	0	0	0	0	0	0	0
Cultus Creek	0	0	1	21	0	0	0	0	0	2	0	0
Hamill Creek	0	0	0	1	1	0	0	0	0	0	0	0
Houston Creek	0	0	0	0	0	0	1	1	0	0	0	0
Kaslo River	0	0	0	0	0	0	7	1	0	0	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	2	0	0	0	0	0	7	0	0
Westfall River	0	0	0	4	0	0	0	0	1	2	1	0
Woodbury Creek	0	0	0	0	0	0	0	0	0	0	0	1

Table B5. Classification of juvenile Bull Trout otoliths using linear discriminant analysis (cross-validated model). These results are from analysis with observations with missing data deleted from the data-set.

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	7	0	0	0	0	0	0	0	0	0	0
Crawford Creek	0	0	24	0	0	0	0	0	0	0	0	0
Cultus Creek	0	0	1	21	0	0	0	0	0	2	0	0
Hamill Creek	0	0	0	1	0	0	0	0	0	1	0	0
Houston Creek	0	0	0	0	0	0	1	1	0	0	0	0
Kaslo River	0	0	0	0	0	0	7	1	0	0	0	0
Midge Creek	0	0	0	0	0	0	1	21	0	0	0	0
Summit Creek	0	0	0	0	0	0	0	0	0	0	0	0
Upper Duncan River	0	0	1	2	0	0	0	0	0	7	0	0
Westfall River	0	0	0	4	0	0	0	0	1	2	1	0
Woodbury Creek	0	0	0	0	0	0	0	0	0	0	0	0

Table B6. Percentage of correct classification of capture location of juvenile Bull Trout based on linear discriminant analysis using imputed data using the MICE method. Model numbers (1-5) refer to the five data-sets with varying values of imputed data to fill missing values.

Location	Un-validated Model					Cross-Validated Model				
	1	2	3	4	5	1	2	3	4	5
Duncan Watershed										
Houston Creek	0%	20%	20%	20%	20%	0%	20%	0%	0%	0%
Upper Duncan River	67%	67%	67%	75%	67%	58%	67%	67%	75%	67%
Westfall River	10%	10%	0%	0%	10%	0%	0%	0%	0%	0%
Kootenay Watershed										
Coffee Creek	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Cooper Creek	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Crawford Creek	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Cultus Creek	85%	88%	85%	88%	85%	85%	85%	85%	88%	85%
Hamill Creek	33%	33%	33%	33%	33%	0%	0%	0%	0%	0%
Kaslo River	78%	89%	78%	78%	89%	78%	78%	78%	78%	78%
Midge Creek	95%	95%	95%	95%	95%	95%	95%	95%	95%	95%
Summit Creek	0%	0%	0%	0%	0%	-	-	-	-	-
Woodbury Creek	100%	100%	100%	100%	100%	-	-	-	-	-
Total	**79%**	**81%**	**79%**	**80%**	**80%**	**77%**	**78%**	**78%**	**79%**	**78%**

Note: "-" hyphen indicates that percentage could not be calculated in leave-one-out cross-validated model because there was only one sample for that location.

Table B7. Classification of natal tributary by linear discriminant analysis (un-validated model) for five different data-sets with different imputed values (indexed by the # symbol and values 1-5). Values are the number of fish predicted for each location. Predicted values that differed depending on the imputed values are shown in bold and highlighted yellow.

Actual Location	#	Predicted Location: Coffee Ck	Cooper Ck	Crawford Ck	Cultus Ck	Hamill Ck	Houston Ck	Kaslo R	Midge Ck	Summit Ck	Upper Duncan R	Westfall R	Woodbury Ck
Coffee Ck	1	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	2	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	3	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	4	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	5	8	0	0	0	0	0	0	0	0	0	0	0
Cooper Ck	1	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	2	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	3	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	4	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	5	0	9	0	0	0	0	0	0	0	0	0	0
Crawford Ck	1	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	2	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	3	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	4	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	5	0	0	25	0	0	0	0	0	0	0	0	0
Cultus Ck	1	0	0	1	**22**	0	0	0	0	0	**3**	0	0
Cultus Ck	2	0	0	1	**23**	0	0	0	0	0	**2**	0	0
Cultus Ck	3	0	0	1	**22**	0	0	0	0	0	**3**	0	0
Cultus Ck	4	0	0	1	**23**	0	0	0	0	0	**2**	0	0
Cultus Ck	5	0	0	1	**22**	0	0	0	0	0	**3**	0	0
Hamill Ck	1	0	0	1	1	1	0	0	0	0	0	0	0
Hamill Ck	2	0	0	1	1	1	0	0	0	0	0	0	0
Hamill Ck	3	0	0	1	1	1	0	0	0	0	0	0	0
Hamill Ck	4	0	0	1	1	1	0	0	0	0	0	0	0
Hamill Ck	5	0	0	1	1	1	0	0	0	0	0	0	0
Houston Ck	1	0	0	0	0	0	**0**	**2**	**3**	0	0	0	0
Houston Ck	2	0	0	0	0	0	**1**	**1**	**3**	0	0	0	0
Houston Ck	3	0	0	0	0	0	**1**	**1**	**3**	0	0	0	0
Houston Ck	4	0	0	0	0	0	**1**	**2**	**2**	0	0	0	0
Houston Ck	5	0	0	0	0	0	**1**	**1**	**3**	0	0	0	0
Kaslo R	1	0	0	0	0	0	**1**	**7**	1	0	0	0	0
Kaslo R	2	0	0	0	0	0	**0**	**8**	1	0	0	0	0
Kaslo R	3	0	0	0	0	0	**1**	**7**	1	0	0	0	0
Kaslo R	4	0	0	0	0	0	**1**	**7**	1	0	0	0	0
Kaslo R	5	0	0	0	0	0	**0**	**8**	1	0	0	0	0
Midge Ck	1	0	0	0	0	0	0	1	21	0	0	0	0
Midge Ck	2	0	0	0	0	0	0	1	21	0	0	0	0

Actual Location	#	Predicted Location											
		Coffee Ck	Cooper Ck	Crawford Ck	Cultus Ck	Hamill Ck	Houston Ck	Kaslo R	Midge Ck	Summit Ck	Upper Duncan R	Westfall R	Woodbury Ck
Midge Ck	3	0	0	0	0	0	0	1	21	0	0	0	0
Midge Ck	4	0	0	0	0	0	0	1	21	0	0	0	0
Midge Ck	5	0	0	0	0	0	0	1	21	0	0	0	0
Summit Ck	1	0	0	0	1	0	0	0	0	0	0	0	0
Summit Ck	2	0	0	0	1	0	0	0	0	0	0	0	0
Summit Ck	3	0	0	0	1	0	0	0	0	0	0	0	0
Summit Ck	4	0	0	0	1	0	0	0	0	0	0	0	0
Summit Ck	5	0	0	0	1	0	0	0	0	0	0	0	0
Upper Duncan R	1	0	0	1	**3**	0	0	0	0	0	**8**	**0**	0
Upper Duncan R	2	0	0	1	**3**	0	0	0	0	0	**8**	**0**	0
Upper Duncan R	3	0	0	1	**3**	0	0	0	0	0	**8**	**0**	0
Upper Duncan R	4	0	0	1	**2**	0	0	0	0	0	**9**	**0**	0
Upper Duncan R	5	0	0	1	**2**	0	0	0	0	0	**8**	**1**	0
Westfall R	1	0	0	0	6	0	0	0	0	**0**	**3**	**1**	0
Westfall R	2	0	0	0	6	0	0	0	0	**0**	**3**	**1**	0
Westfall R	3	0	0	0	6	0	0	0	0	**1**	**3**	**0**	0
Westfall R	4	0	0	0	6	0	0	0	0	**1**	**3**	**0**	0
Westfall R	5	0	0	0	6	0	0	0	0	**1**	**2**	**1**	0
Woodbury Ck	1	0	0	0	0	0	0	0	0	0	0	0	1
Woodbury Ck	2	0	0	0	0	0	0	0	0	0	0	0	1
Woodbury Ck	3	0	0	0	0	0	0	0	0	0	0	0	1
Woodbury Ck	4	0	0	0	0	0	0	0	0	0	0	0	1
Woodbury Ck	5	0	0	0	0	0	0	0	0	0	0	0	1

Table B8. Classification of natal tributary by cross-validated linear discriminant analysis for five different datasets with different imputed values (indexed by the # symbol and values 1-5). Values are the number of fish predicted for each location. Predicted values that differed depending on the imputed values are shown in bold and highlighted yellow.

Actual Location	#	Predicted Location											
		Coffee Ck	Cooper Ck	Crawford Ck	Cultus Ck	Hamill Ck	Houston Ck	Kaslo R	Midge Ck	Summit Ck	Upper Duncan R	Westfall R	Woodbury Ck
Coffee Ck	1	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	2	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	3	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	4	8	0	0	0	0	0	0	0	0	0	0	0
Coffee Ck	5	8	0	0	0	0	0	0	0	0	0	0	0
Cooper Ck	1	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	2	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	3	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	4	0	9	0	0	0	0	0	0	0	0	0	0
Cooper Ck	5	0	9	0	0	0	0	0	0	0	0	0	0

Actual Location	#	Predicted Location											
		Coffee Ck	Cooper Ck	Crawford Ck	Cultus Ck	Hamill Ck	Houston Ck	Kaslo R	Midge Ck	Summit Ck	Upper Duncan R	Westfall R	Woodbury Ck
Crawford Ck	1	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	2	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	3	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	4	0	0	25	0	0	0	0	0	0	0	0	0
Crawford Ck	5	0	0	25	0	0	0	0	0	0	0	0	0
Cultus Ck	1	0	0	1	**22**	0	0	0	**0**	0	**3**	0	0
Cultus Ck	2	0	0	1	**22**	0	0	0	**1**	0	**2**	0	0
Cultus Ck	3	0	0	1	**22**	0	0	0	**0**	0	**3**	0	0
Cultus Ck	4	0	0	1	**23**	0	0	0	**0**	0	**2**	0	0
Cultus Ck	5	0	0	1	**22**	0	0	0	**0**	0	**3**	0	0
Hamill Ck	1	0	0	1	1	0	0	0	0	0	1	0	0
Hamill Ck	2	0	0	1	1	0	0	0	0	0	1	0	0
Hamill Ck	3	0	0	1	1	0	0	0	0	0	1	0	0
Hamill Ck	4	0	0	1	1	0	0	0	0	0	1	0	0
Hamill Ck	5	0	0	1	1	0	0	0	0	0	1	0	0
Houston Ck	1	0	0	0	0	0	**0**	**2**	3	0	0	0	0
Houston Ck	2	0	0	0	0	0	**1**	**1**	3	0	0	0	0
Houston Ck	3	0	0	0	0	0	**0**	**2**	3	0	0	0	0
Houston Ck	4	0	0	0	0	0	**0**	**2**	3	0	0	0	0
Houston Ck	5	0	0	0	0	0	**0**	**2**	3	0	0	0	0
Kaslo R	1	0	0	0	0	0	1	7	1	0	0	0	0
Kaslo R	2	0	0	0	0	0	1	7	1	0	0	0	0
Kaslo R	3	0	0	0	0	0	1	7	1	0	0	0	0
Kaslo R	4	0	0	0	0	0	1	7	1	0	0	0	0
Kaslo R	5	0	0	0	0	0	1	7	1	0	0	0	0
Midge Ck	1	0	0	0	0	0	0	1	21	0	0	0	0
Midge Ck	2	0	0	0	0	0	0	1	21	0	0	0	0
Midge Ck	3	0	0	0	0	0	0	1	21	0	0	0	0
Midge Ck	4	0	0	0	0	0	0	1	21	0	0	0	0
Midge Ck	5	0	0	0	0	0	0	1	21	0	0	0	0
Summit Ck	1	-	-	-	-	-	-	-	-	-	-	-	-
Summit Ck	2	-	-	-	-	-	-	-	-	-	-	-	-
Summit Ck	3	-	-	-	-	-	-	-	-	-	-	-	-
Summit Ck	4	-	-	-	-	-	-	-	-	-	-	-	-
Summit Ck	5	-	-	-	-	-	-	-	-	-	-	-	-
Upper Duncan R	1	0	0	1	**3**	0	0	0	0	0	**7**	**1**	0
Upper Duncan R	2	0	0	1	**3**	0	0	0	0	0	**8**	**0**	0
Upper Duncan R	3	0	0	1	**3**	0	0	0	0	0	**8**	**0**	0
Upper Duncan R	4	0	0	1	**2**	0	0	0	0	0	**9**	**0**	0
Upper Duncan R	5	0	0	1	**2**	0	0	0	0	0	**8**	**1**	0
Westfall R	1	0	0	0	6	0	0	0	0	1	3	0	0

Actual Location	#	Predicted Location											
		Coffee Ck	Cooper Ck	Crawford Ck	Cultus Ck	Hamill Ck	Houston Ck	Kaslo R	Midge Ck	Summit Ck	Upper Duncan R	Westfall R	Woodbury Ck
Westfall R	2	0	0	0	6	0	0	0	0	1	3	0	0
Westfall R	3	0	0	0	6	0	0	0	0	1	3	0	0
Westfall R	4	0	0	0	6	0	0	0	0	1	3	0	0
Westfall R	5	0	0	0	6	0	0	0	0	1	3	0	0
Woodbury Ck	1	-	-	-	-	-	-	-	-	-	-	-	-
Woodbury Ck	2	-	-	-	-	-	-	-	-	-	-	-	-
Woodbury Ck	3	-	-	-	-	-	-	-	-	-	-	-	-
Woodbury Ck	4	-	-	-	-	-	-	-	-	-	-	-	-
Woodbury Ck	5	-	-	-	-	-	-	-	-	-	-	-	-

Note: "-" hyphen indicates that predicted values were not available in leave-one-out cross-validated model because there was only one sample for that location.

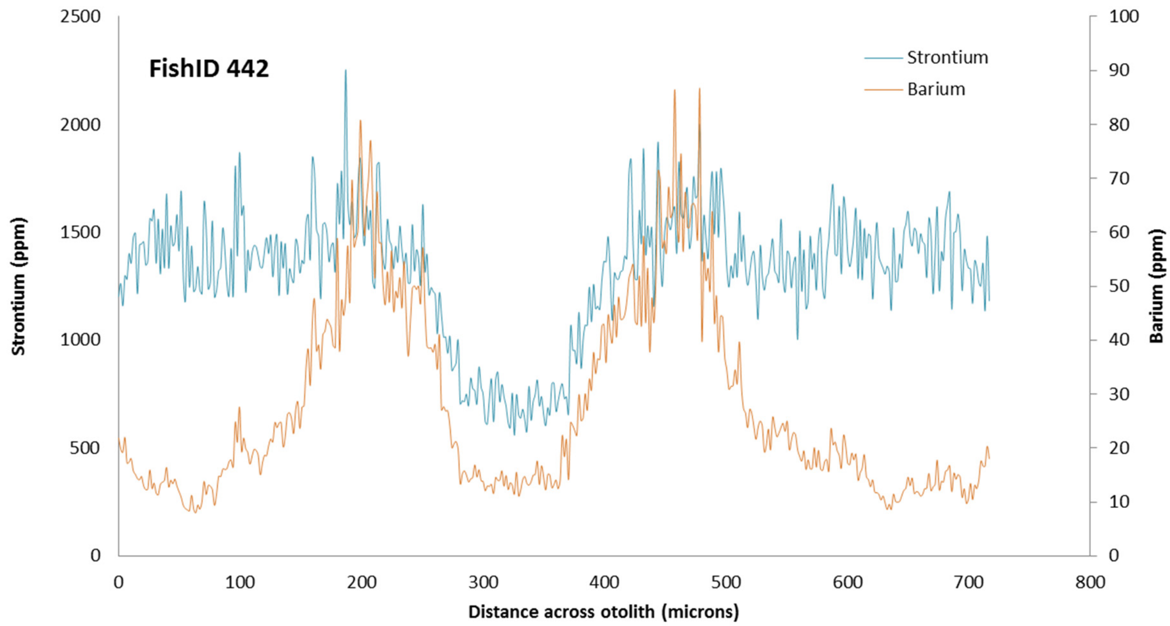

Figure B1. Example of raw laboratory data for elemental concentrations of strontium and barium for a juvenile Bull Trout.

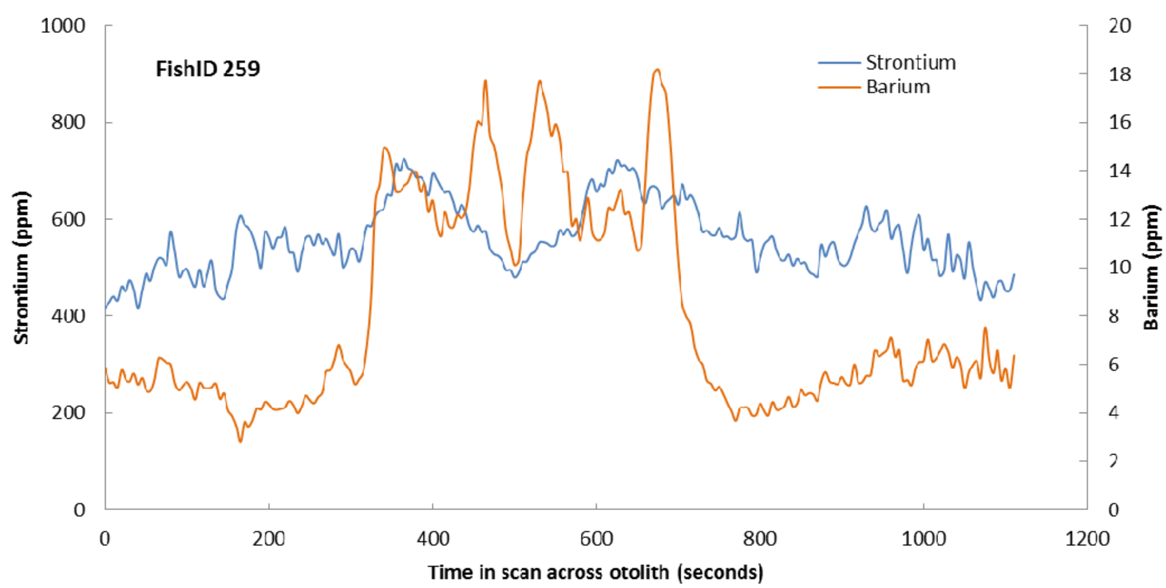

Figure B2. Example of raw laboratory data for elemental concentrations of strontium and barium for an adult Bull Trout.

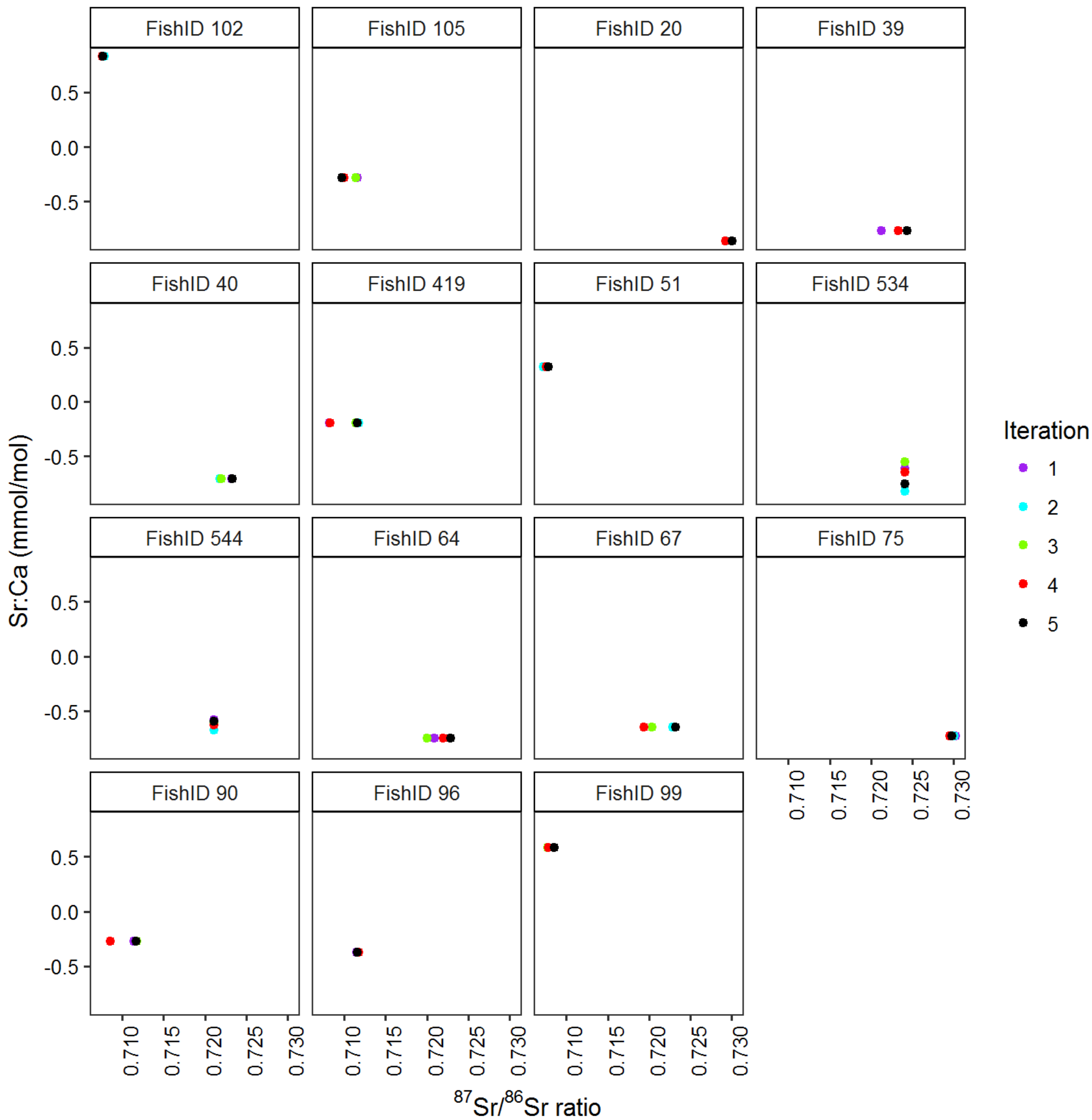

Figure B3. Imputed values of Sr:Ca and $^{87}Sr/^{86}Sr$ ratio for juvenile Bull Trout with missing data. Five iterations of the imputation method were conducted to produce five plausible values for each missing data point. Fish ID 534 and 544 were missing Sr:Ca and Ba:Ca values whereas all the other Fish IDs shown were missing $^{87}Sr/^{86}Sr$ ratio.

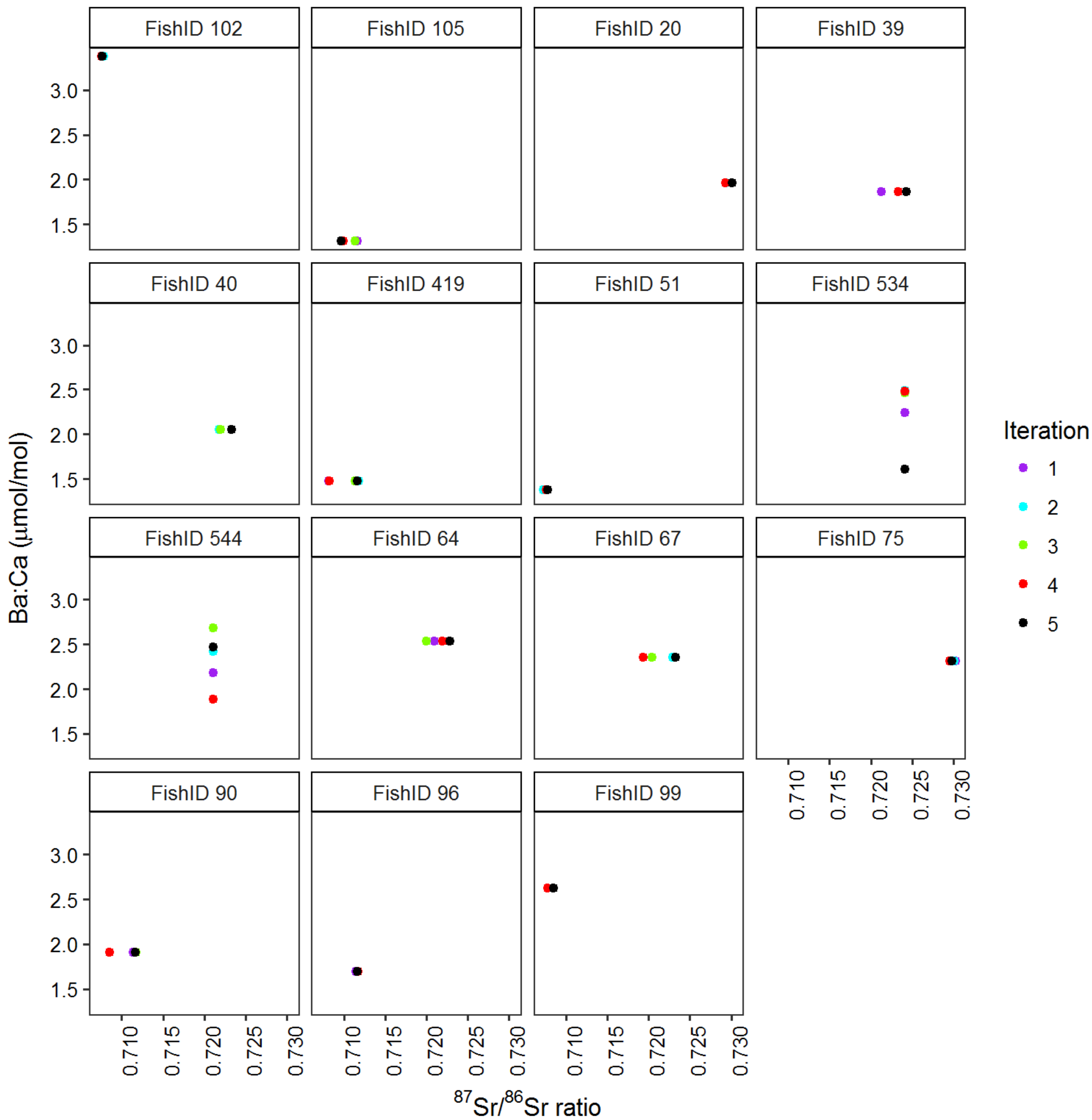

Figure B4. Imputed values of Ba:Ca and $^{87}Sr/^{86}Sr$ ratio for juvenile Bull Trout with missing data. Five iterations of the imputation method were conducted to produce five plausible values for each missing data point. Fish ID 534 and 544 were missing Sr:Ca and Ba:Ca values whereas all the other Fish IDs shown were missing $^{87}Sr/^{86}Sr$ ratio.

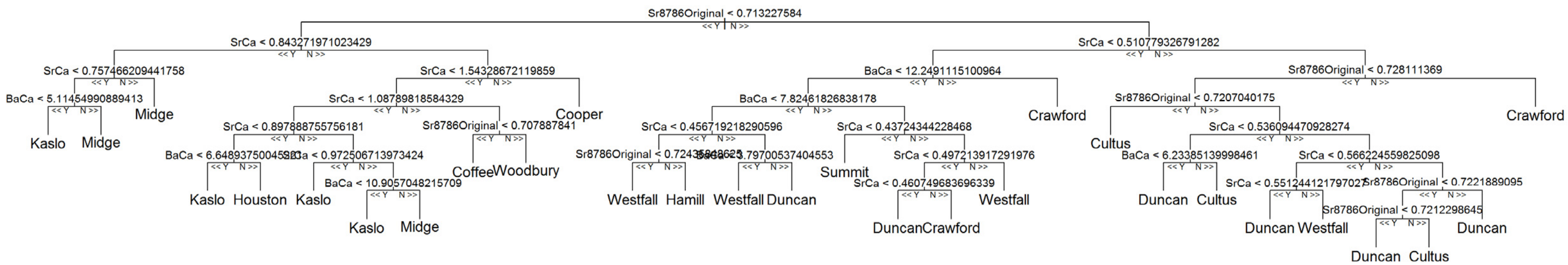

Figure B5. Example of a representative classification tree from the random forest analysis.

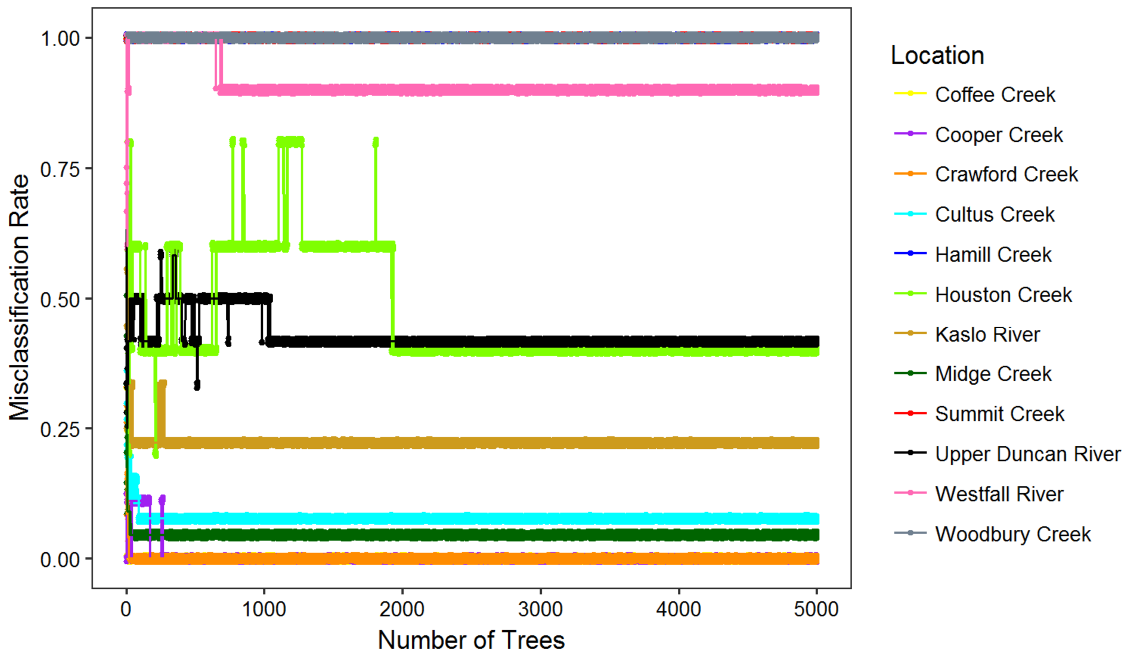

Figure B6. Misclassification rate by number of trees in the random forest analysis predicting juvenile Bull Trout using otolith microchemistry data.

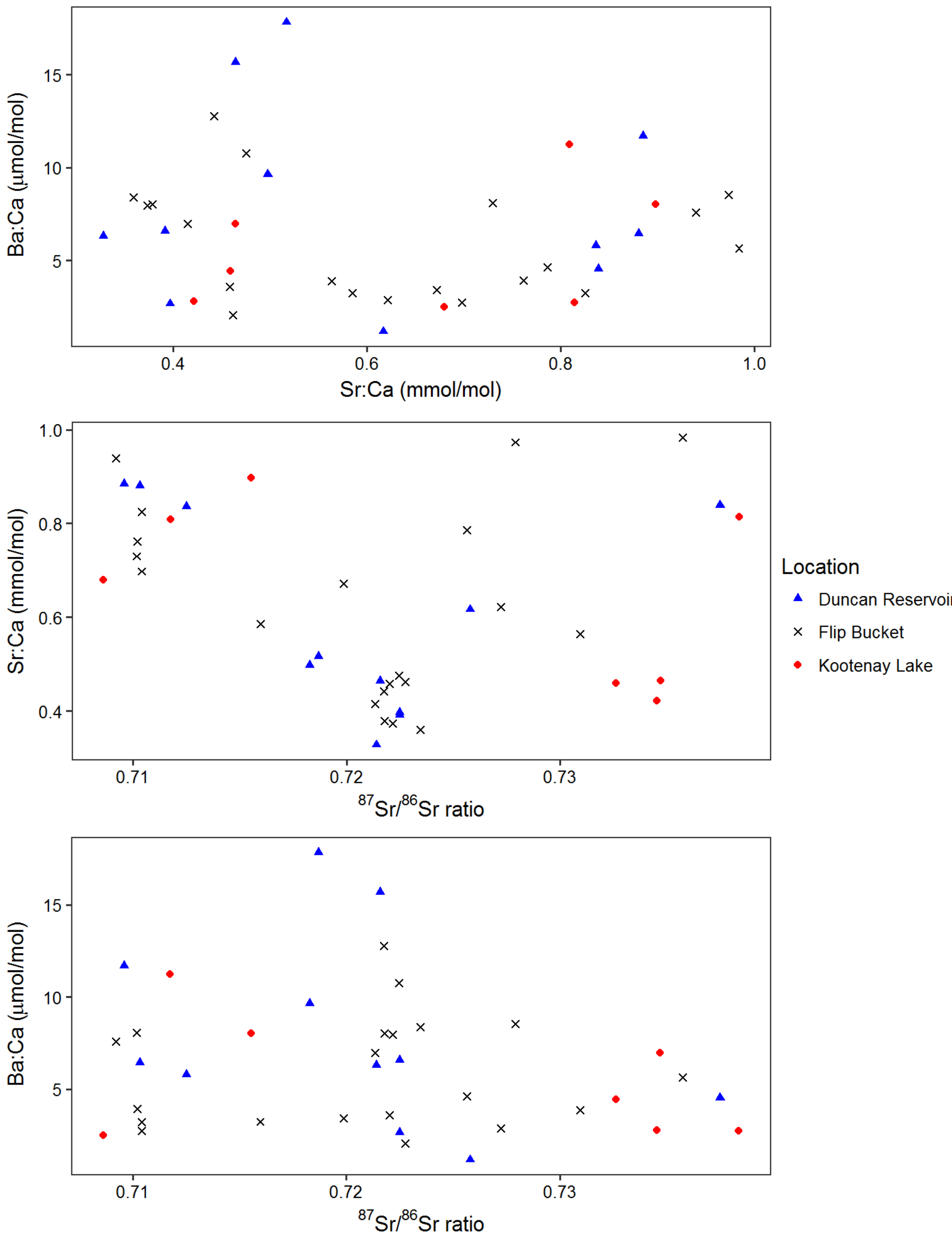

Figure B7. Untransformed values of otolith chemistry predictor variables from adult Bull Trout by capture location. These values correspond to the natal region of the otolith outside of the core.