Duncan Dam Project Water Use Plan

Upper Duncan Bull Trout Migration Monitoring

Reference: DDMMON-5

Year 4 Data Report (2013)

Study Period: 2012-2014

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Upper Duncan Bull Trout Migration Monitoring (DDMMON-5)

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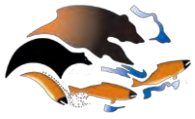

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Executive Summary

The Duncan Dam, operated by BC Hydro, is within the important Bull Trout (*Salvelinus confluentus*) habitat of the Upper Duncan River. To mitigate the effects of the dam in terms of migration for the species, the fish transfer program uses the Duncan Dam flip bucket to allow passage of adult Bull Trout from the lower Duncan River to the Duncan Reservoir upstream. Operation of the fish passage program at Duncan Dam presents operational, safety and fish stranding risks that need to be justified, and operations optimized to ensure the long term success of the program. The Upper Duncan River Bull Trout Migration Monitoring Program (DDMMON #5) is intended to assess the effectiveness of the transfer program and address the following key management question:

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

Otolith microchemistry was used to quantify differences in elemental signatures among Bull Trout spawning tributaries and develop a model to predict the natal origin of adult Bull Trout. The ratios of barium-138 to calciuim-43 (Ba:Ca) and strontium-86 to calcium-43 (Sr:Ca) were used to describe differences in water chemistry, juvenile otolith chemistry, and adult otolith chemistry. Linear regression was used to assess the relationship between Ba:Ca and Sr:Ca in juvenile Bull Trout otoliths and the streams where they were collected. Linear discriminant analysis was used to quantify how well juvenile otolith chemistry could be distinguished based on element ratios, and to develop a model to predict the natal tributaries of adult Bull Trout collected in Kootenay Lake, Duncan Reservoir, and the flip bucket. This report used data collected in 2008-2009 (Year 2), and 2012-2013 (Year 4). The key management question was addressed by using otolith microchemistry to predict the natal origins of adult Bull Trout captured in Kootenay Lake and in the flip bucket, and assessing the percentage of these fish that originated in Duncan Reservoir tributaries.

Sr:Ca and Ba:Ca ratios in water samples had distinct chemical signatures in most of the Duncan and Kootenay watershed tributaries assessed although there was overlap among a few streams (Hamill Creek, Upper Duncan River, and Westfall Creek). Regressions indicated a strong relationship between Ba:Ca and Sr:Ca ratios in water samples and juvenile Bull Trout otoliths in Years 2 and 4.

Analyses suggested that both juvenile and adult otolith chemistries differed between Year 2 and 4, which was likely related to the different laboratories used in these years. Therefore, statistical analyses were done separately for Years 2 and 4. In Year 2, juvenile Bull Trout otolith chemistry was distinct for most tributaries with some overlap among a few of the streams. Using cross-validated linear discriminant analysis, juvenile capture locations were correctly classified for 79% of individuals (Year 2 data). Most the variance (96%) was explained by Sr:Ca with a smaller amount (4%) explained by Ba:Ca. Based on the model using the otolith chemistry of juveniles to predict adult natal origin (Year 2 data only), 69% of adults captured in Kootenay Lake were classified to one of the three natal tributaries in the Duncan Watershed. Fifty percent of the adults captured in Duncan Reservoir and 78% of those captured in the flip bucket were predicted to be from the Duncan Watershed tributaries.

In Year 4, the juvenile Bull Trout otolith data showed some of the same trends (e.g. Poplar and Cooper creeks had the most distinct Ba:Ca and Sr:Ca ratios) but strong stream groupings were lacking and the within-stream variability in element ratios was much greater than in Year 2. This is consistent with the analysis of the same samples between the two labs for Sr:Ca, indicating higher variability in the lab that conducted the Year 4 analysis. Consequently, models using Year 4 data had poor ability to distinguish between natal areas. Linear discriminant analysis correctly classified the capture locations for 41% of the juvenile Bull Trout. Twelve percent of adults

captured in Kootenay Lake were classified as from one of the three tributaries in the Duncan Watershed. Of the adult Bull Trout captured in the flip bucket, 40% were predicted to be from Duncan Watershed tributaries and 60% were predicted to be from the Kootenay Watershed.

The large differences in otolith chemistry results in Years 2 and 4 limit the strength of conclusions that can be drawn relevant to the management questions. Data from Year 2 are thought to be more reliable than Year 4 and provided better classification accuracy for addressing the objectives. The results support the idea that tributaries in the Kootenay and Duncan watersheds have distinct water chemistry that result in distinct elemental ratios in the otoliths of Bull Trout. Although there are uncertainties regarding the proportion of adfluvial Bull Trout from Kootenay Lake that migrate past Duncan Dam to spawn in upstream tributaries, the otolith chemistry data suggest that a substantial portion (>40%) of the adults caught in the flip bucket were reared in tributaries upstream of Duncan Dam. The data available suggest the transfer program is likely important for the recruitment of Bull Trout in Kootenay Lake and Duncan Reservoir.

Recommendations for future study include the following:

- Consistency in the laboratory used for chemical analyses, and their instrumentation, methodology and data processing is crucial to refine models and obtain comparable data among years.

- For streams in which there was significant overlap in element ratios, investigating whether the ratio of ^{88}Sr:^{86}Sr and Li:Ca could help discriminate among streams is recommended. Existing otoliths that were not analyzed for Li (most Year 4 juveniles and adults) or ^{88}Sr (Year 2 data) could be rescanned to analyze for these elements and analyses of new otoliths should also include these isotopes.

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

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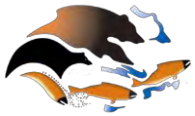

1. INTRODUCTION

The Duncan Dam, operated by BC Hydro, is within the important Bull Trout (*Salvelinus confluentus*) habitat of the Upper Duncan River. To mitigate the effects of the dam in terms of migration for the species, a fish transfer program is maintained to allow passage of adult Bull Trout from the lower Duncan River to the Duncan Reservoir upstream. In order to understand whether this transfer program is effective in allowing populations above and below the dam to contribute to one another, BC Hydro has undertaken the Upper Duncan River Bull Trout Migration Monitoring Program (DDMMON #5) with the consultation of the Duncan Dam Water Use Planning (DDM WUP) Consultative Committee (CC). By aiding in the understanding of the effects of dam operations on Bull Trout population dynamics, the results of this study will guide and direct future management action and Water Use Planning (WUP) processes in the region.

Within this past study period (Year 4 of DDMMON #5), the program continued to assess the effectiveness of the adult Bull Trout transfer program by using otolith microchemistry to determine natal origin of individual Bull Trout captured in the Kootenay Lake system. In particular, the study focused on determining the life history of fish as determined by different migration patterns and rearing location between the tributaries of the Duncan Reservoir, and Kootenay Lake watershed tributaries. In addition, juvenile rearing areas were assessed to determine if differences could be observed between rearing habitats in the Duncan Reservoir versus Kootenay Lake, with the goal to improve the ability to classify the dependence of adult migrating fish on both natal areas and rearing habitat upstream of the dam.

1.1 Project Background

The Duncan River is located within south eastern British Columbia and flows out of the Selkirk and Purcell mountains to the north before entering the northern arm of Kootenay Lake, northeast of the town of Nelson, BC (Figure 1). The Duncan Dam, constructed in 1967 as a part of the Columbia River Treaty process between the US and Canada, is situated on the Duncan River, 12km upstream of the inflow to Kootenay Lake. Duncan Dam is an earth fill and concrete structure 39.7 m high and 792 m long, with two low level outlets and a single spillway (DVH Consulting 2001). Flood control remains the sole purpose of Duncan Dam, and it has never been used for electrical power generation. By damming the Duncan River, the facility has created the Duncan Reservoir, storing up to 1.73 billion m^3 of water (Anon 1986).

For Bull Trout, the Duncan River is an important migration corridor for fish moving between Kootenay Lake and the tributaries of the Duncan River. By constructing Duncan Dam, this migration route was thought to be blocked (Peterson and Withler 1965), at least until Bull Trout were observed at the base of the dam in 1968 by the first senior dam operator (“Dutchie” Wageningen). Following this observation, flows were altered in the spring and summer to facilitate Bull Trout passage into the upstream Duncan Reservoir (O’Brien 1999). Subsequently, fish transfers have occurred annually from late spring (mid-May) to early fall (mid-September) (BC Hydro 2008).

Previous years of this monitoring study have provided a background dataset related to Bull Trout migration behaviour in the upper and lower Duncan systems. Annual

spawning movement of *adfluvial* Bull Trout has been shown to occur from Kootenay Lake to the upper Duncan River, tributaries of the lower Duncan River, the Lardeau River and Trout Lake (O'Brien 1999). As well, mark recapture studies have been conducted during Bull Trout transfers and suggest that spawning adults may pass upstream in later years (Ord *et al* 2000). This suggests that some adults may migrate though Duncan Dam, go back downstream, and migrate back up through the dam to the Duncan Reservoir in later years. Operation of the fish passage program at Duncan Dam presents operational, safety and fish stranding risks that need to be justified, and operations optimized to ensure the long term success of the program. The weir installation poses safety risks for dam operators due to the need for working in water and operations need to be managed during installation. Furthermore, passage operations require up to 24 hours of no flow from the dam, which can result in stranding particularly when Lardeau River flows are too low to backwater the tailrace and downstream Duncan River habitats. Further study regarding the optimization of fish passage operations is being conducted in DDMMON#6.

By testing water and otolith microchemistry, it is possible to more accurately discern population origin and life history differences between groups of fish. This effective technique has been used in the Arrow Lakes Reservoir while studying early rearing life histories and out-migration timing of Bull Trout (Clarke and Telmer 2008). Other research has also demonstrated that strontium (Sr) and barium (Ba) to calcium (Ca) ratios in otoliths are proportionately related to ambient water by use of an incorporation coefficient (Clarke *et al*. 2007; Wells *et al.* 2003). This research shows that the methods used in completing this project may aid in the overall accuracy and efficacy of this work.

Figure 1 Study Area Overview.

1.1. Project Objectives

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

In addition to the specific Request for Proposal objectives the following were completed:

1) Quantify the seasonal, inter-annual and spatial variation in selected stream chemistries;
2) Determine the association of otolith and fin ray chemistries of juvenile Bull Trout with those of their natal streams; and,
3) Evaluate the potential application of otolith/fin ray chemistry for describing Bull Trout movements throughout the Duncan River/Kootenay Lake basin.

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

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

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

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

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

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

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

The program has been designed to test two hypotheses that have been proposed to test the validity of analytical methodology used in this study to facilitate answering the previously defined management questions. In addition, the second hypothesis was proposed to determine whether recruitment is disproportionate between systems, as the program is not designed to identify all of the contributing factors related to Bull Trout recruitment variability in the Kootenay and Duncan systems (BC Hydro 2008). The hypotheses in the RFP terms of reference are as follows:

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

The null hypotheses were interpreted with the following clarifications:

H01*:* As the Kootenay watershed includes the Duncan watershed, for the purposes of this study we define the Kootenay watershed as all adfluvial Bull Trout spawning and rearing areas except those areas above Duncan Dam. In addition to stream chemistries, the water chemistry and otolith microchemistry associated with Duncan Reservoir is of equal importance in determining watershed of origin of Bull Trout that have natal areas above Duncan Dam.

H02*:* Interpreted as follows: "The proportion of Bull Trout that spawn above Duncan Dam has the same proportion of Bull Trout that originated from the natal tributaries above Duncan Dam when compared to a similar proportion from fish that spawn in other areas in the Kootenay basin."

To falsify this hypothesis, it is necessary to find statistical differences between: 1) spawning fish that are in their natal area as a ratio with those that are spawning in non-natal areas that are moving through or that are above Duncan Dam, compared with 2) the spawning populations using natal areas as a ratio with those using non-natal areas that are found spawning in other tributaries below Duncan Dam.

As adult Bull Trout were collected from two areas, Kootenay Lake and the flip bucket at Duncan Dam, some assumptions were required to address the specific hypotheses. First, Bull Trout collected from Kootenay Lake are assumed to be a random sample of adfluvial Bull Trout from the combined Kootenay and Duncan watersheds. This assumes that Kootenay watershed adfluvial life history Bull Trout reared above Duncan Dam, all migrate through Duncan Dam. Bull Trout that rear only above Duncan Dam, whether in tributaries or the reservoir, are not of interest as they do not migrate through Duncan Dam and are not influenced by passage operations. Second, the samples from the flip bucket are assumed to be migrants that will spawn above Duncan Dam and are adfluvial Bull Trout.

For the program outlined to be successful, it was necessary to identify, with low levels of uncertainty, the natal area of fish caught in the flip bucket at Duncan Dam as originating from either above or below the dam. This was completed by determining if post-natal otolith microchemistry can only be associated with tributaries above Duncan Dam and/or

the post-natal chemistry provides indications of passage through or rearing in Duncan Reservoir. This study was originally planned to use these combined data to improve the probability of excluding fish that reared in other tributaries to Kootenay Lake.

This report includes a summary of the water chemistry collected from each of the tributaries and a summary of the otolith micro-chemistry from juvenile fish collected from these tributaries, and adult fish from Duncan Dam flip bucket and Kootenay Lake.

1.2. Study Area

The study area covers an approximate distance of 150 km from the northern end of the upper Duncan River to central Kootenay Lake (Crawford Creek) (Figure 1). Three of the tributaries were identified in the terms of reference (Houston Creek, upper Duncan River, and Westfall River) and were considered the upper Duncan sites. Poplar Creek was chosen to represent the Lardeau system, two more sites were taken from the lower Duncan River (Hamill Creek and Cooper Creek) (Figure 2), three were taken from the north arm of Kootenay Lake (Kaslo River, Woodbury Creek and Coffee Creek), and two sites were taken from central/south Kootenay Lake (Crawford Creek and Midge Creek) (Figure 3). All sites have been sampled previously, with the exception of Midge Creek. Water samples were collected from each location where juvenile Bull Trout were collected.

Figure 2: Duncan System Sample Locations.

Figure 3: Kootenay Lake Sample Locations.

1.3.1 Sample Sites

All sites are identified from the furthest north and move south (Table A1, Appendix A). Photographs of all sample sites are in Appendix B.

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.

2. METHODS

2.1 Field Methods

Sampling Schedule

This report uses data collected in 2008, 2009, 2012, and 2013. The years when water, juvenile otolith, and adult otolith samples were collected are shown in Table 1. In this report, data collected in 2008 and 2009 initially reported in Golder (2010) are grouped and referred to as “Year 2” of the monitoring program because the complete suite of tributaries was sampled over two years (2008-2009). Similarly, data collected in 2012 and 2013 are grouped and referred to as “Year 4”. Laboratory analyses were conducted separately for Reporting Year 2 (hereafter “Year 2”) and Reporting Year 4 (hereafter “Year 4”) data, so these year groupings are appropriate for analysis.

Table 1. Years that water, juvenile, and adult data were collected during the monitoring program.

Data Type	Reporting Year 2[a]	Reporting Year 4[a]
Water	2008, 2009	2013
Juvenile otoliths	2008, 2009	2013
Adult otoliths	2008	2012, 2013

a. Referred to as "Year 2" and "Year 4" in this report

Water Sampling

All samples were collected in sterilized 125 mL high-density polyethylene (HDPE) narrow mouth bottles. Once in the field, samples were labelled with the following:

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

Samples were taken facing upstream with 60 mL filtering syringes equipped with 0.45 µm filters. Filtering syringes were conditioned by triple rinsing, and then the sample bottles were conditioned using the same method but using filtered stream water prior to filling the sample bottle to the neck. Two samples were taken from each tributary, which were treated with vials of 0.5 mL HNO_3 (once added to sample this equaled 0.4% HNO_3 in each sample) for trace metals analysis.

Once the samples were collected, they were stored in a cooler with ice packs for transport along with two field blanks of deionized water (one preserved and one not). At the end of each day, samples were refrigerated until ready to be shipped to the lab. Samples were shipped in coolers with ice packs in an effort to keep the samples as cool as possible for transport.

Juvenile Fish Collection

Juvenile fish were collected using a Smith Root type 12 backpack electro-fisher. All collection activities were carried out as per the Resource Inventory Standards Committee (RISC, 1997) standards and methods for fish collection. Once the fish had been collected from the sample site, they were euthanized using diluted clove oil. Once mortality was confirmed, fish were measured for length (mm) and weight (g). All fish were stored according to tributary, and frozen for transport to the lab where otoliths were subsequently extracted.

Otolith Collection - Adult Fish

Transfer Station Sampling

The weekly visits that coincided with the transfer schedule provided by BC Hydro enabled the collection of adult Bull Trout from the flip bucket. The collection permit allowed for a maximum of 30 adult Bull Trout to be collected each year (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 Ministry of Environment. The fish collected had meristic data recorded (weight, fork length, gender and other information as requested) and the heads were collected. Each head was stored in a plastic bag in a cooler and then frozen until the otoliths were removed. Otolith removals were conducted in the lab after sufficient thawing.

Recreational Bull Trout Fishery Collection Program

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

2.2 Laboratory Methods

Water Chemistry Analysis

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

Otolith Preparation and Analysis

In Year 4 of the study, sagittal otoliths were collected from juvenile bull trout (n = 143) in the upper Duncan River, Houston Creek, Westfall River, Hamill Creek, Poplar Creek, Cooper Creek, Kaslo River, Woodbury Creek, Coffee Creek, Crawford Creek, and Midge Creek. Sagittal otoliths were collected from adult bull trout collected from the Duncan Dam flip bucket (n=48) and from Kootenay Lake (n=33). No heads were returned from the Duncan Reservoir during the head collection program. Individual otoliths were stored in microcentrifuge tubes until processing.

Due to the unavailability of the British Columbia laboratory previously used in this study, a different laboratory in Australia was used for the laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) of the otoliths collected in 2012 and 2013. The

Australian laboratory also processed and analyzed samples that were collected in 2008 but not previously analyzed. Analysis on a subsample of the previously analyzed otoliths was also conducted for quality control and assurance purposes. The lab used in Year 4 was provided the methodology used in Year 2, and attempted to follow those protocols as closely as possible. However, the methods for the preparation and the elemental analysis were slightly different between the two laboratories. Both methods are outlined below.

School of Earth and Ocean Sciences, University of Victoria (Year 2 Laboratory)

The otoliths were extracted and provided to the laboratory. After extraction and initial preparation in epoxy, the otoliths, along with the epoxy covering, were scored with a scalpel and sectioned using a Buehler isomet saw. Secondary epoxy embedding was accompanied by placing the sectioned otolith into acrylic tubing where more epoxy was added to secure the otolith. The otolith core was exposed by polishing with adhesive-backed lapping paper in 320, 600, and 1200 grit sizes (Buehler Carbimet). To achieve a highly polished surface, otoliths were moistened with 0.25 µm Metadi Supreme diamond suspension spray (Buehler mfg.) and polished with 2500 Texmet grit pads (Buehler mfg.). Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) was accomplished at the School of Earth and Ocean Sciences, University of Victoria, using the UP-213 Laser Ablation System (New Wave Research) attached to an X Series II ICP-MS (Thermo Electron Corporation). Concentrations of Strontium (^{86}Sr), Barium (^{138}Ba), Manganese (^{55}Mn), Calcium (^{43}Ca), Magnesium (^{24}Mg), Lithium (^{7}Li) and Zinc (^{66}Zn) were identified in a transect line across the diameter of the otoliths reported in illustrations as time in seconds. Transects ran from the outer edge of the otolith through the core to the opposite edge in a straight line. Prior to scanning, background data was collected for 20 seconds to separate the background signal from otolith elemental chemistry. Finally, PlasmaLab (version 2.5.3.280, Thermo Electron 2003) software was used for data collection and reduction. Operating parameters of LA-ICP-MAS and data filtering are described in Sanborn and Telmer (2003).

Adelaide Microscopy, University of Adelaide (Year 4 Laboratory)

Otoliths were extracted and provided to the laboratory. After arrival at the laboratory, one whole otolith per fish was either mounted onto a microscope slide with a clear setting epoxy resin (Struers) spiked with indium chloride (approximately 30 $\mu g \cdot g^{-1}$) as a resin indicator, or embedded in indium spiked resin before sectioning using a low speed saw. For otoliths mounted onto a microscope slide, they were then polished with progressively finer grades of wet lapping film to expose the otolith nucleus. Embedded otoliths were polished on each side and mounted onto a slide with indium spiked epoxy. Slides were then cleaned in ultrapure water and air dried for 24-h under a laminar flow hood.

Otolith element analysis was performed using a laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) system housed at Adelaide Microscopy, University of Adelaide. The LA-ICP-MS system consisted of a New Wave UP-213 high-performance ultraviolet laser connected to an Agilent 7500cs ICP-MS (see Table 2 for operating conditions). The mounted otoliths were introduced into a sealed ablation chamber with a helium atmosphere (0.82 $l \cdot min^{-1}$). Laser ablation runs were run in time resolved mode and traced a transect path across the diameter of the otoliths. All ablation runs were preceded by a pre-ablated run in order to score the transect path. Pre-ablation

laser settings were: 40 µm laser beam diameter, at a pulse rate of 4 Hz and a scan speed of 30 µm·s^{-1}. Ablation runs followed the pre-ablation path, with a laser setting reconfigured to: 30 µm laser beam diameter, at a pulse rate of 5 Hz and a scan speed of 3 µm·s^{-1}. Prior to scanning, background data was collected for 30 seconds to separate the background signal from otolith elemental chemistry. Transects for ablation runs for juvenile otoliths ran from the left edge of the otolith through the core to the left edge. Transects for adult otoliths, which were much larger, started at the outside edge of the otolith on the sulcus side, ran to the core (center) of the otolith, then turned and ran back to the other edge at an angle of approximately 30-100° from the first transect line.

The elemental isotopes chosen for analysis were: Magnesium (^{24}Mg and ^{25}Mg); Manganese (^{55}Mn); Zinc (^{64}Zn and ^{66}Zn); Strontium (^{86}Sr and ^{88}Sr); Indium (^{115}In); Barium (^{137}Ba and ^{138}Ba); and Calcium (^{43}Ca) which was used as an internal standard. All trace element concentrations were ratioed to ^{43}Ca. A reference standard (National Institute of Standards and Technology: NIST 612) was analyzed after every tenth otolith ablation to correct for machine drift. Elemental concentrations in parts per million (ppm) were determined using GLITTER v5·3 (www.glitter-gemoc.com). All data processing was performed using Excel (Microsoft).

Table 2. Details of the operating parameters of the New Wave Nd Yag 213 nm UV laser and Agilent 7500cs inductively coupled plasma mass spectrometer (ICP-MS) used to analyze sectioned otoliths.

Laser		
	Wavelength	213 nm
	Mode	Q-switch
	Frequency	5 Hz
	Laser power	75%
	Carrier gas	Ar (0.90 - 0.95 l·min^{-1})
	Beam energy	0.6 – 0.7 mJ
	Beam density	7 – 8 J·cm^{-2}
	Spot size	30 µm
ICP-MS		
	Optional gas	He (60.0%)
	Cone	Pt
	Detection modes	Pulse and analogue
	Dwell Times	^{24}Mg, ^{25}Mg, ^{55}Mn, ^{64}Zn, ^{66}Zn, ^{86}Sr, ^{88}Sr, ^{137}Ba & ^{138}Ba (300 ms); ^{43}Ca (100 ms); and ^{115}In (50 ms)

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 rearing in the natal stream during the first summer of growth. For age-0 juveniles sampled in their natal stream, the entire otolith outside the core represents the chemistry of the natal stream. For adults, the portion representing the natal tributary was immediately outside the core but inside of the region corresponding to when juveniles migrated downstream to larger tributaries the lakes or reservoirs.

Profile data of the LA-ICP-MS scans were graphed and provided by the laboratories, showing the element concentrations on the vertical axis and elapsed time during the scan on the horizontal axis. The core was identified by an abrupt change in element concentrations (strontium and barium) on each side of the core. For adults, the outmigration of juveniles from the natal stream was identified as an abrupt change in elemental concentrations. The values corresponding to the natal rearing area were extracted from the profile scans in spreadsheet software and averaged. Mean values of elements for the natal area were the unit of analysis for both juveniles and adults.

For adults, otolith chemistry during the lake residency period was also of interest, to assess whether the elemental signatures would differ among capture locations. Relatively stable element concentrations near the outer edge of the otolith, as indicated by flat lines on the graphs, were selected to represent chemistry at the capture location for each adult from Year 4. These values were averaged for each fish for the analysis of adult otolith chemistry at the capture location. Analysis of the outer edge of otoliths collected from adults in Year 2 was not conducted.

In Years 2 and 4 of the study, a portion of juvenile and adult Bull Trout showed sudden changes to extremely low concentrations of Sr and Ba in parts of the otolith. Review of the literature concerning otolith chemistry suggested that these sections likely reflect layers deposited as a different crystalline form of calcium carbonate. Teleost fish otoliths are typically composed primarily of the 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 areas of interest (natal origin or adult capture location) were not included in the analyses.

Bull Trout Ageing

Otoliths were used to age bull trout as per methods developed at the Pacific Biological Station Ageing Unit (DFO) (D. Gillespie, DFO pers.comm. April 2008). The otoliths were aged using images. Annual growth in otolith sections consisted of an adjacent pair of hyaline (translucent in transmitted light; associated with diminished growth during winter and counted as the annulus) and opaque (dark; rapid summer growth) bands (DeVries & Frie 1996). The otolith sample was read by two different readers and if the two ages differed for a fish, the sample was read a third time and assigned a final.

In addition, the laser scan of the element Zinc (^{64}Zn) was examined for a subsample of otoliths and fin rays to determine if variations in density reflect rates of incorporation corresponding to seasons. These data and the supplementary visual inspections provided age data that corresponded to changing trace metal signatures to recreate life histories stages associated with environmental changes in trace metal water chemistry.

2.3 Statistical Analysis

All analyses used ^{86}Sr (hereafter “Sr”) and ^{138}Ba (hereafter “Ba”) to describe and compare the chemistry of water and otoliths samples. Other metals (lithium,^{7}Li, and manganese,^{55}Mn) were considered but exploratory analyses indicated that these

elements did not significantly improve discrimination among streams (see Results) so they were not used in the final analysis.

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

The relationship between elemental ratios of water samples and juvenile otoliths was described using linear regression. A separate regression was conducted for Year 2 and Year 4. The units of observation for water samples were mean values from fall sampling. The units of observation for juvenile otoliths were individual fish, where values were means of multiple scanned segments from the portion of the otolith representing the natal area.

Multivariate analysis of variance (MANOVA) was used to test for differences in water chemistry among capture locations. The two response variables were Sr:Ca ratio and Ba:Ca ratio. Separate models were used to assess differences among capture locations for juvenile and adult Bull Trout. Otoliths chemistry was analyzed at a different lab in Year 4 than in Year 2. As a control quality check to ensure results from the two labs were directly comparable, Sr:Ca and Ba:Ca were compared using a block design MANOVA where laboratory was the fixed effect and capture location (stream) was the block effect, using all of the Year 2 and Year 4 data. As an additional quality control check, a subsample (n=35) of the juvenile Bull Trout samples that were analyzed in Year 2 were re-analyzed by the new lab in Year 4, using the second sagittal otoliths from these fish. Paired samples from individual fish analyzed at both labs were compared for Sr:Ca and Ba:Ca ratios using a block design MANOVA where laboratory was the fixed effect and the individual fish was the block effect. If MANOVAs suggested significant differences, ANOVAs were conducted to test which of the two response variables differed. For all analyses, effects were considered significant if the p-value was less than 0.05.

Linear discriminant analysis (LDA) was used to describe differences in otolith chemistry among capture locations and develop a predictive model of the natal area of Bull Trout. Leave-one-out classification was used to cross-validate models. Split-sample LDA was used to predict the natal origins of adult Bull Trout based on the LDA model of juvenile Bull Trout of known origin. First, the analysis was conducted using only Year 2 juvenile and adult data, as was done in the previous Golder (2010) report. Because MANOVA and graphical assessment indicated that juvenile otolith chemistry data from Year 2 and Year 4 were different (see Results), these data sets could not be combined in the predictive model. A separate set of LDAs (unvalidated, leave-one-out cross-validated, and split sample) was conducted for juvenile and adult Bull Trout otoliths collected and analyzed in Year 4. In addition, the model using Year 2 juveniles was used to predict the natal origin of adults captured in Year 4 using split-sample LDA. The natural logarithms of Ba:Ca and Sr:Ca were used in MANOVA and linear discriminant analyses to better meet assumptions of normality. All analyses were conducted using R version 3.0.2 (R Core Team 2013) and LDA was conducted using the MASS package (Venables and Ripley 2002).

3. RESULTS

3.1 Water Chemistry

Ratios of Ba:Ca and Sr:Ca varied among tributaries, especially for Poplar and Cooper creeks, which had much higher values of Sr:Ca and Ba:Ca than the other tributaries in all three years of sampling (Figure 4). Ba:Ca ratios were considerably lower at Crawford Creek than at Poplar and Cooper Creeks, but they were consistently higher than at the remaining sites for all three years of sampling (Figure 4). Water chemistry was similar for Hamill, Houston, Upper Duncan, and Westfall creeks. Samples from Woodbury, Kaslo, and Coffee creeks consistently had the lowest Ba:Ca ratios (Figure 4). The Ba:Ca and Sr:Ca ratios were not different among sampling years (MANOVA; P=0.98; Figure 4). Water samples from Houston Creek, Upper Duncan River and Westfall River were not analyzed for ultra-low trace metals therefore were not used for 2013 analysis. Average elemental ratios (mmol/mol) measured by ICP-MS for the systems examined are provided in Appendix D. The values are provided as an element:Ca ratio.

Lithium (^{7}Li) was also plotted against the Sr:Ca ratio to assess whether it differed among tributaries and might be used to improve discrimination among tributaries that had similar ratios of Ba:Ca and Sr:Ca (Figure E1, Appendix E). In particular, it was explored to determine whether lithium could be used to distinguish between Hamill and Crawford creeks, which are Kootenay tributaries, and Houston Creek, Westfall Creek, Upper River Duncan River, which are in the Duncan watershed. Houston Creek had a greater Li:Ca ratio than all other streams. However, Li:Ca did not help discriminate between Hamill and Crawford creeks, and the Duncan tributaries (Westfall and Upper Duncan). Similar exploratory graphical analysis was conducted for manganese (^{55}Mn) but this element did not help discriminate among tributaries and consequently was not used in analyses.

Raw water chemistry data from 2008 to 2013 are provided in Attachment A.

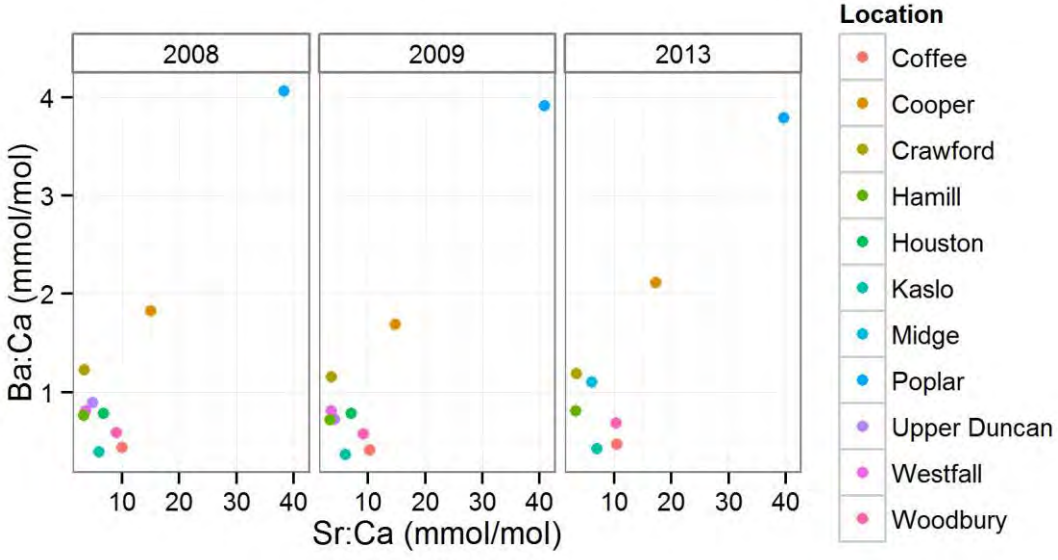

Figure 4. Water chemistry ratio for each tributary by year. Values are means from fall sampling dates.

3.2 *Juvenile Otolith Chemistry*

The relationship between the Ba:Ca ratio in juvenile Bull Trout otoliths and water samples from their capture location was significant in Year 2 (both $P<0.001$; Figure 5). The regression explained a similar amount of variation in otolith Ba:Ca in Year 2 ($y = 5.2x + 5.8$; $r^2 = 0.51$) and 2013 ($y = 6.7x + 3.1$; $r^2 = 0.54$). The otolith Ba:Ca values from Cooper Creek in Year 2 were much larger than predicted by the regression but this was not observed in Year 4 (Figure 5).

There was a strong relationship between Sr:Ca ratio in juvenile Bull Trout otoliths and water samples in Year 2 and Year 4 (both $P<0.001$; Figure 6). The regression explained a greater amount of the variation in otolith Sr:Ca values in Year 2 ($y = 0.12x + 0.11$; $r^2 = 0.98$) than in Year 4 ($y = 0.14x + 0.32$; $r^2 = 0.73$) because of greater variability in otolith values in Year 4 (Figure 6).

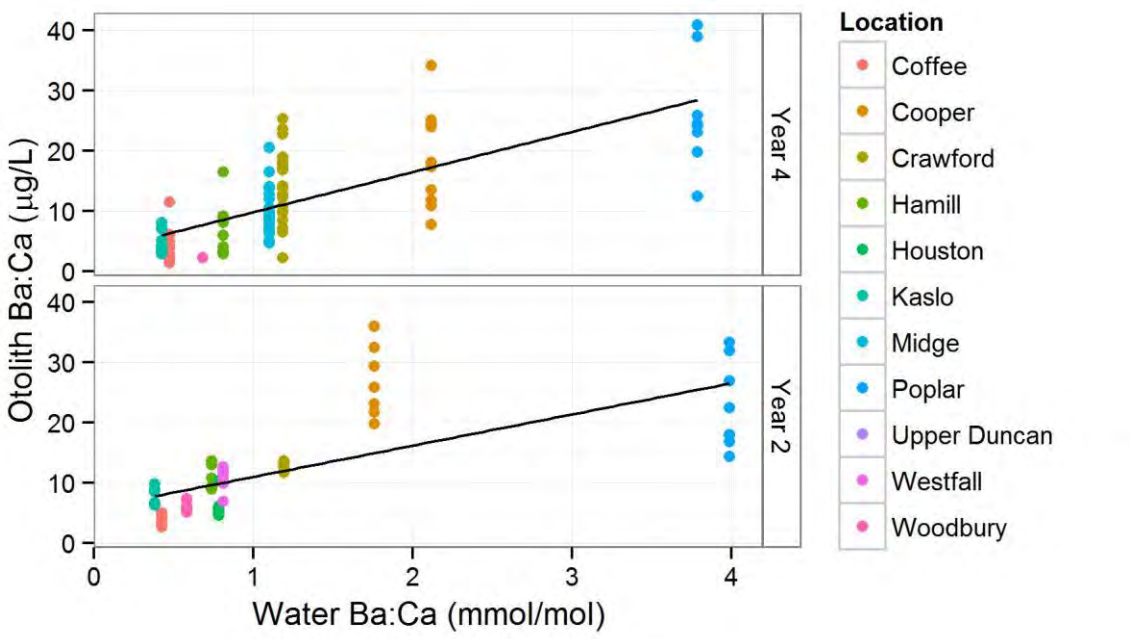

Figure 5. Relationship between the Ba:Ca ratio in juvenile Bull Trout otoliths and water samples by year.

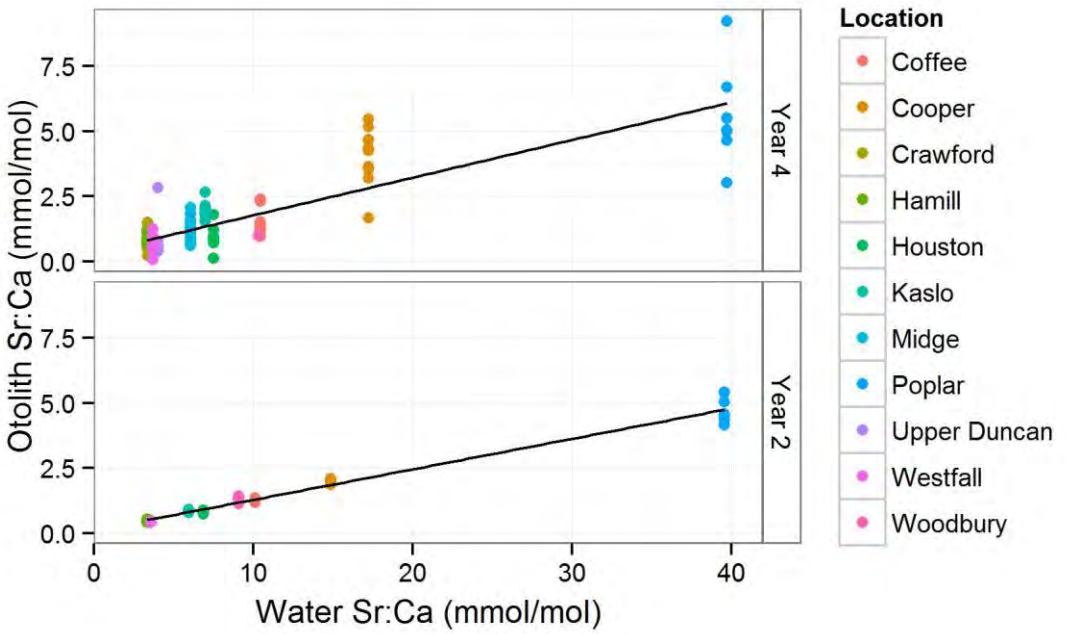

Figure 6. Relationship between the Sr:Ca ratio in juvenile Bull Trout otoliths and water samples by year.

In Year 2, juvenile Bull Trout otolith chemistry data were fairly well grouped by capture location, with Cooper and Poplar having the most distinct chemistry in terms of Sr:Ca and Ba:Ca (Figure 7). There was overlap between some of the tributaries, especially for Hamill, Houston, and Westfall Creeks, as well as the Upper Duncan River. In Year 4, there was much more within stream variation in otolith chemistry and strong stream groupings were lacking. Some of the trends observed in Year 2 were also observed in Year 4, including higher Sr:Ca in Poplar and Cooper creeks, and greater Ba:Ca in Crawford Creek compared to most others tributaries, but there was much more variation in ratios within each stream. MANOVA indicated that juvenile Bull Trout otolith chemistry ratios differed significantly between Year 2 and Year 4 (P=0.001; Figure 7) while accounting for stream to stream variation as a block effect. Block design ANOVA indicated that the Sr:Ca ratio was significantly greater (P=0.03) and the Ba:Ca ratio was significantly lower (P=0.02) in Year 4 than in Year 2. These analyses compared fish sampled in Year 2 (collected in 2008 and 2009 and analyzed in Victoria, BC, Canada) to those sampled in Year 4 (collected in 2013 and analyzed in Adelaide, Australia).

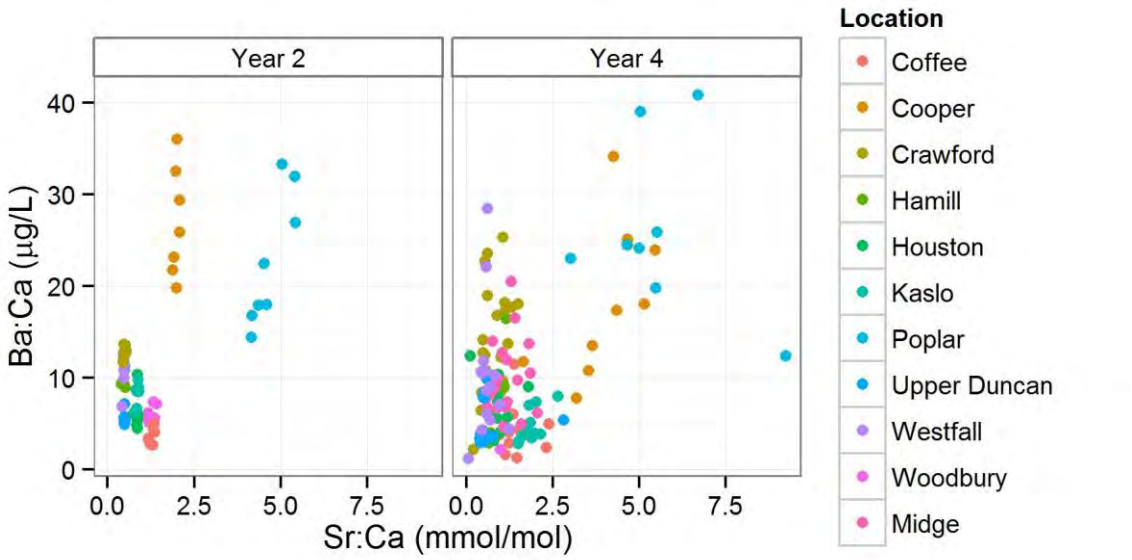

Figure 7. Natal otolith chemistry of juvenile Bull Trout by capture location.

The otolith chemistry of juvenile Bull Trout captured in Year 2 and analyzed at both laboratories was also compared (Figure 8). The Ba:Ca and Sr:Ca ratios in juvenile Bull Trout otoliths differed significantly by laboratory while accounting for the repeated measures on individual fish as a blocking effect (MANOVA; $P<0.001$). Both Ba:Ca and Sr:Ca were greater in the laboratory used in Year 2 than the one used in Year 4 ($P<0.001$).

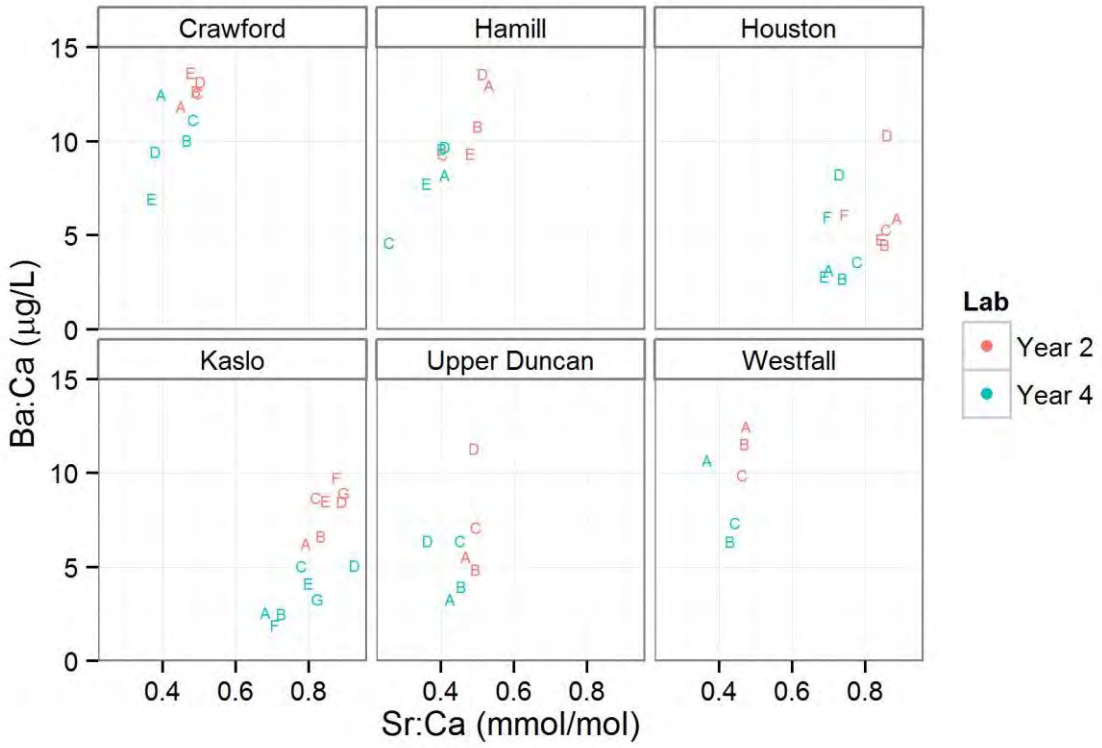

Figure 8. Comparison of otolith chemistry for juvenile Bull Trout captured in Year 2 and analyzed at two different labs. Letters show samples from the same fish.

The MANOVA results discussed above indicate differences in juvenile otolith chemistry between Year 2 and Year 4 and that these differences were related to the laboratories used, not changes in chemistry among years. For this reason, juvenile Bull Trout otolith chemistry data from Year 2 and Year 4 were not combined for linear discriminant analyses.

For juvenile Bull Trout captured in Year 2, otolith Ba:Ca and Sr:Ca ratios differed significantly among capture locations (MANOVA; $P<0.001$). Linear discriminant analysis correctly classified the capture locations for 84% of individual fish, based on the unvalidated model (Table 3; Figure 9). The coefficients of discriminants indicated that the first discriminating function (LD1) was most related to Sr:Ca and the second discriminating function (LD2) was more related to Ba:Ca. Most of the between-group variance was explained by LD1 (95%) with a smaller percentage explained by LD2 (5%). Based on linear discriminant scores, Cooper and Poplar were the most distinct, whereas there was the greatest overlap was between Hamill, Houston, and Westfall creeks, and Upper Duncan River (Figure 9). Using the leave-one-out cross-validated LDA, juvenile capture locations were correctly classified in 79% of cases (Table 3). Classification tables showing the predictions of LDA models by capture location are provided in Table E1 (Appendix E).

Table 3. Percentage of correct classification of capture location of juvenile Bull Trout captured in Year 2 (2008-2009), based on linear discriminant analysis.

Capture Location	Unvalidated Model	Cross-Validated Model
Coffee	100%	100%
Cooper	100%	100%
Crawford	57%	57%
Hamill	67%	50%
Houston	71%	71%
Kaslo	71%	71%
Poplar	100%	100%
Upper Duncan	86%	86%
Westfall	86%	57%
Woodbury	100%	86%
Total	**84%**	**79%**

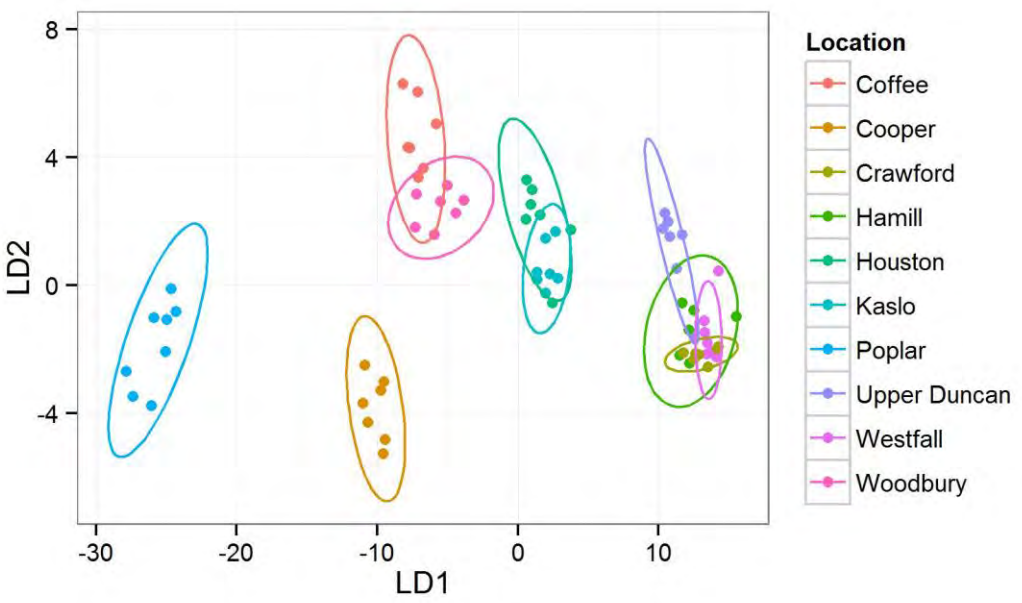

Figure 9. Discriminant scores for natal otolith chemistry of juvenile Bull Trout captured in tributaries of Duncan Reservoir and Kootenay Lake in Year 2 (2008-2009). Ellipses show 95% confidence intervals of the discriminant scores.

For juvenile Bull Trout in Year 4, otolith Ba:Ca and Sr:Ca ratios differed significantly among capture locations (MANOVA: $P<0.001$). Both Ba:Ca and Sr:Ca differed among capture locations (ANOVA; both $P<0.001$).

Models using the Year 4 juvenile Bull Trout otolith data had poor ability to distinguish between natal areas (Figure 10). Linear discriminant analysis correctly classified the capture locations for 44% of the juvenile Bull Trout, based on the unvalidated model (Table 4). The coefficients of discriminants indicated that the first discriminating function (LD1) was most related to Sr:Ca and the second discriminating function (LD2) was more related to Ba:Ca. Most of the between-group variance was explained by LD1 (69%) with a smaller percentage explained by LD2 (31%). As in the Year 2 analysis, Cooper and Poplar creeks were the most distinct from other creeks based on discriminant scores. Using the leave-one-out validated LDA, juvenile capture locations were correctly classified in 41% of cases. Classification tables showing the predictions of LDA models by capture location are provided in Table E2 (Appendix E).

Table 4. Percentage of correct classification of capture location of juvenile Bull Trout captured in Year 4 (2013), based on linear discriminant analysis.

Capture Location	Unvalidated Model	Cross-Validated Model
Coffee	50%	50%
Cooper	50%	40%
Crawford	59%	55%
Hamill	0%	0%
Houston	0%	0%
Kaslo	83%	75%
Midge	55%	55%

Poplar	63%	50%
Upper Duncan	62%	62%
Westfall	0%	0%
Woodbury	0%	n/a*
Total	**44%**	**41%**

* only one juvenile otolith sample was available so a leave-one-out cross-validation was not possible for Woodbury Creek.

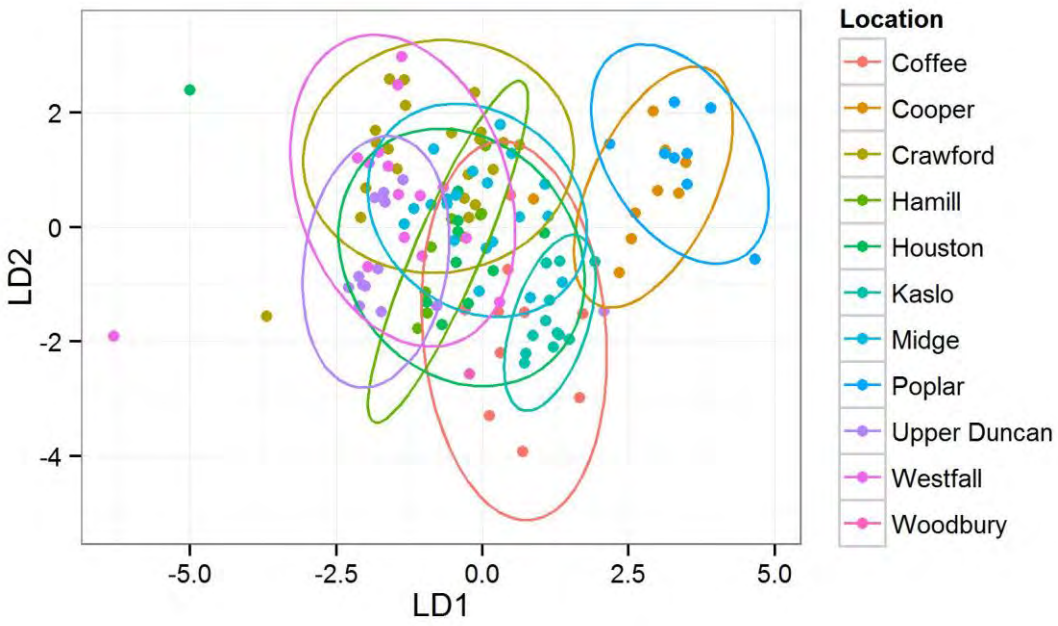

Figure 10. Discriminant scores for natal otolith chemistry of juvenile Bull Trout captured in tributaries of Duncan Reservoir and Kootenay Lake in Year 4 (2013). Ellipses show 95% confidence intervals of the discriminant scores.

Overall, the LDA models indicate better ability to classify natal origin using the Year 2 juvenile Bull Trout data than with data from Year 4. This difference was related to a much larger variation in chemistry ratios within tributaries in Year 4 than in Year 2 (Figure 7). The variability in both Sr:Ca and Ba:Ca was greater in Year 4, but the difference was most pronounced in the Sr:Ca ratio. As the Sr:Ca ratio had most of the discriminating power in the LDA models (based on model coefficients and variance explained), the high variability in Sr:Ca in Year 4 reduced predictive accuracy of the models, as shown by the difference is classification accuracy between Tables 3 and 4.

3.3 Adult Natal Area Designations

The Ba:Ca and Sr:Ca ratios of adult Bull Trout in Year 2 did not differ among the Duncan Lake, Kootenay Lake, and the flip bucket capture locations (MANOVA; P=0.4; Figure 11).

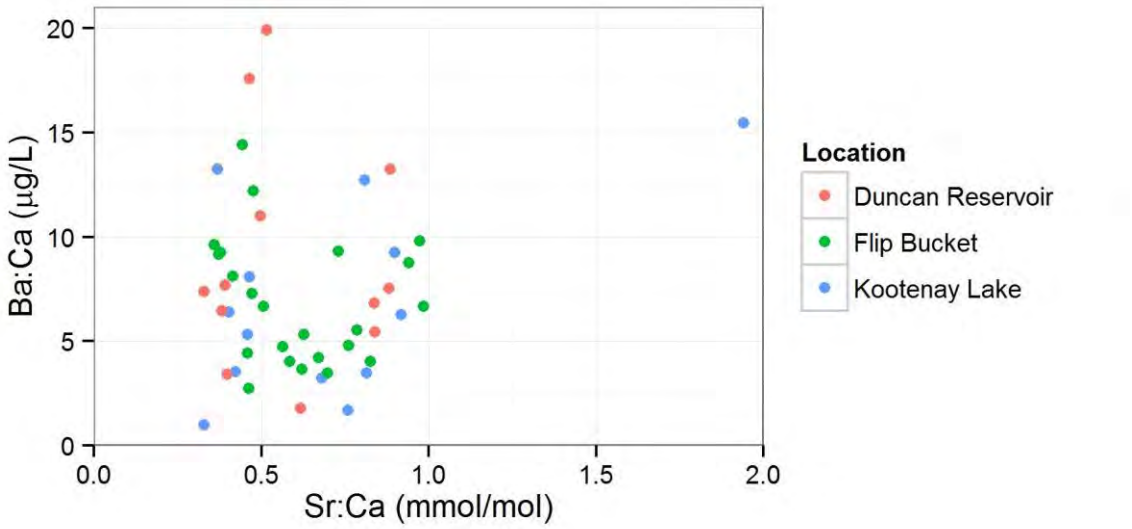

Figure 11. Natal otolith chemistry ratios by capture location for adult Bull Trout in Year 2 (2008).

Based on the split-sample LDA that used otolith chemistry of juveniles to predict adult natal origin (Year 2 data only), 69% of adults captured in Kootenay Lake were classified to one of the three natal tributaries in the Duncan Watershed (Table 5). Fifty percent of the adults captured in Duncan Reservoir and 78% of those captured in the flip bucket were predicted to be from the Duncan Watershed tributaries.

Table 5. Classification of the natal origin of adults captured in Year 2 (2008) by capture location, using predictive model of juveniles captured in Year 2 (2008-2009).

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

The Ba:Ca and Sr:Ca ratios in the natal portion of adult Bull Trout otoliths collected in Year 4 were significantly different between the Kootenay Lake and flip bucket capture locations (MANOVA; *P*=0.005). The Sr:Ca ratio was significantly greater in Kootenay Lake than the flip bucket (*P*=0.001; Figure 12) but the Ba:Ca ratio was not different between capture locations (*P*=0.8).

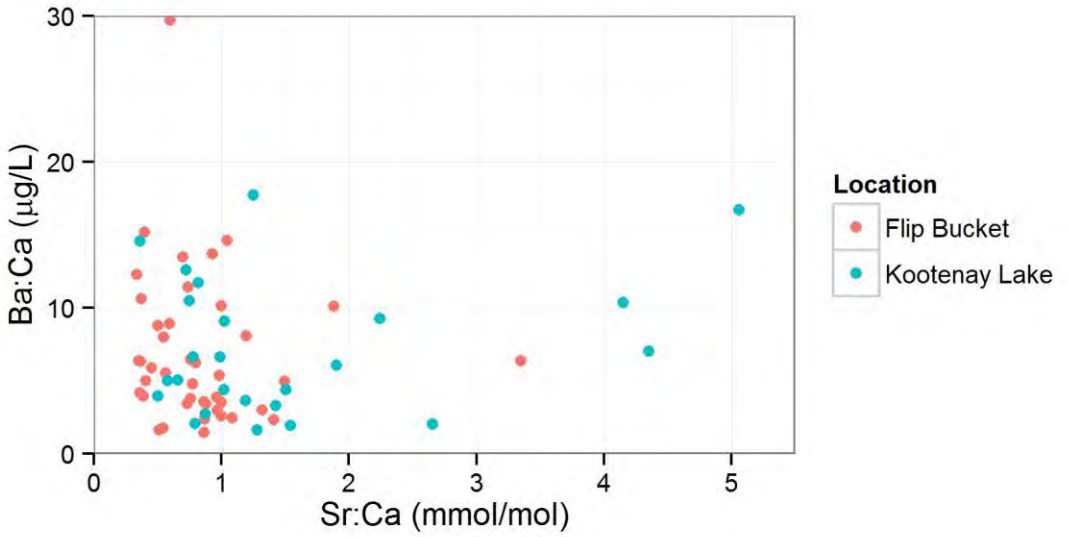

Figure 12. Natal otolith chemistry of adult Bull Trout from Year 4 (2012-2013).

Based on the split-sample LDA that used otolith chemistry of juveniles to predict adult natal origin (Year 4 only), 12% of adults captured in Kootenay Lake were classified as from one of the three tributaries in the Duncan Watershed (Table 6). Of the 42 adult Bull Trout captured in the flip bucket, 40% were predicted to be from Duncan Watershed tributaries and 60% were predicted to be from the Kootenay Watershed.

Table 6. Classification of the natal origin of adults captured in Year 4 (2012-2013) by capture location, using predictive model of juveniles captured in Year 4 (2013).

	Adult Capture Location			
	Flip Bucket		Kootenay Lake	
Predicted Natal Tributary	**#**	**%**	**#**	**%**
Houston	0	0	0	0
Upper Duncan	10	24	3	12
Westfall	7	17	0	0
Total Duncan Watershed	**17**	**40**	**3**	**12**
Coffee	10	24	6	24
Cooper	0	0	2	8
Crawford	7	17	5	20
Hamill	0	0	0	0
Kaslo	2	5	4	16
Midge	6	14	5	20
Poplar	0	0	0	0
Woodbury	0	0	0	0
Total Kootenay Watershed	**25**	**60**	**22**	**88**

Because the cross-validated juvenile Year 4 LDA had poor predictive ability (41%), an alternative split-sample LDA was conducted using otolith chemistry of Year 2 juveniles to predict natal origin of Year 4 adults. The model predicted that 24% of adults captured in

Kootenay Lake and 55% of those captured in the flip bucket were from one of the three tributaries in the Duncan Watershed (Table 7).

Predictions using either Year 2 or Year 4 juvenile otolith data to classify the natal origin of adults captured in Year 4 (Tables 6 and 7) both differed from the results using juvenile and adult data from Year 2 (Table 5). The majority of adults captured in Kootenay Lake in Year 4 were classified as originating in one of the Kootenay watershed tributaries (76% and 88%; Tables 6 and 6). Only 31% of adults captured in Year 2 were classified as Kootenay watershed origin (Table 5).The predicted origin of adult Bull Trout captured in the flip bucket was similar in all three models (40-55% Duncan Watershed origin).

Table 7. Classification of the natal origin of adults captured in Year 4 (2012-2013) by capture location, using predictive model of juveniles captured in Year 2 (2008-2009).

	Adult Capture Location			
	Flip Bucket		Kootenay Lake	
Predicted Natal Tributary	**#**	**%**	**#**	**%**
Houston	10	24	3	12
Upper Duncan	7	17	2	8
Westfall	6	14	1	4
Total Duncan Watershed	**23**	**55**	**6**	**24**
Coffee	6	14	6	24
Cooper	1	2	1	4
Crawford	1	2	0	0
Hamill	1	2	0	0
Kaslo	6	14	6	24
Poplar	1	2	4	16
Woodbury	3	7	2	8
Total Kootenay Watershed	**19**	**45**	**19**	**76**

MANOVA indicated that adult Bull Trout otolith chemistry ratios differed significantly between Year 2 and Year 4 (P<0.001) while accounting for stream to stream variation as a block effect. Block design ANOVA indicated that the Sr:Ca ratio (P<0.001) was different but the Ba:Ca ratio was not (P=0.3). Sr:Ca ratios of adult Bull Trout were larger and more variable in Year 4 than in Year 2 (Figure 13).

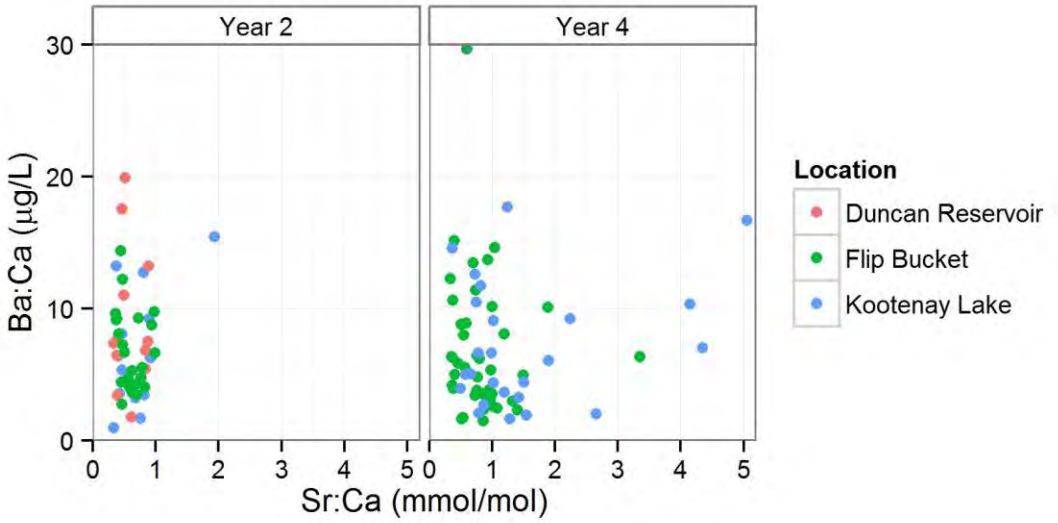

Figure 13. Natal otolith chemistry ratios for adult Bull Trout by study year and capture location.

3.4 Adult Otolith Chemistry at Capture Location

The outer edge of adult Bull Trout otoliths from Year 4 were analyzed to determine if there were differences among individuals that might correspond to adult residency in different water bodies. Adults were only collected from Kootenay Lake and the flip bucket in Year 4. Adult otoliths from Duncan Reservoir were not available in Year 4 because no heads were obtained from the recreational fishery collection program. Otolith chemistry ratios did not differ between adults captured in Kootenay Lake and the flip bucket (MANOVA; *P*=0.6). Sr:Ca ratios were more variable in adults captured in Kootenay Lake (n=16) than those captured in the flip bucket (n=26) whereas Ba:Ca ratios were very similar among capture locations (Figure 14). Because otolith chemistry did not differ among locations, LDAs to classify adults based on chemistry ratios were not performed. Analysis of the outer edge of otoliths collected from adults in Year 2 was not conducted but raw data from LA-ICP-MS are available and the sections representing the chemistry at the capture location could be summarized in future years, if desired. Capture information for adult Bull Trout from Year 4 are provided in Appendix C.

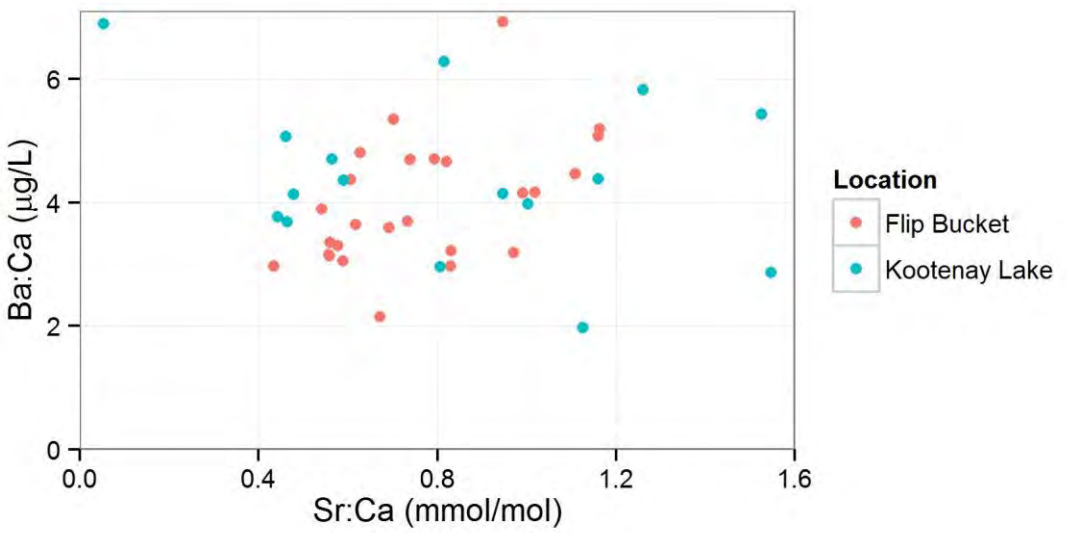

Figure 14. Otolith chemistry ratios from the outer portion of otolith representing recent adult residency for Bull Trout capture in 2013.

4. DISCUSSION

4.1 Stream Chemistry

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

4.2 Otolith Analysis

Analyses suggested that otolith chemistry data from Year 2 and Year 4 of the sampling program were not comparable, which limits conclusions that can be drawn relevant to the management questions. Multiple analyses suggested that both juvenile and adult otolith chemistries differed between Year 2 and 4, after accounting for repeated sampling on study tributaries or individual Bull Trout. The Ba:Ca ratio in otoliths was lower in Year 4 than in Year 2. The Sr:Ca ratio in otoliths was greater in Year 4 than Year 2 for some tributaries (Poplar and Cooper; Figure 7) but the reverse was true for several others (Figure 8). In addition, the variability in Sr:Ca ratios within streams was much greater in Year 4 than Year 2. As the Sr:Ca ratio was the variable that had the most power for discriminating among tributaries (96% of the explained variance in 2008 juveniles), the high variability in Sr:Ca in Year 4 greatly reduced the utility of the models for classifying adults of unknown origin. Paired otolith samples from the same individual juvenile Bull Trout that were analyzed in the two different laboratories that were used in Year 2 and the Year 4 suggested that the differences were related to the laboratories,

not the variation in chemistries among sampling years. This was further supported by water chemistry data, which was similar in 2008, 2009 and 2013, and showed a strong relationship with juvenile otolith chemistry in all years (although the relationship was stronger in Year 2 than in Year 4).

With regard to the management questions, data from Years 2 and 4 are discussed and interpreted in the sections below. More weight is put on the Year 2 data, which are thought to be more reliable for several reasons:

- Juvenile otolith data from Year 2 correlated more closely with the stream chemistry data than Year 4 otolith data. The large increase in the variability of Sr in otoliths in Year 4 was not observed in water chemistry data.

- Large variability in the Sr:Ca ratio in salmonid otoliths within a stream (e.g. ~3-9 mmol/mol in Poplar Creek) has not been reported in published studies from other locales.

- The Year 2 data provide better predictive ability than the Year 4 data. Models using data from Year 4 provide little information to help answer management questions.

Although Year 2 data are primarily used to address management questions at this stage, strong conclusions cannot be drawn because of the conflicting results between data sets.

One of the primary management objectives of this monitoring program was to determine if adfluvial Bull Trout in Kootenay Lake were spawned and reared in tributaries upstream of Duncan Dam. The conclusion that can be drawn from the model using Year 2 data is that a majority of adult Bull Trout captured in the flip bucket (78%) and Kootenay Lake (69%) originated from tributaries in the Duncan Watershed (Table 3). If this is true, then passage of fish through Duncan Dam is important component for recruitment of the Kootenay Lake Bull Trout population. Based on the cross-validated classification accuracy from Year 2 juveniles, the classification accuracy is assumed to be 79%.

Results from models using data from Year 4 (2012-2013) differed in that the majority (88%) of adults captured in Kootenay Lake were classified into natal origins in the Kootenay Watershed and only a small percentage (12%) classified into Duncan natal tributaries. However, the classification accuracy based on the Year 4 juveniles was low (41%), suggesting large uncertainty associated with these predictions. In fact, two of the three Duncan tributaries had 0% classification accuracy (Table 4), suggesting that assignment of adults to Duncan natal tributaries would be underestimates. Regardless of which years' data and models are interpreted, a substantial portion (>40%) of the Bull Trout captured in the flip bucket were classified as originating from tributaries upstream of Duncan Dam. Because fish caught in the flip bucket are assumed to have been residing in Kootenay Lake and were migrating upstream, this suggests a large portion of Duncan origin Bull Trout reside in Kootenay Lake and use the flip bucket for upstream passage.

The large differences in Sr:Ca and Ba:Ca ratios measured by the different laboratories used in Year 2 and Year 4 were not expected. Both laboratories were contacted to

discuss potential reasons for the differences. Although the same general approach was followed by both laboratories, and the Year 4 laboratory attempted to mimic the methodology followed in Year 2, the model of LA-ICP-MS instrument used and some methodological details differed which likely contributed to differences in elemental ratios. For instance, in Year 2, raw data outputs from the LA-ICP-MS for otoliths were filtered using an 11-point running average prior to conversion to ppm (Sanborn and Telmer 2003), whereas the Year 4 lab filtered data using a running average after conversion into ppm, which could have contributed to better precision in the Year 2 results (B. Gillanders, pers. comm). Another potential reason for differences is that particular LA-ICP-MS instruments can measure very different concentrations of certain elements depending on the materials it has previously analyzed, which can affect the background levels of elements (B. Gillanders, University of Adelaide, pers. comm.). Although it was not possible to identify the particular reason for large differences in measured element concentrations between laboratories, these differences highlight the important of consistency in instrumentation and protocols during multi-year monitoring programs using otolith and water microchemistry.

Adult Bull Trout in Year 4 (collected in 2012 and 2013) had much more variable and greater Sr:Ca ratios than those in Year 2 (Figure 13). The tributaries of Kootenay Lake, especially Poplar and Cooper creeks, had relatively high Sr:Ca ratios, whereas the three Duncan tributaries (Houston, Upper Duncan, and Westfall) all had low Sr:Ca. This likely explains why the high Sr:Ca ratios for adult Bull Trout in Year 4 resulted in large percentage classification to Kootenay watershed tributaries, and lower percentage assign to Duncan tributaries. Because the highly variable Sr:Ca ratios in Bull Trout otoliths in Year 4 may be questionable, these results should be interpreted with caution.

Exploratory analyses of Li and Mn for this report and the previous Golder (2010) report did not suggest strong discriminating power among tributaries based on these elements. However, these elements were only available for a subset of the data (e.g., not for 2008 juveniles and only for some of the 2012-2013 adults). Further analysis of the Li in otoliths is warranted in future years, to assess whether this element may help distinguish some of the streams that are currently prone to misclassification using the models. In addition, the ratio of ^{88}Sr:^{86}Sr has been used to distinguish between populations of Rainbow Trout (Gibson-Reinemer et al. 2009) and could be investigated as an explanatory variable in future years of this monitoring program. Mn concentrations in Year 4 were very low, with values near the background levels read by the LA-ICP-MS instrument, and did not indicate substantial variation among streams. The results do not suggest Mn is a useful element for improving classification of otoliths in the study area.

4.2.1 Adult Otolith Chemistry at Capture Location

Water samples analyzed in Year 2 suggested differences in water chemistry ratios among sample sites in Kootenay Lake and Duncan Reservoir (Golder 2010). If these differences were also observed in the outer portion of adult otoliths, then the otolith signatures from recent adult residency could potentially be used to assess movements of adults between Kootenay Lake and Duncan Reservoir. For instance if a fish with a Kootenay Lake type signature were captured in Duncan Reservoir, then it would indicate migration upstream through the flip bucket. Unfortunately, adult Bull Trout were not captured in Duncan Reservoir in Year 4 to assess differences in otolith chemistry compared to Kootenay Lake. There were no differences between the chemistry ratios of

the outer portion of otolith of Bull Trout from Kootenay Lake and the flip bucket, which is not surprising, as most of the fish in the flip bucket are assumed to have been residing in Kootenay Lake.

Large differences were not observed among capture locations in the elemental signatures in the outer edge otoliths from adult Bull Trout in Year 4. However, exploratory analyses in Golder (2010) showed large variations in otolith chemistry during the post-natal period of Bull Trout, although interpretation and the precise areas of residency based on these signatures was uncertain. Water samples corresponding to adult otolith chemistry were not taken from Kootenay Lake or Duncan Reservoir in 2012 and 2013, and only a few locations were sampled in earlier years. Additional water sampling to help characterize and distinguish the chemistry in these waterbodies is warranted and would help further interpretation and analysis of chemistry profiles during the post-natal period of Bull Trout. However, based on the results of Year 4, analysis of the outer edge of adult otoliths is not likely to be useful in determining migrations of post natal fish from Duncan tributaries into Kootenay Lake

5. CONCLUSIONS

The overall management question of this monitoring program is:

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

Although there are uncertainties regarding the proportion of adfluvial Bull Trout from Kootenay Lake that migrate past Duncan Dam to spawn in upstream tributaries, the otolith chemistry data suggest that a substantial portion of the adults caught in the flip bucket were reared in tributaries upstream of Duncan Dam. Because of the conflicting otolith chemistry data it is not possible to draw strong conclusions, but the data available suggest the transfer program is likely important for the recruitment of Bull Trout in Kootenay Lake and Duncan Reservoir.

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

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

The distinct water chemistries among most of the sampled tributaries and their strong association with the otolith chemistry of juvenile Bull Trout provide support for rejecting null hypothesis H01. The results from Year 4 highlight the importance of maintaining consistent laboratories and protocols among years in order to develop predictive models of natal origin based on these distinct stream chemistries.

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

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

Results from Year 2 of the study provided estimates of the proportion non-natal and natal Bull Trout in the samples of adults captured in the Kootenay and Duncan watersheds. These results suggest that the Duncan watershed may contribute disproportionately to recruitment in the Duncan and Kootenay systems because a majority of adult fish captured in the flipbucket were predicted to have natal origins in Duncan watershed tributaries. However, otolith chemistry data from Year 4 were highly variable and did not allow reliable estimates of natal origin. Because of the conflicting results and large variability in recent chemistry data, statistical tests of the differential proportion of non-natal fish were not possible and H02 cannot be addressed at this time.

After the above hypotheses have been addressed, the final management question identified in the terms of reference is:

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

The current monitoring program is not designed to address this question. The results suggest that the continued operation of the transfer program is important for Bull Trout populations, 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.

6. RECOMMENDATIONS

Overall, the results suggest that otolith microchemistry is an effective way to classify the natal origin of Bull Trout and address other management questions related to the recruitment and life-history of Bull Trout in the Duncan and Kootenay watersheds. There remains uncertainty in the classification of several streams, which overlapped in terms of their microchemisty based on the Year 2 data, and these issues were compounded by highly variable and conflicting chemistry data obtained in Year 4. Based on these findings, the following recommendations for future study are offered:

- Sampling of adults from Duncan Reservoir is needed in order to test the management hypothesis regarding differential proportions of natal versus non-natal Bull Trout in Duncan Reservoir and Kootenay Lake.

- Consistency in the laboratory used for chemical analyses, and their instrumentation, methodology and data processing is crucial to refine models and obtain comparable data among years. A blind test is suggested to determine replication and variance of known common samples among laboratories be performed, prior to laboratory selection for future analyses. Rescanning existing otoliths collected in 2012 and 2013 from a new laboratory or, if possible, use of the existing laboratory, should help resolve this issue.

- Collect additional water samples including pre and post stratification samples from Duncan Reservoir and the north arm of Kootenay Lake, the Lardeau River and the lower Duncan River. These samples could help determine whether significant differences in water chemistry exist among areas used by adult Bull Trout, and whether further analysis of post-natal portions of adult otoliths is warranted.

- For streams in which there was significant overlap in element ratios, investigating whether the ratio of ^{88}Sr:^{86}Sr and Li:Ca is recommended to determine if these isotopes may help discriminate among streams. Existing otoliths that were not analyzed for Li (most Year 4 juveniles and adults) or ^{88}Sr (Year 2 data) could be rescanned to analyze for these elements and analyses of new otoliths should also include these isotopes.

7. REFERENCES

Anonymous. 1986. Annual Report to the Governments of the United States and Canada. Columbia River Treaty Permanent Engineering Board, Washington D.C. and Ottawa Ont. 49 p.

BC Hydro. 2008. Duncan Dam Water Use Plan, Monitoring Program Terms of Reference: Upper Duncan River Bull Trout Migration Monitoring-Appendix 6. 13 p.

Clarke, A.D, and K.H Telmer. 2008. Arrow Reservoir Bull Trout: Microchemical analysis of otoliths to determine stock structure, migration timing, and location of spawning and rearing habitats. Prepared for: James Baxter and Steve Arndt. Fish & Wildlife Compensation Program, Nelson, BC.

Clarke, A.D., K.H. Telmer, and J.M. Shrimpton. 2007. Habitat use and movement patterns for a fluvial species, the Arctic grayling, in a watershed impacted by a large reservoir: evidence from otolith microchemistry. Journal of Applied Ecology 44: 1156-1165.

Devries D.R and R.V. Frie. 1996. Determination of age and growth. In: B.R. Murphy & D.W. Willis (eds) Fisheries Techniques 2nd edn. Bethesda, MD: American Fisheries Society, pp. 483-512.

DVH Consulting. 2001. Duncan Dam Status of Knowledge Report, Fisheries Assessment and Planning Study. Report prepared for BC Hydro, Kootenay Generation Area, Castlegar, BC.

Elsdon, T.S., B.K. Wells, S.E. Campana, B.M. Gillanders, C.M. Jones, K.E. Limburg, D.H. Secor, S.R. Thorrold, and B.D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology 46: 297-330.

Gauldie, R.W. 1996. Effects of temperature and vaterite replacement on the chemistry of metal ions in the otoliths of *Oncorhynchus tshawytscha*. Canadian Journal of Fisheries and Aquatic Sciences 53: 2015–2026.

Gibson-Reinemer, D.K., B.M Johnson, P.J. Martinez, D.L. Winkleman, A.E. Koenig, and J.D. Woodhead. 2009. Elemental signatures in otoliths of hatchery rainbow trout (*Oncorhynchus mykiss*): distinctiveness and utility for detecting origins and movement. Canadian Journal of Fisheries and Aquatic Sciences 66: 513-524.

Golder Associates Ltd (Golder). 2010. Upper Duncan Bull Trout migration monitoring – Final Report March 2010. Report prepared for BC Hydro, Castlegar, BC. Golder Report No. 09-1480-0051: 49 pp. + 8 apps.

O'Brien, D, 1999. The Duncan Bull Trout telemetry project (1995-1997). Prepared for the Columbia Basin Fish and Wildlife Compensation Program, Nelson, BC.

Ord, D., R. Olmsted, D. den Biesen, G. Birch. 2000. Migration Timing and Abundance Trends in Adfluvial Bull Trout at Duncan Dam, British Columbia: Results of 1995-2000 Tagging Studies. Prepared by BC Hydro, Kootenay Generation Area, Castlegar, BC.

Peterson, G.R. and I.L.Withler. 1965. Effect on fish and game species of the development of Duncan Dam for hydro-electric purposes. Management Publication 8, British Columbia Fish and Wildlife Branch. 70 p.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Resource Inventory Standards Committee (RISC). 1997. Fish Collection Methods and Standards. Version 4.0., January 1997. http://ilmbwww.gov.bc.ca/risc/pubs/aquatic/fishcol/index.htm

Sanborn, M and K. Telmer, K. 2003. The spatial resolution of LA-ICP-MS line scans across heterogeneous materials such as fish otoliths and zoned minerals. Journal of Analytical Atomic Spectrometry 18: 1231-1237.

Venables, W.N. and B.D. Ripley. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Wells, B.K., Rieman, B.E., Clayton, J.L., Horan, D.L., and Jones, C.M. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: The potential application of hard-part chemistry to describe movements in freshwater. Transactions of the American Fisheries Society 132: 409-424.

Yoshinaga, J., A. Nakama, M. Morita, and J.S. Edmonds. 2000. Fish otolith reference material for quality assurance of chemical analyses. Marine Chemistry 69: 91-97.

Appendix A – Site Locations and UTM Coordinates

Table A1. Water Chemistry and Juvenile Bull Trout Collection Locations, 2013

Basin	Sample Location[a]	Juvenile Bull Trout Collected	Bank[b]	UTM Coordinates		
				Zone	Easting	Northing
Upper Duncan River	Upper Duncan River	Yes	RUB	11U	483681	5648552
Duncan Reservoir	Houston Creek	Yes	LUB	11U	483579	5648542
	Westfall River	Yes	RUB	11U	485870	5625830
	Poplar Creek	Yes	RUB	11U	491337	5584815
Kootenay Lake (North Arm)	Hamill Creek	Yes	LUB	11U	503757	5561121
	Cooper Creek	Yes	LUB	11U	502705	5560725
Kootenay Lake	Kaslo River	Yes	RUB	11U	506725	5528471
Kootenay Lake (North Arm)	Woodbury Creek	Yes	RUB	11U	506621	5513654
	Coffee Creek	Yes	LUB	11U	505778	5504832
Kootenay Lake (South Arm)	Crawford Creek	Yes	LUB	11U	513352	5502186
	Midge Creek	Yes	RUB	11U	514003	5469193

[a]Sample locations are identified from the furthest north moving south.
[b]RUB=Right bank as viewed facing upstream; LUB=Left bank as viewed facing upstream

Appendix B – Site Photographs

Plate 1: Upper Duncan River 13 September 2013

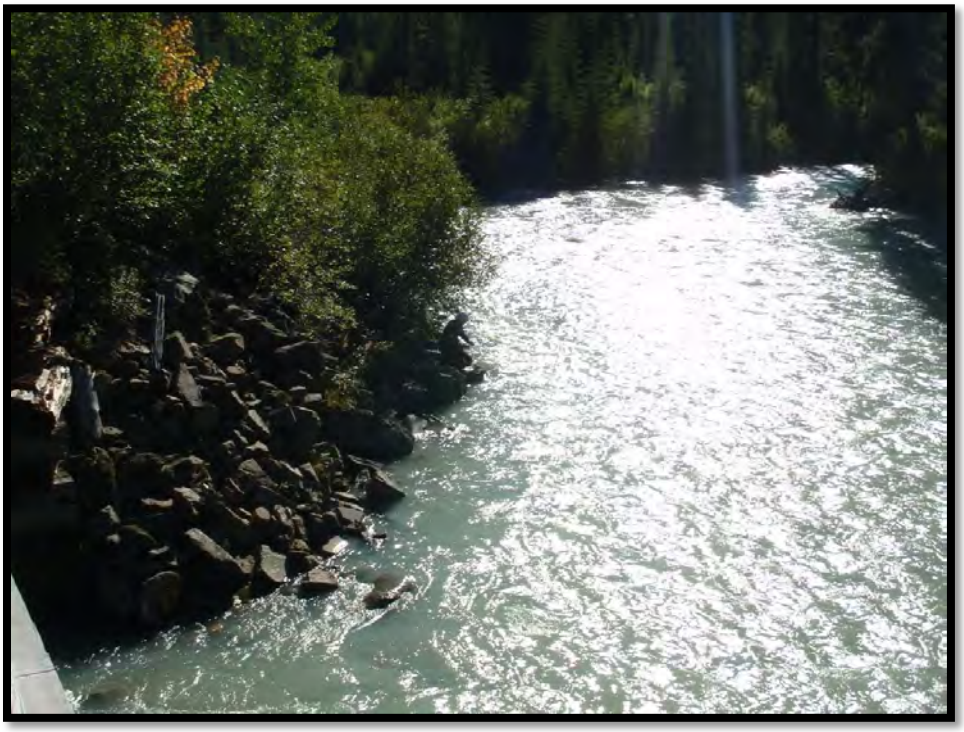

Plate 2: Houston Creek 13 September 2013

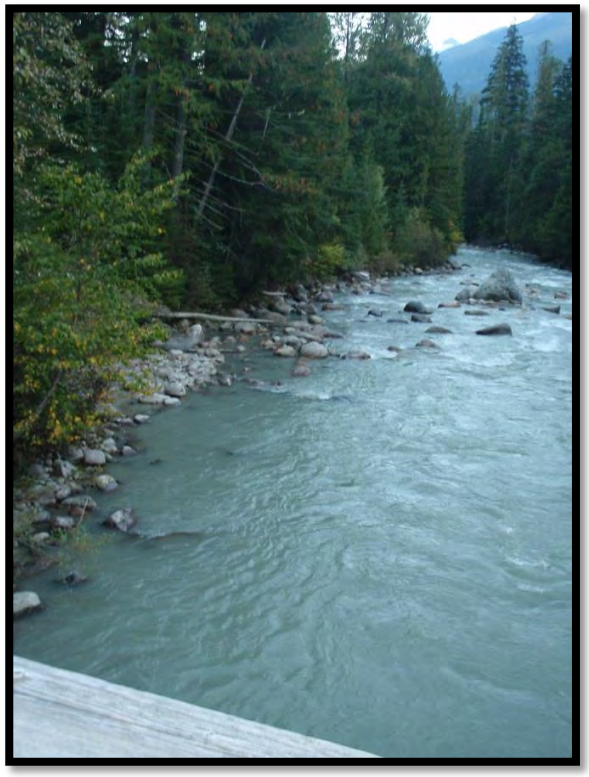

Plate 3: Westfall River 13 September 2013

Plate 4: Hamill Creek 4 September 2013

Plate 5: Poplar Creek 4 September 2013

Plate 6: Cooper Creek 4 September 2013

Plate 7: Kaslo River 5 September 2013

Plate 8: Woodbury Creek 4 September 2013

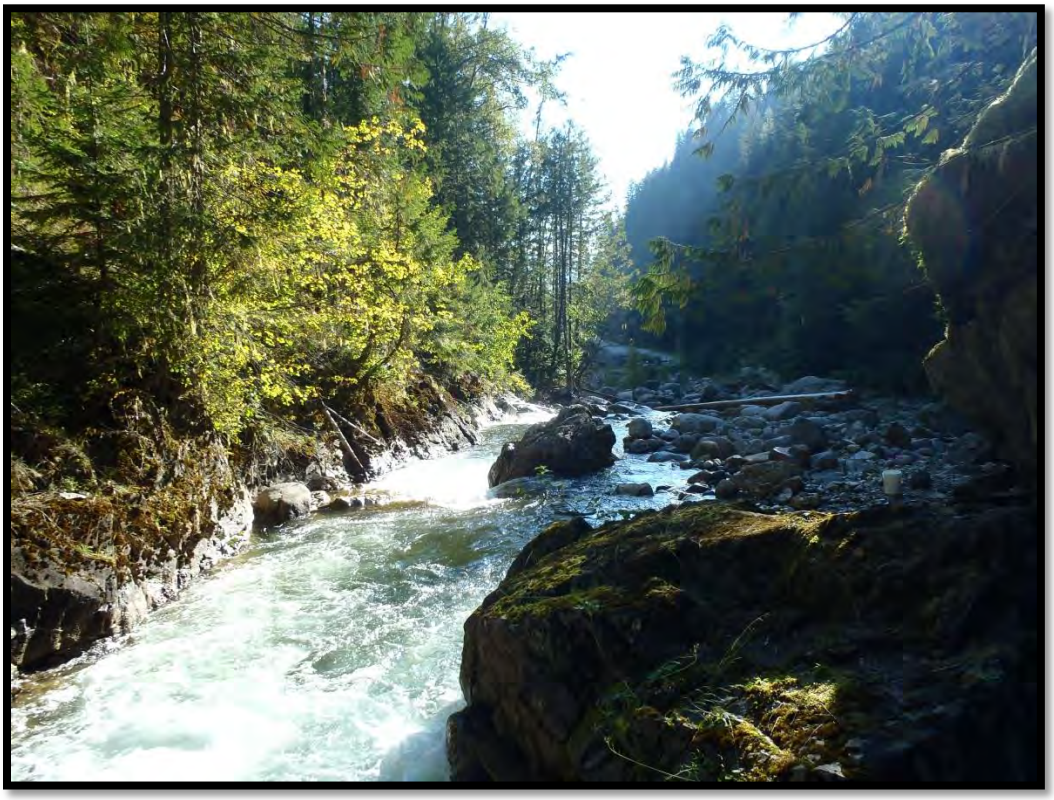

Plate 9: Coffee Creek 4 September 2013

Plate 10: Crawford Creek 5 September 2013

Plate 11: Midge Creek 5 September 2013

Appendix C – Bull Trout Data

Table C1. Kootenay Lake Adult Bull Trout Data

Capture Date	Fish ID Number	Fork Length (mm)	Weight (g)	Sex	Age
30-Aug-12	306	445	680	Unknown	7
30-Aug-12	310	445	907	Unknown	6
31-Aug-12	311	572	2268	Unknown	7
08-May-13	322	711	4309	Female	6
26-May-13	323	508	1361	Female	4
26-May-13	326		2722	Female	9
26-May-13	335	508	1361	Unknown	8
01-Apr-13	324	635	2041	Unknown	6
01-Apr-13	334	NR[a]	NR	Unknown	8
13-May-13	325	660	3175	Female	6
13-May-13	328	686	2722	Female	6
12-May-13	327	813	5443	Female	7
12-May-13	367	710	4536	Unknown	7
12-May-13	369	813	6804	Unknown	7
15-May-13	329	559	1361	Female	9
07-May-13	330	610	1814	Female	7
05-May-13	331	635	2268	Female	6
12-Jun-13	332	838	5897	Male	5
21-May-13	336	660	2722	Unknown	9
04-May-13	333	NR	3629	Unknown	5
02-May-13	368	711	2722	Female	5
02-Sep-12	309	686	2268	Female	4
02-Sep-12	317	749	4082	Unknown	6
05-Sep-12	318	660	2722	Female	5
08-Sep-12	307	533	1361	Female	4
08-Sep-12	313	660	2155	Female	7
11-Sep-12	314	483	907	Unknown	5
11-Sep-12	315	584	2608	Unknown	6
01-Oct-12	316	686	3175	Female	4
05-Oct-12	320	737	3402	Unknown	7
19-Oct-12	312	NR	3741	Unknown	6
01-Aug-12	308	483	907	Unknown	5
01-Aug-12	319	NR	NR	Unknown	5

Appendix D – Average Elemental Ratios

Table D1. Average elemental ratios (mmol/mol) measured by ICP-MS for the systems examined in 2013. The values are provided as an element:Ca ratio.

Sample Location	Month	Sr:Ca (mmol/mol) 2008	Sr:Ca (mmol/mol) 2009	Sr:Ca (mmol/mol) 2013	Ba:Ca (mmol/mol) 2008	Ba:Ca (mmol/mol) 2009	Ba:Ca (mmol/mol) 2013
Duncan Basin							
Upper Duncan River	September	4.90	4.15	4.00	0.90	0.72	Not Available
Houston Creek	September	6.72	6.98	7.50	0.79	0.78	Not Available
Westfall River	September	3.58	3.59	3.70	0.81	0.81	Not Available
Hamill Creek	September	3.27	3.39	3.31	0.76	0.72	0.81
Poplar Creek	September	38.27	40.86	39.72	4.06	3.91	3.79
Cooper Creek	September	15.00	14.72	17.24	1.83	1.69	2.12
Kootenay Basin							
Kaslo River	September	5.93	5.98	7.00	0.40	0.37	0.43
Woodbury Creek	September	8.95	9.17	10.32	0.59	0.58	0.68
Coffee Creek	September	9.99	10.27	10.44	0.44	0.41	0.47
Crawford Creek	September	3.43	3.56	3.35	1.23	1.16	1.19
Midge Creek	September	Not Available	Not Available	6.05	Not Available	Not Available	1.10

Appendix E – Supplementary Results

Table E1. Classification of juvenile Bull Trout captured in 2008 and 2009 (Year 2) based on linear discriminant analysis.

Known Capture Location	Predicted Capture Location									
	Coffee	Cooper	Crawford	Hamill	Houston	Kaslo	Poplar	Upper Duncan	Westfall	Woodbury
Unvalidated Model										
Coffee	7	0	0	0	0	0	0	0	0	0
Cooper	0	7	0	0	0	0	0	0	0	0
Crawford	0	0	4	1	0	0	0	0	2	0
Hamill	0	0	1	4	0	0	0	0	1	0
Houston	0	0	0	0	5	2	0	0	0	0
Kaslo	0	0	0	0	2	5	0	0	0	0
Poplar	0	0	0	0	0	0	8	0	0	0
Upper Duncan	0	0	0	1	0	0	0	6	0	0
Westfall	0	0	1	0	0	0	0	0	6	0
Woodbury	0	0	0	0	0	0	0	0	0	7
Cross-Validated Model										
Coffee	7	0	0	0	0	0	0	0	0	0
Cooper	0	7	0	0	0	0	0	0	0	0
Crawford	0	0	4	1	0	0	0	0	2	0
Hamill	0	0	2	3	0	0	0	0	1	0
Houston	0	0	0	0	5	2	0	0	0	0
Kaslo	0	0	0	0	2	5	0	0	0	0
Poplar	0	0	0	0	0	0	8	0	0	0
Upper	0	0	0	1	0	0	0	6	0	0
Westfall	0	0	3	0	0	0	0	0	4	0
Woodbury	1	0	0	0	0	0	0	0	0	6

Table E2. Classification of juvenile Bull Trout captured in 2013 (Year 4) based on linear discriminant analysis.

Known Capture Location	Predicted Capture Location									
	Coffee	Cooper	Crawford	Hamill	Houston	Kaslo	Poplar	Upper Duncan	Westfall	Woodbury
Unvalidated Model										
Coffee	5	0	0	0	0	3	2	0	0	0
Cooper	0	5	0	0	0	1	1	3	0	0
Crawford	0	0	13	0	0	0	6	0	1	2
Hamill	0	0	1	0	0	0	4	0	3	0
Houston	1	0	0	0	0	0	6	0	2	1
Kaslo	2	0	0	0	0	10	0	0	0	0
Midge	0	0	6	0	0	2	11	0	0	1
Poplar	0	3	0	0	0	0	0	5	0	0
Upper	0	0	1	0	0	1	0	0	8	3
Westfall	1	0	9	0	0	0	1	0	4	0
Woodbury	1	0	0	0	0	0	0	0	0	0
Cross-Validated Model										
Coffee	5	0	0	0	0	3	2	0	0	0
Cooper	0	4	0	0	0	1	1	4	0	0
Crawford	0	0	12	0	0	0	7	0	1	2
Hamill	0	0	1	0	0	0	4	0	3	0
Houston	1	0	0	0	0	0	6	0	2	1
Kaslo	2	0	0	0	0	9	1	0	0	0
Midge	0	0	6	0	0	2	11	0	0	1
Poplar	0	4	0	0	0	0	0	4	0	0
Upper	0	0	1	0	0	1	0	0	8	3
Westfall	1	0	9	0	0	0	1	0	4	0
Woodbury	0	0	0	0	0	0	0	0	0	0

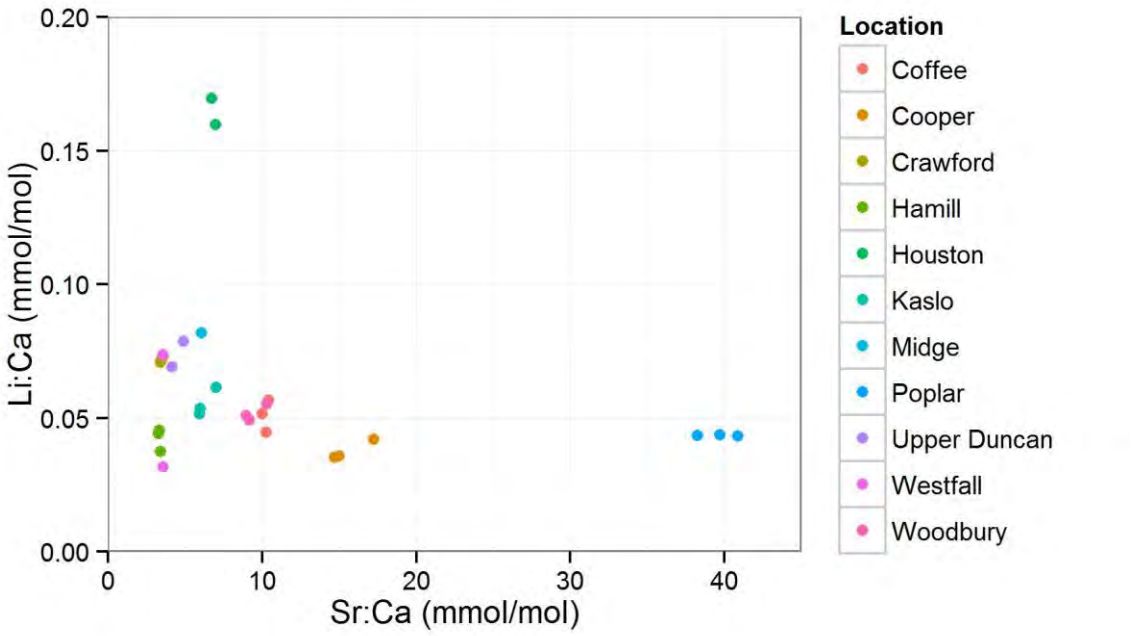

Figure E1. Water chemistry ratios in Kootenay and Duncan watershed tributaries, showing lithium to calcium ratio as an additional potential variable to separate streams. Data are mean values from fall sampling in 2008, 2009, and 2013.