Site C Clean Energy Project

Fisheries and Aquatic Habitat Monitoring and Follow-up Program

Fish Otolith and Fin Ray Microchemistry Study

Construction Years 5 and 6 (2019 and 2020)

TrichAnalytics Inc.

May 3, 2022

TrichAnalytics Inc.

Site C Clean Energy Project

Fish Otolith and Fin Ray Microchemistry Study (2019 and 2020)

Prepared for

BC Hydro

Site C Clean Energy Project

1111 West Georgia St, 9th floor

Vancouver, BC V6E 4G2

By

TrichAnalytics Inc.

#207, 1753 Sean Heights

Saanichton, BC

V8M 0B3

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EXECUTIVE SUMMARY

Construction of the BC Hydro Site C Clean Energy Project (the Project) continued in 2021, which will be the third hydroelectric dam on the Peace River near the town of Fort St. John in northeastern British Columbia (BC). BC Hydro developed the Site C Fisheries and Aquatic Habitat Monitoring and Follow-up Program (FAHMFP) in accordance with Provincial Environmental Assessment Certificate Condition No. 7 and Federal Decision Statement Condition Nos. 8.4.3 and 8.4.4 for the Project. Monitoring Programs, Mon-1b and Mon-2, of the FAHMFP continued to collect otoliths and fin rays from select fish species, including those considered herein, from the Peace River and its tributaries. For the purposes of this study, the sampled otoliths and fin rays collected in 2019 and 2020 were used to determine possible recruitment sources to the Peace River of key indicator fish species.

The main objectives of the study were to determine the recruitment sources for three fish species and differentiate recruitment sources between upstream and downstream of the Project. The three fish species included Arctic Grayling, Bull Trout, and Rainbow Trout. Otoliths and fin rays were collected in 2019 and 2020, and were analyzed in 2021 using a Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS) along the temporal growth axis from edge (capture location) to core (natal signal).

A total of 40 elements were examined for possible analysis and inclusion in modelling; however, it was determined that only barium (as a molar ratio to calcium, Ba:Ca) and strontium (Sr:Ca) could ultimately be used in the model, with zinc (Zn) providing the annual/seasonal signal for support in finding the first summer region in the tissue. For the other elements not used, the reasons for exclusion were specific to the element and included: 1) not readily detected in the water or was not available at all locations; 2) water concentrations did not vary among locations; 3) element was not detected or did not vary in the fish tissues of these species; 4) literature review suggests the element concentration in fish tissue is not driven by water concentrations, but rather physiology or diet; or 5) there was not a significant relationship between water concentration and fish tissue concentration, and therefore, no incorporation coefficient could be calculated.

A tiered approach to a nested Quadratic Discriminant Analysis (nQDA) was used to develop recruitment source prediction models for each species – tissue combination. The first tier was to predict the watershed, and the second tier was to predict a stream within a watershed (if that watershed had more

than one stream used in the model, e.g., Halfway River watershed). The models were trained and developed using water and tissue capture chemistries, and were tested for accuracy using confusion matrices. The models were then validated using natal regions of <1 year old fish of each species, as these have known natal origins. This tested the accuracy of new data and examined existence and patterns of model overfitting. Finally, life history graphs were created for the validation data to help visualize tissue chemistries over time from fish with known natal origins and captures. The test data (i.e., fish captured with unknown natal origins) were then entered into the model to predict their recruitment source. Overall, the models along with life history graphs of the test data fish provided recruitment information for all but two fish (classified as "unknown" origin). The main findings from the otolith and fin ray microchemistry of the fish captured in the study area are presented below:

- Arctic Graylings captured in the Peace River recruited from the Moberly, Halfway and Beatton River watersheds, which is similar to what has been previously reported for this study area. These conclusions are predominantly based on the fin ray analysis and model due to the higher sample size and higher accuracy.
- Bull Trout fin rays were also used to derive overall predictions for recruitment sources, as more fin rays were available than otolith samples. Streams in the Halfway River watershed (mainly Chowade River and Cypress Creek) were the dominant recruitment sources for Peace River captures, both upstream and downstream of the Project. Moberly River provided a less prominent source of Bull Trout to the Peace River upstream of the Project and the Pine River was predicted as the only potential recruitment source of Bull Trout downstream of the Project. These conclusions echo those reported previously for Bull Trout.
- There were clear similarities between recruitment sources for Rainbow Trout reported here for 2019/2020 and those reported previously. The Rainbow Trout captured in the Halfway River watershed also originated from there. The Rainbow Trout captured in Farrell Creek, either recruited from Farrell Creek or recruited from other upstream sources, such as Halfway River watershed and possibly, Dinosaur Reservoir. The Peace River-captured Rainbow Trout (n=3) were all predicted to have recruited from sources upstream of the Project: one from Maurice Creek, one from Colt Creek (in Halfway River watershed), and one from Farrell Creek.

ACKNOWLEDGEMENTS

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TrichAnalytics Inc. would like to acknowledge Golder Associates Ltd. and BC Hydro for providing the historic water chemistry data from 2008-2018, and fish otolith and fin ray samples for the 2019-2020 study period. D. Ford (Golder Associates Ltd.) also provided valuable information throughout on various watersheds and fish species.

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1. INTRODUCTION

1.1. Background

Construction of the BC Hydro Site C Clean Energy Project (the Project) continued in 2021, which will be the third hydroelectric dam on the Peace River near the town of Fort St. John in northeastern British Columbia (BC). BC Hydro developed the Site C Fisheries and Aquatic Habitat Monitoring and Follow-up Program (FAHMFP; BC Hydro 2015) in accordance with Provincial Environmental Assessment Certificate, Schedule B, Condition No. 7 and Federal Decision Statement Condition Nos. 8.4.3 and 8.4.4 for the Project. Monitoring Programs, Mon-1b and Mon-2, of the FAHMFP continued to collect otoliths and fin rays from select fish species, including those considered herein, from the Peace River and its tributaries. For the purposes of this study, the sampled otoliths and fin rays collected in 2019 and 2020 were used to determine possible recruitment sources to the Peace River of key indicator fish species.

This Fish Otolith and Fin Ray Microchemistry Study, alongside previous studies (Clarke et al. 2011; Earthtone and Mainstem 2013; TrichAnalytics 2020) amounts to the largest and most comprehensive examination of fish natal and summer rearing locations using otolith and fin ray microchemistry ever conducted in BC. This report extends previous baseline studies and fisheries monitoring work conducted during construction in 2019 and 2020 that focused on identifying potential spawning and early rearing locations of fish in the Peace River watershed.

Otolith and fin ray microchemistry using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) has been used as a standard tool to characterize natal sources and migration of freshwater fish species (Wells et al. 2003; Clarke et al. 2007a, b; Gibson-Reinemer et al. 2009). Collection of fin rays is preferred by BC Hydro over otolith sampling for monitoring programs that require microchemistry analysis (BC Hydro 2015) due to the non-lethal sampling nature of fin rays. Otoliths are, however, opportunistically collected from fish that inadvertently succumb from sampling under the FAHMFP studies (BC Hydro 2015).

Barium and/or strontium (as molar ratios to calcium) are commonly used for prediction modelling of fish recruitment and migration due to the fact that these elements provide a reflection of water chemistry over time in the fish structures (i.e., otoliths and fin rays) (Ziegeweid 2021; Secor 1992; Linley et al. 2016; Farrell and Campana 1996; Radtke 1989). As these structures are highly stable, the uptake of these elements and ultimate accretion in the calcium-based tissues is permanent, and has been shown to be highly correlative with the concentrations in the aquatic environment (Linley et al. 2016; Bath et

al. 2000). Specifically, for Site C and surrounding geographic area, the following four previous studies have been conducted with these two elements:

- Clarke et al. (2011) demonstrated that barium and strontium in otoliths were useful for differentiating Arctic Grayling and Mountain Whitefish from the Peace, Halfway, and Moberly rivers; however, some fish had unknown recruitment sources due to unique chemistry.
- In 2011, six species of fish (i.e., Arctic Grayling, Bull Trout, goldeye, mountain whitefish, Rainbow Trout and walleye) in the area were studied using otoliths, and recruitment sources were predicted for 16 waterbodies. Again, however, there were still unknown sources identified (Earthtone and Mainstream 2012).
- Earthtone and Mainstream (2013) expanded on Earthtone and Mainstream (2012) by including more water chemistry data to help identify unknown source regions. Otoliths from the same six species of fish were analyzed and modeled to identify recruitment sources from 45 locations.
- TrichAnalytics (2020) continued with analysis and modelling of the same six fish species from samples collected from 2014 to 2018, using otoliths and included fin rays for the first time as a non-lethal alternative. Fin ray analysis was deemed as effective as otoliths in characterizing recruitment sources.

Results using otolith and fin ray microchemistry, in addition to previous findings, will help support other monitoring programs in the FAHMFP. Specifically, microchemistry results will be combined with other fish habitat data sources (e.g., genetics, radio telemetry, fish capture locations) in a weight-of-evidence approach to provide insight on life history[1] and recruitment sources of key indicator fish species in the Peace River. Ultimately, information from all monitoring approaches will be used to answer management questions and test management hypotheses for these key fish species (BC Hydro 2015).

1.2. Study Objectives

The main objectives of the current study were to determine the recruitment sources of three fish species and differentiate recruitment sources between upstream and downstream of the Project. Recruitment sources, in this study, are defined as either natal or first summer stream locations or both. Specifically, the main objective, taken directly from the FAHMFP (BC Hydro 2015), is: "*Microchemistry will be used to estimate the proportion of each species that were spawned and reared upstream versus downstream of*

[1] Life history, in the context of this report, is defined as the history of a fish's movement within or among streams over its lifetime using otolith and fin ray microchemistry.

the Project" (BC Hydro 2015); most specifically for Bull Trout, Rainbow Trout, and Arctic Grayling. Additional objectives include finding opportunities to improve upon previous modelling approaches, and to identify gaps where further fish sampling of specific species, waterbodies, or water samples could also help improve the future modeling approach.

The key fish species in the 2019/2020 microchemistry study include: Arctic Grayling, Bull Trout, and Rainbow Trout. All fish were captured between 2019 and 2020 in the Peace River between Peace Canyon Dam and Many Islands, Alberta, as well as from tributaries of the Peace River, between Peace Canyon Dam and the Project.

Activities to meet the study objectives included:

1) Compilation of water chemistry data from 2008 to 2018.
2) Selection of elements for LA-ICP-MS analysis and modelling.
3) Compilation of capture chemistry data, obtained from the outer edges of otoliths and fin rays, from previous studies.
4) Analysis of otoliths and fin rays from samples collected in 2019/2020 for barium, strontium, and calcium using LA-ICP-MS along the temporal growth axis from the structure edge (capture location) to the core (natal/maternal signal).
5) Calculation of species/tissue incorporation coefficients using new and previously collected data.
6) Construction of species- and tissue-specific models to predict recruitment sources using water and tissue chemistry data collected from the study area from 2008 to 2020, where available.
7) Model assessment, validation and confusion matrices to determine model prediction accuracy overall and within specific waterbodies.
8) Development of life-history graphs for visual depiction of fish recruitment and migration over the course of their lives to help support model predictions.
9) Identification of gaps and/or opportunities for improvement in the modelling in future work.

2. RATIONALE FOR ELEMENTS SELECTED FOR MICROCHEMISTRY ANALYSIS

As requested by BC Hydro, TrichAnalytics Inc. was asked to provide rationale for the elements selected for LA-ICP-MS analysis of fin rays and otoliths for use in the habitat prediction modelling program. During discussions with BC Hydro, TrichAnalytics Inc. understood that following regulatory review of

the TrichAnalytics (2020) report, it was suggested that the fish habitat model to predict recruitment sources could potentially be expanded to include other elements, aside from strontium (Sr:Ca), and barium (Ba:Ca). The purpose of using additional elements in the model would be to further differentiate between various watercourses that have overlapping Sr:Ca and Ba:Ca chemistries, and thereby provide improved predictability power in the fish habitat model. This section is a summary of the approach and findings, and the ultimate rationale for the final selection of elements to move forward with in the recruitment models.

2.1. Scope of Work

This part of the project focused on assessing the feasibility in developing a more complex model that would improve differentiation among the various waterbody chemistries. The following tasks were completed to assess the feasibility of changing the modeling approach for the current report:

- Available water chemistry data (from 2016 to 2019) for the Peace River watershed and its tributaries were compiled and reviewed to characterize which elements could be available for use in the model: i) what elements were routinely collected in the waterbodies; ii) what elements are routinely detected in water chemistry; iii) what is the variability of elemental concentrations within a waterbody; and iv) what is the variability of elemental concentrations among the different waterbodies.
- Identified elements actually are detected and accumulate in the calcium-based otolith and fin ray structures using LA-ICP-MS analysis of previous fish structures collected in 2014-2018;
- Reviewed the literature to identify potential elements other researchers have determined to be successful and unsuccessful in predicting freshwater fish habitat;
- Assess linear relationship between water concentrations and tissue concentrations – a requirement for the calculation of water to fish tissue incorporation coefficients.

2.2. Results

2.2.1. Water Chemistry

A total of 40 elements have been measured in water samples collected from various waterbodies from 2016-2019 as part of Site C environmental monitoring. Of those, 24 elements show either considerable chemistry data missing for some key waterbodies, or the elements were consistently below detection limits. These elements include: antimony, arsenic, beryllium, bismuth, boron, cesium, chromium, cobalt, lead, mercury, molybdenum, phosphorus, potassium, rubidium, silver, sulfur, tellurium, thallium, thorium, tin, titanium, tungsten, vanadium, zirconium, and zinc). Other than zinc, these elements are excluded from further consideration in the laser ablation inductively coupled plasma mass spectrometer

(LA-ICP-MS) analysis and habitat modelling (zinc was maintained as it is useful for navigating temporal regions within the tissue structure – see Section 2.2.2). Therefore, the 15 elements (plus zinc) remaining under consideration include: aluminum, barium, cadmium, calcium, copper, iron, lithium, magnesium, manganese, nickel, selenium, silicon, sodium, strontium, and uranium.

2.2.2. Fin Ray and Otolith Microchemistry

Based on water chemistry, there are potentially 15 elements detected showing at least some variability among locations. It was important to determine if these elements could be detected in the two fish structures prior to moving forward with their inclusion in the analysis and modelling. Therefore, we analyzed three Bull Trout fin rays and three Rainbow Trout otoliths for these elements to determine if they can be detected in calcium-based structures. Silicon could not be analyzed, as the standard (NIST 612) used is predominantly made of this element and thus, measuring it could cause permanent damage to the mass spectrometer. Zinc was substituted as the 15th element, because it is a good marker for seasonal/annual growth in the fish structures (even though it is not readily detected in the water samples).

Eight of 15 elements were detected in both fin rays and otoliths, including barium, calcium, iron, magnesium, manganese, sodium, strontium, and zinc. Three of 15 elements were detected in otoliths, but not fin rays: copper, lithium, and nickel. Four of 15 elements were not detected in either fin rays or otoliths and include aluminum, cadmium, selenium, and uranium. Therefore, these last four elements will not be considered further for inclusion in the study.

2.2.3. Literature Review

The published literature and white paper reports were reviewed to identify elements other researchers have used successfully or unsuccessfully to differentiate fish recruitment sources. Most of the literature focused on using otoliths for recruitment predictions and migration monitoring, but the few studies that used fin rays provided similar conclusions for usefulness.

Otoliths and fin rays typically consist of calcium carbonate, organic matrix, and trace elements. These tissues grow incrementally, where seasonal variations in the ratio of calcium carbonate to organic matrix result in the growth bands (Neilson and Geen 1985; Rice et al. 1985; Hoie et al. 2008). The growing season (opaque zones) contains higher concentrations of the organic matrix, while the tissue deposited during the winter (translucent zones) are more mineral rich (Beckman and Wilson 1995).

Over 50 elements have been detected in fish otoliths (Hussy et al. 2020). Most studies assume that element incorporation into the fish structure will be directly proportional to concentrations in the environment (i.e., water), but this is an erroneous assumption. There are many other factors, such as diet, and physiological changes/aspects, that can also have significant influence on the structural chemistry. There are also three main ways that elements can be incorporated into an otolith or fin ray structure that should be considered: 1) randomly trapped in the matrix; 2) substituted for calcium in the matrix; or 3) bound to organic matrix constituents.

Most studies have focused on strontium (Sr) and barium (Ba) for recruitment source modelling. These elements have similar ionic radii to calcium and compete for calcium binding sites in the matrix. Water and otolith Sr:Ca have demonstrated strong positive correlations in many experimental studies (Hussy et al. 2020). Additionally, otolith Ba concentrations directly reflect water concentrations (Hussy et al. 2020). However, Bath et al. (2000) found that Ba saturation in the fish structure can occur, where an increased water concentration of Ba may not be directly proportional to the Ba concentration measured in the otolith or fin ray.

Another element that binds to the mineral fraction of the fish structure is manganese (Mn), possibly replacing calcium, so may also work as a good environmental tracer (Sturrock et al. 2015; Thorrold and Shuttleworth 2000; Dorval et al. 2007; Mohan et al. 2012). However, Friedrich and Halden (2010) and Limburg et al. (2015) suggest there may also be physiological influence in Mn accumulation in some species, evidenced by highest Mn in the core and decreasing concentrations towards the edge of the fish structure. Looking at previous analysis (TrichAnalytics 2020) where Mn was analyzed, but not reported, Rainbow Trout otoliths clearly show oscillations in Mn, with some indication there may be influence of age. Similar patterns were also observed in otoliths from Arctic Grayling and Bull Trout. Bull Trout fin ray concentrations and patterns of Mn appear less oscillatory and lower in concentration than otoliths, but visible.

There are conflicting reports for lithium (Li) and magnesium (Mg) as being useful for environmental tracing. These two elements were hypothesized as being trapped in the matrix only, and not directly related to environmental concentrations or physiological processes (Hussy et al. 2020). Alternatively, Wells et al. (2003), Marohn et al. (2009), Miller (2011), Bani et al. (2020) and Woodcock et al. (2012) report that otolith Mg is likely driven by physiological processes, but not water concentrations. In another scenario, Clarke and Telmer (2008) successfully used both Li and Mg (along with Ba, Sr, and

Mn) in their habitat modeling with Bull Trout otoliths, and Mg was also included in recruitment source modelling (along with Ba, Sr, and Sr isotope ratios) of Chinook Salmon using fin rays (Linley et al. 2016).

Transition metals and elements that readily bind to sulfur, such as copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn), will primarily bind to the organic fraction of the fish structures (Izzo et al. 2016; Thomas et al. 2017; Hussy et al. 2020), and are under more physiological control. They would, therefore, be less useful as environmental tracers. For example, Zn does not seem to be influenced by water concentration in the lab or the field (Ranaldi and Gagnon 2008), and neither does Cu (Hanson and Zdanowicz 1999; Milton et al. 2000). Rather, Zn is more reflective of seasonality of diet and growth, as opposed to water concentration (Halden et al. 2000; Halden and Friedrich 2008).

Finally, some elements are almost never included in fish recruitment modeling. Bani et al. (2020) suggest that phosphorus (P), potassium (K), and sodium (Na) are most likely associated with physiological factors, such as maturation and spawning activity, as opposed to environmental concentrations. Hussy et al. (2020) concluded similarly based on a review of various elemental accumulation patterns in fish otoliths.

2.2.4. Element Selection for LA-ICP-MS

Through the culmination of data and information gathered from water chemistry, fin ray and otolith analysis, and the literature we proposed to analyze for the following seven elements: calcium (Ca), barium (Ba), lithium (Li), magnesium (Mg), manganese (Mn), strontium (Sr), and zinc (Zn) (Table 1). However, all seven elements will not be used for modeling. Zinc and calcium will not be included in the model. For zinc, most water chemistry is below detection limits, so habitats cannot be differentiated based on this element. Additionally, zinc is not considered a good environmental tracer. Rather, zinc is a very useful element for measuring age (i.e., oscillates intra-annually with the growing/feeding season) and thereby, helps with identification of different temporal regions (e.g., core, first summer). Calcium will only be used to normalize the other elemental concentrations (using element:Ca ratios). Ba:Ca, Sr:Ca, Mn:Ca, Mg:Ca, and Li:Ca were successfully used in modelling habitat for Bull Trout (otoliths) in British Columbia (Clarke and Telmer 2008), and four of the elements (excluding lithium) were successful for modelling habitat use in catfish (fin rays; Avigliano et al. 2020) and American eels (otoliths; Benchetrit et al. 2015). Lithium was not detected in the three Bull Trout fin rays that we analyzed for this phase, so may only be useful for modeling otoliths. The rationale for inclusion or exclusion of elements is also provided in Table 1.

Table 1. Summary of rationale for including or not including elements in the analysis of fin rays and otoliths for habitat prediction modeling.

Element	Water Variability	Detectable in Rainbow Trout Otoliths	Detectable in Bull Trout Fin Rays	Literature Comments*	Reason for not including	Reason for including
Aluminum	yes	no	no	rarely used	not detected in tissue	n/a
Barium	yes	yes	yes	good environmental tracer	n/a	environmental tracer, detected, variable
Cadmium	yes	no	no	rarely used	not detected in tissue, low water concentrations	n/a
Calcium	yes	yes	yes	used to correct elements	n/a	used to correct elements
Copper	yes	yes	no	rarely used	not detected in fin rays, is not variable	n/a
Iron	only Kobes, Beatton differ from others	yes	yes	rarely used, physiological	haven't seen evidence of use as environmental tracer	n/a
Lithium	yes	yes	no	environment, but not often	n/a	could work as environmental tracer, not detected in fin rays though

Element	Water Variability	Detectable in Rainbow Trout Otoliths	Detectable in Bull Trout Fin Rays	Literature Comments*	Reason for not including	Reason for including
Magnesium	only Kobes, Lynx differ from others	yes	yes	environment and growth	n/a	could work as environmental tracer, limited variability in water and tissue
Manganese	yes	yes	yes	environment and growth	n/a	could work as environmental tracer, variability in water and tissue
Nickel	only Kobes, Beatton, Cache differ from others	yes	no	contaminant exposure, rarely used	not detected in fin rays, low water concentrations	n/a
Selenium	yes	no	no	contaminant exposure	not detected in tissue, low water concentrations	n/a
Sodium	yes	yes	yes	rarely used, physiological	not used as environmental tracer, only for reproductive status	n/a
Strontium	yes	yes	yes	good environmental tracer	n/a	environmental tracer, detected, variable
Uranium	yes	no	no	rarely used, physiological	not detected in tissue	n/a

Element	Water Variability	Detectable in Rainbow Trout Otoliths	Detectable in Bull Trout Fin Rays	Literature Comments*	Reason for not including	Reason for including
Zinc	no variability, not detected in most samples	yes	yes	diet and growth	n/a	ageing, identifying first summer, will not be used in model

* See reference section

2.2.5. Linearity Assessment for Water and Tissue Concentrations

The most common way to assess if the environment (i.e., water) is the main driver of fish tissue (i.e., otolith and fin ray) concentration/molar ratios is to statistically examine the relationship between the two matrices (Gibson-Reinemer et al. 2009). This relationship is required for modelling because the slope becomes the "incorporation coefficient" for use in the calculation of the Element:Ca ratio in unknown regions of the fish tissue (e.g., core).

As expected, Ba:Ca and Sr:Ca had significant linear relationships between water and both otoliths and fin rays (Examples: Rainbow Trout otolith Figure 1; Bull Trout fin ray Figure 2). There were no significant relationships with any other element, namely lithium (Example: Arctic Grayling otolith Figure 3), magnesium (Example: Arctic Grayling otolith Figure 4), or manganese (Example: Arctic Grayling otolith Figure 5; Rainbow Trout otolith Figure 6), suggesting for these fish species at least, these elemental concentrations are not driven by environmental concentrations, but rather diet or physiology or both. Therefore, they cannot be included in the recruitment modelling of these fish species.

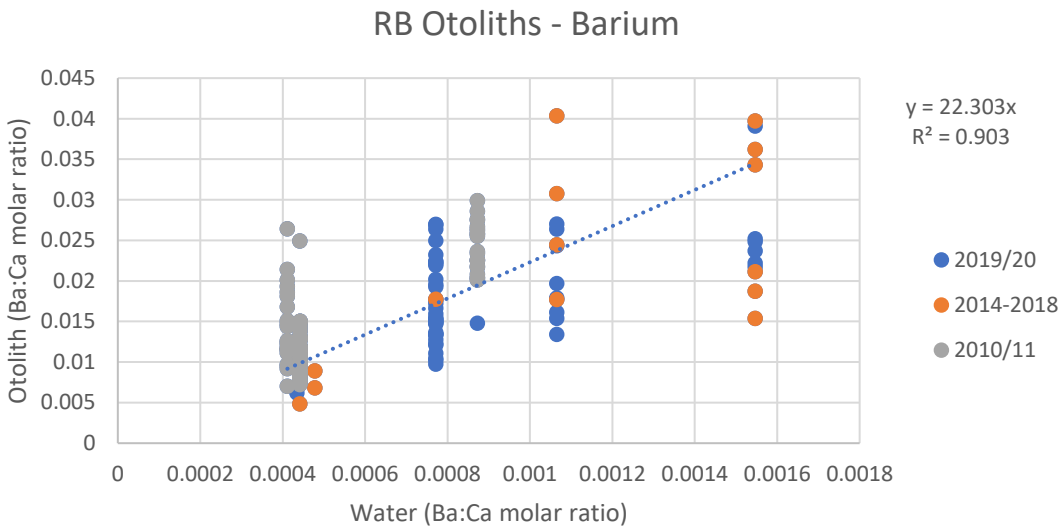

Figure 1. Linear relationship between water and Rainbow Trout fin ray Barium:Calcium (Ba:Ca).

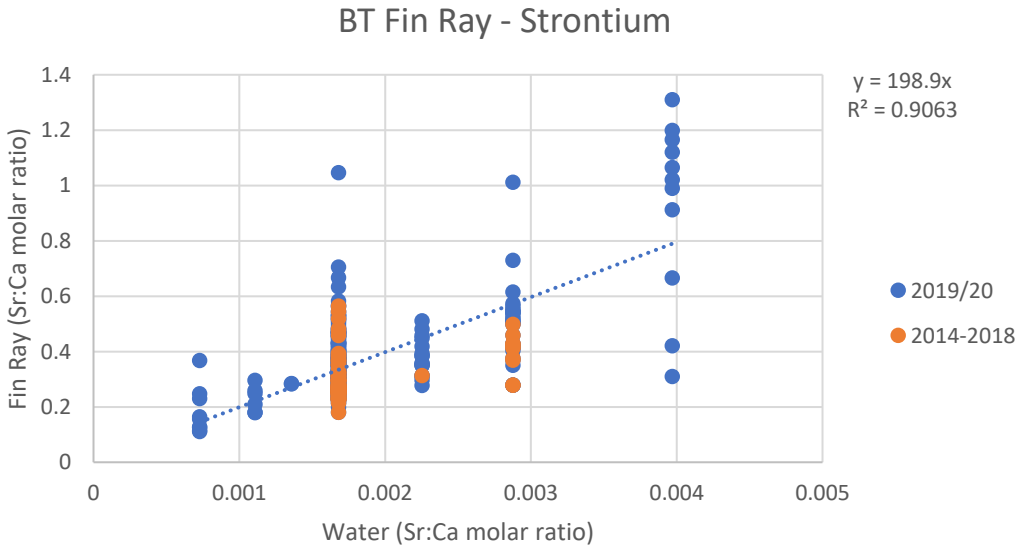

Figure 2. Linear relationship between water and Bull Trout fin ray Strontium:Calcium (Sr:Ca).

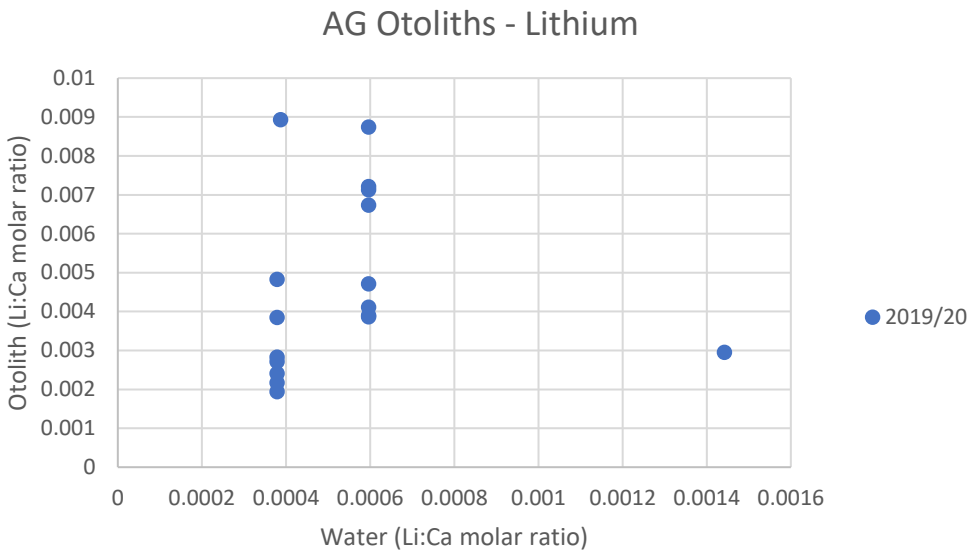

Figure 3. No relationship between water and otolith Lithium:Calcium (Li:Ca) in Arctic Grayling.

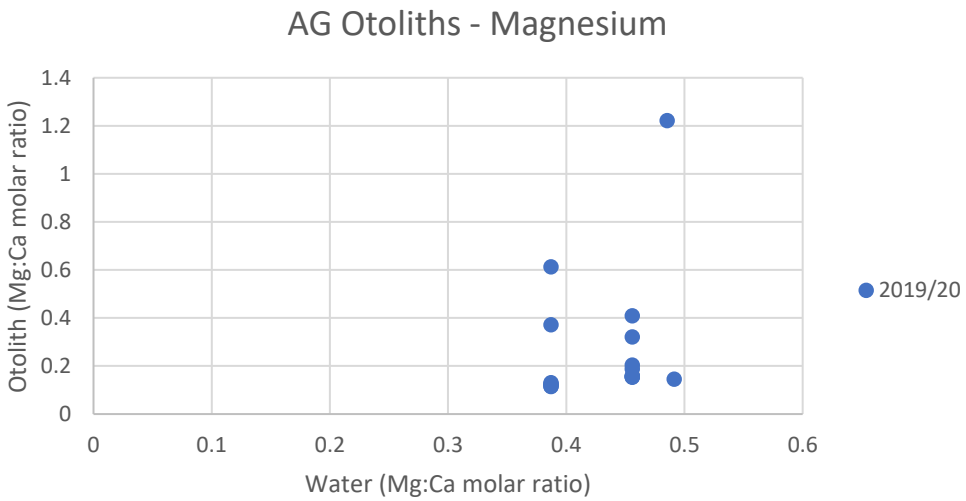

Figure 4. No relationship between water and otolith Magnesium:Calcium (Mg:Ca) in Arctic Grayling.

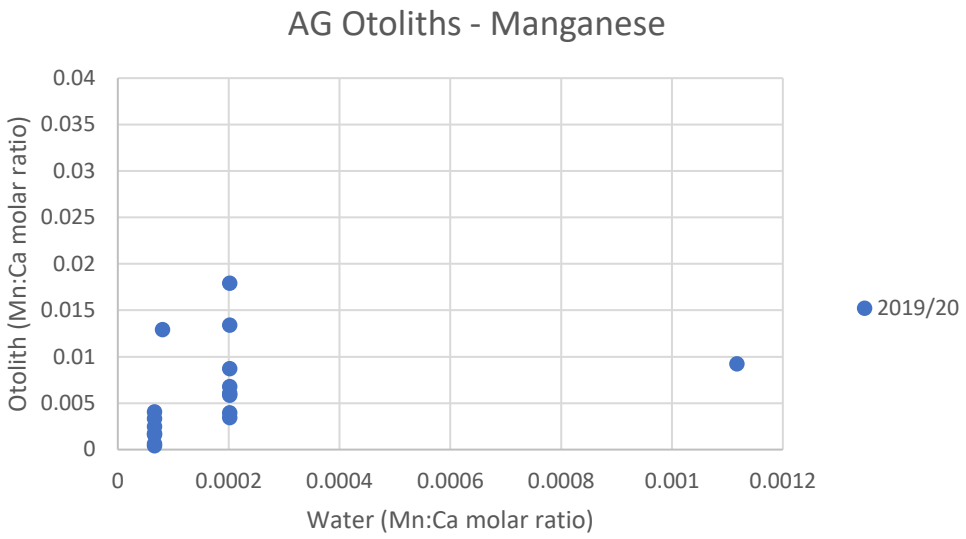

Figure 5. No relationship between water and otolith Manganese:Calcium (Mn:Ca) in Arctric Grayling.

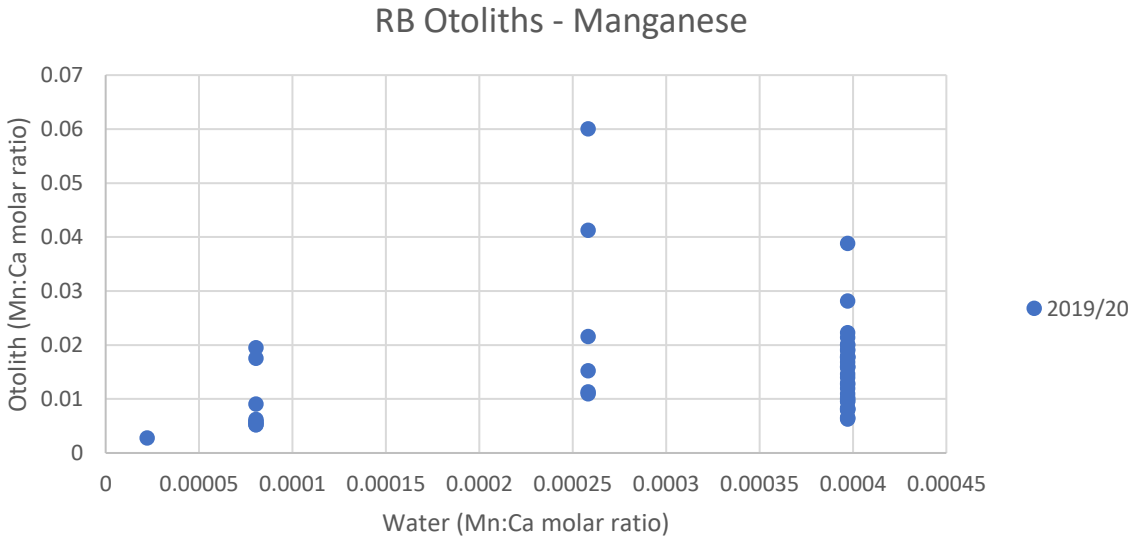

Figure 6. No relationship between water and otolith Manganese:Calcium (Mn:Ca) in Rainbow Trout.

2.3. Summary

While some of the 40 elements evaluated have been successfully used for other species and geographies, based on this assessment only Ba:Ca and Sr:Ca can be confidently used for the fish recruitment prediction models. The rationale for excluding the remaining elements is summarized below:

- 25 out of the 40 elements were consistently missing water chemistry data for at least one critical water body, or water concentrations were consistently below detection limits – these included (antimony, arsenic, beryllium, bismuth, boron, cesium, chromium, cobalt, lead, mercury, molybdenum, phosphorus, potassium, rubidium, silver, tellurium, thallium, thorium, tin, titanium, tungsten, vanadium, zinc, and zirconium).
- Silicon was removed from consideration, as the standard used for tissue microchemistry cannot be analyzed for silicon (would cause permanent damage to the mass spectrometer due high concentrations).
- Copper, lithium, and nickel were removed from consideration because they could only be detected in otoliths, but not fin rays, in the fish species considered here, and there was little to no variability in the otolith concentrations.
- Aluminum, cadmium, selenium, and uranium were removed from consideration because they were not detected in either otoliths or fin rays in the fish species considered here.
- Phosphorus, potassium, and sodium were removed from consideration because previous studies in the literature (e.g., Hussy et al. 2020) suggest these elemental concentrations in fish tissues are largely driven by physiological parameters, as opposed to water concentrations in their environment.
- Manganese, magnesium, and lithium were removed from consideration because there was no significant, positive, linear relationship between water and tissue, which is necessary to calculate incorporation coefficients.

3. METHODS

3.1. Surface Water Chemistry Data

BC Hydro provided surface water chemistry data from the Peace River watershed and its tributaries from 2008 to 2018 (Golder 2009; Clarke et al. 2011; Earthtone and Mainstream 2013). Water sample

strontium [Sr] and barium [Ba] concentrations were converted to molar ratios (Ba:Ca and Sr:Ca) using the associated calcium [Ca] concentration in the water sample via equations 1 and 2.

Sr:Ca (mol/mol) = ([Sr] / 87.62) / ([Ca] / 40.078) Eqn. 1

Ba:Ca (mol/mol) = ([Ba] / 137.327) / ([Ca] / 40.078) Eqn. 2

Molar ratios were used (instead of raw concentrations) as barium and strontium concentrations in otoliths and fin rays are proportionate to the Ba:Ca and Sr:Ca ratios in the water the fish reside (Clarke et al. 2007a).

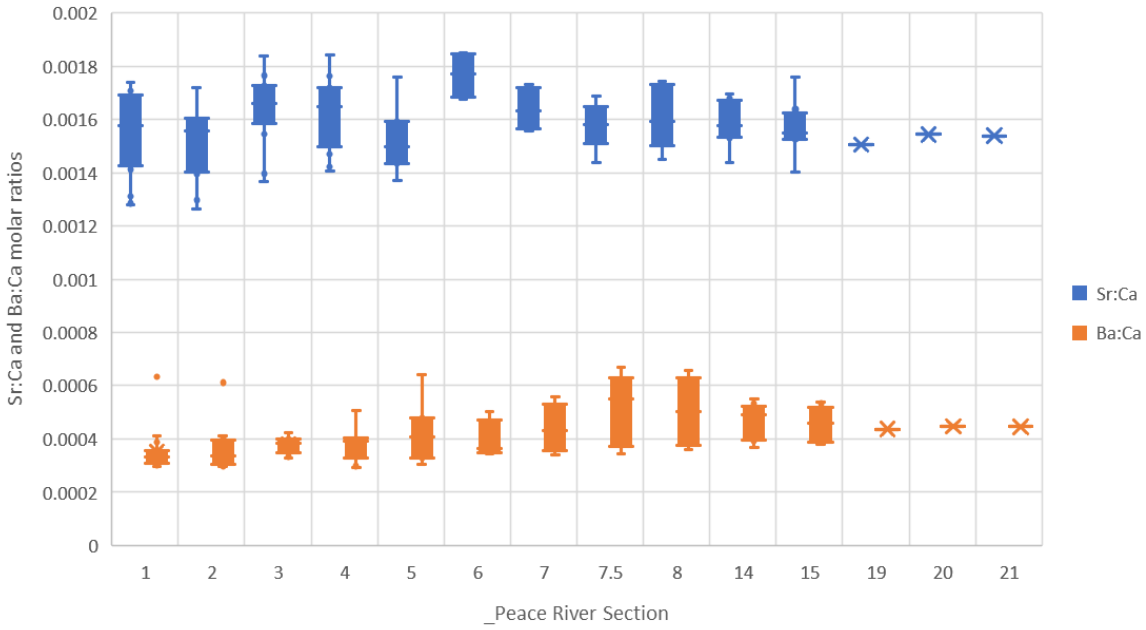

Figure 7. Variability in Sr:Ca and Ba:Ca molar ratios in various sections of the Peace River.

Surface water chemistry was first used to calculate incorporation coefficients from water to the fish tissues, i.e., fin rays and otoliths, of each species (see Section 3.4). Where multiple years of water chemistry data were available for a location, results demonstrated low interannual variability (Earthtone and Mainstream 2013; TrichAnalytics 2020). Therefore, results were averaged across years, within a location, to obtain one value per location for each elemental ratio (Table 2). In the Peace River, multiple locations within the river have been sampled; however, due to low variability

spatially (Figure 7, with an exception to Section 6 at the confluence with Pine River where Sr:Ca is slightly elevated compared to other sections) these data were also combined and averaged for the calculation of the incorporation coefficients. For other waterbodies that had multiple sampling locations, such as Cypress Creek which had spatial variability, the data had to be combined due to low sample number. It should be noted that the individual water chemistry data was only combined to calculate incorporation coefficients, but not for the actual recruitment model where individual water chemistry values were used.

Table 2. Water chemistry results (molar ratio) for each sampling location used to calculate fish tissue incorporation coefficients.

Location	Number of Water Samples	Ba:Ca (avg +/- 1SD)	Sr:Ca (avg +/- 1SD)
Peace R.	33	0.000442 ± 0.000115	0.00168 ± 0.00012
Dinosaur Res.	9	0.000443 ± 0.000134	0.00170 ± 0.000056
Maurice Creek	2	0.000872 ± 0.000159	0.001358 ± 0.000102
Farrell Cr.	6	0.000772 ± 0.000217	0.00122 ± 0.000129
Halfway R. (mainstem)	15	0.000411 ± 0.000057	0.00225 ± 0.000376
Chowade Cr.	3	0.000435 ± 0.000151	0.00397 ± 0.00186
Colt Cr.	4	0.00107 ± 0.000062	0.000727 ± 0.000127
Kobes Cr.	1	0.00155	0.000902
Cypress Cr.	8	0.00478 ± 0.000145	0.00288 ± 0.000735
Fiddes Cr.	2	0.000532 ± 0.000018	0.00225 ± 0.000179
Moberly R.	10	0.00126 ± 0.000241	0.00111 ± 0.000057
Beatton R.	4	0.000594 ± 0.000146	0.00169 ± 0.000023
Pine R. (mainstem)	5	0.000666 ± 0.000108	0.00152 ± 0.00015
Wolverine Cr.	1	0.000668	0.00168
Fellers Cr.	1	0.000327	0.00091
Callazon Cr.	1	0.000602	0.00211
Burnt Cr.	1	0.000663	0.00141

3.2. Fish Otolith and Fin Ray Samples

Fish were sampled by Golder Associates Ltd. (Golder) at various locations from 2019 to 2020 through monitoring programs Mon-1b, Task 2c (Site C Reservoir Tributaries Fish Population Indexing Survey) and Mon-2, Task 2a (Peace River Large Fish Indexing Survey) of the FAHMFP (BC Hydro 2015). All sampling reports are available here: https://sitecproject.com/document-library/environmental-and-socio-economic-plans-and-reports Fin rays were the preferred samples collected from captured fish, while otoliths were collected opportunistically from fish that succumbed to the sampling method employed. Sampling methods to remove the fish tissues followed Mackay et al. (1990) and are also briefly described in TrichAnalytics (2020).

Ninety-five otoliths and 348 fin rays were provided to TrichAnalytics for microchemistry analysis (Table 3). Samples were provided from the following species: Arctic Grayling, Bull Trout, and Rainbow Trout. Details of fish otolith and fin ray sample collection locations from 2019 and 2020, including field-measured fish length (fork length), are provided in Appendix A. Maps of capture locations are provided for each species in Section 4.

Table 3. Microchemistry sample composition by species and sample type for fish captured in 2019 and 2020.

Species	Scientific Name	Number of Otoliths	Number of Fin Rays
Arctic Grayling (AG)[(1)]	*Thymallus arcticus*	37	102
Bull Trout (BT)[(1)]	*Salvelinus confluentus*	8	223
Rainbow Trout (RB)[(1)]	*Oncorhynchus mykiss*	50	23
	Total	95	348

Note: (1) 28 AG, 6 BT, and 20 RB samples contain both an otolith and fin ray collected from the same fish.

3.3. Otolith and Fin Ray Microchemistry

Otoliths and fin rays were prepared for analysis by TrichAnalytics following similar methods to Clarke et al. (2007b) and TrichAnalytics (2020). Briefly, structures were sectioned using a sterilized razor blade or mini handsaw and embedded in epoxy (otoliths embedded sulcus-side up). The epoxy cured for over eight hours before further preparation. The samples were first polished with 320-grit adhesive-backed lapping paper close to the core and by 600-grit lapping paper to expose the core. Final sanding was

conducted using 1,200-grit lapping paper to remove micro-scratches and further polished using 0.24 µm diamond suspension spray on a polishing pad.

Otolith and fin ray microchemistry analyses were conducted by TrichAnalytics using an NWR-213 (New Wave Research Inc.) laser ablation (LA) instrument and an iCAP RQ series (ThermoFisher Scientific) inductively coupled mass spectrometer (ICP-MS). For otoliths, the laser ablation settings using a line scan were as follows: Power – 60%; Frequency – 20 Hz; Speed – 5 µm/s; Spot size – 30 µm. For fin rays, which were considerably smaller than the otoliths, the laser ablation settings were adjusted to the following: Power – 40%; Frequency – 20 Hz; Speed – 5 µm/s; Spot size – 5 µm. Line scans were plotted and run from the edge of each otolith and fin ray through the core, and to the other edge, where possible (some otoliths were received broken).

An external standard reference material SRM 612 (NIST 2012) was used to calibrate the concentration of the samples (otoliths and fin rays) for barium (Ba), strontium (Sr), and calcium (Ca). Calcium was used as an internal correction standard (40% for otoliths; 27% for fin rays; 8.5% for SRM 612). Elemental concentrations (mg/kg) in the otolith or fin ray samples were calculated using equation 3.

$$\text{Concentration ([Ba] or [Sr])} = [(\text{signal} - \text{background})/\text{sensitivity}] * (40\%/\text{Ca in sample \%}) \quad \text{Eqn. 3}$$

where "signal" is the counts per second (cps) of Ba or Sr in the sample; "background" is the cps of Ba or Sr prior to initiating the laser; "sensitivity" is the calibration slope for Ba or Sr as determined by the SRM 612 standard; 40% is the calcium content in an otolith (exchange this value for 27% for calculating concentrations of Ba or Sr in fin ray samples); and "Ca in sample (%)" is the calculated concentration of calcium in the sample. Data was collected through the ICP-MS using Qtegra™ software (ThermoFisher Scientific), Version 2.8.3170.309, and processed using R software (Version 4.0.0).

Otolith and fin ray barium and strontium concentrations were converted to micromoles (mmol) and corrected to calcium (converted to mol) to obtain Sr:Ca and Ba:Ca (mmol/mol) molar ratios using equations 4 and 5:

$$\text{Sr:Ca (mmol/mol)} = ([\text{Sr}] * 10^3 / 87.62) / (400{,}000 / 40.078) \quad \text{Eqn. 4}$$

$$\text{Ba:Ca (mmol/mol)} = ([\text{Ba}] * 10^3 / 137.327) / (400{,}000 / 40.078) \quad \text{Eqn. 5}$$

where 400,000 is the calcium correction for an otolith (exchange this value for 270,000 for calcium correction for a fin ray sample).

Through the line scan analysis from edge to core, the entire life history (e.g., natal habitat, migration) can be revealed and can span many years depending on the age of the fish. This information was used to create life history graphs to support the model predictions by providing a bigger picture of fish movement over time. However, for the purpose of this study, the main objective was to determine the recruitment source for each fish. Therefore, we focused on obtaining an average chemical signature in two regions (core and first summer). We also collected the average signature in the edge region to help with the calculation of incorporation coefficients and for use in the model. The three regions (Zymonas and McMahon 2009; Earthtone and Mainstream 2013; TrichAnalytics 2020), included:

- Edge – represents the waterbody at the time of fish capture (25 µm region)
- First summer – first peak of zinc (Zn) concentrations after the core in the otolith and the first opaque (dark) region after the core in fin rays before the first winter annulus. This region represents the first summer rearing location of the fish (25 µm region).
- Core – small region in the center of the otolith or fin ray, representing the maternal yolk incorporation into the juvenile otolith/fin ray structure and, hence, the waterbody occupied by the mother when spawning (20 µm region). This region is inferred to represent the natal stream/river that the fish recruited from (Earthtone and Mainstream 2013).

3.4. Determination of Incorporation Coefficients

The capture (edge) chemistry of the fin ray or otolith was compared to the average water chemistry (from Section 3.1, Table 2) at the capture location to derive incorporation coefficients for each element-species-structure combination using linear regression fitted through the origin. To improve the accuracy of incorporation coefficient from previous calculations on these fish species, capture chemistries from the current study (2019 and 2020) were integrated with capture chemistries from previous studies (from 2010 to 2011: Earthtone and Mainstream 2013; and from 2014 to 2018: TrichAnalytics 2020) (Table 4). By adding these samples, the number of locations increased for the fish tissue combination, thereby increasing the number of calibration points along the incorporation coefficient slope. Data from previous studies included:

- Arctic Grayling otoliths: n=157 (2010 to 2011)
- Rainbow Trout otoliths: n=112 (2010 to 2011) and n=15 (2014 to 2018).
- Bull Trout otoliths: n=57 (2010 to 2011) and n=11 (2014 to 2018).
- Bull Trout fin rays: n=141 (2014 to 2018)

Table 4. Otolith and Fin Ray Samples Used for the Calculation of the Incorporation Coefficients

Waterbody	Otoliths			Fin Rays		
	2010-2011	2014-2018	2019-2020	2010-2011	2014-2018	2019-2020
Peace R.	RB, AG, BT	RB, AG	AG, BT		BT	RB, AG, BT
Dinosaur Res.	RB					
Maurice R.	RB, BT		RB			RB, BT
Farrell Cr.		RB	RB, BT			RB
Halfway R.	RB, AG, BT					
Chowade Cr.		BT	RB, BT			BT
Colt Cr.		RB	RB, AG			RB, AG, BT
Kobes Cr.		RB	RB			RB
Cypress R.		RB, BT	BT		BT	BT
Fiddes Cr.		BT	BT		BT	BT
Moberly R.	AG	AG	AG			AG
Beatton R.			AG			AG
Pine R.	AG, BT					
Wolverine Cr.	BT					
Fellers Cr.	BT					
Callazon Cr.	BT					
Burnt R.	BT					

The incorporation coefficients were then used to correct other regions/concentrations of the otolith or fin ray for model development/training, validation, predictions, and life-history graphs.

3.5. Stream Recruitment Prediction

Stream recruitment prediction was completed through formal statistical modelling informed by insight gleaned from life-history graphs.

3.5.1. Statistical Modelling

3.5.1.1. Fish Sample Groups

In combination with available water chemistry, the 2019 to 2020 otoliths and fin ray samples were used to develop stream-occupancy models, which were then applied to unknown origin data to predict stream occupancy. Specifically, capture chemistry data from all 2019 to 2020 otoliths and fin ray samples were used to train the stream models ('training data"; see Section 3.5.1.4); thereafter, these samples were divided into two groups: 1) known recruitment source ("validation data"; see Section 3.5.1.5); and 2) unknown recruitment source ("test data"; see Section 3.5.1.6).

Validation samples were used to assess the prediction accuracy of the model. For Arctic Grayling, the validation samples included 28 otoliths (Appendix Table B1) and 23 fin rays (Appendix Table B2) and only included fish captured in the Moberly River (regardless of size) and those fish samples with fork length <100 mm when captured in a tributary (D. Ford, pers. comm.). For Bull Trout, the validation samples included six otoliths (Appendix Table B3) and 11 fin rays (Appendix Table B4), and only included fish with age-1 fork lengths less than those provided in Table 5. For Rainbow Trout, the validation samples included 36 otoliths (Appendix Table B5) and 15 fin rays (Appendix Table B6), and samples included those with age-1 fork lengths less than those provided in Table 6.

Table 5. The maximum length (mm) of an age-1 Bull Trout in various streams of the Halfway River watershed in 2019 and 2020.

Year	Stream		
	Chowade River	Cypress Creek	Fiddes Creek
2019	120	122	89
2020	123	114	121

Table 6. The maximum length (mm) of an age-1 Rainbow Trout in various waterbodies in 2019 and 2020 (values are approximate as there was overlap between age-1 and age-2 fish – D. Ford, pers. comm.).

Year	Stream			
	Colt Creek	Kobes Creek	Farrell Creek	Maurice Creek
2019	114	118	120	-
2020	111	116	108	126

3.5.1.2. Modelling Approach

Nested Quadratic Discriminant Analysis (nQDA) was used to predict stream recruitment sources based on Ba:Ca and Sr:Ca molar ratios. Quadratic structures were used (in preference to linear) owing to the persistent heterogeneity of variances across streams (James et al., 2013). Modelling was completed separately for each species (Arctic Grayling, Bull Trout, and Rainbow Trout) and tissue type (otolith and fin ray). Stream recruitment models were developed using training and validation data. These models were ultimately used to predict stream recruitment for fish samples with unknown origins (test data). Diagnostic statistics were calculated during model training and validation to understand the predictive

power of the nQDA framework, explore patterns of inaccurate predictions (e.g., relationships between observed and predicted streams), and to investigate model overfitting. Life-history graphs were reviewed to depict model predictions using the natal region only in the context of an individual's entire lifetime chemistry dynamics.

3.5.1.3. Model Structure

The nested aspect of the modelling reflects two levels of recruitment prediction: watershed (tier 1), and stream within watershed (tier 2; Figure 8). For each species-tissue combination, QDA models were developed to first predict watershed recruitment; watershed-specific stream QDA models were also developed for watersheds represented by more than one stream (i.e., Beatton and Halfway rivers for Arctic Grayling; and Halfway River for Rainbow Trout and Bull Trout).

Stream recruitment modelling proceeded in the following stepwise manner:

1) Predict watershed recruitment using the relevant species-tissue watershed model (tier 1)
2) Predicting steam occupancy (tier 2) using one of two approaches depending on the watershed predicted in step '1)':
    a. when the predicted watershed is represented in the data by a single stream (e.g., Maurice Creek), the predicted stream was recorded as the predicted watershed
    b. when the predicted watershed was represented by multiple streams (e.g., Halfway River), stream recruitment was predicted using the relevant watershed-specific stream QDA model.
3) Compare model recruitment prediction with life history graph (tier 3) to provide confirmation or further insight to support a final recruitment prediction (see Section 3.5.3).

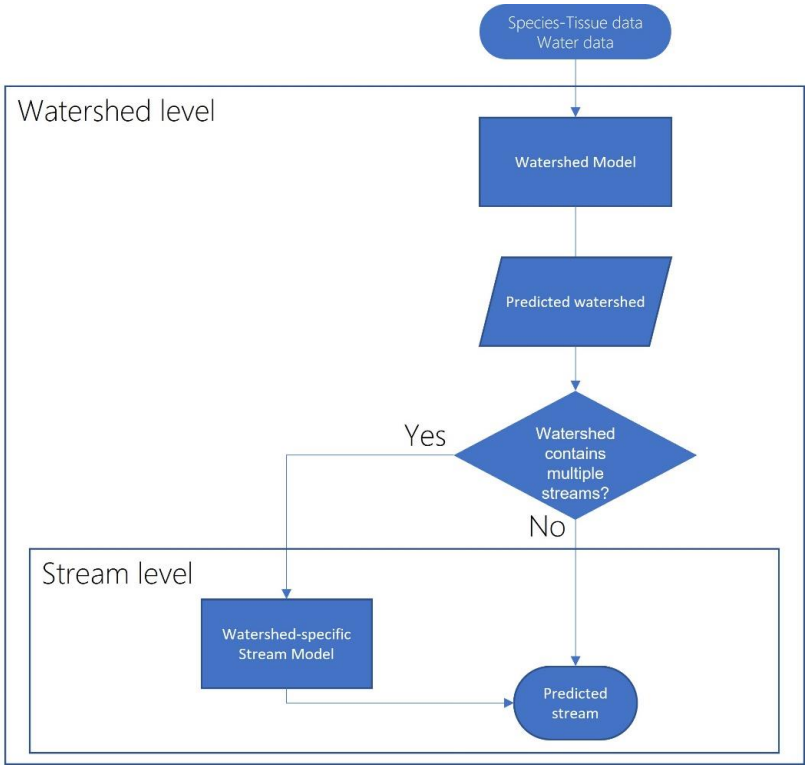

Figure 8. Schematic representation of the nQDA modelling structure used to predict stream recruitment.

Since this process was completed separately for each species-tissue combination, six watershed-level models (three species; two tissue types), and eight watershed-specific stream-level models (Beatton and Halfway River watersheds for Arctic Grayling otoliths and fin ray; Halfway River watershed for both trout species' otoliths and fin rays) were developed.

3.5.1.4. Model Training

Models were trained using molar ratios calculated for water samples and the relevant species and tissue samples (i.e., capture chemistry). The following information for each model was obtained:

1) Probability of occupancy for each watershed
2) Predicted watershed occupancy, i.e., the watershed with the highest probability of occupancy (obtained in step 1)
3) For samples predicted to occupy a multi-stream watershed: probability of occupancy of each stream within the predicted watershed

4) Predicted stream occupancy. For samples predicted to occupy a single-stream watershed, the predicted stream = the predicted watershed; for those predicted to occupy a multi-stream watershed, stream occupancy was deemed to be the stream within that watershed with the highest probability of occupancy (obtained in step 3).

Prediction accuracy and confusion matrices were used to understand the predictive power of the nQDA framework with respect to the training data. Accuracy was calculated as the rate of correct predictions (i.e., the proportion of streams correctly predicted) for each species and tissue, separately for tissue and water data. Patterns of inaccurate predictions were explored using confusion matrices (again, separately for tissue and water data), which show the pairwise frequencies of predicted vs observed stream occupancy. Here, a model with 100% accuracy would show all frequencies along the matrix diagonal, revealing a perfect match between predicted and observed streams. In contrast, a model framework with poor prediction accuracy would show frequencies scattered throughout the matrix. More realistic, intermediate conditions arise when some sites are accurately predicted while some are repeatedly confused with a subset of other sites. Exploration of these patterns helps to explain underlying mechanisms driving model inaccuracy (such as a similarity in underlying chemistry in different streams), and the acceptability of some inaccuracies (e.g., within-watershed inaccuracies may be more tolerable than cross-watershed inaccuracies).

3.5.1.5. Model Validation

The nQDA models were validated using the validation data described in Section 3.5.1.1 and Appendix B. For each record, median Ba:Ca and Sr:Ca molar ratios were estimated across sample sections assigned as representing the (1) natal and (2) first-summer periods. These values were used to predict natal and first-summer stream recruitment source through the nQDA framework described above, and predicted recruitment source was compared to the known source for each individual fish. Model overfitting (that is, when a model accurately predicts the data used to train it, but poorly predicts new data) was inferred by calculating the prediction accuracy rate for these validation data, separately for each species-tissue model, and comparing results to those obtained for the training data. A large reduction in prediction accuracy from the training to validation data would indicate model overfitting; similar rates would indicate the model is able to predict new data as well as the data used to train it (and, hence, is not overfitted). Evidence of model overfitting is highlighted and explored with insight gleaned from life-history graphs.

3.5.2. Stream Recruitment Predictions for Test Data

The nQDA models were used to predict recruitment sources in test data (i.e., individuals with unknown natal origins), and modified where appropriate based on insight obtained from life-history graphs. Median natal and first summer molar ratio signatures were estimated following the same methods used for validation data and used to predict stream occupancy during these early life stages. Life-history plots were generated for test data to visualize their lifetime movement and contextualize their occupancy of natal streams relative to their capture location (see Section 3.5.3). As these three fish species spend their first summer in their natal streams, only the predicted natal habitat is reported as the recruitment source.

3.5.2.1. Model Limitations

It should be noted that there are limitations in the model predictions, other than the uncertainties described above as measured in the confusion matrix. The most notable limitation is the spatial scale over which this study occurs. The species studied have the potential to move over long distances in the Peace River and tributaries, including hundreds of kilometers in the Peace River, and on the order of several hundred to thousands of kilometers of tributary streams. Given the large number of streams over this spatial scale, it is possible that water chemistry overlaps among streams that are sampled or not sampled for water chemistry. There is also the potential for fish to recruit to the Peace River from locations upstream of Peace Canyon Dam. While most tributaries between Peace Canyon and the dam site that have known recruitment for these species have been sampled for structures and water chemistry, not all locations have been sampled. For example, Needham Creek in the Halfway River watershed is considered a significant source of Bull Trout to the Peace River, with the second or third highest redd counts among the six Halfway River tributaries monitored (https://sitecproject.com/sites/default/files/Mon-1b-Task-2b-Peace-River-Bull-Trout-Spawning-Assessment-2020-Annual-Report.pdf). However, there is only one water sample available from this creek (from 2012), and to date, no Bull Trout samples captured from Needham Creek are available for analysis. Given all of these factors, it is possible that recruitment locations have been 'missed' (i.e., not sampled for structures or water chemistry) or mis-assigned. If a fish had an origin from one of these locations and we sampled its structure, the model may either mis-assign the fish to another location included in the model, or classify it as 'unknown'.

Other limitations may include 1) the under-representation of some waterbodies due to low numbers of data for inclusion in the model, 2) spatial or temporal heterogeneity of water chemistry that is

uncharacterized within a waterbody due to low sample number, 3) variability among fish individuals of the same species in the uptake and accretion of barium or strontium into their bony structures, and 4) as discussed previously, overlap in barium and/or strontium chemical signatures among potential recruitment sources.

As the model evolves over time with increased sample availability and increased representation of possible recruitment sources, some of these limitations may be reduced.

3.5.3. Life-history Graphs

3.5.3.1. Graph Structure

Life-history graphs were developed for each fish (separately for otoliths and fin rays) to contextualize natal stream predictions. The approach here is adapted from Earthtone and Mainstream (2013), Clarke et al. (2015) and TrichAnalytics (2020). In this report graphs depict smoothed changes in consecutive Ba:Ca and Sr:Ca molar ratios over an individual fish's lifetime, superimposed over (1) 95% ellipses of molar ratios for each stream from the model-training data, and (2) points showing all natal molar ratios from validation samples of the same species and tissue type. Training-data ellipses and validation natal-data points were colour coded by site. The overlap of the individual's smoothed molar ratios with these ellipses and points provides a visual depiction of stream occupancy over time and, hence, a geospatial "life history". As conducted previously, median molar ratios for natal, first summer, and capture periods were added as points to facilitate visual inferences of stream occupancy during these specific periods within the context of the individual's broader life history.

3.5.3.2. Interpretation

Life history graphs were used for two purposes: 1) to visualize migration to and from different waterbodies for each individual fish; and 2) to provide a third tier of assessment for fish recruitment predictions. Life history graphs are used to support or not support the model predictions (which use quadratic discriminant function analysis – see Section 3.5.2), as the model itself only predicts based on a very small (4 second or 20 µm) analysis of the core region to predict natal recruitment, whereas the life history graph includes all data, including the natal region used in the model. Additionally, there are a few considerations when using the life history graphs to help further elucidate the model predictions, and include some of the following:

- proximity or occurrence within stream ellipses,
- the variability in the life history chemistry over time (e.g., highly variability/movement or stable),

- the similarity or difference in natal chemistry relative to capture chemistry, and
- migration (or not) from a watershed from place of capture (e.g., if the fish left the watershed it was captured in, it would have to have occupied the Peace River at one point in its life).

4. RESULTS AND DISCUSSION

4.1. Incorporation Coefficients

The Ba:Ca and Sr:Ca incorporation coefficients for each species and tissue are provided in Table 7 with figures provided in Appendix C. Arctic Grayling otolith coefficients were similar to what has been previously reported at 0.043 (Ba:Ca) and 0.402 (Sr:Ca) (Earthtone and Mainstream 2013), 0.035 (Ba:Sr) and 0.371 (Sr:Ca)(TrichAnalytics 2020), and 0.0484 (Ba:Ca) and 0.346 (Sr:Ca) (Clarke et al. 2007b). This is the first report that has calculated Arctic Grayling fin ray incorporation coefficients for this monitoring program. Appendix Figures C1 (otoliths) and C2 (fin rays) highlight the derivation of the Arctic Grayling incorporation coefficients.

Table 7. Incorporation coefficients from water to otolith or water to fin ray for each fish species.

Species	Tissue	Ba:Ca	Sr:Ca
Arctic Grayling	Otolith	0.0406	0.359
	Fin ray	0.106	0.318
Bull Trout	Otolith	0.00990	0.247
	Fin ray	0.0301	0.199
Rainbow Trout	Otolith	0.0223	0.210
	Fin ray	0.0762	0.226

Bull Trout otolith incorporation coefficients (Table 7) were similar to Earthtone and Mainstream (2013) at 0.014 (Ba:Ca) and 0.287 (Sr:Ca), and with TrichAnalytics (2020) Ba:Ca at 0.010. Clarke and Telmer (2008) reported 0.0155 (Ba:Ca) and 0.296 (Sr:Ca) for Bull Trout otoliths. Fin ray coefficients were similar with TrichAnalytics (2020) at 0.023 (Ba:Ca) and 0.163 (Sr:Ca). Appendix Figures C3 (otoliths) and C4 (fin rays) highlight the derivation of the Bull Trout incorporation coefficients.

Rainbow Trout otolith incorporation coefficients were similar to what has been previously reported (Earthtone and Mainstream 2013) at 0.026 (Ba:Ca) and 0.250 (Sr:Ca), as well as TrichAnalytics (2020) at

0.180 (Sr:Ca). The TrichAnalytics (2020) Ba:Ca coefficient was considerably lower at 0.012, likely due to the low sample number used for the previous calculation. This is the first report that has calculated Rainbow Trout fin ray incorporation coefficient for this monitoring program. Appendix Figures C5 (otoliths) and C6 (fin rays) highlight the derivation of the Rainbow Trout incorporation coefficients.

The addition of more fish samples (i.e., capture chemistries on tissue) from more locations (i.e., water chemistries) in the future would facilitate further fine-tuning of incorporation coefficient calculations, particularly for species-tissue combinations where sample sizes and/or number different locations are currently low, such as for Arctic Grayling (both otoliths and fin rays) and Rainbow Trout (fin rays).

4.2. Arctic Grayling Stream Recruitment

4.2.1. Otoliths

In total, 37 Arctic Grayling otoliths were collected in 2019 and 2020 (Table 8 and Figure 9.):

Table 8. Number and fork length of Arctic Grayling fish captured with otolith samples.

Capture Watershed	Capture Site	Number of Otoliths Collected	Fork Length (mm) range
Beatton River	Beatton River mainstem	1	190
	Bratland Creek	14	54 - 96
	Laprise Creek	5	51 - 60
Halfway River	Colt Creek	1	50
Moberly River	Moberly River mainstem	8	52 - 170
Peace River	Upstream of Project	3	337 - 362
	Downstream of Project	5	99 - 206

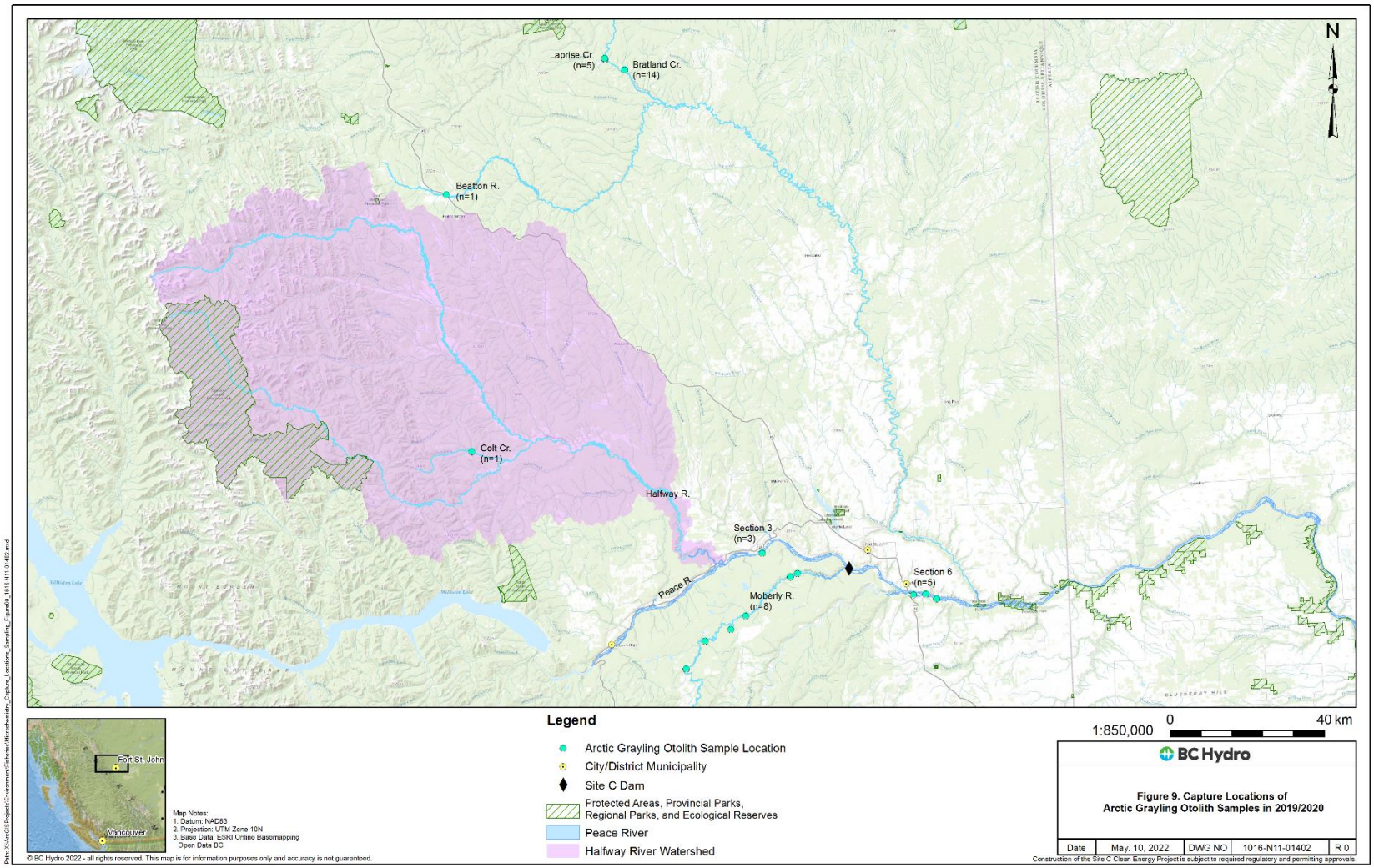
Laprise Cr.
(n=5)
Bratland Cr.
(n=14)
Beatton R.
(n=1)
Colt Cr.
(n=1)
Halfway R.
Section 3
(n=3)
Section 6
(n=5)
Peace R.
Moberly R.
(n=8)
N
Legend
Arctic Grayling Otolith Sample Location
City/District Municipality
Site C Dam
Protected Areas, Provincial Parks, Regional Parks, and Ecological Reserves
Peace River
Halfway River Watershed
1:850,000
0
40 km
Fort St. John
Vancouver
Map Notes:
1. Datum: NAD83
2. Projection: UTM Zone 10N
3. Base Data: ESRI Online Basemapping
Open Data BC
© BC Hydro 2022 - all rights reserved. This map is for information purposes only and accuracy is not guaranteed.
BC Hydro
Figure 9. Capture Locations of
Arctic Grayling Otolith Samples in 2019/2020
Date
May. 10, 2022
DWG NO
1016-N11-01402
R 0
Construction of the Site C Clean Energy Project is subject to required regulatory and permitting approvals.

Figure 9.

4.2.1.1. Arctic Grayling Otolith Model Performance

Water chemistry results (n=48) from seven waterbodies, and otolith capture chemistries (n=77) from seven waterbodies, for a total of 125 chemistries from 9 waterbodies (i.e., prior locations[2]), were used to train the Arctic Grayling recruitment source model (Figure 10). Further, the subset of 28 AG otoliths used for validation purposes were input into the model to test prediction accuracy and over-fitting, and visually assessed using the life-history graphs for verification.

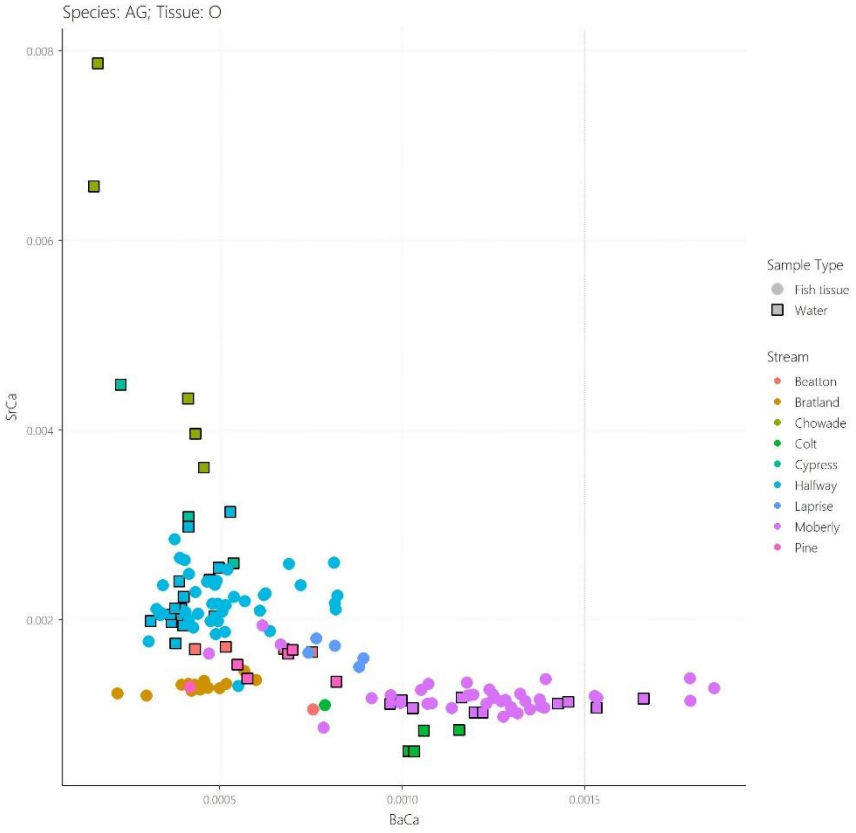

Figure 10. Water and otolith capture chemistries used in the Arctic Grayling otolith model.

The Arctic Grayling otolith model had varied predictive power across streams, with particularly high accuracy for watersheds represented by a relatively large sample size (>10 samples; Table 9). Model

[2] Prior location is defined in this report as a waterbody that is known to be (at least, potentially) used by a fish species (e.g., known natal habitat, recruiting source, previous capture location).

validation was limited by the geographic spread of Arctic Grayling otoliths; however, general comparisons of stream-specific accuracy rates for training and validation data indicate that the nQDA model performed similarly for both (training and validation) data. Validation prediction accuracy was unexpectedly low, even at the watershed level (tier 1), for natal regions for fish from Bratland and Laprise creeks in the Beatton River watershed. Of the 11 Bratland Creek natal samples that were misclassified at the watershed level, two were classified as being from Halfway River, and nine from Moberly River (Table 10). Similarly, the three misclassified Laprise Creek samples were predicted to have come from the Moberly River watershed. These results could indicate overfitting of the nQDA watershed-level model for the Beatton River watershed, possibly driven by an overlap in otolith chemistry between Arctic Grayling fry from Beatton and Moberly River watersheds that was not reflected in the training data.

Table 9. Prediction Accuracy (% correctly classified stream and watershed) and sample sizes for Arctic Grayling otoliths in training data, and validation data.

Observed Occupancy		Training Data						Validation Data				
		Water			Tissue			Natal		1st Summer		
		% correct		n	% correct		n	% correct		% correct		n
Watershed	Stream	Stream	Watershed		Stream	Watershed		Stream	Watershed	Stream	Watershed	
Halfway	Halfway	100	100	13	97	100	39					0
	Colt	75	75	4	0	0	1	0	0	0	0	1
	Chowade	100	100	5			0					0
	Cypress	42	100	7			0					0
Moberly	Moberly	100	100	10	100	100	34	100	100	100	100	8
Pine	Pine	0	0	5	50	50	2					0
Beatton	Beatton	25	50	4	0	0	1					0
	Bratland			0	100	100	12	14	21	50	85	14
	Laprise			0	80	80	5	20	40	100	100	5
	Overall	73	83	48	95	96	94	39	46	71	89	28

Note: Grey cells give sample sizes for water and tissue training data, and validation data.

Table 10. Confusion matrix for AG otoliths (correct prediction in gray cell) – capture locations shown in columns, predicted occupancy shown in rows. Number outside bracket is predicted natal, and number inside bracket is predicted first summer.

Predicted Locations		Capture Locations			
Watershed	Stream	Halfway R.	Moberly R.	Beatton R.	
		Colt Ck.		Bratland Ck.	Laprise Ck.
Halfway R.	Colt Ck.			1 (1)	
	Chowade R.			1 (1)	
Moberly R.			8 (8)	9 (0)	3 (0)
Beatton R.	Bratland Ck.			2 (7)	
	Laprise Ck.				1 (5)
	Mainstem	1 (1)		1 (5)	1 (0)
Total		1	8	14	5

Colt Creek had low prediction accuracy in both the training data and validation data, where the fish was predicted to have recruited from Beatton River mainstem. It is notable that Colt Creek had the lowest sample size (n=5) in the training data (and comprised of water as opposed to tissue chemistry). Hence, the model was built on relatively limited data for this site, which may have contributed to the misclassification of the Colt Creek otolith validation sample.

Life-history graphs for all Arctic Grayling otolith validation data can be found in Appendix E, with representation from Bratland Creek, Laprise Creek and Moberly Creek below. The fish captured in Bratland Creek (AG# 1406) was also correctly predicted to have recruited from Bratland Creek, and the life history indicates a tight, low variability in otolith chemistry (Figure 11). However, AG# 1411, which was also captured in Bratland Creek, was incorrectly predicted to have recruited from Moberly River. The life-history graph for this fish highlights where its natal prediction clearly overlapped with the Moberly River ellipse, suggesting not all variability in Bratland Creek was captured in the training data, which would have led to overfitting for this site and potentially explaining the model misclassification (Figure 12). Additionally, the spread of Bratland Creek natal predictions for the validation fish (indicated in Figure 12 by the dark blue dots) relative to the dark blue ellipse representing the Bratland Creek training data, support the inference of model overfitting for this site.

The fish, AG# 1283, captured in Laprise Creek was correctly predicted to have recruited from Laprise Creek, and the life history indicates a tight, low variability in otolith chemistry (Figure 13). However, AG# 1229, which was also captured in Laprise Creek, was incorrectly predicted to have recruited from Moberly River. Like some of the Bratland Creek captured fish, the life-history graph for this Arctic

Grayling shows overfitting for Laprise Creek (light orange points and ellipse), and a clearer overlap with the Moberly River training data (light purple ellipse) than the Laprise Creek training data (Figure 14).

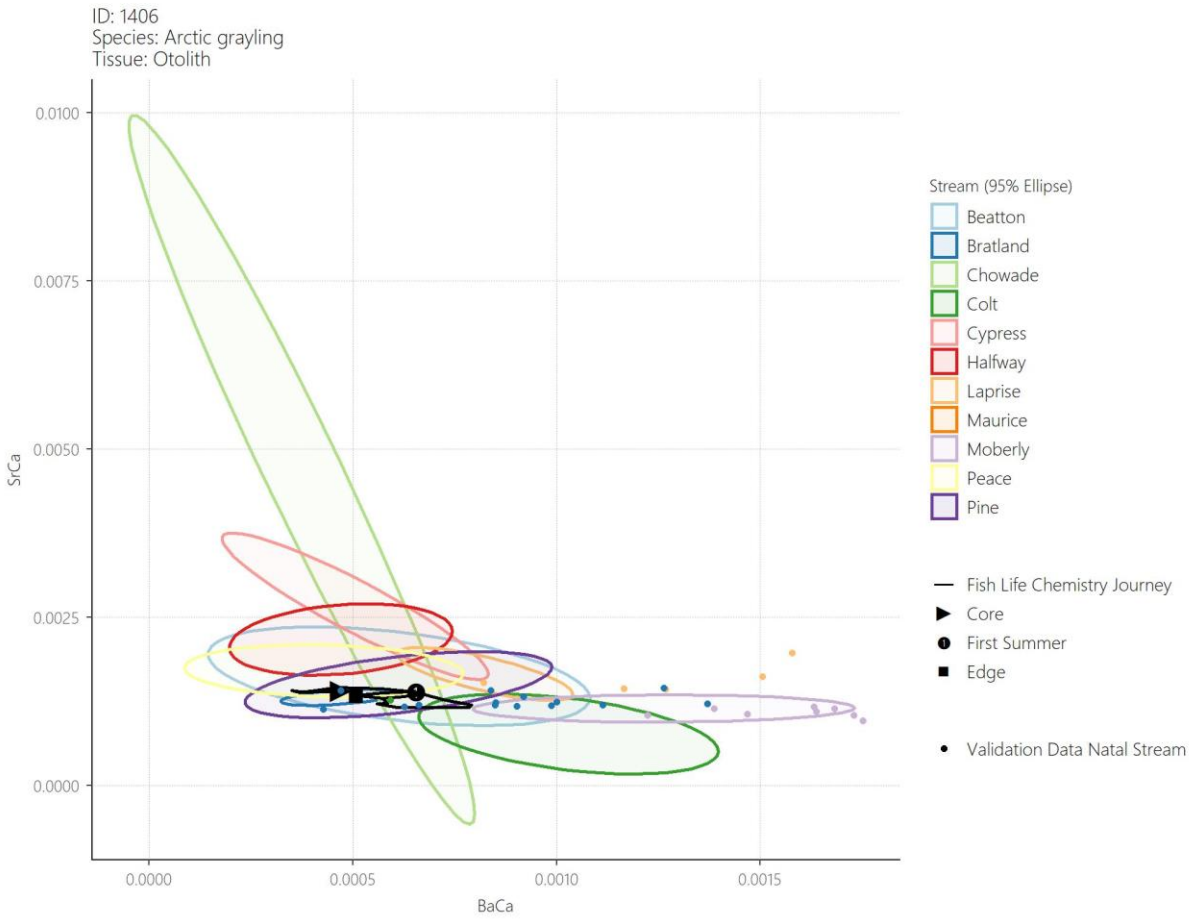

Figure 11. Representative life-history chemistry used for model validation for Bratland Creek (AG#1406, 93 mm).

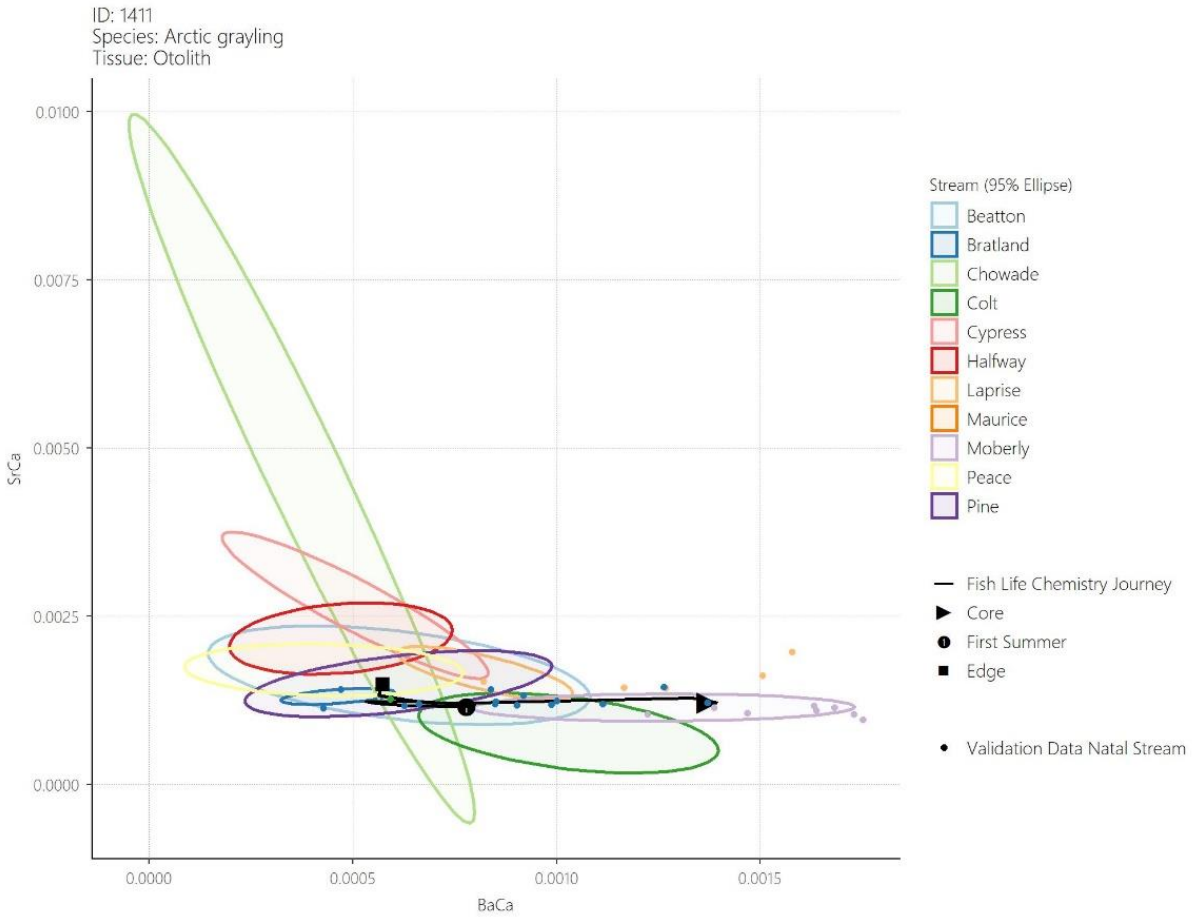

Figure 12. AG#1411 (65 mm) captured in Bratland Creek was incorrectly predicted to have recruited from Moberly River due to model overfitting. The life history graph suggests the natal region is aligned with Bratland Creek validation data (dark blue dots).

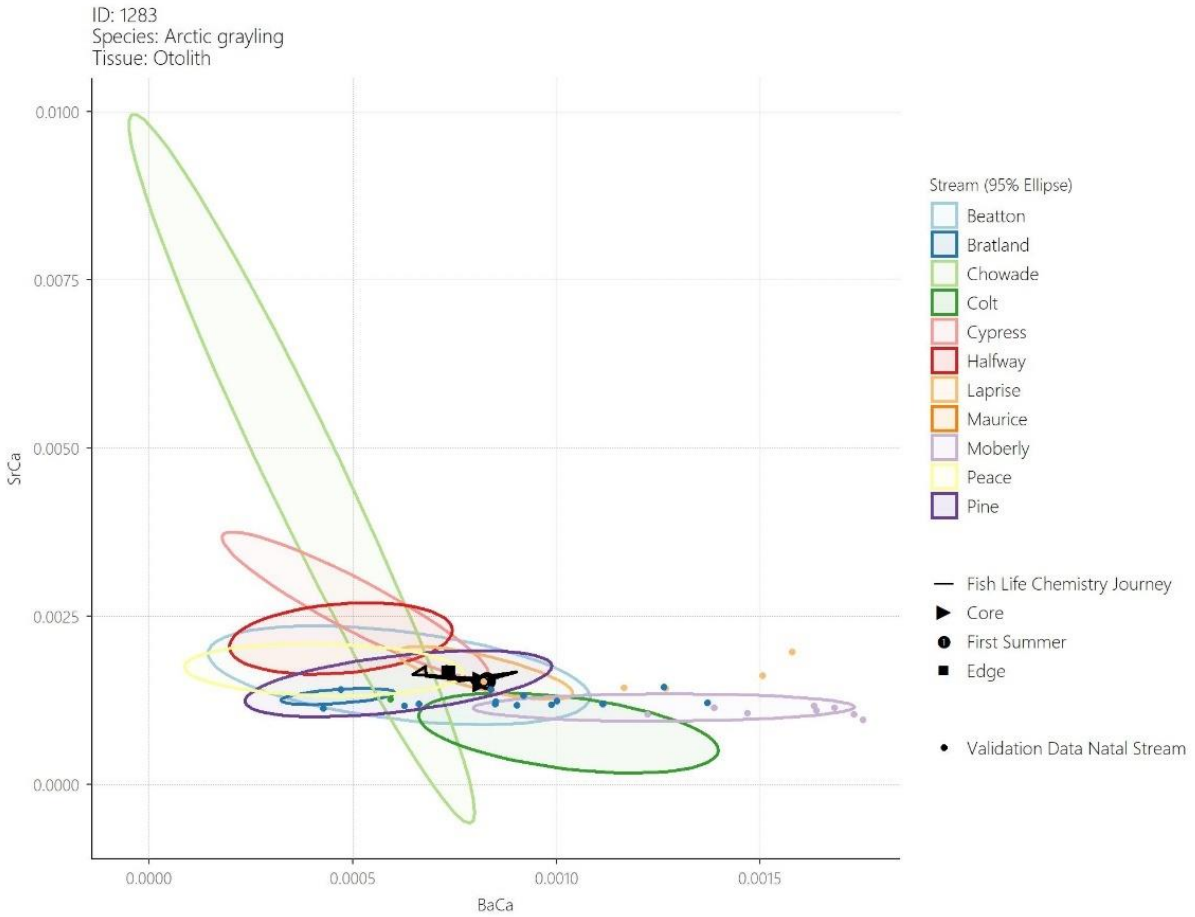

Figure 13. Representative life-history chemistry used for model validation for Laprise Creek (AG#1283, 56 mm).

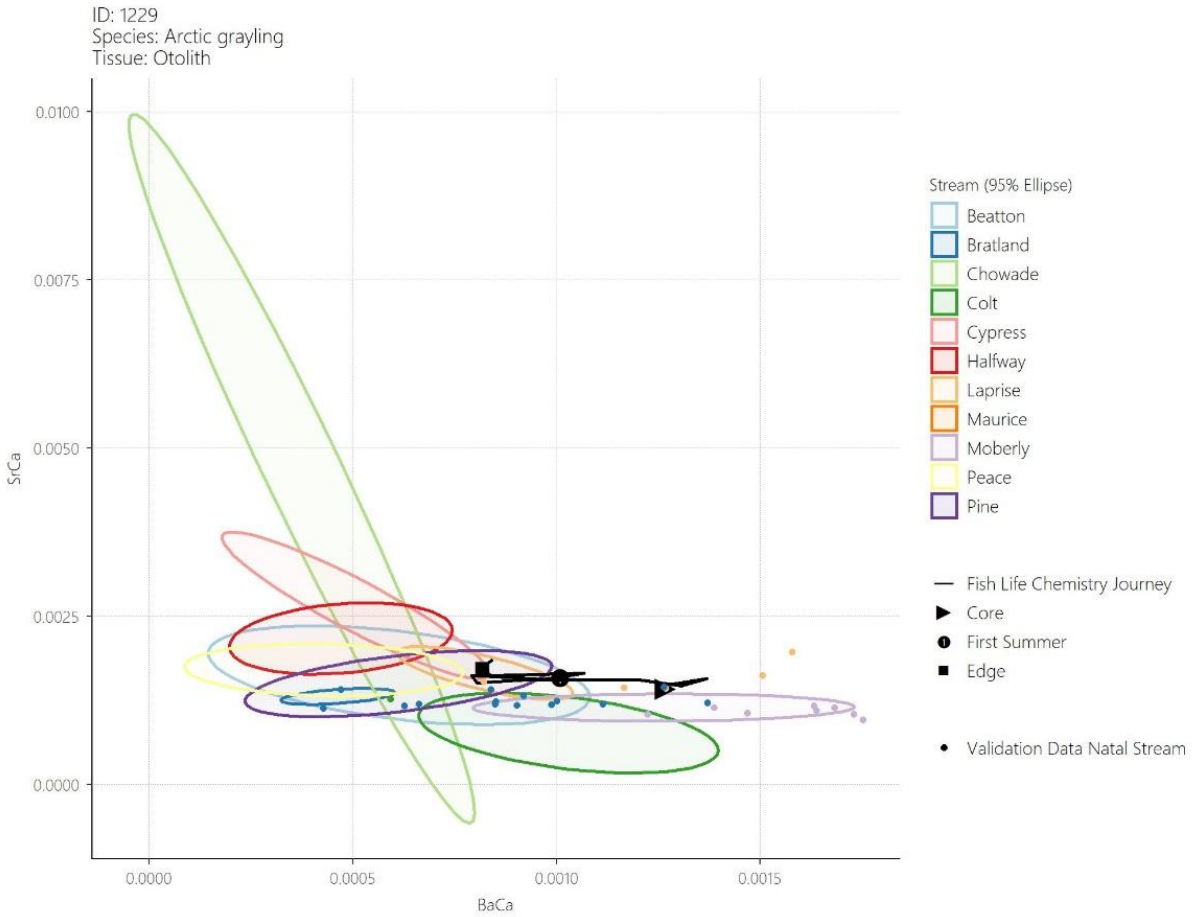

Figure 14. AG#1229 (57 mm) captured in Laprise Creek was incorrectly predicted to have recruited from Moberly River due to model overfitting. The life history graph suggests the natal region is aligned with Laprise Creek validation data (orange dots).

All Moberly River-captured fish were correctly predicted to have recruited from Moberly River. Fish AG #2025 (Figure 15) and AG #3375 (Figure 16) demonstrate how the life-history chemistry and all natal predictions for this species (light purple dots) generally fall within the Moberly River region (light purple ellipse) indicating little model overfitting for this stream.

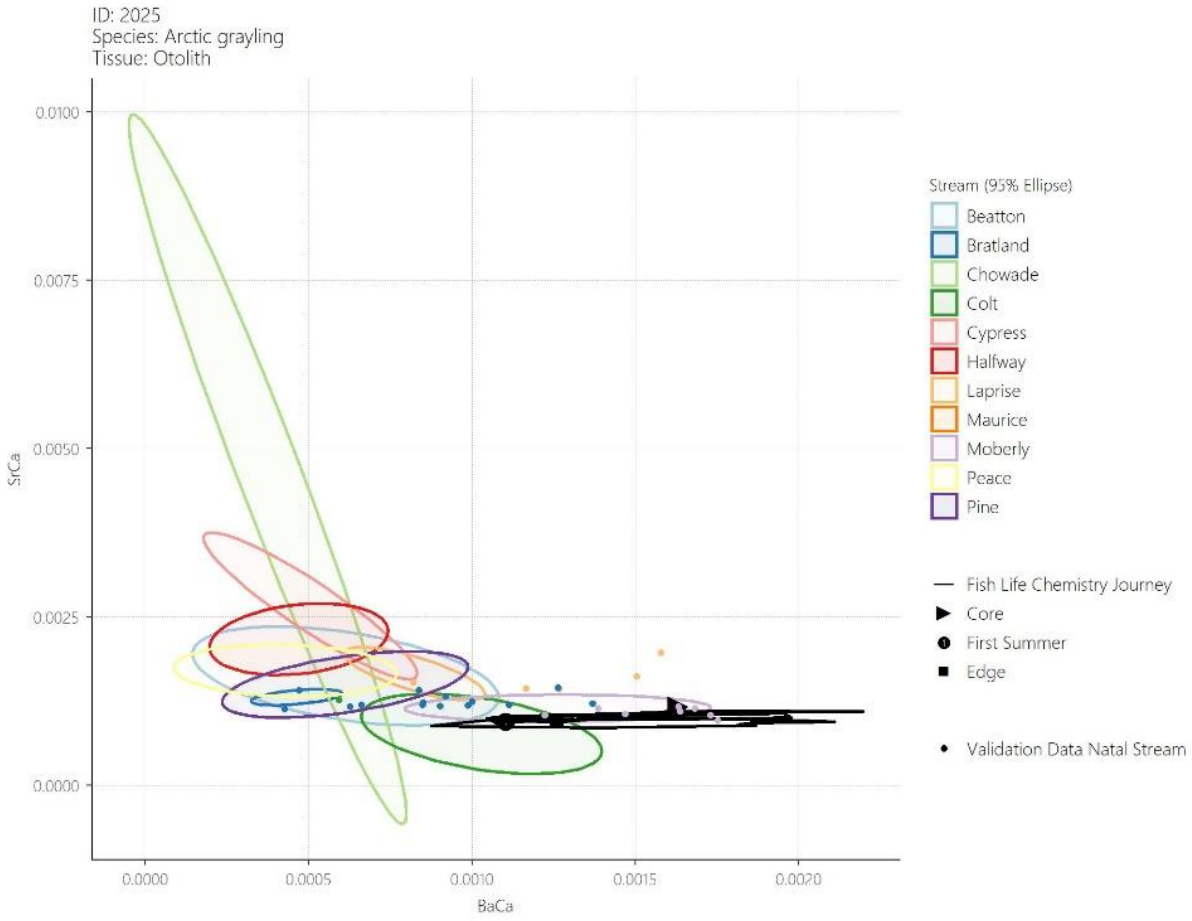

Figure 15. Representative life-history chemistry used for model validation for Moberly River (AG#2025, 170 mm).

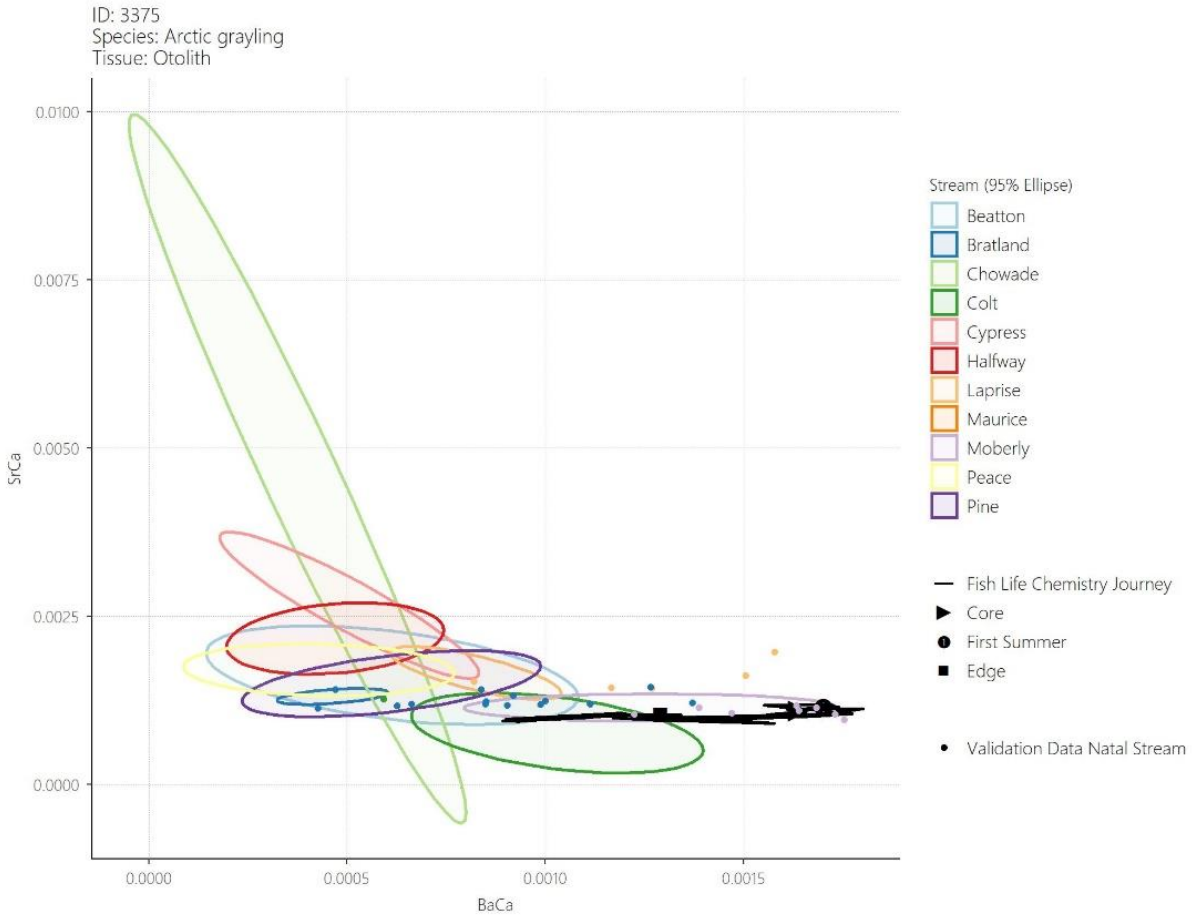

Figure 16. Representative life-history chemistry used for model validation for Moberly River (AG#3375, 166 mm).

The life-history graphs for fish captured from Colt Creek highlight a dissimilarity between the data used to train and validate the model. For example, while the fish chemistry changes little over the life of fish AG #1124 (Figure 17), indicating it remained within or near its natal habitat until capture, the chemistry is more similar to Bratland Creek than Colt Creek.

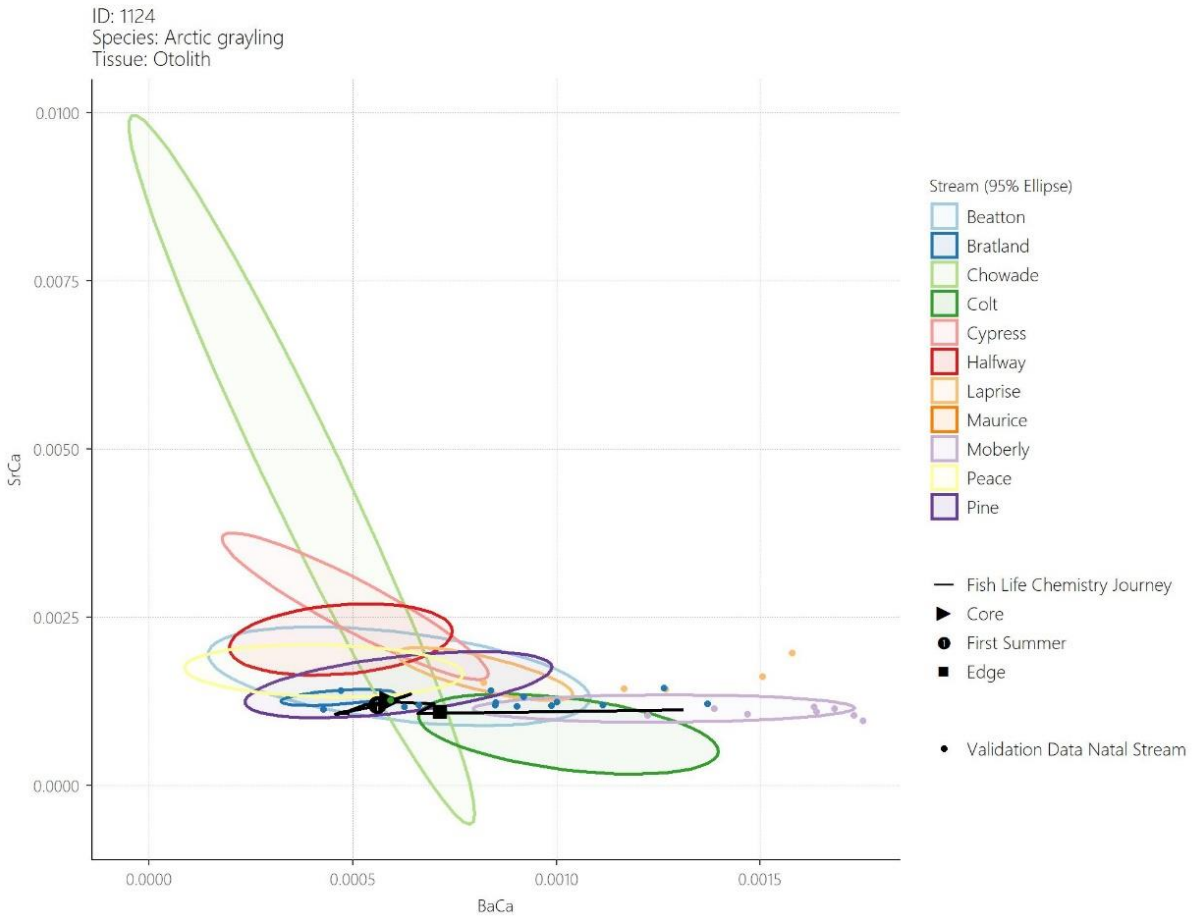

Figure 17. Life-history chemistry of Colt Creek captured AG #1124 (50 mm). While the edge chemistry is within the Colt Creek ellipse (dark green ellipse), the natal and first summer is more similar to Bratland Creek (dark blue ellipse).

4.2.1.2. Prediction of Stream Occupancy in Test Data

Of the nine unknown-origin Arctic Grayling otoliths, eight were captured in the Peace River. Three Arctic Grayling were captured in the Peace River upstream of the Project, where two were predicted to have recruited from Moberly River and one from Chowade River in the Halfway River watershed (Table 11). Of the five Arctic Grayling captured in the Peace River downstream of the Project, four were predicted to have recruited from Moberly River (80%), and the other one from Chowade River. The fish captured in Beatton River, was predicted to have originated from Moberly River.

Table 11. Model-predicted Arctic Grayling recruitment sources based on otolith structures. Only capture locations (along the top) and streams included in model (rows) are presented in the table.

Predicted Location		Capture Location			
Watershed	Stream	Beatton R.	Peace R.		Total
		Mainstem	Upstream	Downstream	
Halfway R.	Colt Ck.				
	Chowade R.		1	1	2
	Cypress Ck.				
	Mainstem				
Moberly R.		1	2	4	7
Pine R.					
Beatton R.	Bratland Cr.				
	Laprise Cr.				
	Mainstem				
Total		1	3	5	9

Based on the validation data it is apparent that natal Arctic Grayling sources from Bratland and Laprise creeks were more similar to the Moberly River training data. Indeed, here, seven out of nine (78%) fish were predicted to have recruited from Moberly River, but like the validation data may have recruitment sources in the Beatton River watershed instead. For example, the life-history and the natal region chemistry of the fish captured in Beatton River, AG #14, is closer to that of Bratland Creek (Figure 18). Additionally, the lack of variability in the chemistry (i.e., indicating it remained in or near its natal stream until capture) suggests this fish likely recruited from the Beatton River or a tributary (e.g., Bratland Creek) within that watershed.

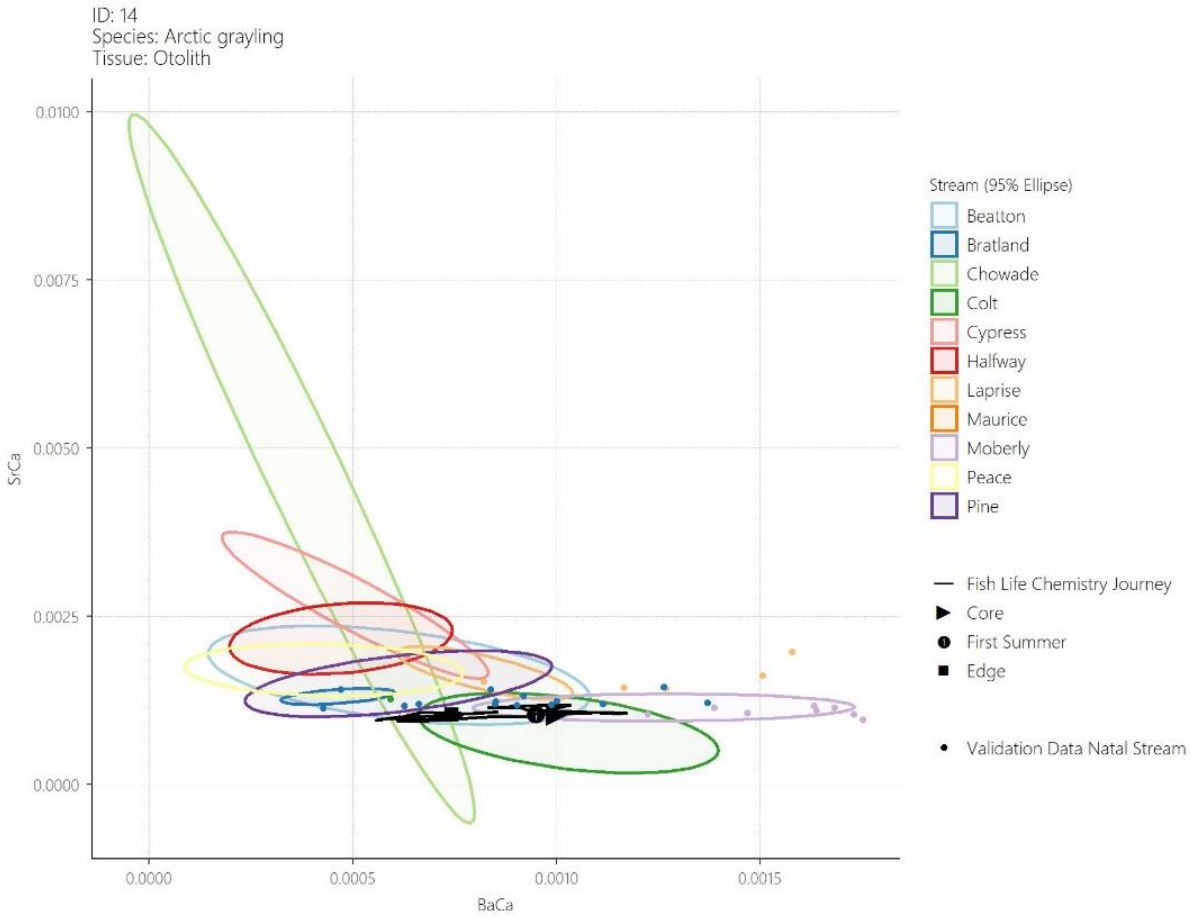

Figure 18. Life-history chemistry for AG #14 (190 mm) predicted by the model to have recruited from Moberly River, but chemistry more closely resembles Bratland Creek validation data (dark blue dots). Additionally, the chemistry suggests this fish remained in this watershed until its capture in Beatton River.

Another fish, AG #5350, which was also predicted to have recruited from Moberly River has life-history chemistry more similar to Bratland Creek (Figure 19). Alternatively, AG# 2777 was predicted to have recruited from Moberly River and the life-history graph supports this prediction (i.e., the natal area is higher in Ba:Ca; Figure 20). Further, the life-history graph suggests there may be regions within the Moberly River with even higher Ba:Ca than what was used to train, or validate the model, as implied by the life-history chemistry 'path' extending beyond the right-hand extent of the light purple ellipse.

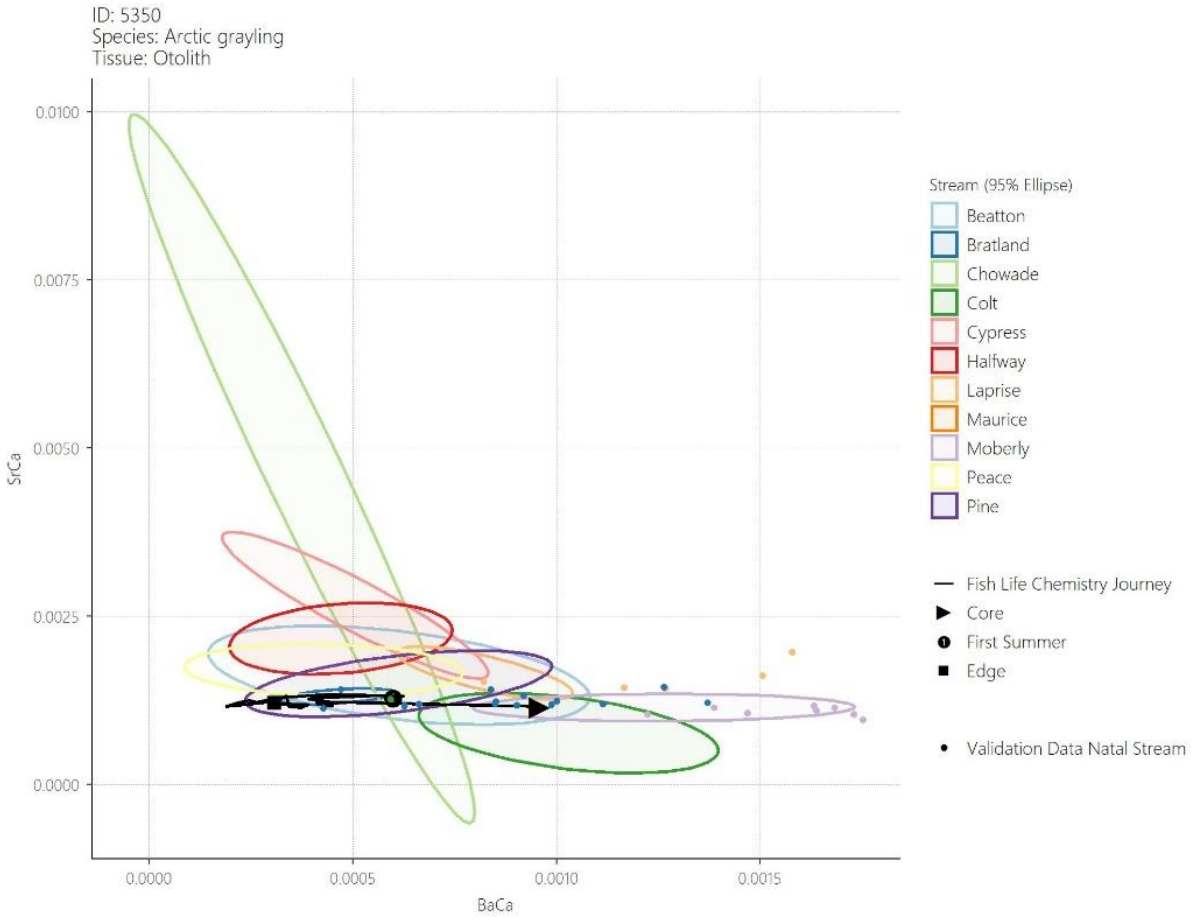

Figure 19. Life-history chemistry for AG #5350 (189 mm) predicted by model to have recruited from Moberly River, but chemistry more closely resembles Bratland Creek (training data: dark blue ellipse; validation data: dark blue dots).

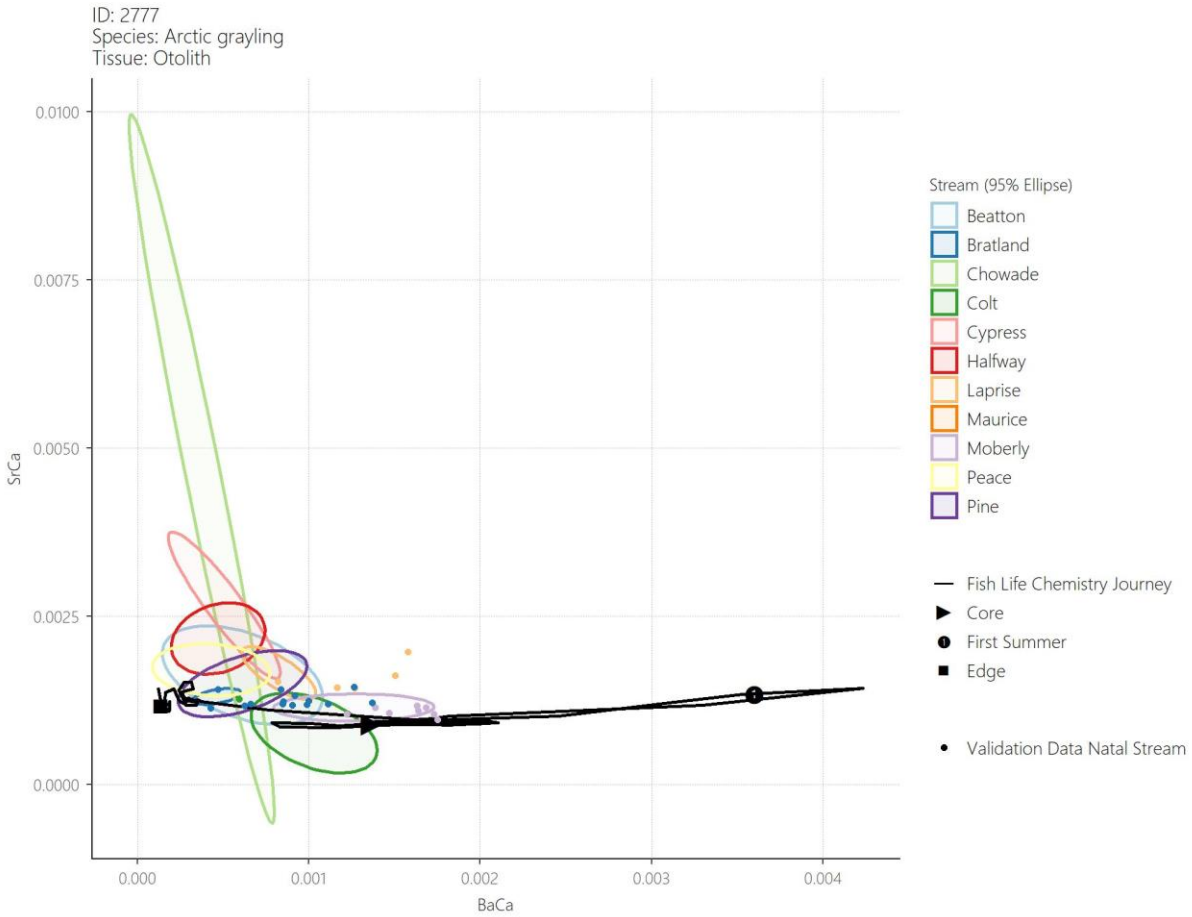

Figure 20. Life-history chemistry for AG #2777 (361 mm) predicted by model to have recruited from Moberly *River. Notice how the path extends along the Ba:Ca axis beyond the training (light purple ellipse) and validation data (light purple dots) for Moberly River.*

The AG #5391, captured in the Peace River, was predicted to have recruited from Chowade River is also more similar to Bratland Creek chemistry than Chowade River (higher Sr:Ca; Figure 21). The validation data indicated that the overfitting of Bratland Creek led to some misclassification of natal predictions as Chowade River, in addition to the Moberly River examples given above.

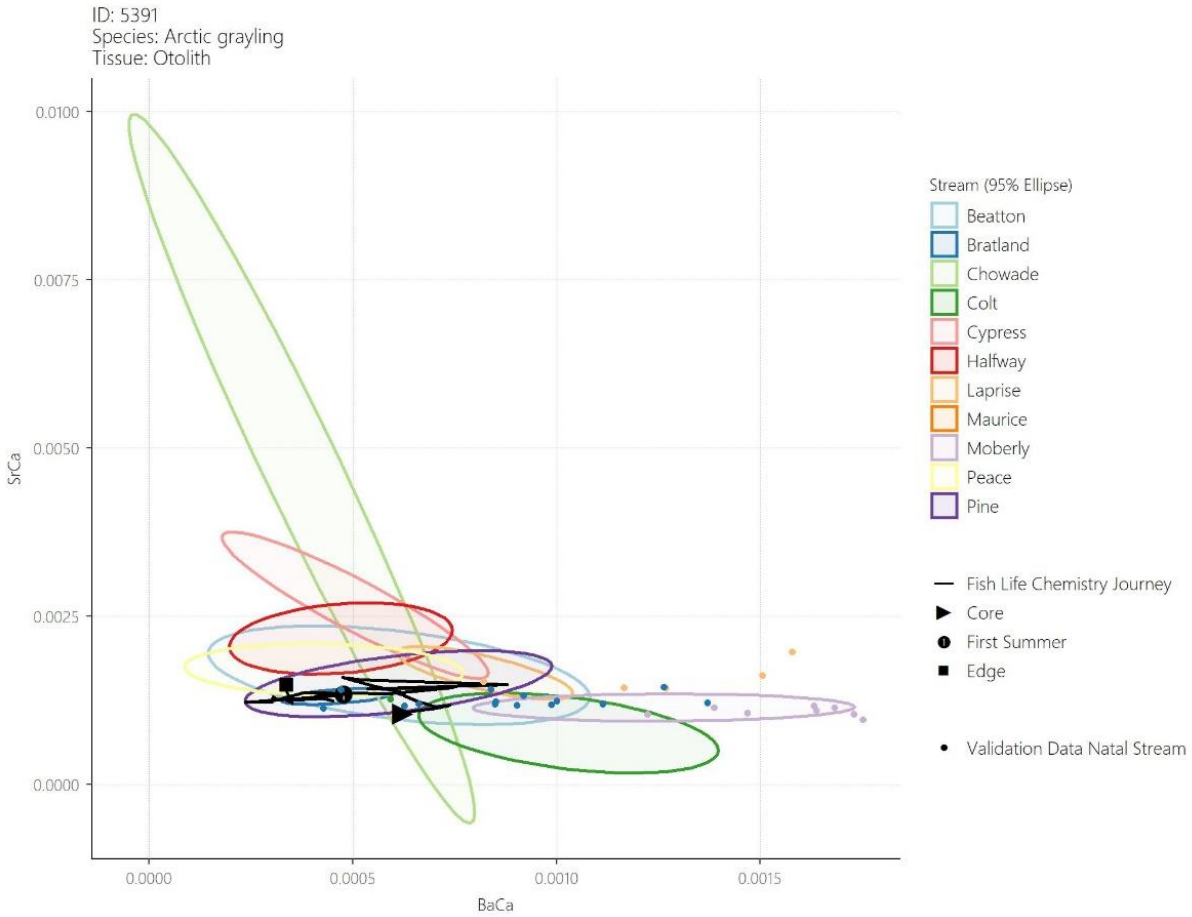

Figure 21. Life-history chemistry for AG #5391 (204 mm), captured in the Peace River, was predicted by model to have recruited from Chowade River, but chemistry resembles Bratland Creek (training data: dark blue ellipse; validation data: dark blue dots).

4.2.2. Fin Rays

In total, 102 Arctic Grayling fin rays were collected in 2019 and 2020 (

Table 12 and Figure 22):

Table 12. Number and fork length of Arctic Grayling fish captured with fin ray samples.

Capture Watershed	Capture Site	Number of Fin Rays Collected	Fork Length (mm) range
Beatton River	Beatton River mainstem	9	190 - 271
	Bratland Creek	16	47 - 222
	Laprise Creek	6	51 - 60
Halfway River	Colt Creek	1	50
Moberly River	Moberly River mainstem	1	274
Peace River	Upstream of Project (including Site C fishway)	26	265 - 386
	Downstream of Project	43	96 - 391

4.2.2.1. Arctic Grayling Fin Ray Model Performance

Water chemistry results (n=48) from seven waterbodies, and fin ray capture chemistries (n=30) from four waterbodies, for a total of 78 chemistries from nine waterbodies (i.e., prior locations), were used to train the model (Figure 23). Further, the sub-set of 23 Arctic Grayling fin rays used for validation purposes were input into the model to test prediction accuracy, and visually assessed using the life-history graphs.

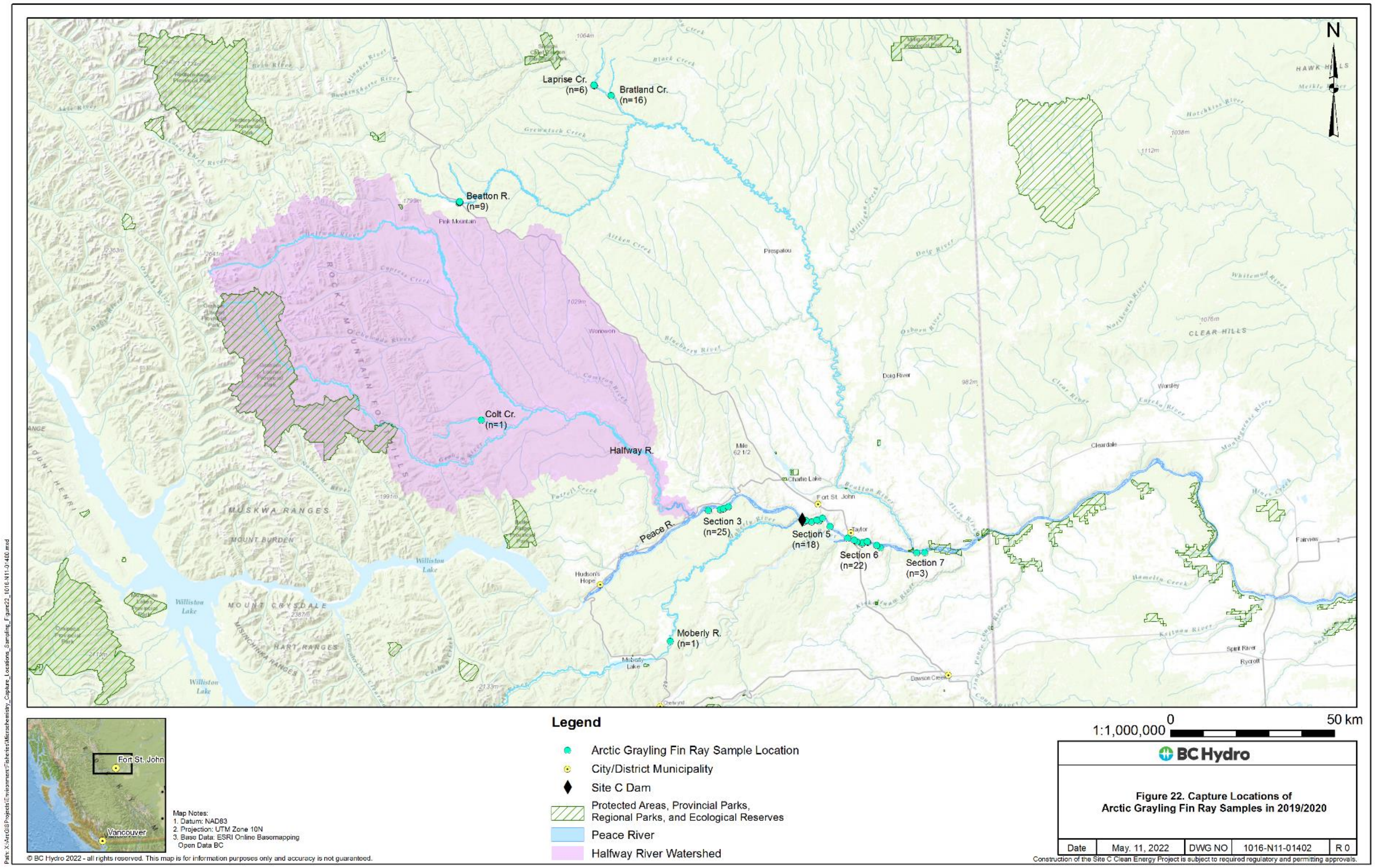
N
Laprise Cr.
(n=6)
Bratland Cr.
(n=16)
Beatton R.
(n=9)
Colt Cr.
(n=1)
Halfway R.
Peace R.
Section 3
(n=25)
Section 5
(n=18)
Section 6
(n=22)
Section 7
(n=3)
Moberly R.
(n=1)
Fort St. John
Hudson's Hope
Taylor
Dawson Creek
Legend
Arctic Grayling Fin Ray Sample Location
City/District Municipality
Site C Dam
Protected Areas, Provincial Parks, Regional Parks, and Ecological Reserves
Peace River
Halfway River Watershed
1:1,000,000
0
50 km
BC Hydro
Figure 22. Capture Locations of Arctic Grayling Fin Ray Samples in 2019/2020
Date
May. 11, 2022
DWG NO
1016-N11-01402
R 0
Map Notes:
1. Datum: NAD83
2. Projection: UTM Zone 10N
3. Base Data: ESRI Online Basemapping
Open Data BC
© BC Hydro 2022 - all rights reserved. This map is for information purposes only and accuracy is not guaranteed.
Construction of the Site C Clean Energy Project is subject to required regulatory and permitting approvals.

Figure 22.

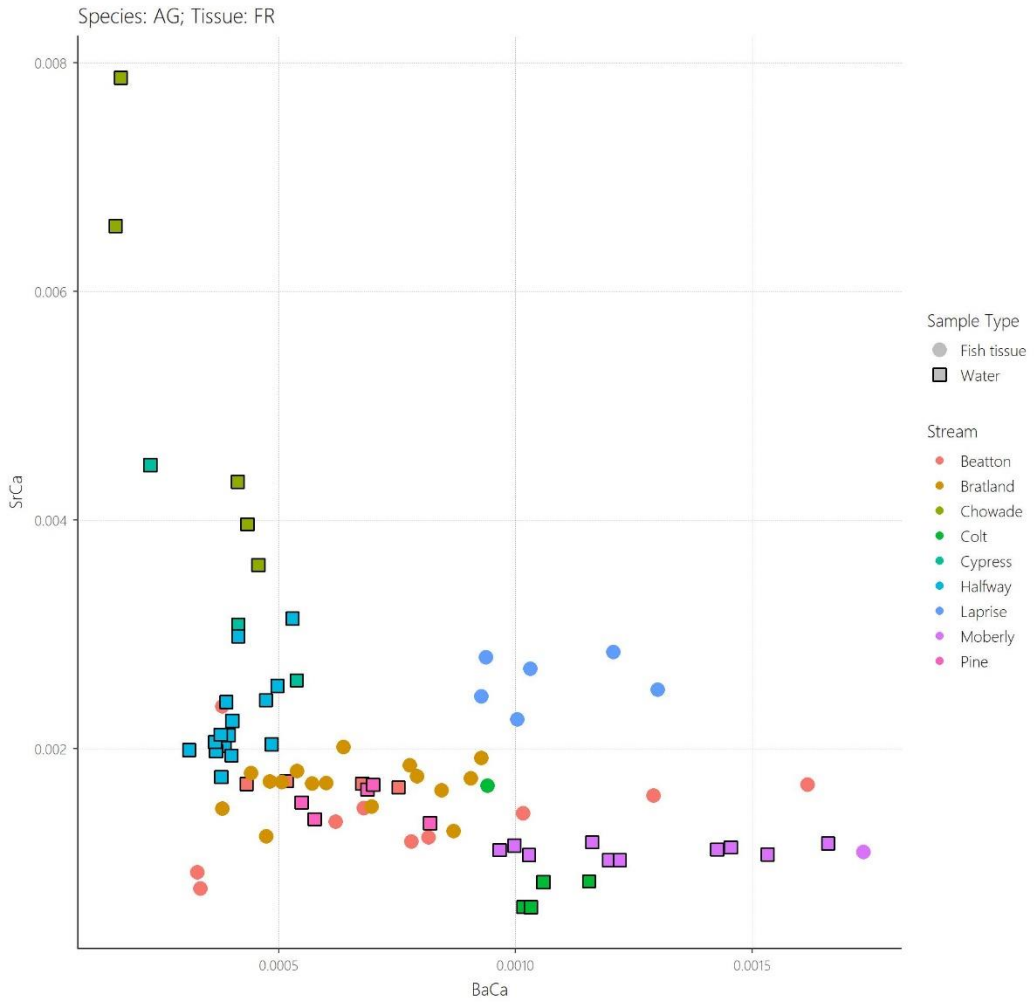

Figure 23. Water and fin ray capture chemistries used in the Arctic Grayling fin ray model.

The Arctic Grayling fin ray model yielded accurate predictions across the data used to train it (Table 13), with stream- and watershed-level accuracies exceeding 80% for water and tissue samples from all locations. Model validation was again restricted by spatially limited samples, with only two from outside Beatton River. Within the Beatton River watershed, prediction accuracies were high, especially at the watershed level (Table 14). As with otoliths, AG #1124 from Colt Creek could not be accurately predicted.

Table 13. Prediction Accuracy (% correctly classified stream and watershed) and sample sizes for Arctic Grayling fin rays in training data, and validation data.

Observed Occupancy		Training Data						Validation Data				
		Water			Tissue			Natal		1st Summer		
		% correct		n	% correct		n	% correct		% correct		n
Watershed	Stream	Stream	Watershed		Stream	Watershed		Stream	Watershed	Stream	Watershed	
Halfway	Halfway	85	92	13			0					0
	Colt	100	100	4			0	0	0	0	0	1
	Chowade	100	100	5			0					0
	Cypress	86	86	7			0					0
Moberly	Moberly	100	100	10	100	100	1	100	100	100	100	1
Pine	Pine	0	0	5			0					0
Beatton	Beatton	0	100	4	86	86	7					0
	Bratland			0	81	94	16	67	100	80	100	15
	Laprise			0	100	100	6	100	100	100	100	6
	Overall	75	85	48	87	94	30	74	96	83	96	23

Note: Grey cells give sample sizes for water and tissue training data, and validation data.

Table 14. Confusion matrix for Arctic Grayling fin rays (correct prediction in gray cell) – capture locations shown in columns, predicted occupancy shown in rows.

Predicted Location		Capture Location			
Watershed	Stream	Halfway R.	Moberly R.	Beatton R.	
		Colt Ck.		Bratland Ck.	Laprise Ck.
Halfway R.	Colt Ck.				
	Chowade R.				
Moberly R.			1 (1)		
Beatton R.	Bratland Ck.			10 (12)	
	Laprise Ck.			3 (1)	6 (6)
	Mainstem	1 (1)		2 (2)	
Total		1	1	15	6

Life-history graphs for Arctic Grayling fin ray validation data can be found in Appendix E, with examples for Moberly River, Laprise Creek and Bratland Creek provided below. The fish, AG #1101, was captured in Moberly and the characteristically high Ba:Ca signature is apparent in this fish's life

history (Figure 24). The AG #1279 was captured in Laprise Creek and spent its entire life there (Figure 25), while AG #1413 recruited from Bratland Creek and remained there until capture (Figure 26).

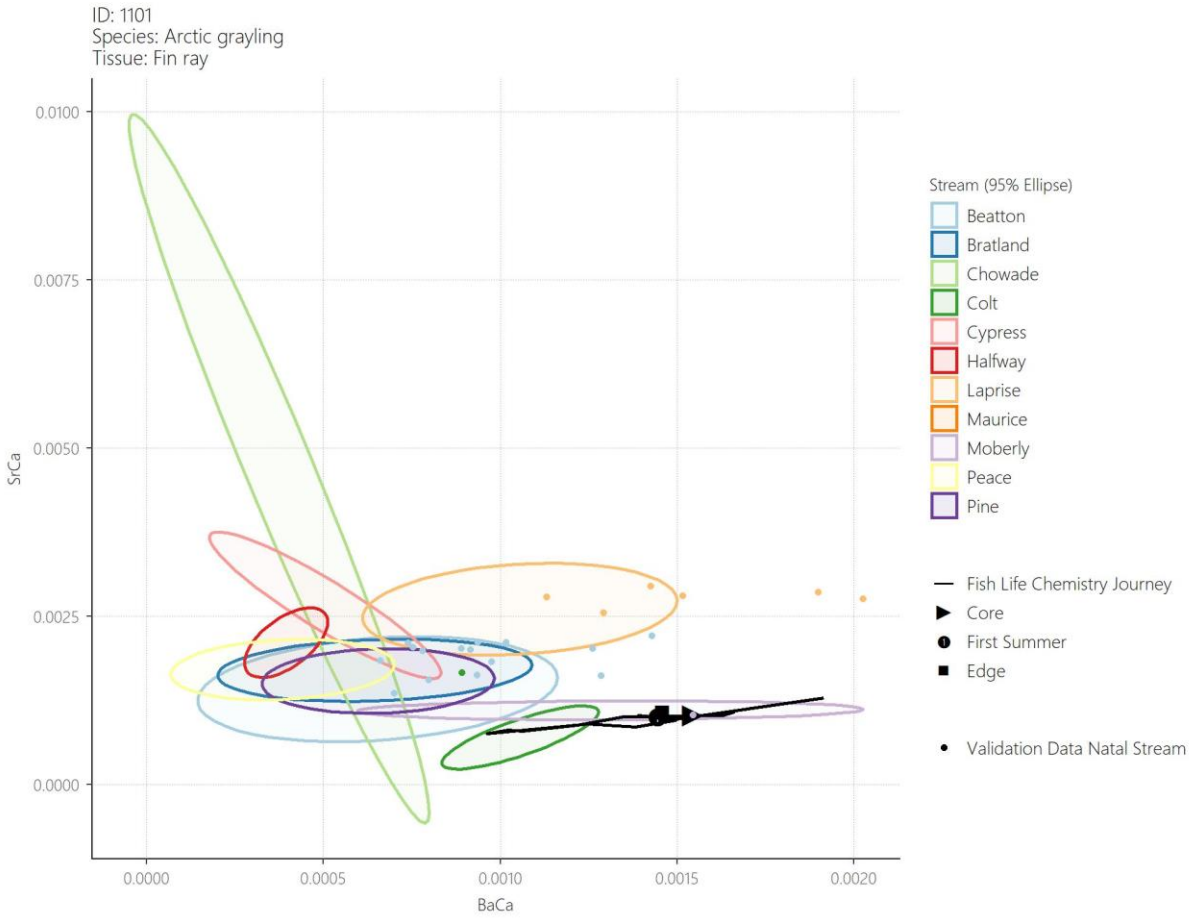

Figure 24. Representative life-history chemistry used for model validation for Moberly River (AG#1101, 274 mm).

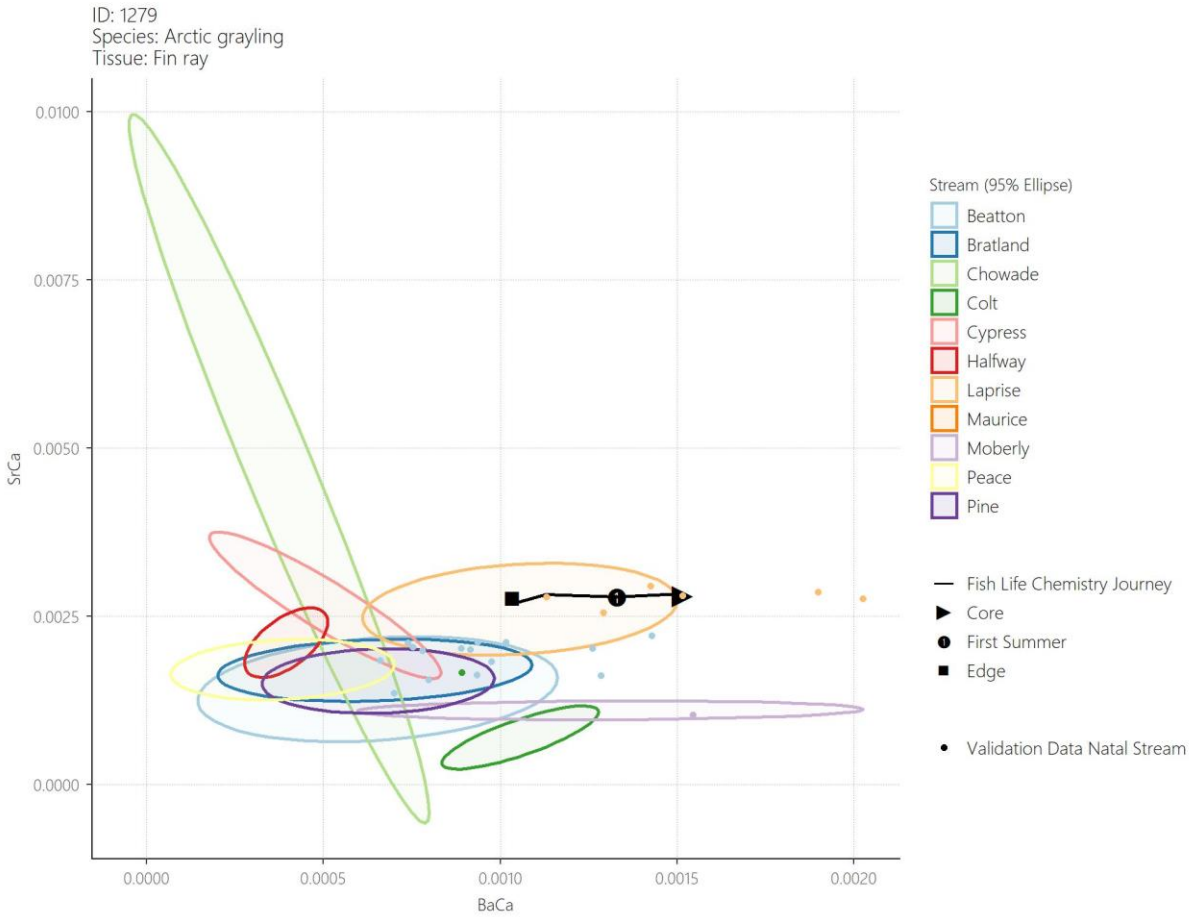

Figure 25. Representative life-history chemistry used for model validation for Laprise Creek (AG #1279, 56 mm).

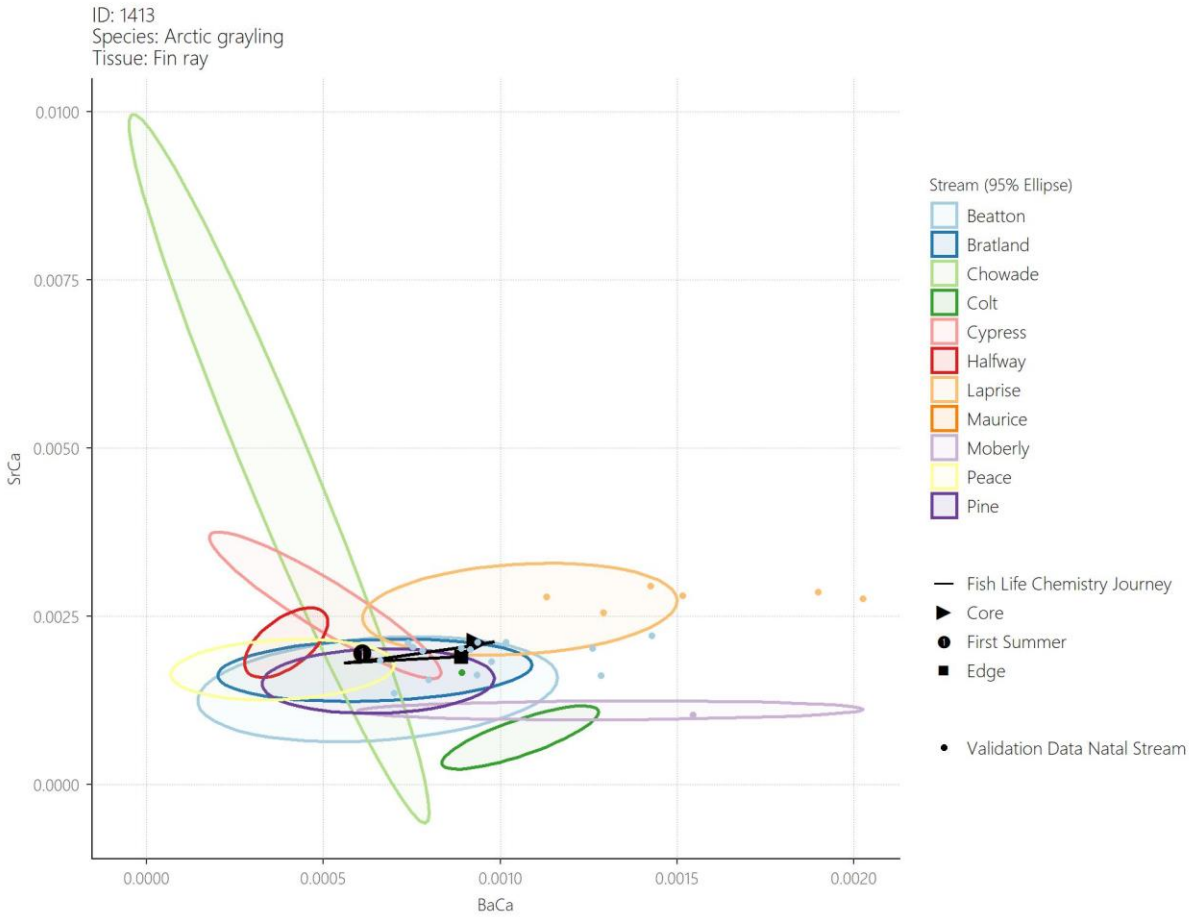

Figure 26. Representative life-history chemistry used for model validation for Bratland Creek (AG#1413, 64 mm).

There was some model overfitting for Bratland Creek Arctic Grayling, where natal chemistry in the validation samples had higher Ba:Ca than in the model training data. This may have led to erroneous predictions of Laprise Creek as a recruitment source instead of Bratland Creek. For example, AG #1406 was captured in Bratland Creek but was predicted by the model to have recruited from Laprise Creek (Figure 27).

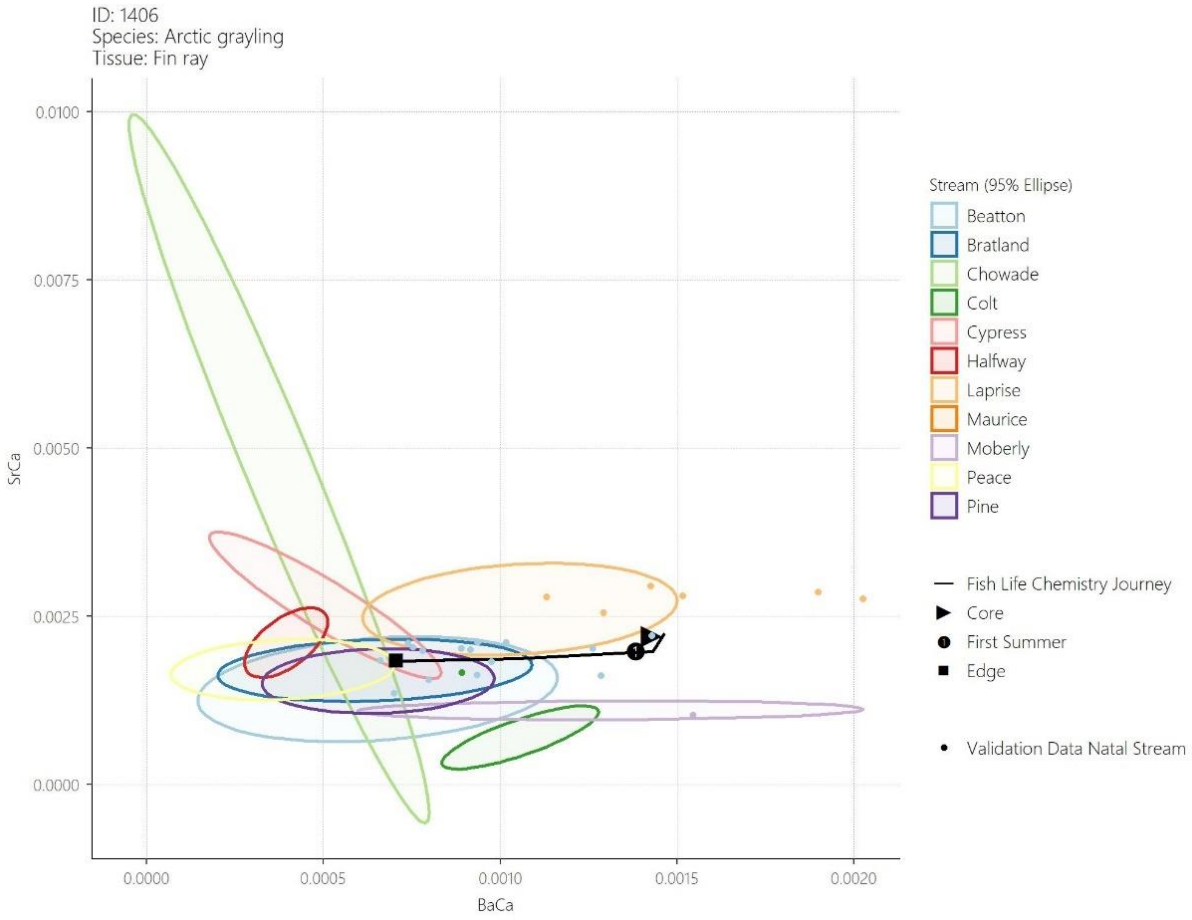

Figure 27. Example of how a Bratland Creek Arctic Grayling (AG#1406, 93 mm) may have instead been predicted by the model to have recruited from Laprise Creek (orange ellipse).

As with the otolith, AG #1124 captured in Colt Creek could not be accurately predicted, as there was a disconnect between the model chemistry for this location (which was mainly based on water chemistry data) and the one natal validation sample (Figure 28).

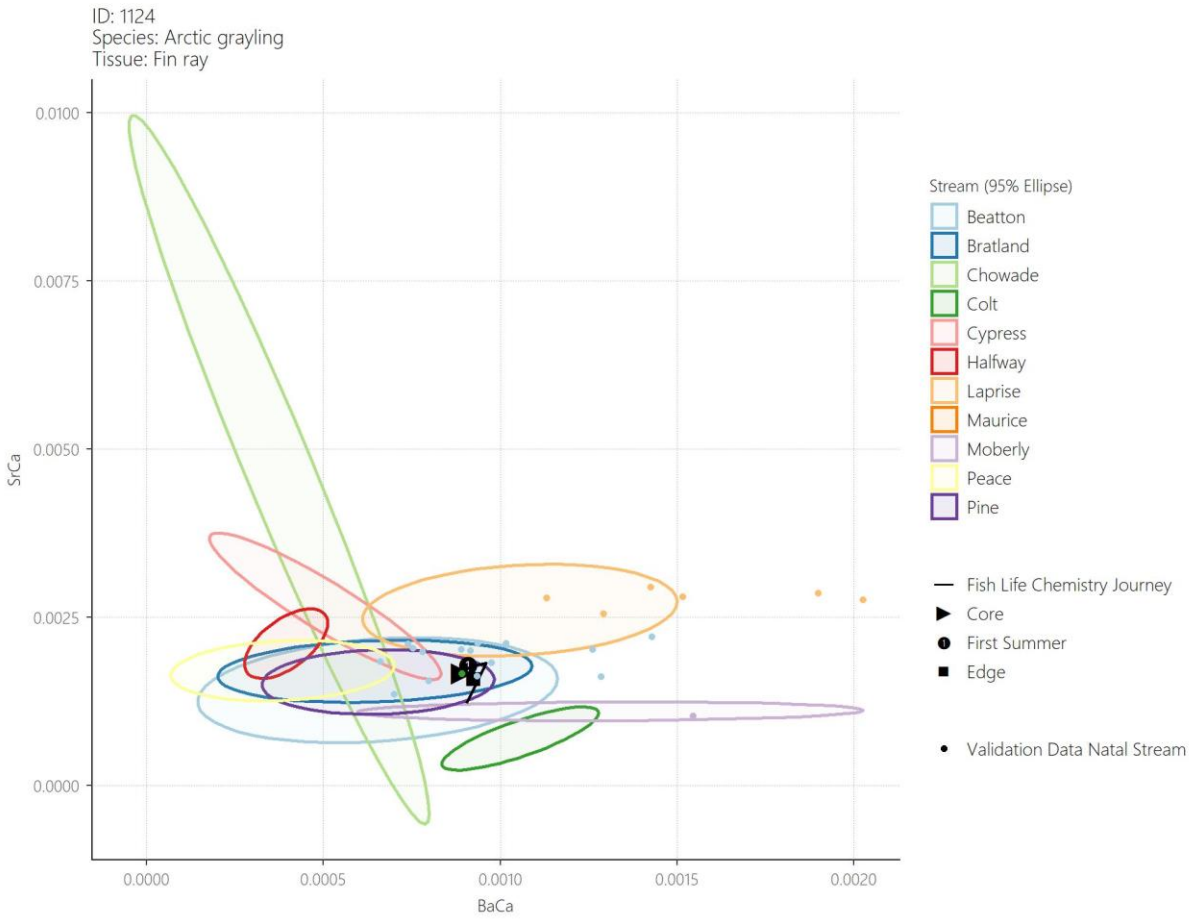

Figure 28. The Colt Creek captured fish (AG#1124, 50 mm) was erroneously predicted to have recruited from Bratland Creek (dark blue ellipse). Note that life history is not similar to Colt Creek training data (dark green ellipse).

4.2.2.2. Prediction of Stream Occupancy in Test Data

For the 79 unknown-origin fin rays, 69 were captured in the Peace River, and 10 were captured within the Beatton River watershed (one from Bratland Creek, and nine from the Beatton River mainstem). The sample captured in Bratland Creek was also predicted to have originated from Bratland Creek (Table 15). Those captured in the Beatton River mainstem were predicted to have originated from Bratland Creek (n=1), Laprise Creek (n=2), and Beatton River (n=5), with one fish predicted from Moberly River; however, this latter fish (AG #19) is more likely to have recruited from the Beatton River watershed based on the life history (Figure 29), as the Ba:Ca signature is lower than typical for Moberly fish (Figure 24).

Table 15. Model predicted Arctic Grayling recruitment sources based on fin ray structures. Only capture locations (along the top) and streams included in model (rows) are presented in the table.

Predicted		Observed					
Watershed	Stream	Beatton R.			Peace R.		Total
		Bratland Ck.	Laprise Ck.	Mainstem	Upstream	Downstream	
Halfway R.	Colt Ck.						
	Chowade R.					1	1
	Cypress Ck.				1	6	7
	Mainstem				3	7	10
Moberly R.				1	4	2	7
Pine R.							
Beatton R.	Bratland Cr.	1		1	4	15	21
	Laprise Cr.			2	2	9	13
	Mainstem			5	12	3	20
Total		1		9	26	43	79

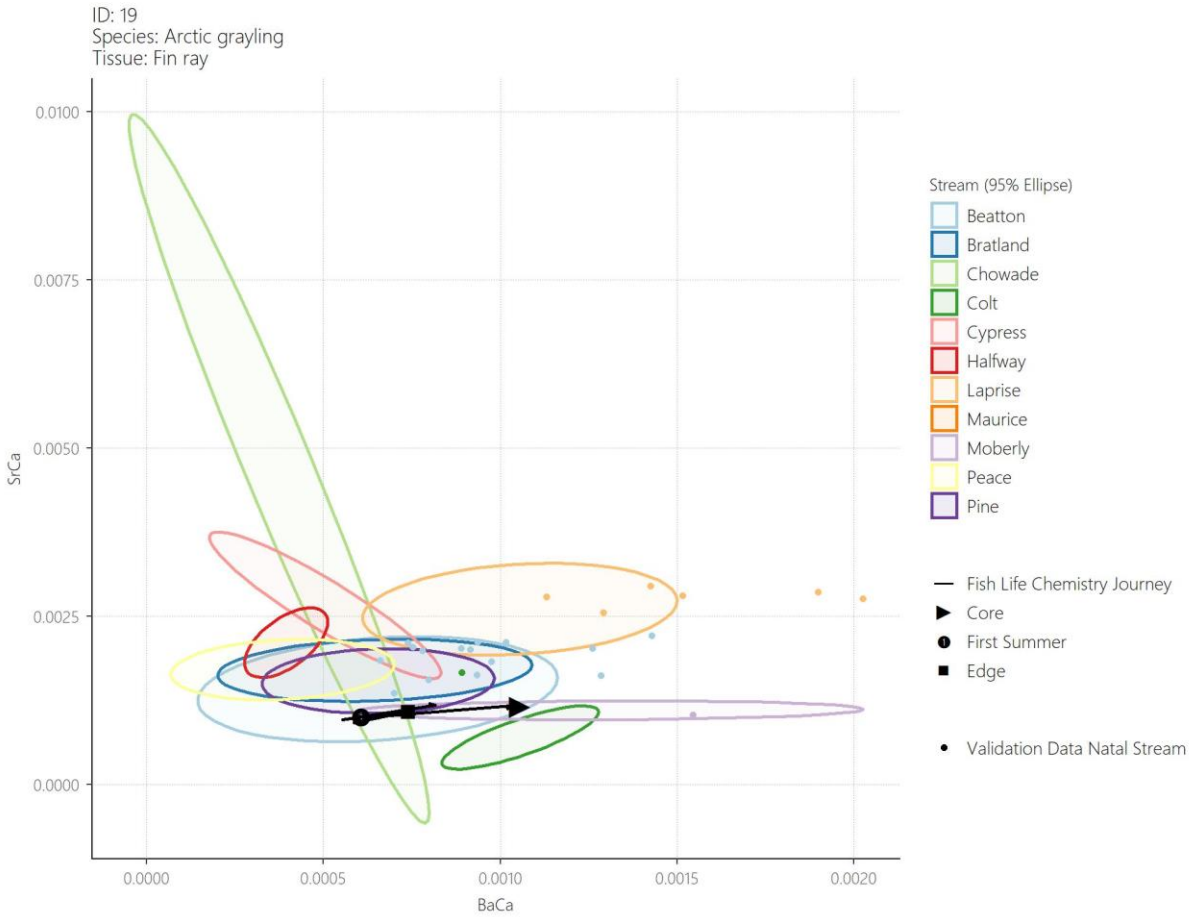

Figure 29. Life-history chemistry for AG#19 (222 mm) predicted by the model to have recruited from Moberly (light purple ellipse and dots), but chemistry more closely resembles Beatton River (light blue ellipse and points).

The Arctic Grayling samples captured upstream and downstream of the Project site were predicted to originate from numerous waterbodies within the Halfway River, Moberly River, and Beatton River watersheds. Most of the Arctic Grayling captured upstream Peace River were predicted to have recruited from the Beatton River watershed (18/26; 69%), with most predicted to have recruited from the mainstem (46%) and 23% from either Bratland or Laprise creeks. The model predicted that 15% recruited from Moberly River, and another 15% from Halfway River watershed (one from Cypress Creek and three from the mainstem); however, AG #5138 life-history chemistry appears more similar to

Moberly River (higher Ba:Ca ratio) than Beatton River, which was predicted by the model (Figure 30). Therefore, Moberly River recruitment may be slightly higher at 19% of total upstream Peace River captured Arctic Grayling. Additionally, the life history of AG #FC-TUF-2020031-1 (caught in the Site C fishway) appears more similar to Pine River (Figure 31), as opposed to Bratland Creek (predicted by model), due to low amounts of chemical variability over its life history and a pattern dissimilar to what is typical of Bratland Creek fish (Figure 26). Therefore, Pine River may be a small recruitment source for Arctic Grayling to the Peace River; however, the model data for Pine River is based on only five water chemistry samples and no Arctic Grayling tissues.

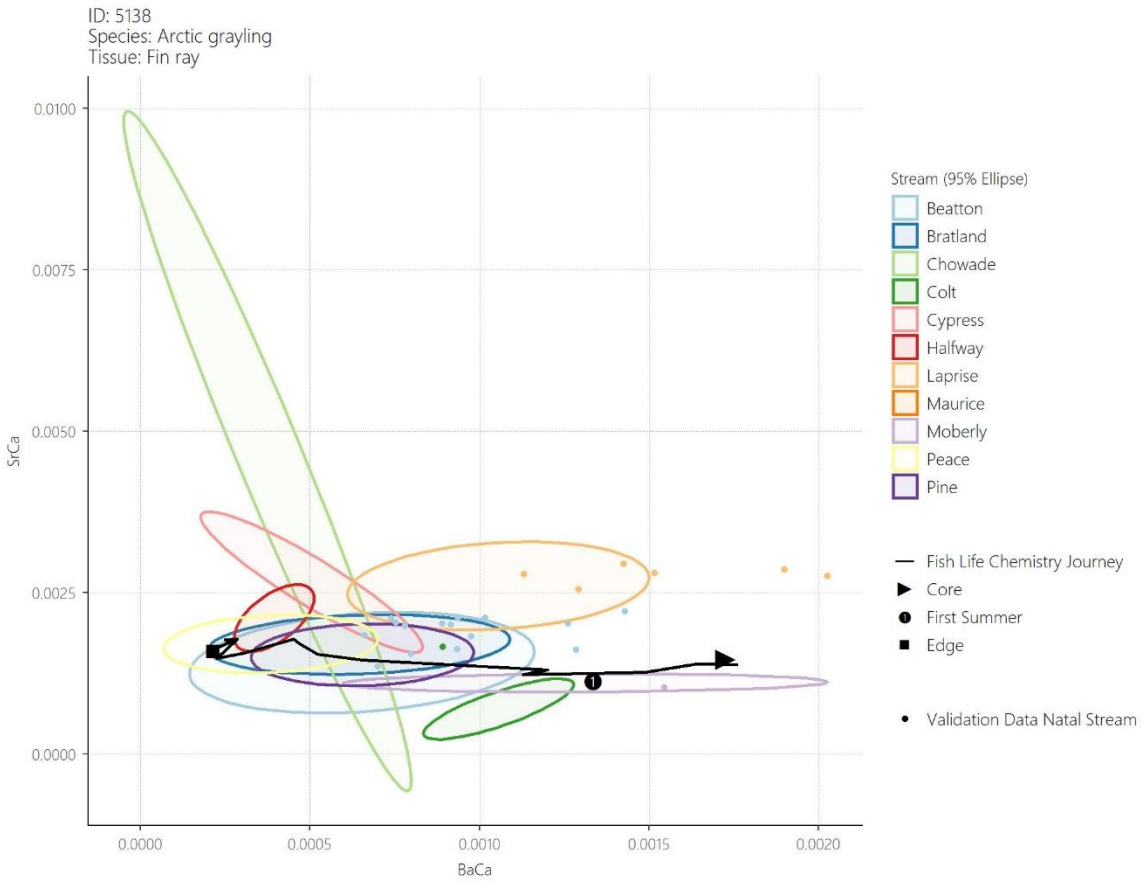

Figure 30. Life-history chemistry for AG #5138 (345 mm) predicted by model to have recruited from Beatton River (light blue ellipse and points), but natal and first summer chemistry more closely resembles Moberly River (light purple ellipse and dots).

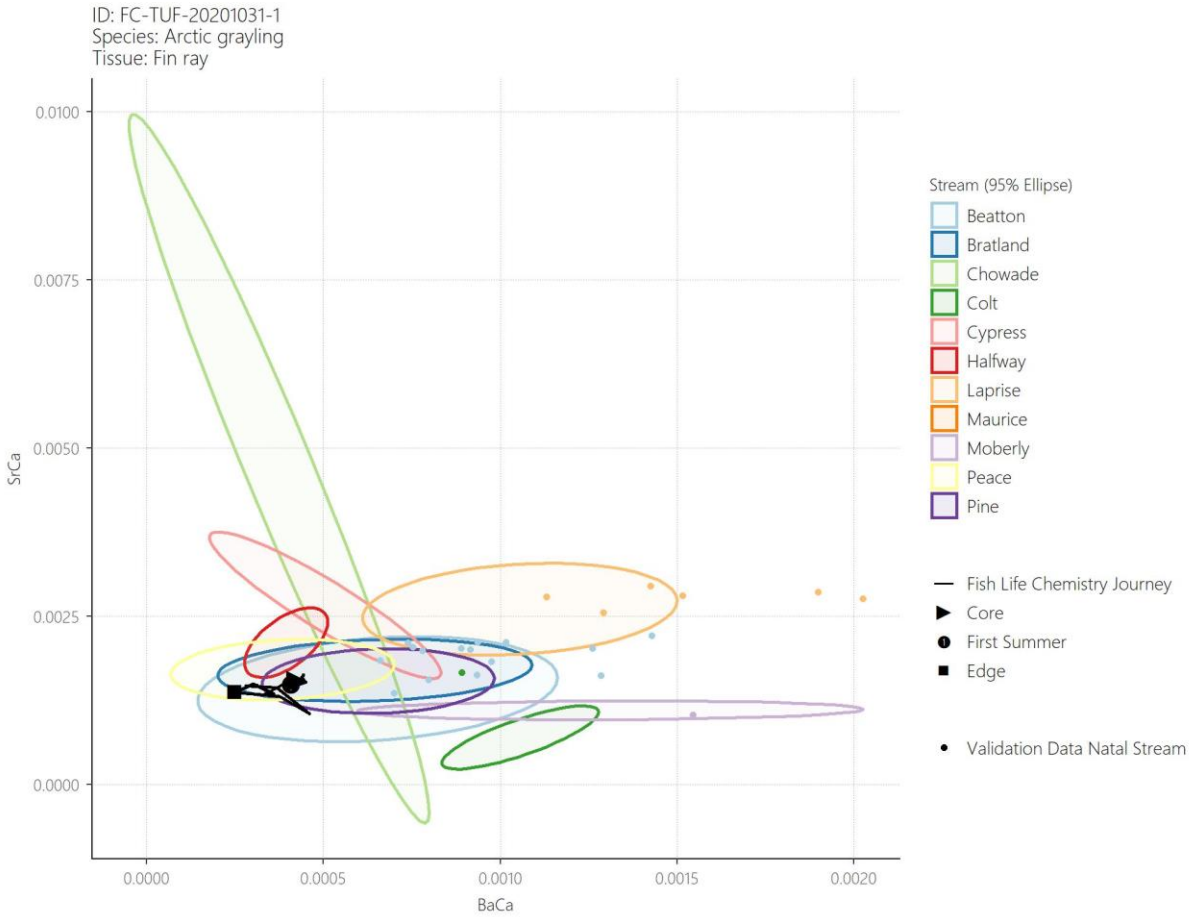

Figure 31. Life-history chemistry for AG#FC-TUF-2020031-1 (265 mm) predicted by model to have recruited from Bratland Creek (dark blue ellipse), but combination of lack of variability in chemistry over time and the dissimilar pattern to other fish in Bratland Creek, suggests a different recruitment source – possibly, Pine River (dark purple ellipse).

Arctic Grayling captured in the Peace River downstream (n=43) of the Project had similar predictions for recruitment as Peace River upstream, where 63% were predicted to have recruited from the Beatton River watershed (n=27), with most coming from Bratland Creek (35%) and Laprise Creek (21%), as opposed to the mainstem (7%). The Halfway River (33%), particularly Cypress Creek (14%) and the mainstem (16%), were the next dominant recruitment sources for the Peace River downstream Arctic Grayling. One fish was predicted to have recruited from Chowade River (AG #432); however, upon inspection of the life-history chemistry (Figure 32), this fish likely recruited from Laprise Creek, because Chowade Creek chemistry is dominated by higher Sr:Ca than the chemistry of this fish.

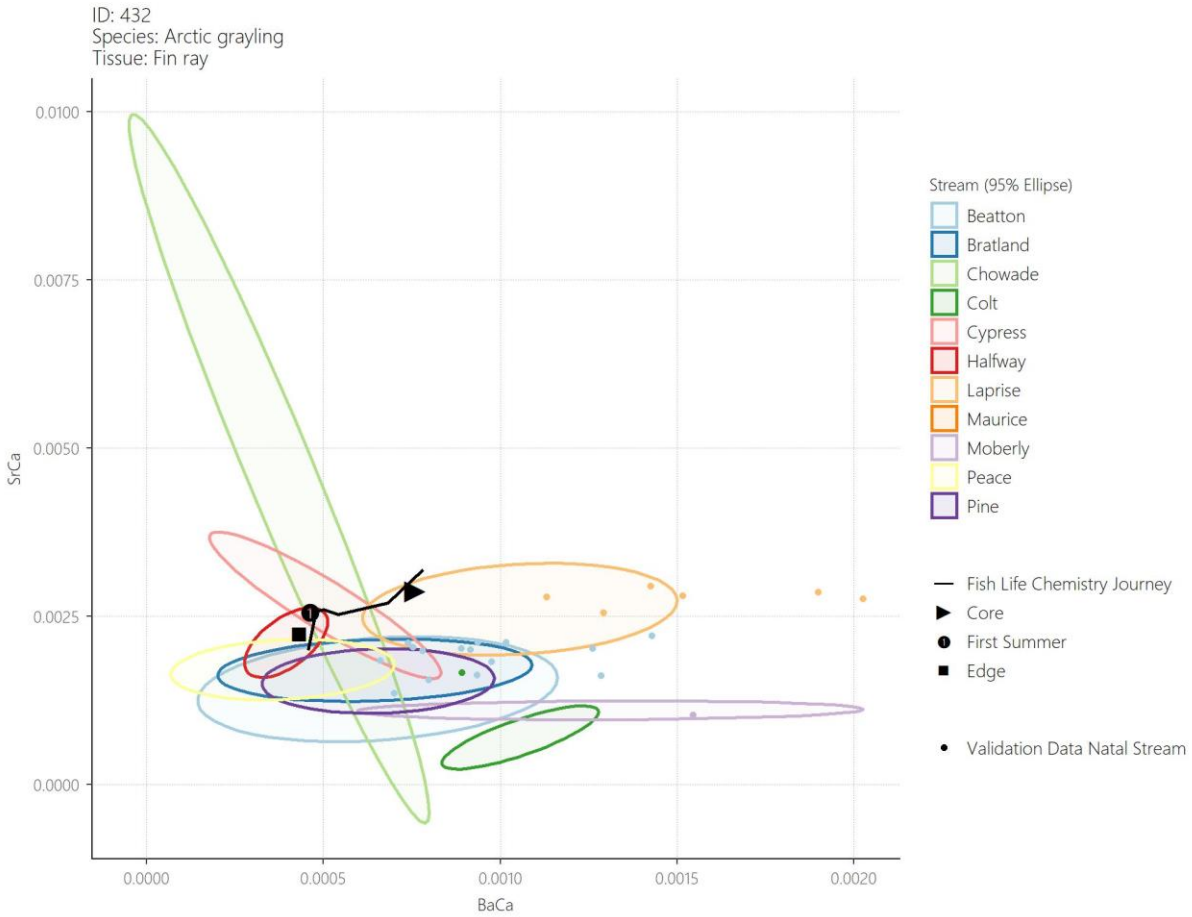

Figure 32. Life-history chemistry for AG#432 (204 mm) predicted by model to have recruited from Chowade River, but life-history chemistry pattern more closely resembles Laprise Creek (light orange ellipse and points) with migration into the Peace River (yellow ellipse) where it was ultimately captured.

Arctic Grayling fin ray modelling has been developed based on water chemistry for all prior locations, except for the Beatton River watershed. In this way, there is less confidence in the model output for recruitment predictions for fish from (for example) Moberly River or Halfway River watershed. Using life-history chemistries to help facilitate improved characterization for those waterbodies, there were some discrepancies with the model predictions: 29 out of 79 (37%) for stream predictions and 23 (29%) for watershed predictions. Discrepancies are presented in Table 16, and life-history graphs are

in Appendix E. Main discrepancies focused on differentiating Moberly River and Beatton River (mainstem and tributaries) – the model has difficulty differentiating these waterbodies, but the life-history chemistries suggest the difference is that Moberly River is dominated by a strong Ba:Ca signal which is particularly characteristic of that location. Additionally, some predictions focused on Bratland and Laprise creeks as recruitment sources, but the life-history graphs suggested the sources could be from Cypress Creek or Halfway River mainstem. Unfortunately, no tissues are available from these locations to inform this possibility.

Table 16. Discrepancies between model predictions and life-history graphs for Arctic Grayling fin rays.

Fish ID	Capture Location	Model Prediction	Life History Graph
2719	Peace	Beatton	Bratland
5484	Peace	Beatton	Bratland
2647	Peace	Beatton	Moberly
2775	Peace	Beatton	Moberly
2776	Peace	Beatton	Moberly
2777	Peace	Beatton	Moberly
5138	Peace	Beatton	Moberly
5209	Peace	Beatton	Moberly
4889	Peace	Bratland	Beatton
3064	Peace	Bratland	Cypress
5351	Peace	Bratland	Cypress
5630	Peace	Bratland	Cypress
2955	Peace	Bratland	Halfway
3688	Peace	Bratland	Halfway
5360	Peace	Bratland	Halfway
5391	Peace	Bratland	Halfway
5750	Peace	Bratland	Halfway
FC-TUF-20201031-1	Peace	Bratland	Pine
432	Peace	Chowade	Laprise
1747	Peace	Halfway	Laprise
13	Beatton	Laprise	Bratland
1192	Bratland	Laprise	Bratland
4529	Peace	Laprise	Bratland
519	Peace	Laprise	Cypress
894	Peace	Laprise	Cypress
4418	Peace	Laprise	Cypress
19	Beatton	Moberly	Beatton
5208	Peace	Moberly	Beatton
4551	Peace	Moberly	Bratland

4.2.3. Summary of Recruitment Sources for Arctic Grayling

Arctic Graylings captured in the Peace River recruited from the Moberly River, and the Halfway River and Beatton River watersheds. Following reassignment of recruitment source based on life history graphs (Table 16), results suggest that Arctic Grayling captured in Peace River upstream of the Project had fairly equal sources of upstream and downstream recruitment. The upstream sources recruited from Moberly River and the Halfway River watershed (mainly from the mainstem and Cypress Creek). The recruitment sources downstream of the Project all recruited from the Beatton River watershed (primarily Bratland Creek). Grayling captured in Peace River downstream of the Project had slightly greater recruitment from upstream sources, mostly from Halfway River watershed (similarly, the mainstem and Cypress Creek) and less (approximately 5%) from Moberly River. The remaining recruitment came from Beatton River watershed (approximately 45%), and a possible recruitment from Pine River. These conclusions are predominantly based on the fin ray analysis and model due to the higher sample size (n=79 compared to n=9 for otoliths) and were informed by life-history chemistries, which helped overcome challenges with model overfitting and additional discrepancies between validation data and test data.

These Arctic Grayling recruitment sources appear consistent among studies. For Arctic Grayling otoliths collected from 2010 to 2012, the dominant recruitment source was predicted as Moberly River for both upstream and downstream captured grayling (Earthtone and Mainstream 2013). Additionally, there was minor recruitment contributions from Halfway River watershed (mostly Cameron River, which is not in our current recruitment model), Beatton River (mostly mainstem), and Pine River (mainstem only), with 36 (23%) unknown recruitment sources (Earthtone and Mainstream 2013). Only three Arctic Grayling were captured from 2014 to 2018 (including only two from Peace River) and all were predicted to have recruited from the Moberly River (TrichAnalytics 2020).

4.3. Bull Trout Stream Recruitment

4.3.1. Otoliths

In total, 8 Bull Trout otoliths were collected in 2019 and 2020 (Table 17 and Figure 33):

Table 17. Number and fork length of Bull Trout fish captured with otolith samples.

Capture Watershed	Capture Site	Number of Otoliths Collected	Fork Length (mm) range
Halfway River	Chowade River	4	81 - 179
	Cypress Creek	2	84 - 116
	Fiddes Creek	1	83
Peace River	Downstream of Project	1	822

4.3.1.1. Bull Trout Otolith Model Performance

Water chemistry results (n=48) from eight waterbodies, and otolith capture chemistries (n=49) from six waterbodies, for a total of 97 chemistries from eight waterbodies (i.e., prior locations), were used to train the model (Figure 34). Further, the sub-set of six Bull Trout otoliths used for validation purposes were input into the model to test model overfitting, and then visually assessed using the life-history graphs.

The Bull Trout otolith model varied greatly among locations in its predictive accuracy for the samples on which it was trained (Table 18; Figure 34). While most water samples were accurately classified, especially at the watershed level, the model was less reliable for otoliths, where only 39% of streams and 69% of watersheds were accurately predicted. Pine River had a low prediction accuracy at 45% due to lack of differentiation among other sites, particularly Halfway River tributaries. The watershed was accurately predicted (100%) for training samples within the Halfway River watershed for all streams, except for Fiddes Creek (50% accurate).

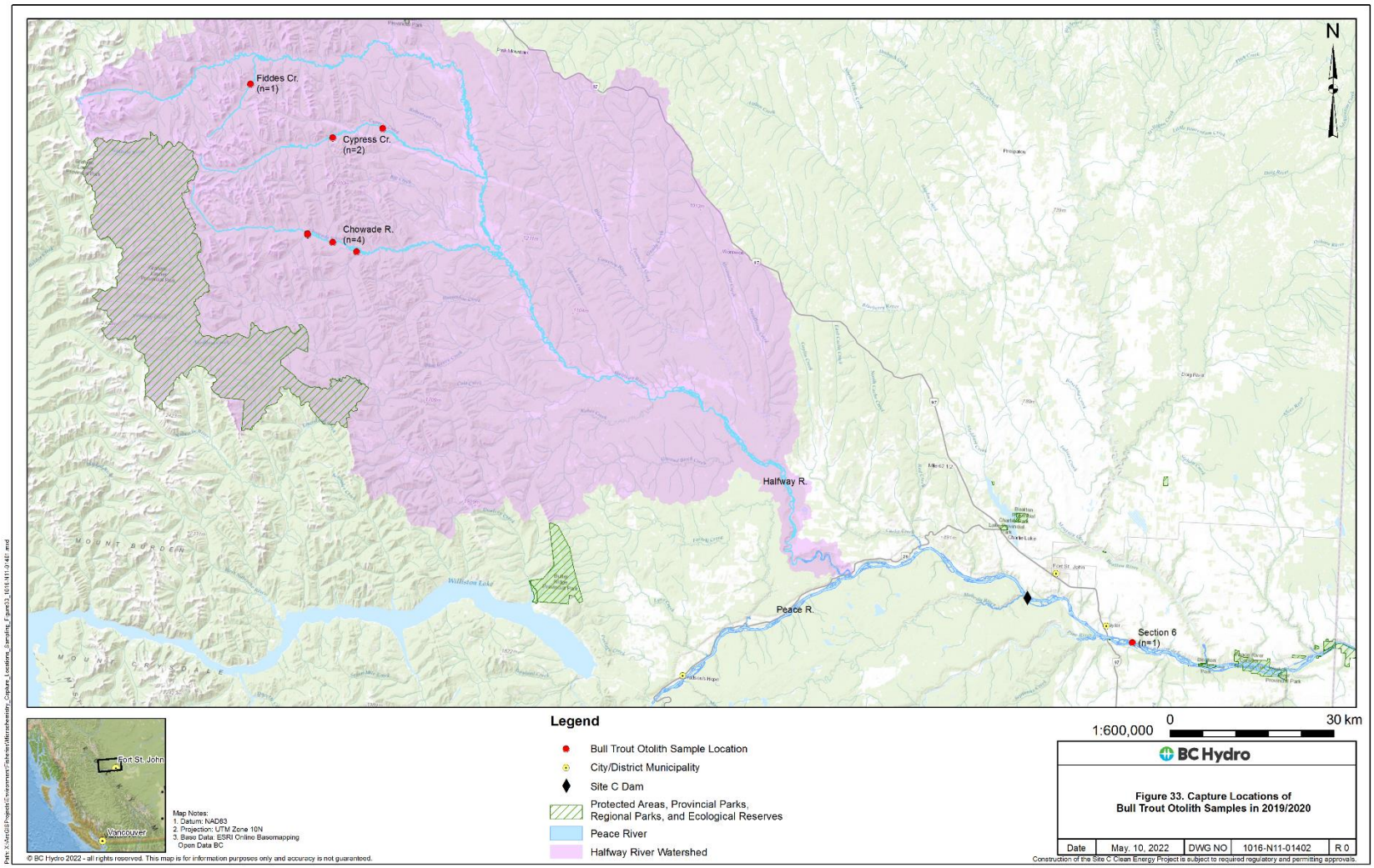
N
Fiddes Cr.
(n=1)
Cypress Cr.
(n=2)
Chowade R.
(n=4)
Halfway R.
Peace R.
Section 6
(n=1)
Fort St. John
Vancouver
Legend
Bull Trout Otolith Sample Location
City/District Municipality
Site C Dam
Protected Areas, Provincial Parks, Regional Parks, and Ecological Reserves
Peace River
Halfway River Watershed
1:600,000
0
30 km
BC Hydro
Figure 33. Capture Locations of Bull Trout Otolith Samples in 2019/2020
Date
May. 10, 2022
DWG NO
1016-N11-01402
R 0
Map Notes:
1. Datum: NAD83
2. Projection: UTM Zone 10N
3. Base Data: ESRI Online Basemapping
Open Data BC
© BC Hydro 2022 - all rights reserved. This map is for information purposes only and accuracy is not guaranteed.
Construction of the Site C Clean Energy Project is subject to required regulatory and permitting approvals.

Figure 33.

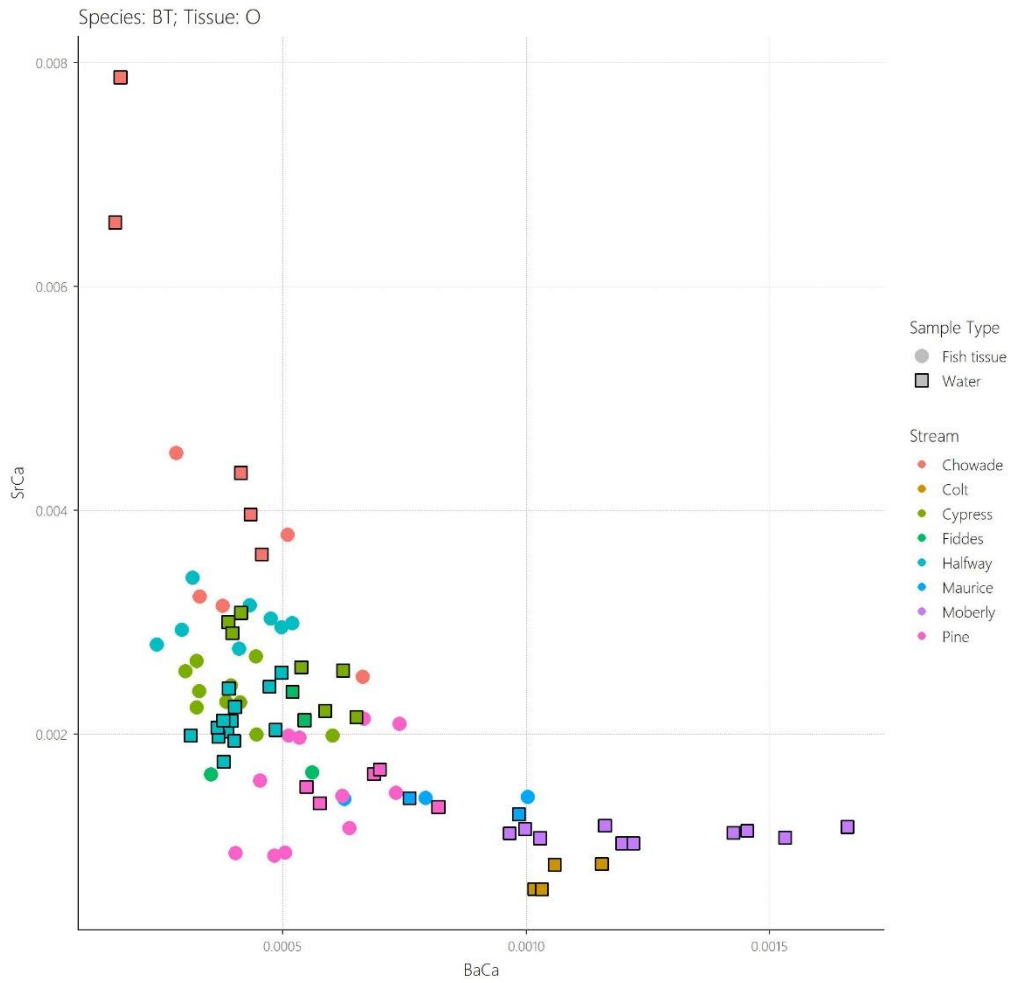

Figure 34. Water and tissue chemistry data used to develop and train the Bull Trout otolith model.

Very few validation samples were available (n = 6), and only from the Halfway watershed, thereby limiting definitive conclusions regarding model overfitting. Overall and within specific sites, prediction accuracies in the validation data fell within the ranges obtained for the training data suggesting limited, if any, overfitting, at least for the sites represented in the validation data. Life-history graphs for Bull Trout validation samples are provided in Appendix E, with examples for Chowade River (Figure 35) and Cypress Creek (Figure 36) provided below.

Table 18. Prediction Accuracy (% correctly classified stream and watershed) and sample sizes for Bull Trout otoliths in training data, and validation data.

Observed Occupancy		Training Data						Validation Data				
		Water			Tissue			Natal		1st Summer		
		% correct		n	% correct		n	% correct		% correct		n
Watershed	Stream	Stream	Watershed		Stream	Watershed		Stream	Watershed	Stream	Watershed	
Maurice	Maurice	50	50	2	0	0	2					0
Halfway	Halfway	77	100	13	75	100	8					0
	Chowade	100	100	5	40	100	5	100	100	67	100	3
	Colt	100	100	4			0					0
	Cypress	57	100	7	10	100	10	50	50	100	100	2
	Fiddes	0	100	2	0	50	2	0	0	0	100	1
Moberly	Moberly	100	100	10			0					0
Pine	Pine	80	80	5	45	45	22					0
	Overall	79	96	48	39	69	49	67	67	67	100	6

Note: Grey cells give sample sizes for water and tissue training data, and validation data.

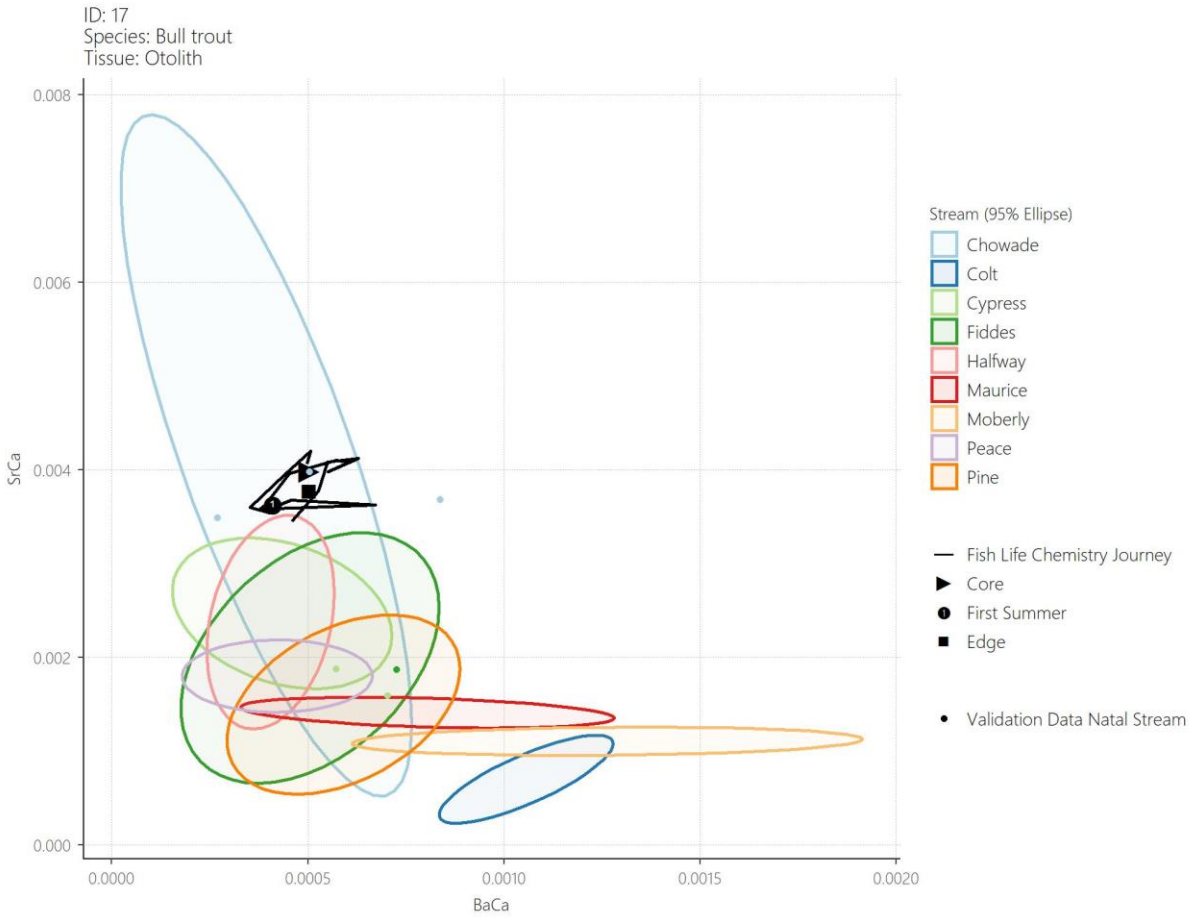

Figure 35. Representative life-history chemistry used for model validation for Chowade River (BT#17, 81 mm).

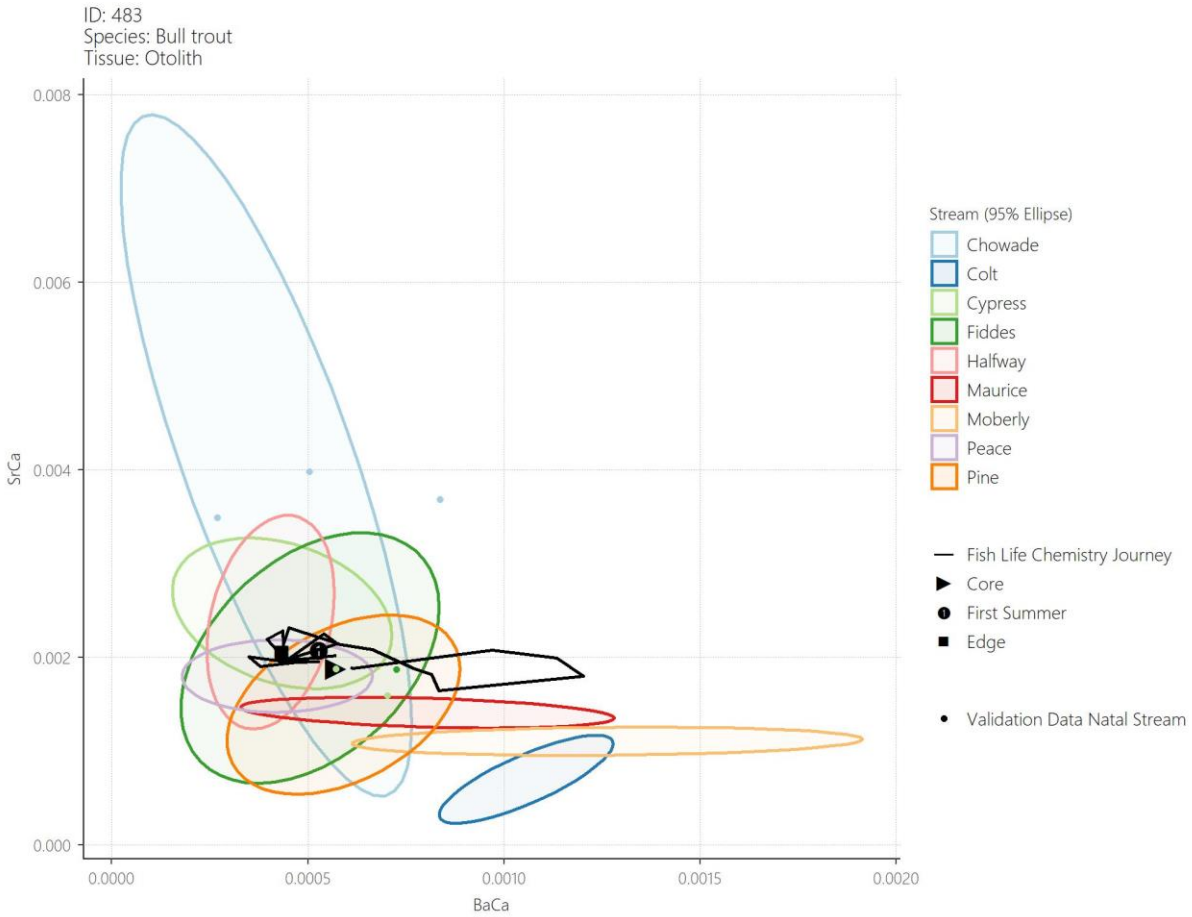

Figure 36. Representative life-history chemistry used for model validation for Cypress Creek (BT #483, 116 mm).

4.3.1.2. *Prediction of Stream Occupancy in Test Data*

Only two Bull Trout otoliths had unknown origins: BT #225 was caught in Chowade River of the Halfway River watershed; and BT #5390 was caught in the Peace River downstream. The nQDA models predicted that the Chowade River-captured Bull Trout recruited from the Pine River (#225); however, inspection of this fish's life-history graph (Figure 37) suggests this individual never left the Halfway River watershed, and more likely recruited from Fiddes Creek. The model predicted that the Bull Trout captured in the Peace River recruited from Cypress Creek of the Halfway River watershed (Figure 38).

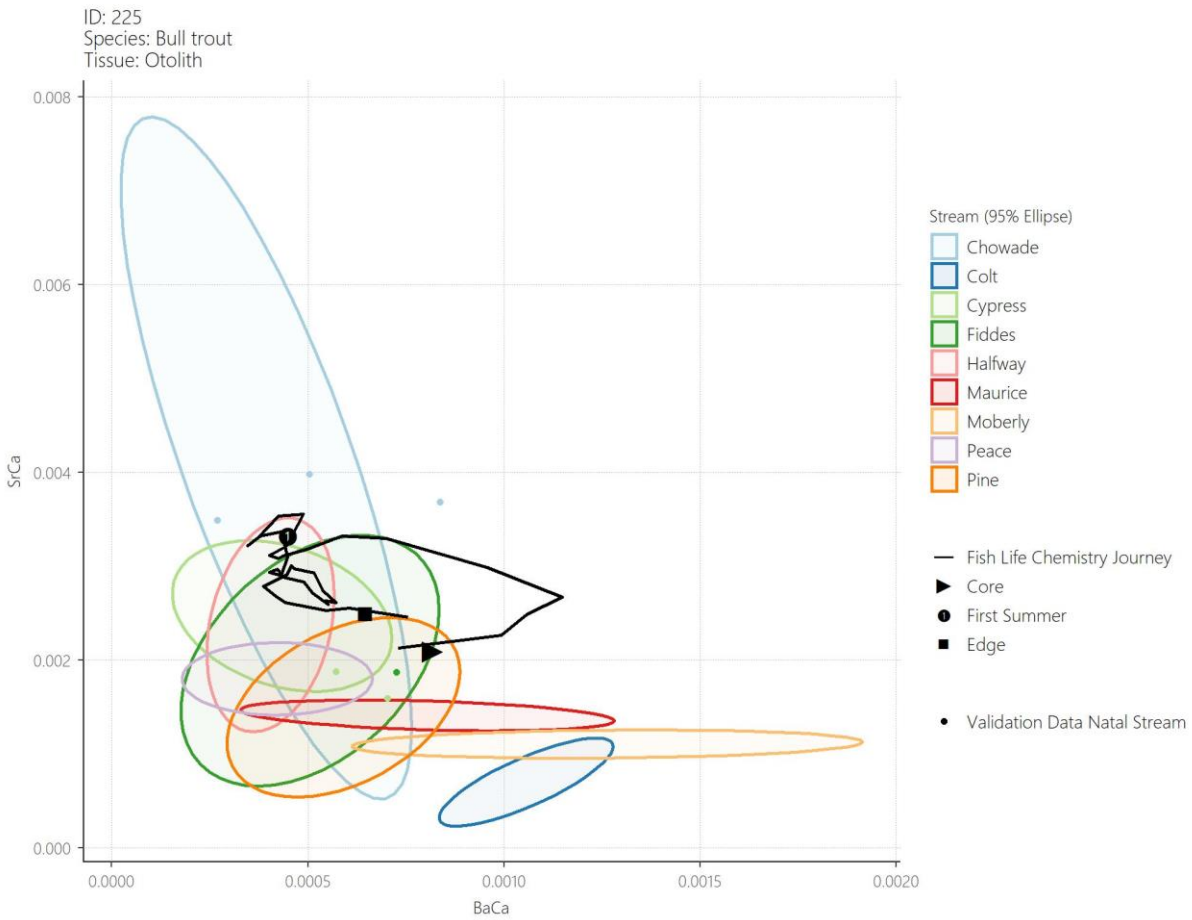

Figure 37. Life-history chemistry for BT#225 (179 mm) predicted by the model to have recruited from Pine River, but chemistry suggests it probably recruited from Fiddes Creek (dark green ellipse) and then migrated to Chowade River, where it remained until capture.

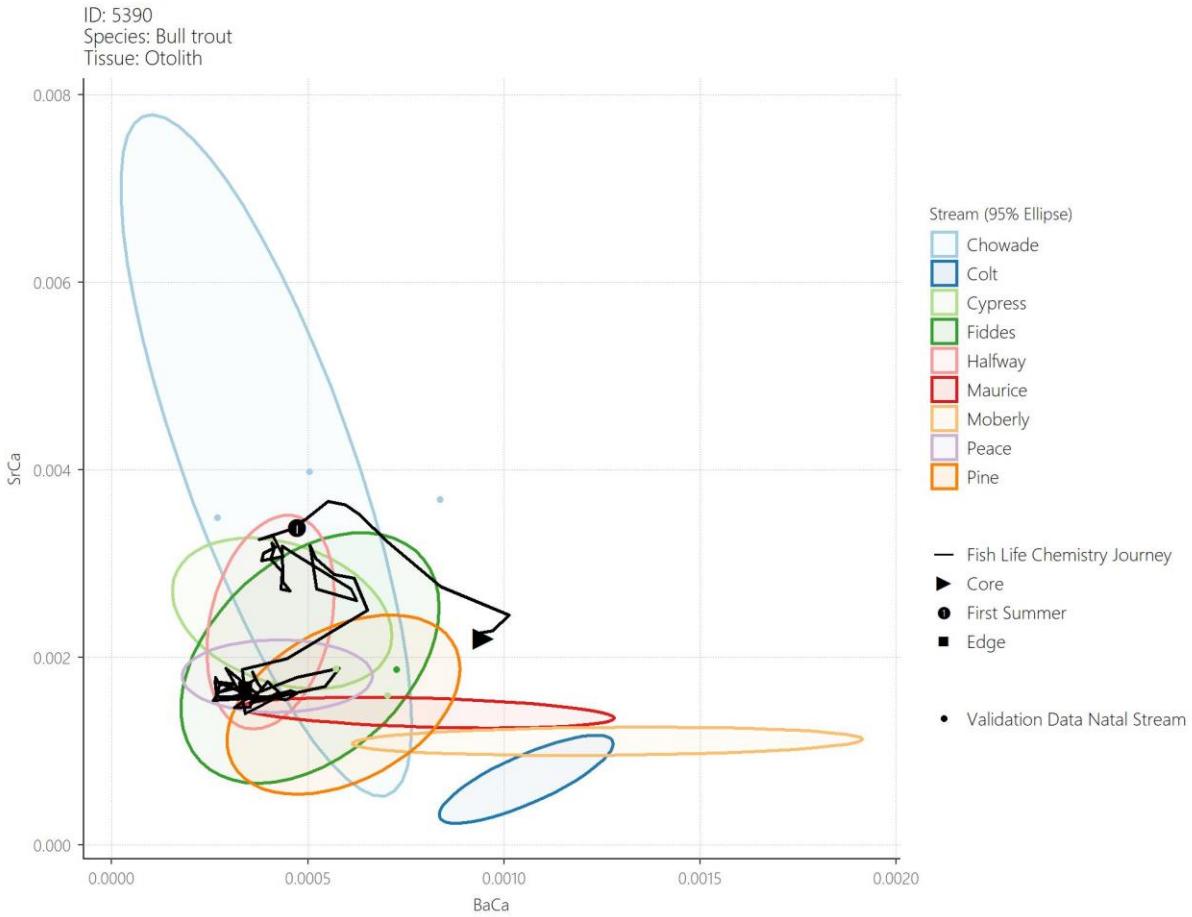

Figure 38. Life-history chemistry for BT #5390 (822 mm) predicted by model to have recruited from Cypress Creek prior to migration and ultimate capture in the Peace River. The natal chemistry appears to be more similar to Pine River or an unknown source, but first summer is similar to Cypress Creek.

4.3.2. Fin Rays

In total, 223 Bull Trout fin rays were collected in 2019 and 2020 (Table 19 and Figure 39):

Table 19. Number and fork length of Bull Trout fish captured with fin ray samples.

Capture Watershed	Capture Site	Number of Fin Rays Collected	Fork Length (mm) range
Halfway River	Chowade River	12	95 - 603
	Colt Creek	9	138 - 218
	Cypress Creek	21	84 - 534
	Fiddes Creek	15	83 - 193
Maurice Creek	Maurice Creek mainstem	2	230 - 256
Moberly River	Moberly River mainstem	6	231 - 425
Peace River	Upstream of Project	86	137 - 822
	Downstream of Project	72	190 - 865

4.3.2.1. Bull Trout Fin Ray Model Performance

Water chemistry results (n=46) from seven waterbodies, and fin ray capture chemistries (n=79) from six waterbodies, for a total of 125 chemistries from eight waterbodies (i.e., prior locations), were used to build and train the model (Figure 40). Further, the sub-set of 11 Bull Trout fin rays used for validation purposes were input into the model to test prediction accuracy, and visually assessed using the life-history graphs.

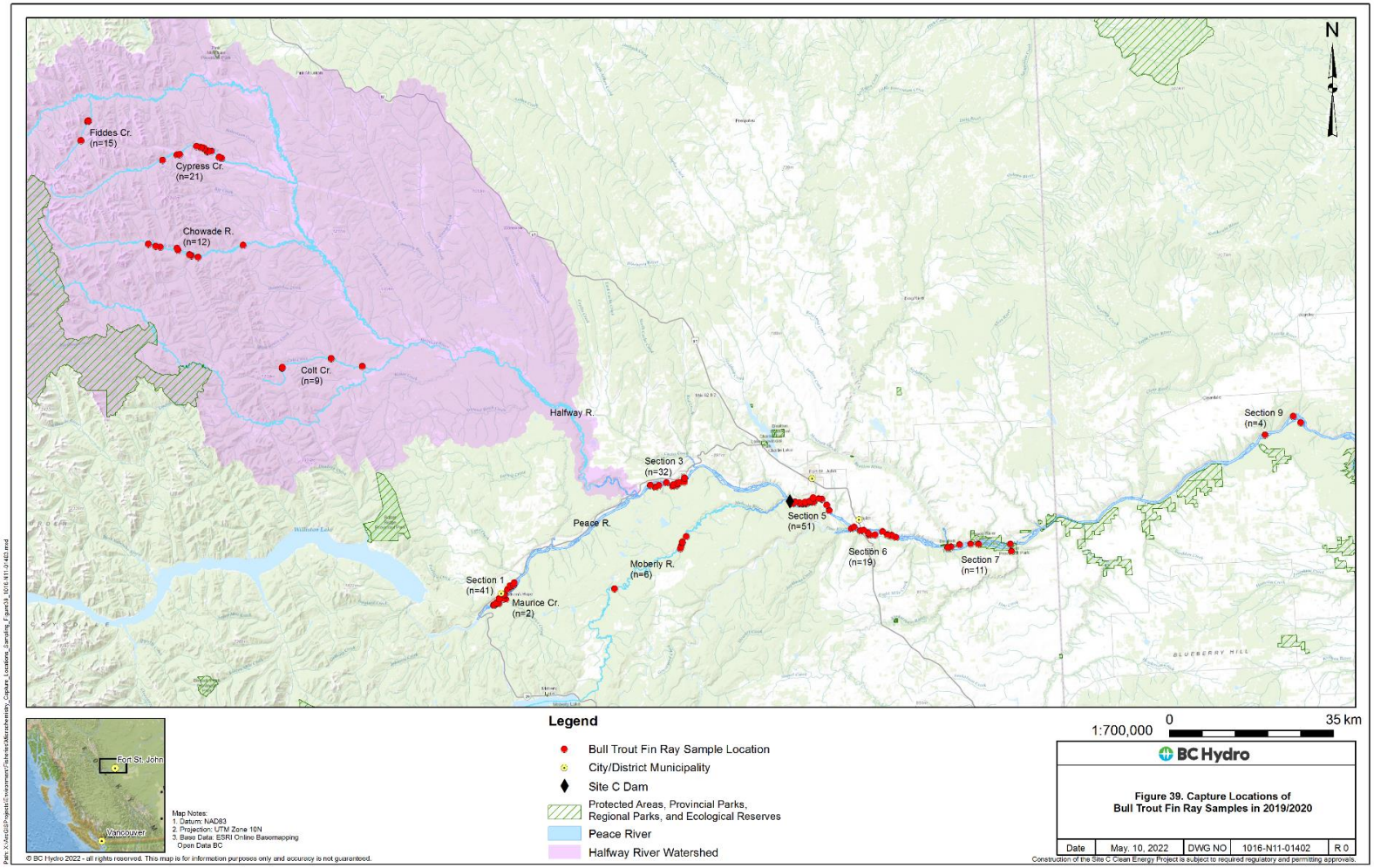
N
Fiddes Cr.
(n=15)
Cypress Cr.
(n=21)
Chowade R.
(n=12)
Colt Cr.
(n=9)
Halfway R.
Section 3
(n=32)
Peace R.
Section 1
(n=41)
Maurice Cr.
(n=2)
Moberly R.
(n=6)
Section 5
(n=51)
Section 6
(n=19)
Section 7
(n=11)
Section 9
(n=4)
Fort St. John
Vancouver
Map Notes:
1. Datum: NAD83
2. Projection: UTM Zone 10N
3. Base Data: ESRI Online Basemapping
Open Data BC
© BC Hydro 2022 - all rights reserved. This map is for information purposes only and accuracy is not guaranteed.
Legend
Bull Trout Fin Ray Sample Location
City/District Municipality
Site C Dam
Protected Areas, Provincial Parks,
Regional Parks, and Ecological Reserves
Peace River
Halfway River Watershed
1:700,000
0
35 km
BC Hydro
Figure 39. Capture Locations of
Bull Trout Fin Ray Samples in 2019/2020
Date
May. 10, 2022
DWG NO
1016-N11-01402
R 0
Construction of the Site C Clean Energy Project is subject to required regulatory and permitting approvals.

Figure 39.

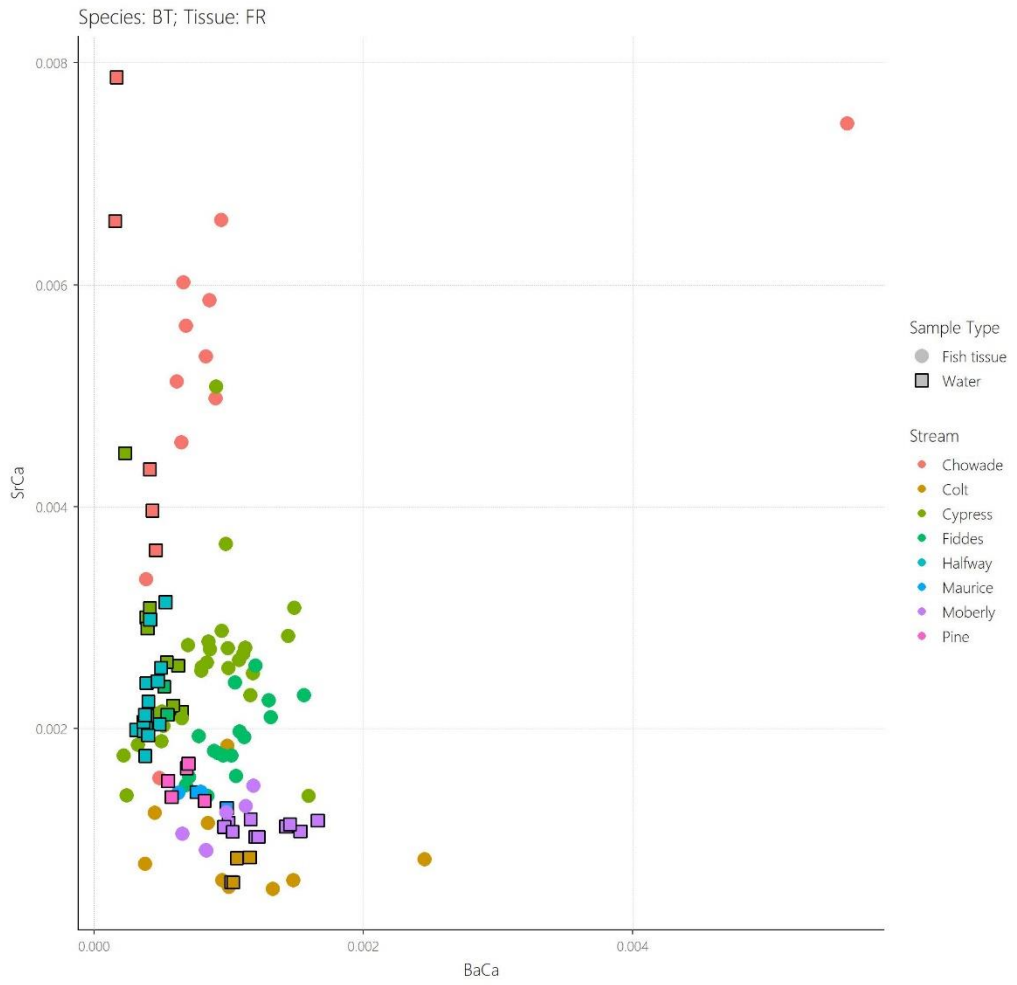

Figure 40. Water and tissue chemistry data used to develop and train the Bull Trout fin ray model.

Accuracy results obtained from the Bull Trout fin ray model were similar to those for Bull Trout otoliths (Figure 40). Prediction accuracies were generally high (>70%) among training data, especially for water samples, with the exceptions of Maurice Creek (water and fin rays) and Moberly River (fin rays) where accuracies were ≤50% (Table 20).

Table 20. Prediction Accuracy (% correctly classified stream and watershed) and sample sizes for Bull Trout fin rays in training data, and validation data.

Observed Occupancy		Training Data						Validation Data				
		Water			Tissue			Natal		1st Summer		
		% correct		n	% correct		n	% correct		% correct		n
Watershed	Stream	Stream	Watershed		Stream	Watershed		Stream	Watershed	Stream	Watershed	
Maurice	Maurice	50	50	2	50	50	4					0
Halfway	Halfway	85	100	13			0					0
	Chowade	100	100	4	82	91	11	100	100	100	100	2
	Colt	75	75	5	63	69	16					0
	Cypress	86	100	7	81	100	27	100	100	100	100	3
	Fiddes			0	63	81	16	0	100	17	100	6
Moberly	Moberly	100	100	10	40	40	5					0
Pine	Pine	80	80	5			0					0
	Overall	87	93	46	70	82	79	45	100	55	100	11

Note: Grey cells give sample sizes for water and tissue training data, and validation data.

Validation data were limited in number and to the Halfway River watershed. Prediction accuracies were 100% among validation samples, except for the classification of Fiddes Creek samples, which were consistently predicted as Cypress Creek, also within the Halfway River watershed. Life-history graphs for Bull Trout fin ray validation samples are provided in Appendix E, with examples for Chowade Creek (Figure 41), Cypress Creek (Figure 42), and Fiddes Creek (Figure 43) provided below. It is discernible in these figures how the validation data, based on core chemistries (shown in figures as colored dots), are dissimilar to the data used to train the model (based on water and edge chemistries; ellipses) especially for Fiddes Creek (dark green ellipse and dots). This dissimilarity between natal and capture chemistries from the same stream/location, may indicate that natal regions in the waterbody have different chemistry from the capture location in the same waterbody. The natal chemistry appears to have either elevated Ba:Ca, Sr:Ca or both relative to capture chemistry (particularly for Fiddes Creek).

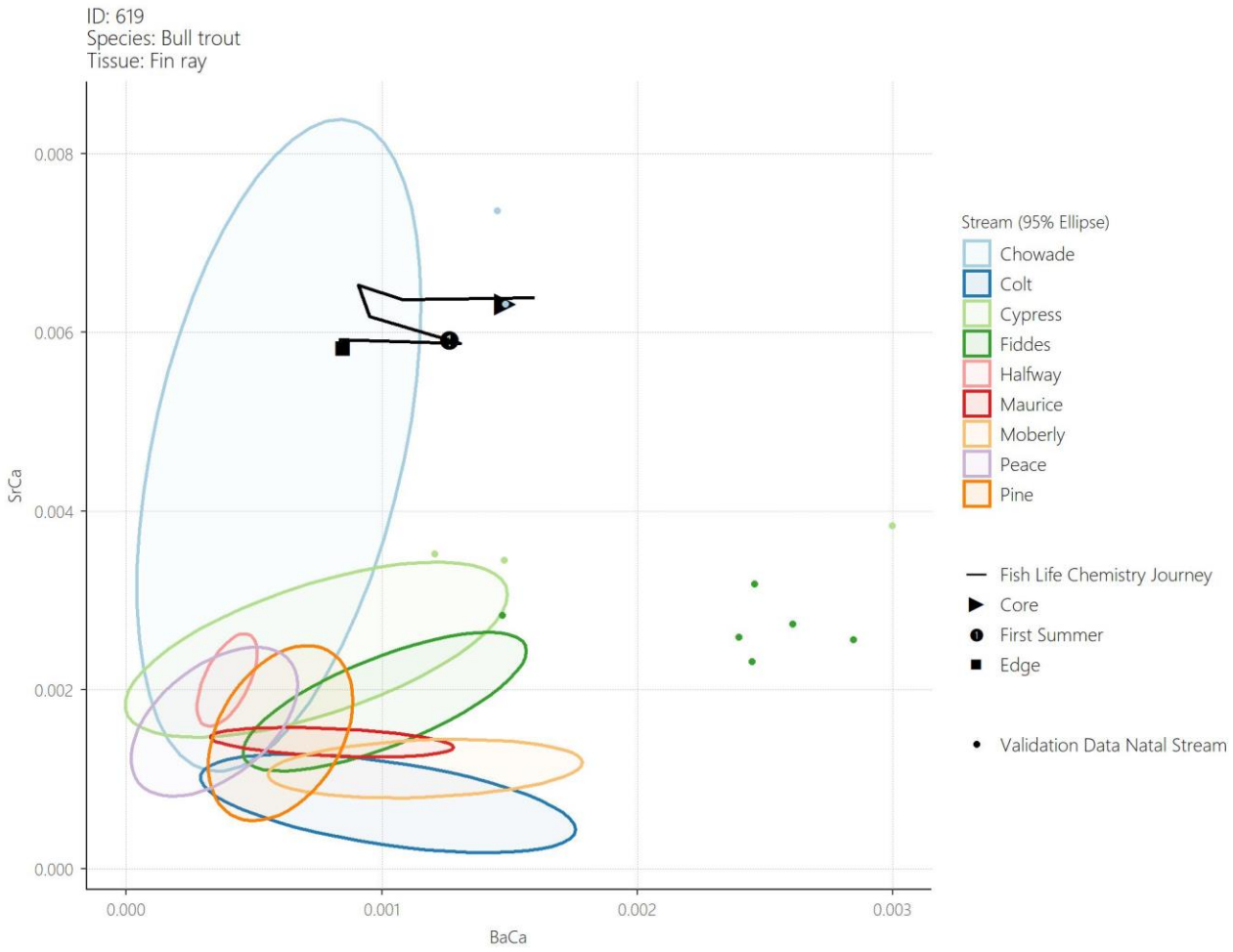

Figure 41. Life-history chemistry for BT#619 (120 mm) predicted by the model to have recruited from Chowade River, where it was captured.

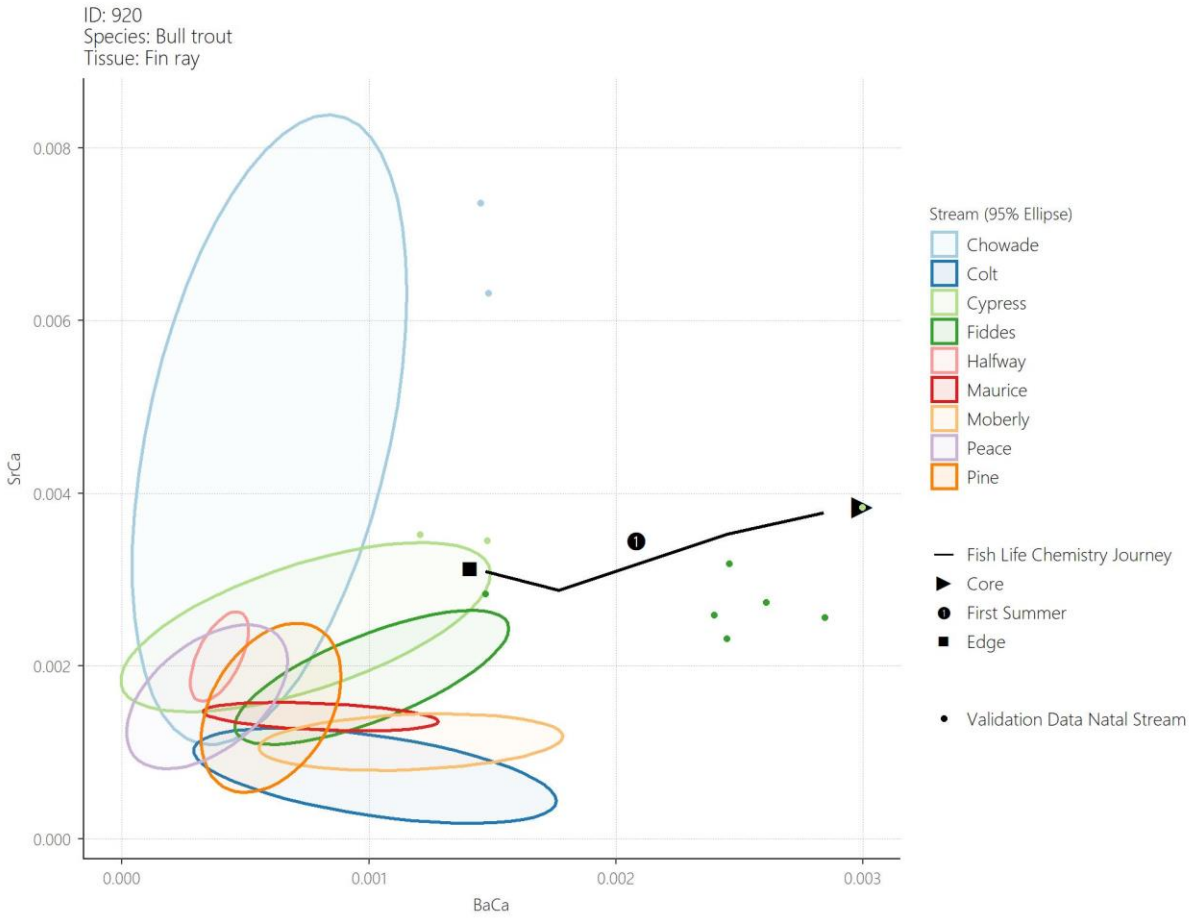

Figure 42. Life-history chemistries for BT #920 (84 mm) predicted by model to have recruited from Cypress Creek, where it was also captured. Note that natal and first summer chemistries are elevated in particularly Ba:Ca relative to the capture chemistry and model (light green ellipse).

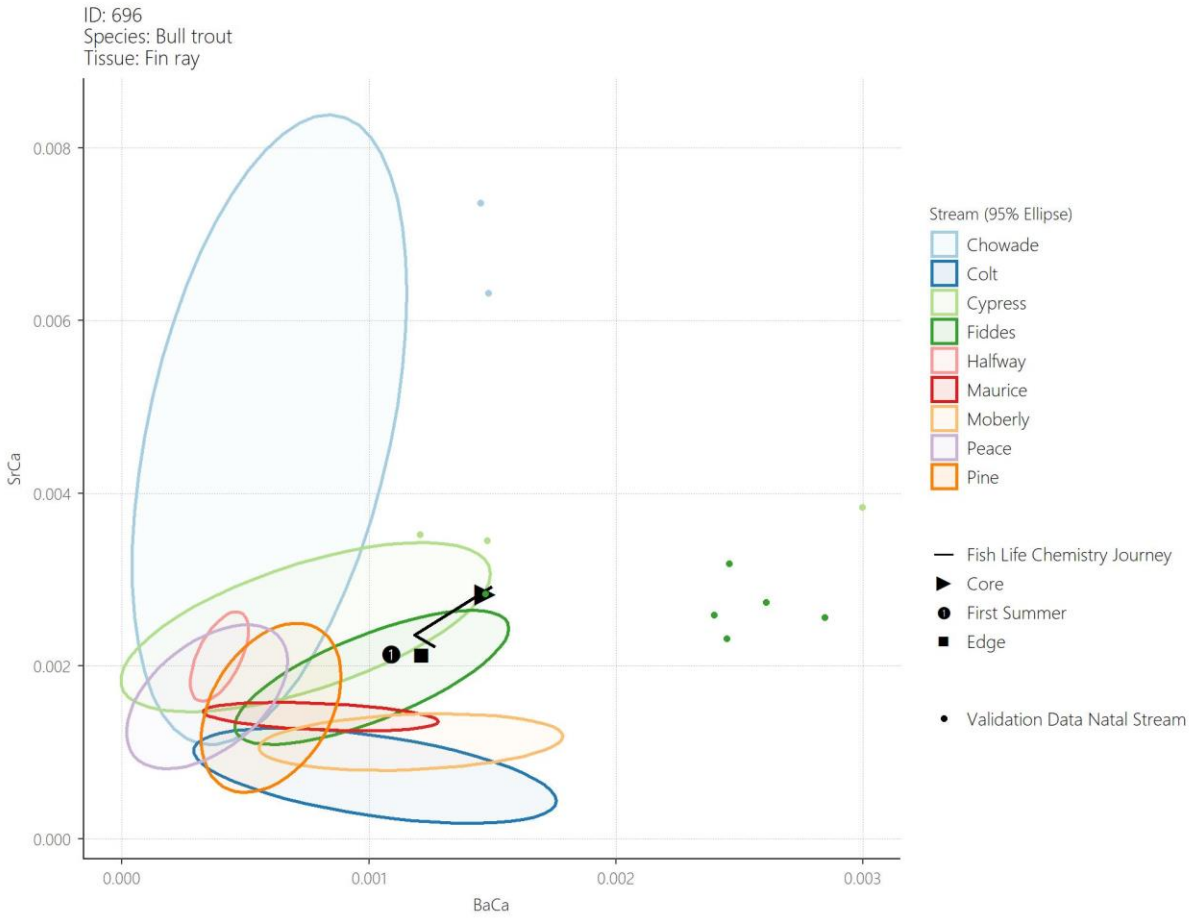

Figure 43. Life-history chemistry for BT 696 (83 mm) predicted by the model to have recruited from Fiddes Creek, where it was captured.

Further, the validation data for Fiddes and Cypress creeks are similar, at least relative to training data. The BT #351 is an example of a fish that was predicted to have recruited from Cypress Creek, but the life-history graph suggests that it more likely recruited from Fiddes Creek (Figure 44), which coincides with its capture location (Fiddes Creek) and fork length (121 mm).

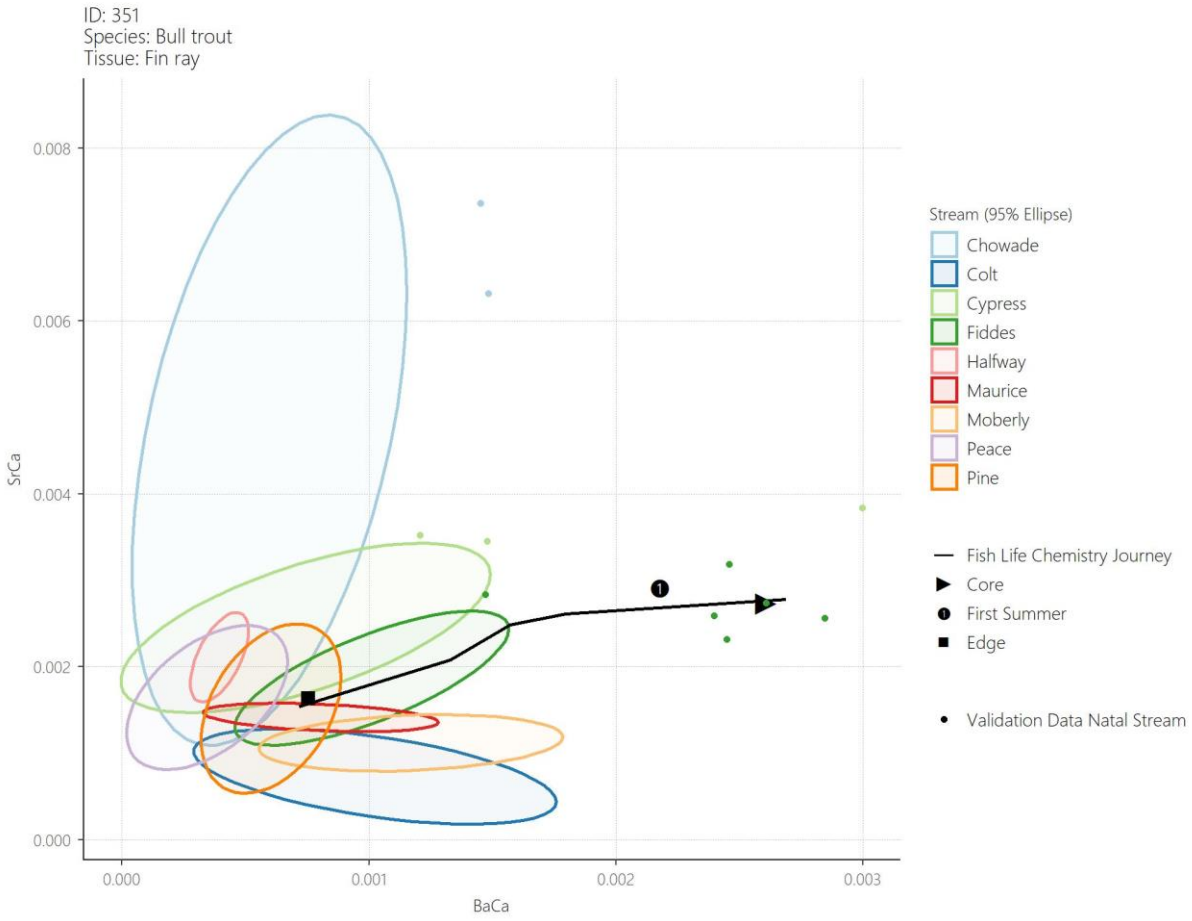

Figure 44. Life-history chemistry for BT 351 (121 mm) predicted by the model to have recruited from Cypress Creek. However, based on the chemistry pattern, it more likely recruited from Fiddes Creek (notice similarity of natal chemistry to validation data – dark green dots) where it was captured in Fiddes Creek.

4.3.2.2. Prediction of Stream Occupancy in Test Data

Of the 210 Bull Trout fin rays with unknown origins, 75% (n=157) were from fish captured in the Peace River (Table 21); the remaining samples came from fish caught in the Halfway River watershed (total: n=45; Cypress Creek: n=17; Chowade River: n=10; Colt and Fiddes creeks: n=9 each), Moberly River (n=6) and Maurice Creek (n=2).

The two Bull Trout captured in Maurice River were predicted to have recruited from Chowade Creek in the Halfway River watershed. Of the six Bull Trout captured in Moberly River, only one was predicted to have recruited from Moberly River. The other five recruited from Cypress Creek (n=2) and Chowade River (n=2) in the Halfway River watershed, and Pine River (n=1). Overall, the 45 Bull Trout captured in Halfway River tributaries were predicted to have originated from the same or another tributary of the Halfway River from where it was recruited.

Table 21. Model predicted Bull Trout recruitment sources based on fin ray structures. Only capture locations (along the top) and streams included in model (rows) are presented in the table.

Predicted Location		Capture Location								
			Halfway R.					Peace R.		Total
Watershed	Stream	Maurice Ck.	Colt Ck.	Fiddes Ck.	Chowade R.	Cypress Ck.	Moberly R.	Upstream	Downstream	
Maurice Ck.										
Halfway R.	Colt Ck.		5					3	2	10
	Fiddes Ck.		1	6		1		4	8	20
	Chowade R.	2			8		2	32	37	81
	Cypress Ck.		3	3	2	16	2	26	35	87
	Mainstem							3		3
Moberly R.							1		2	3
Pine R.							1	3	2	6
Total		2	9	9	10	17	6	71	86	210

There was very little difference in recruitment sources predicted between Bull Trout captured upstream or downstream of the Project in the Peace River. It was predicted that 96% of the Bull Trout captured upstream of the Project recruited from the Halfway River watershed, mostly Chowade River (45%) and Cypress Creek (37%). Similarly, 95% of the Bull Trout captured downstream recruited from the Halfway River watershed, again mainly from Chowade River (43%) and Cypress Creek (41%). Smaller recruitment sources were predicted for Moberly (1%) and Pine (3%) rivers. Examples of life-history chemistries are presented below in Figure 45 to Figure 50.

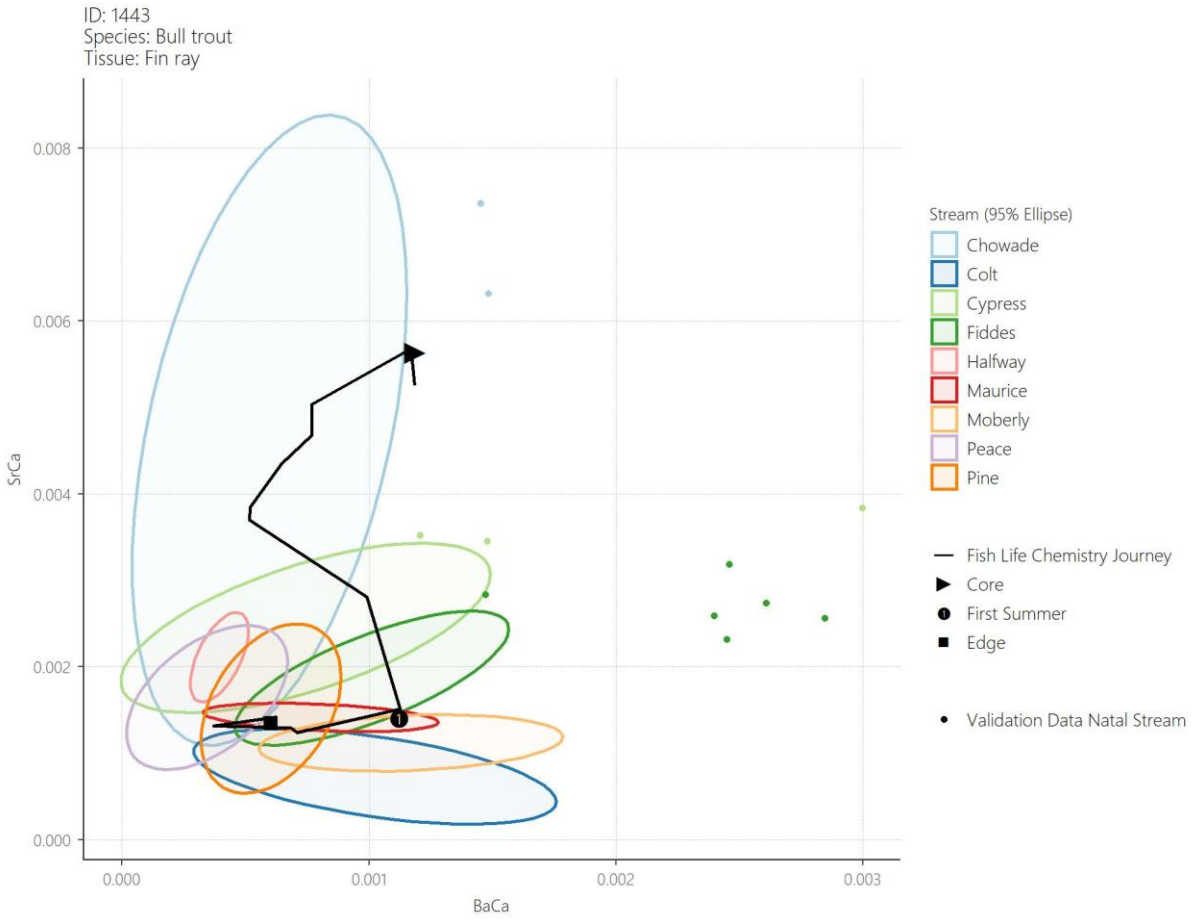

Figure 45. Life-history chemistry for BT#1443 (256 mm) predicted by the model to have recruited from Chowade River, where it then migrated to Maurice Creek until capture.

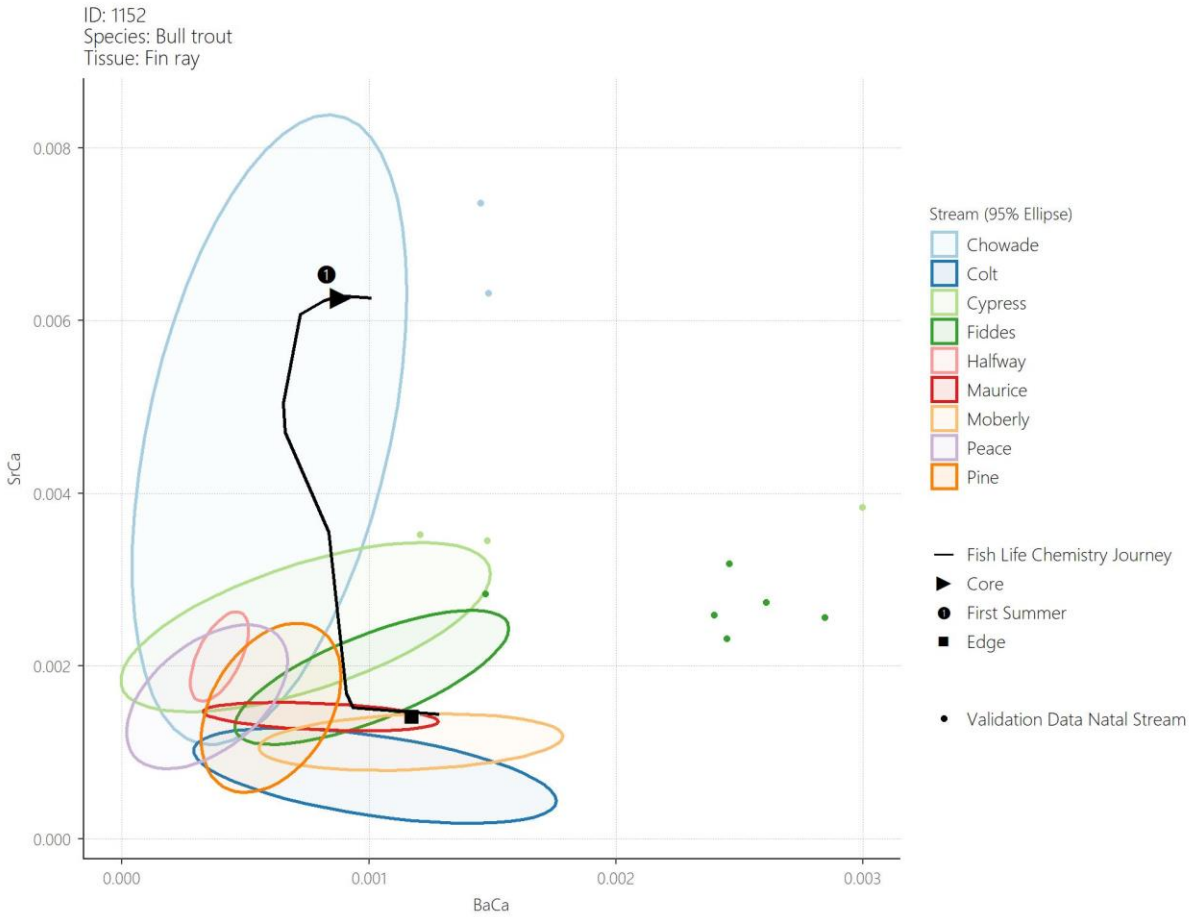

Figure 46. Life-history chemistry for BT #1152 (231 mm) predicted by the model to have recruited from Chowade River, where it then migrated to Moberly River until capture;

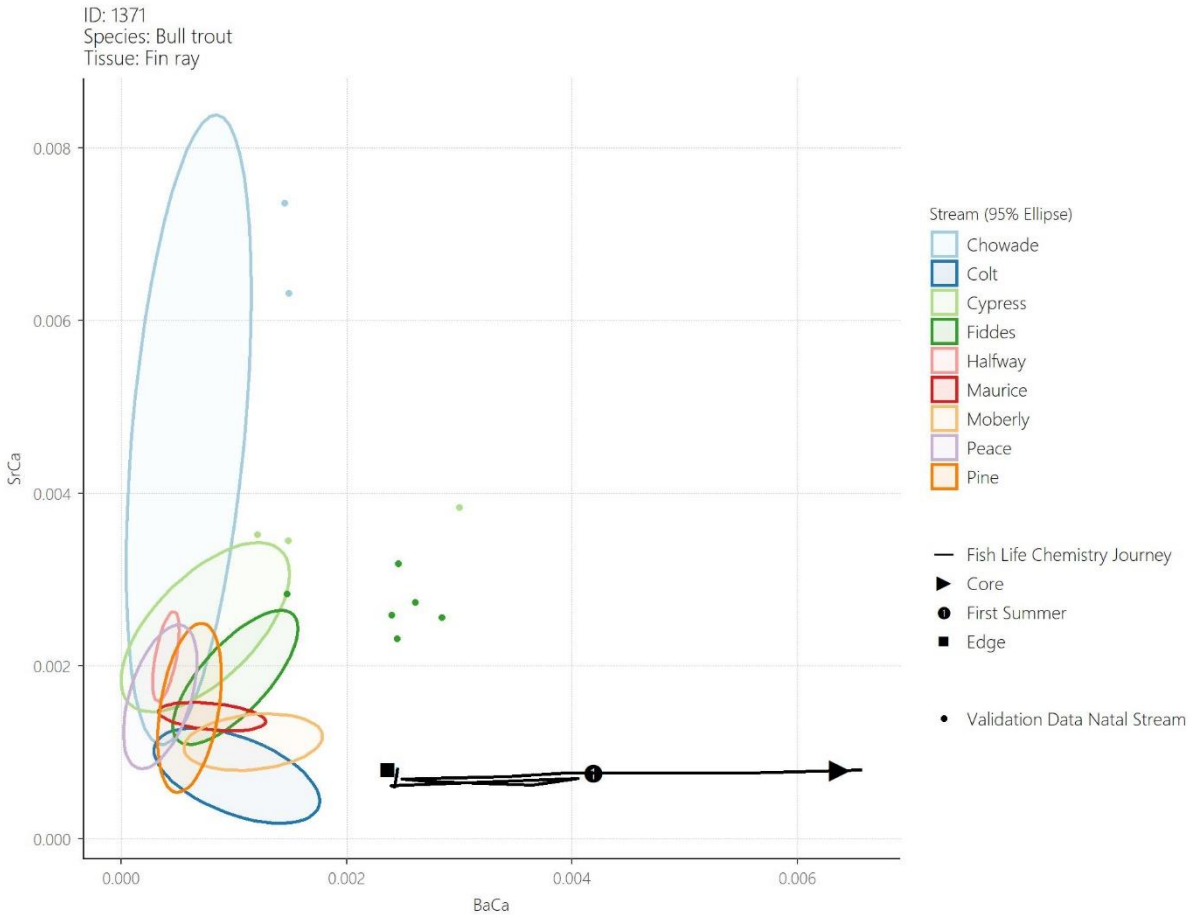

Figure 47. Life-history chemistry for BT#1371 (171 mm) predicted to have remained in Colt Creek its whole life. This is a good example of how Colt Creek fin ray tissue chemistry is different (higher Ba:Ca) from the data used to build and train the model.

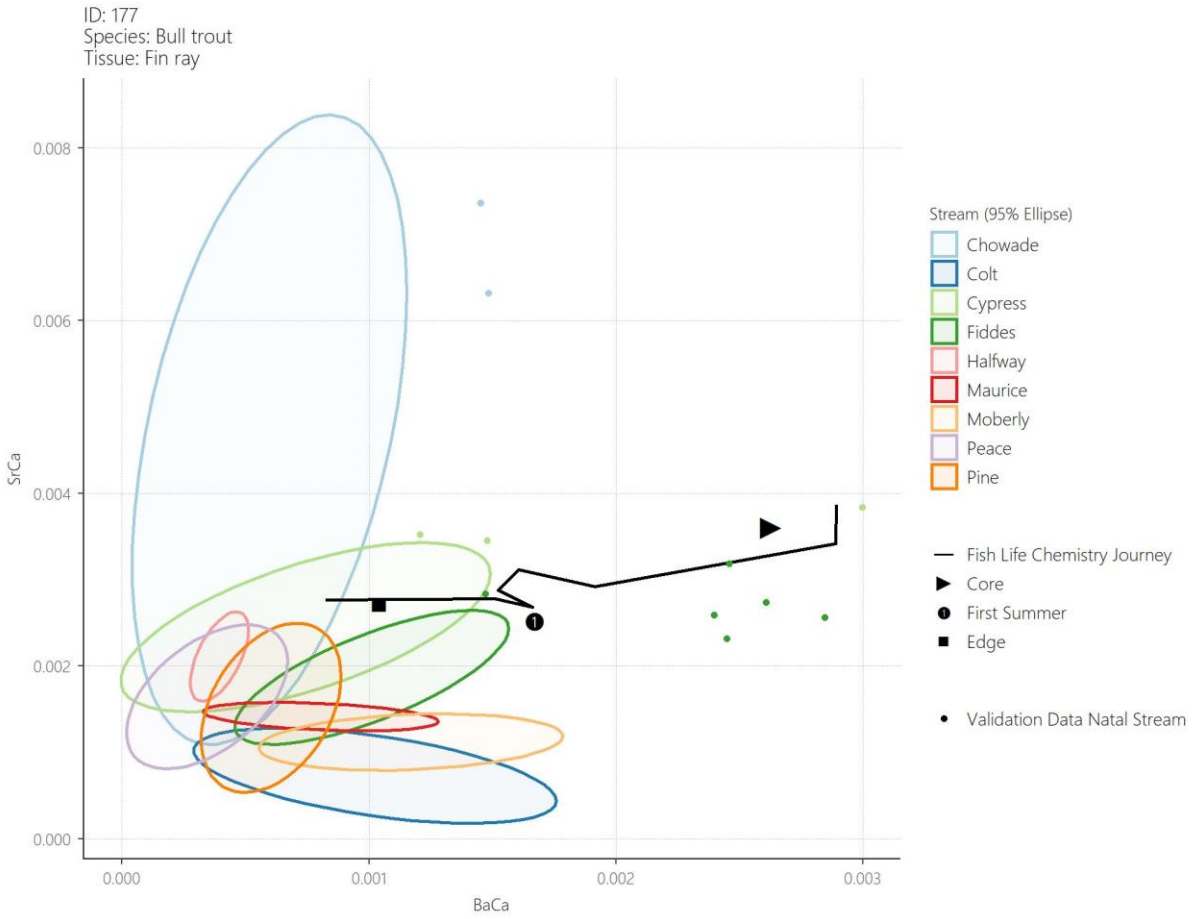

Figure 48. Life-history chemistry for BT 177 (126 mm) predicted to have remained in Cypress Creek its whole life.

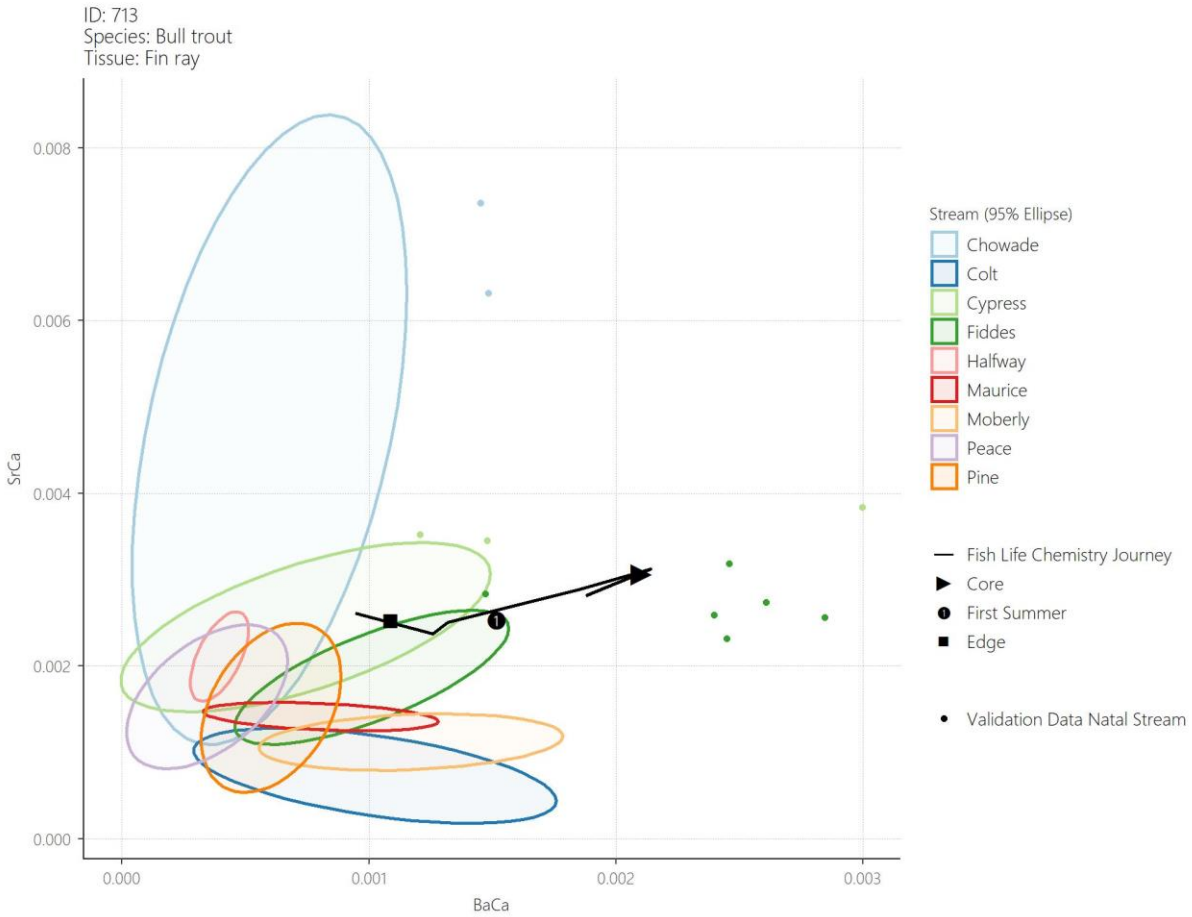

Figure 49. Life-history chemistry for BT#713 (120 mm) predicted to have remained in Fiddes Creek its whole life.

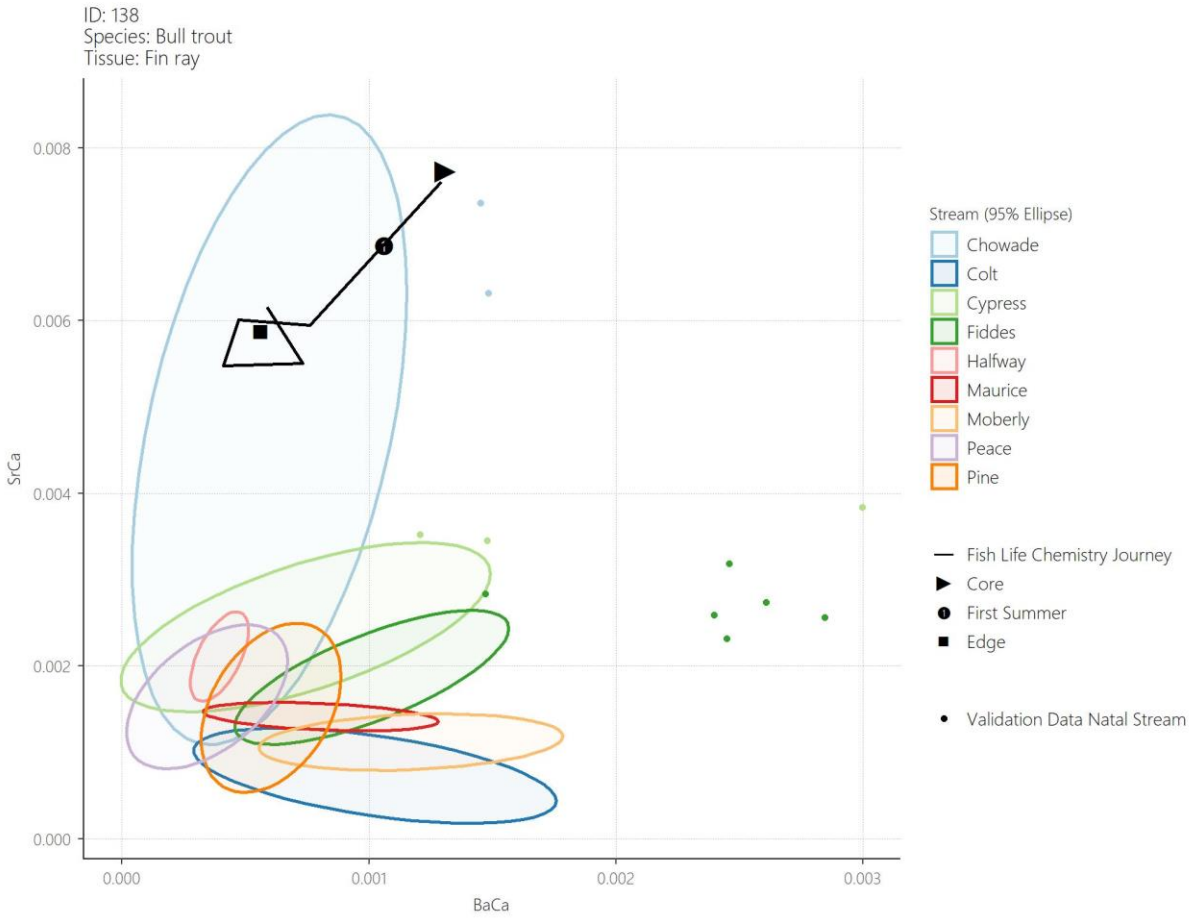

Figure 50. Life-history chemistry for BT#138 (128 mm) predicted to have remained in Chowade River its whole life.

While these figures show excellent alignment between model predictions and life history graphs, there were numerous discrepancies (Table 22). There was 19% (42/210) at the stream level, and 5% (10/210) at the watershed level, indicating some mismatch between the model training data and test data. The model misclassified four individuals as originating from Colt Creek: three were more likely from Moberly River due to elevated Ba:Ca signatures (e.g., Figure 51), and one may have recruited from Pine River with life history signatures with lower Ba:Ca and slightly higher Sr:Ca ratios (Figure 52). There were also discrepancies between the model and the life history for four Bull Trout captured in Cypress Creek: two (BT #668 and BT #5658) had life-history chemistries more closely resembling

Moberly River due to the high Ba:Ca signature (Figure 53), and BT #2744 and BT #5553 had life-history chemistries unlike any other fish captured, so may be from unknown sources (Figure 54). The model also predicted that one of the BT captured in Peace River downstream originated from Fiddes Creek, but is possibly from the Pine River, as the signature had lower Ba:Ca ratio than other fish from Fiddes Creek (Figure 55). Alternatively, BT #2480 was predicted to have recruited from Pine River but may be from Fiddes Creek. Life-history graphs for all Bull Trout fin rays are provided in Appendix E.

Table 22. Discrepancies between the model predictions and life-history chemistries for Bull Trout fin rays

Fish ID	Capture Location	Model Prediction	Life History Graph
2833	Peace	Chowade	Cypress
3832	Peace	Chowade	Cypress
3911	Peace	Chowade	Cypress
4899	Peace	Chowade	Cypress
620	Peace	Chowade	Cypress
887	Peace	Chowade	Cypress
1856	Peace	Chowade	Cypress
3554	Peace	Chowade	Cypress
5390	Peace	Chowade	Halfway
3735	Peace	Colt	Halfway
1371	Colt	Colt	Kobes
2228	Peace	Colt	Moberly
2952	Peace	Colt	Moberly
8135	Peace	Colt	Moberly
6095	Peace	Colt	Pine
191	Peace	Cypress	Chowade
2083	Peace	Cypress	Chowade
207	Chowade	Cypress	Fiddes
1122	Colt	Cypress	Fiddes
334	Fiddes	Cypress	Fiddes
365	Fiddes	Cypress	Fiddes
713	Fiddes	Cypress	Fiddes
4277	Peace	Cypress	Fiddes
4841	Peace	Cypress	Fiddes
5195	Peace	Cypress	Fiddes
1172	Peace	Cypress	Fiddes
2794	Peace	Cypress	Fiddes
6094	Peace	Cypress	Fiddes
2298	Peace	Cypress	Halfway
3716	Peace	Cypress	Halfway
3833	Peace	Cypress	Halfway
2531	Peace	Cypress	Halfway
2924	Peace	Cypress	Halfway
3976	Peace	Cypress	Halfway
668	Peace	Cypress	Moberly
5658	Peace	Cypress	Moberly
2744	Peace	Cypress	Unknown
5553	Peace	Cypress	Unknown
1349	Colt	Fiddes	Colt
931	Cypress	Fiddes	Cypress
5645	Peace	Fiddes	Pine
2480	Peace	Pine	Fiddes

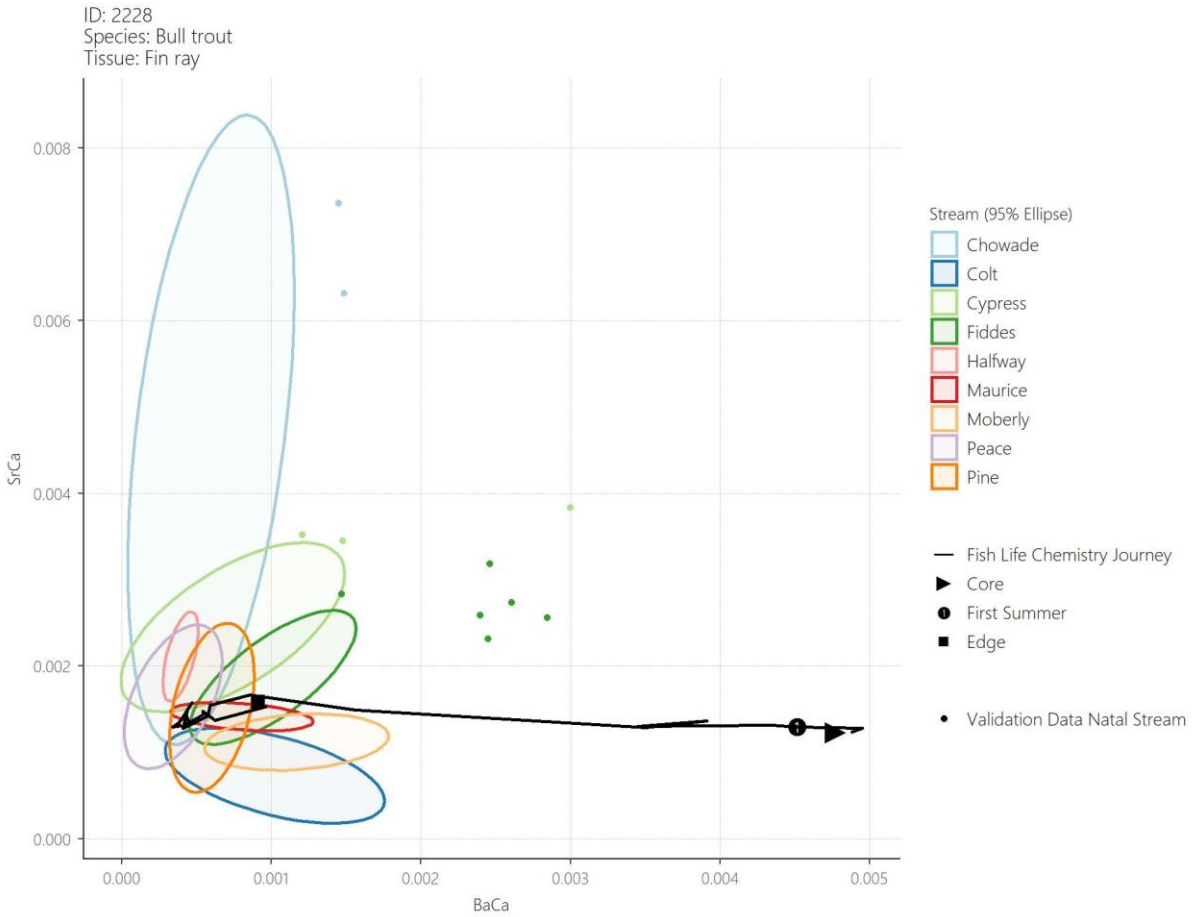

Figure 51. Life-history chemistry for BT#2228 (578 mm) predicted by the model to have recruited from Colt *Creek, however the life-history chemistry suggests Moberly River is the likely recruitment source (as Moberly River has slightly higher Sr:Ca than Colt Creek).*

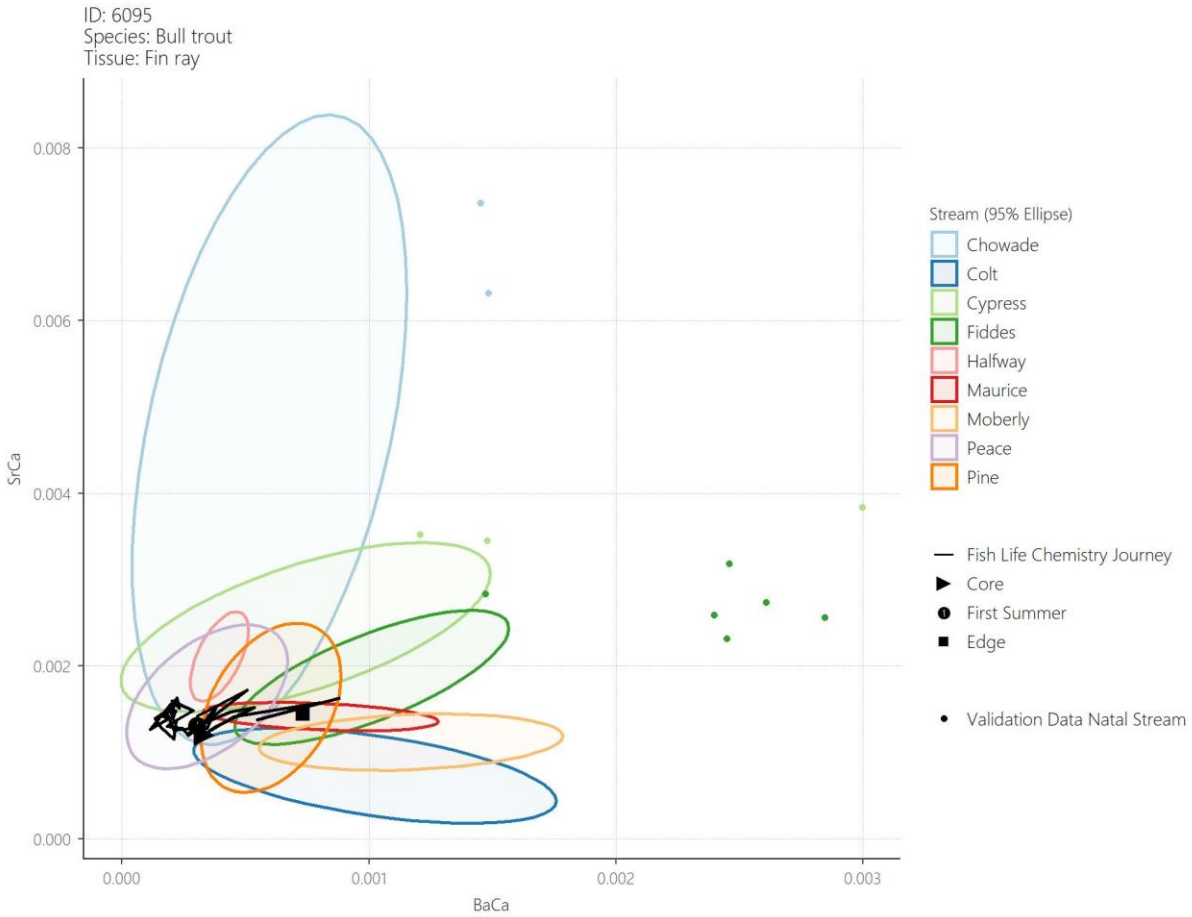

Figure 52. Life-history chemistry for BT#6095 (735 mm) predicted by the model to have recruited from Colt Creek, however the life-history chemistry suggests Pine River may be the likely recruitment source (Colt Creek chemistry has higher Ba:Ca and slightly lower Sr:Ca).

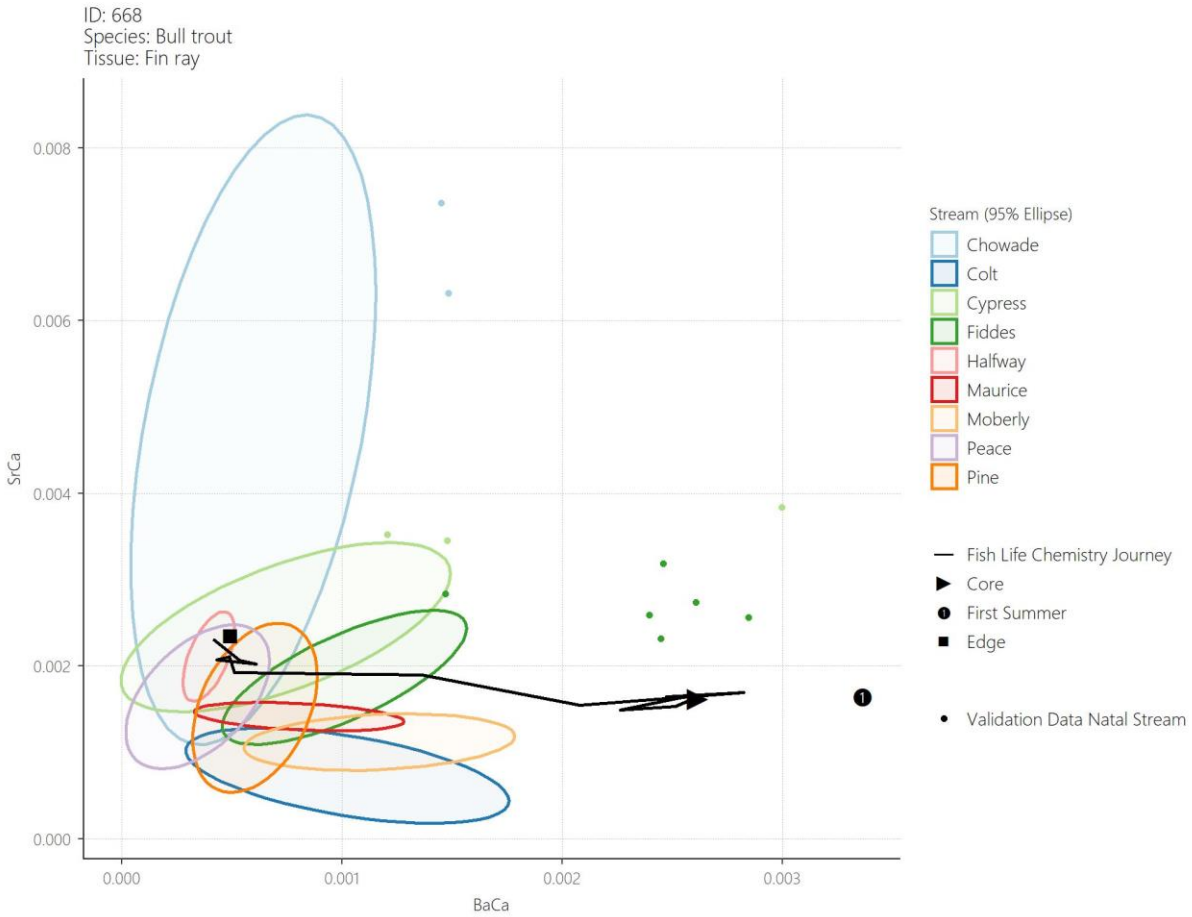

Figure 53. Life-history chemistry for BT#668 (296 mm) predicted by the model to have recruited from Cypress Creek, however the life-history chemistry suggests Moberly River is the likely recruitment source.

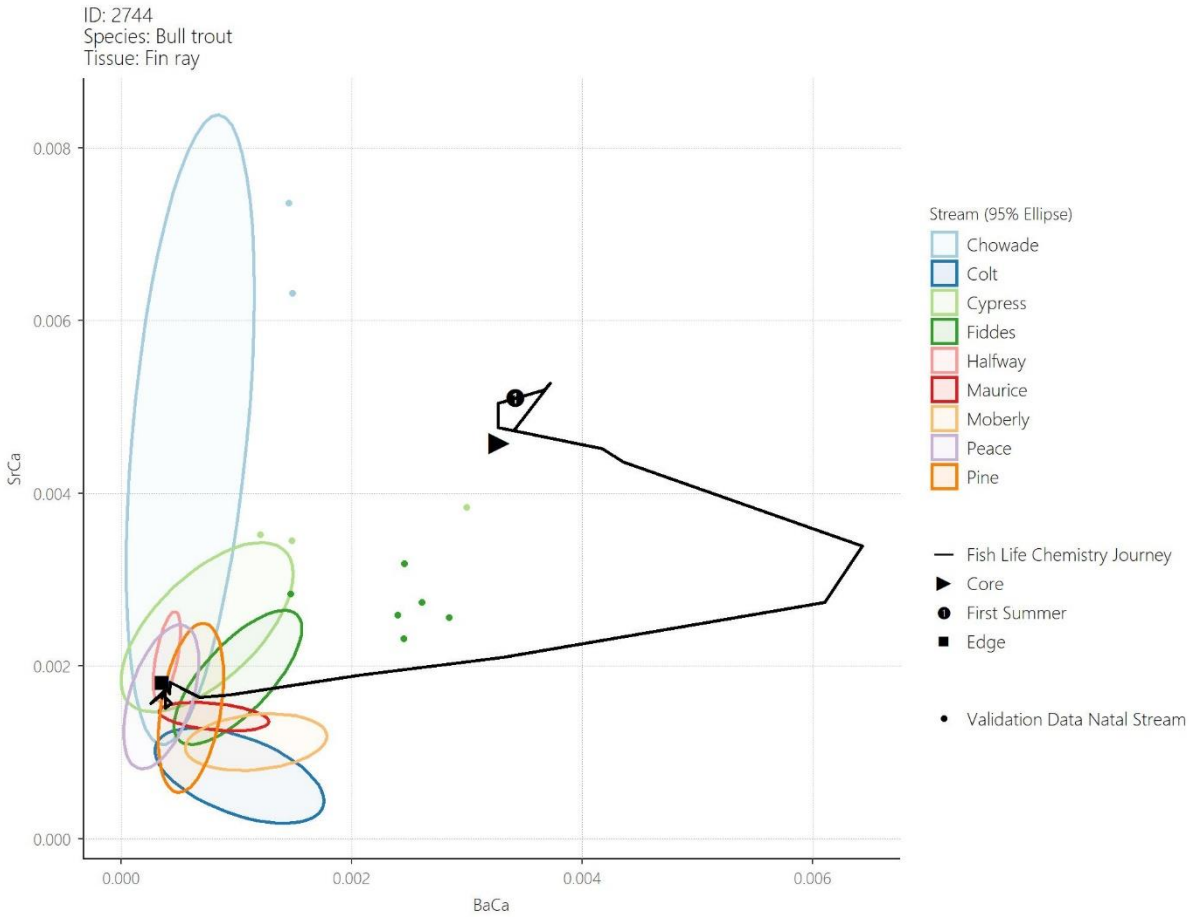

Figure 54. Life-history chemistry of *BT#2744 (374 mm) predicted to have recruited from Cypress Creek, however the life history is unlike any other Bull Trout life histories or any location chemistries, suggest*ing a potentially *unknown recruitment source.*

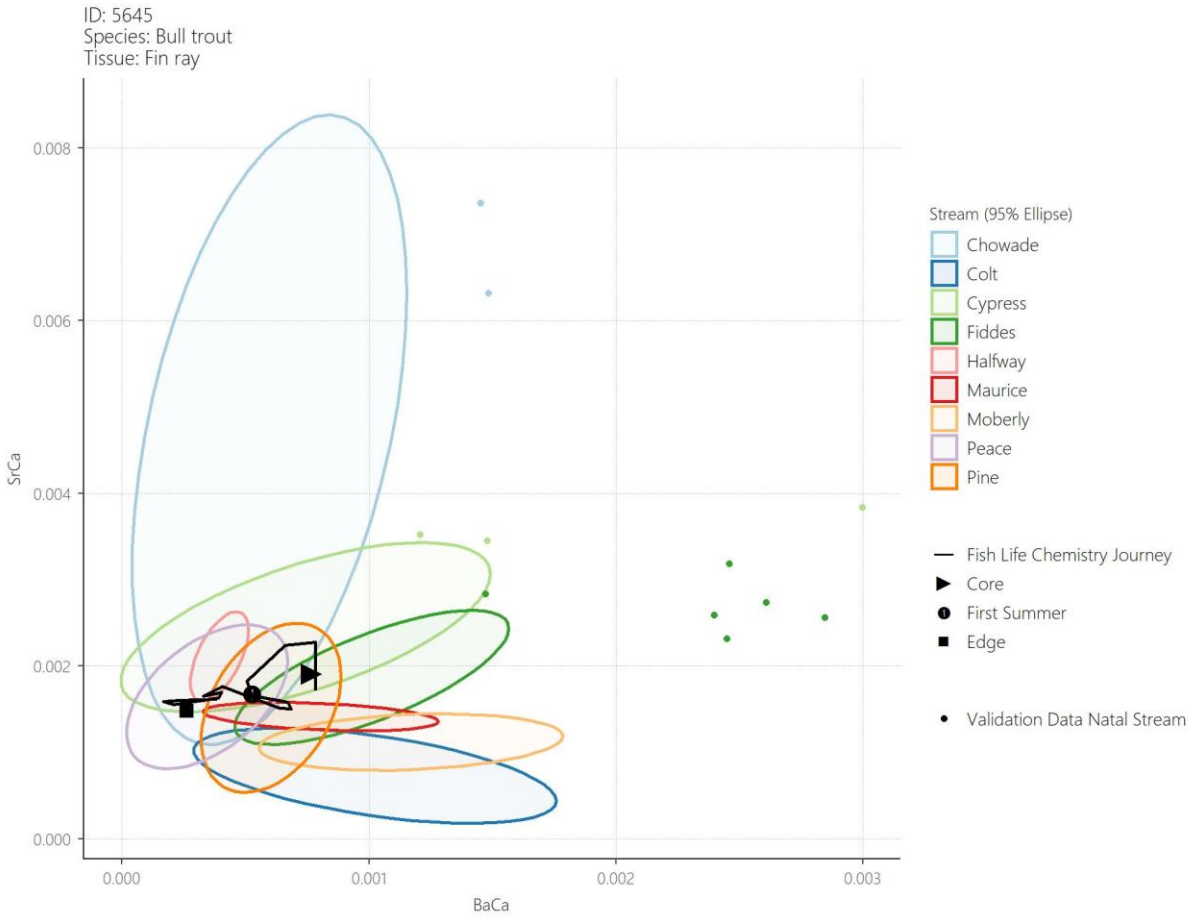

Figure 55. Life-history chemistry of *BT#5645 (290 mm) predicted to have recruited from Fiddes Creek, but life history suggests Pine River (Fiddes Creek Bull Trout have considerably higher Ba:Ca).*

4.3.3. Summary of Recruitment Sources for Bull Trout

As with Arctic Grayling, fin rays were predominantly used to derive overall predictions for the Bull Trout recruitment sources, as more fin rays were available than otoliths (n=210 versus n=2 otoliths). Streams in the Halfway River watershed (mainly Chowade River and Cypress Creek) were the dominant recruitment sources (>95%) for both upstream and downstream Peace River captures. Moberly River offered a small upstream source (only 1% predicted), and the Pine River was predicted as the only potential downstream recruitment source (3%).

These conclusions echo those reported previously for Bull Trout. Both Earthtone and Mainstream (2013) and TrichAnalytics (2020) also predicted Halfway River watershed as the dominant recruitment source for upstream and downstream captured Bull Trout. Earthtone and Mainstream (2013) also predicted recruitment from tributaries in the Halfway River that were not considered in the present model (i.e., Cameron River and Turnoff Creek). Other sources were Gething Creek (7%), which was also not included in the model of the present study, and 17% of their predictions were unknown sources. The only other sources predicted for Bull Trout by TrichAnalytics (2020) was Moberly River (4%) and Pine River (5%; as well as 4% from unknown sources). This study reported <1% unknown recruitment sources.

4.4. Rainbow Trout Stream Occupancy

4.4.1. Otoliths

In total, 50 Rainbow Trout otoliths were collected in 2019 and 2020 (Table 23 and Figure 56):

Table 23. Number and fork length of Rainbow Trout fish captured with otolith samples.

Capture Watershed	Capture Site	Number of Otoliths Collected	Fork Length (mm) range
Halfway River	Colt Creek	8	31 - 86
	Chowade River	1	139
	Kobes Creek	6	33 - 156
Farrell Creek	Farrell Creek mainstem	34	35 - 154
Maurice Creek	Maurice Creek mainstem	1	90

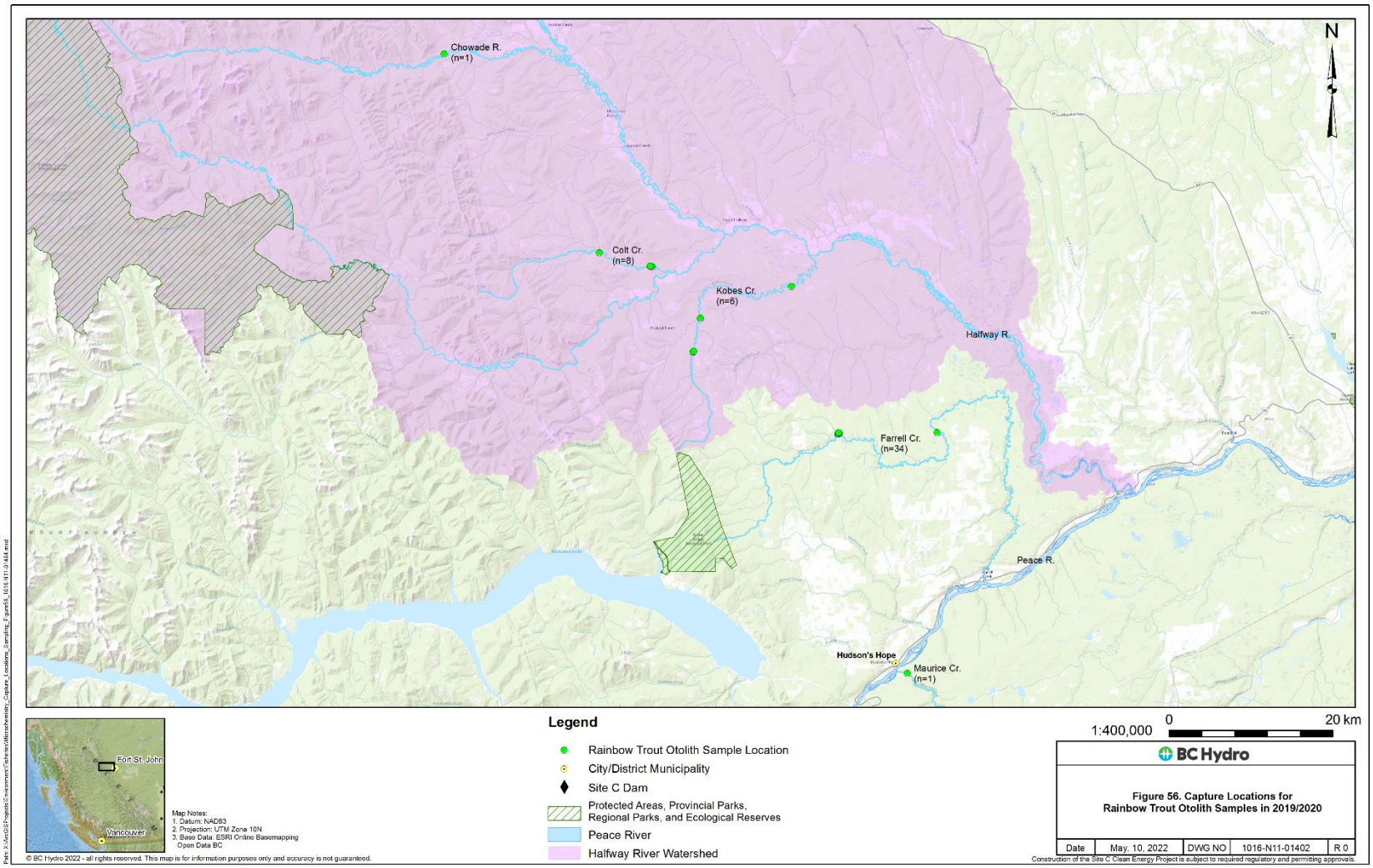
Chowade R.
(n=1)
Colt Cr.
(n=8)
Kobes Cr.
(n=6)
Halfway R.
Farrell Cr.
(n=34)
Peace R.
Hudson's Hope
Maurice Cr.
(n=1)
N
Legend
Rainbow Trout Otolith Sample Location
City/District Municipality
Site C Dam
Protected Areas, Provincial Parks, Regional Parks, and Ecological Reserves
Peace River
Halfway River Watershed
1:400,000
0
20 km
BC Hydro
Figure 56. Capture Locations for Rainbow Trout Otolith Samples in 2019/2020
Date
May. 10, 2022
DWG NO
1016-N11-01402
R 0
Fort St. John
Vancouver
Map Notes:
1. Datum: NAD83
2. Projection: UTM Zone 10N
3. Base Data: ESRI Online Basemapping
Open Data BC
© BC Hydro 2022 - all rights reserved. This map is for information purposes only and accuracy is not guaranteed.
Construction of the Site C Clean Energy Project is subject to required regulatory and permitting approvals.

Figure 56.

4.4.1.1. Rainbow Trout Otolith Model Performance

Water chemistry results (n=45) from seven waterbodies, and Rainbow Trout otolith capture chemistries (n=134) from eight waterbodies, for total of 179 chemistries from eight waterbodies (i.e., prior locations), were used to train the model (Figure 57). Further, the subset of 35 Rainbow Trout otoliths used for validation purposes were input into the model to assess model overfitting.

The Rainbow Trout otolith model contained stream- and watershed-level prediction accuracies for the training data that varied among sites (Table 24). Stream-level prediction accuracy exceeded 80% overall for the water training samples, with some potential challenges with accuracy for Halfway River mainstem (62%). Among otoliths in the training data, stream-level accuracies were generally higher for sites outside the Halfway watershed (≥93%) compared to those within it (≤55%).

Validation samples were limited to four locations, with most from Farrell Creek (Table 24). Within the Halfway River watershed, stream differentiation appeared poor (<20%) and did not improve at the watershed level. Errors in the predictions associated with each location is provided in Table 25. While Farrell Creek accuracy was good for samples from Farrell Creek (>74%), this location was over predicted for Colt and Kobes creeks, within the Halfway River watershed. The sample from Maurice Creek was predicted to have recruited from Kobes Creek (natal) and Farrell Creek (first summer). Results suggest model overfitting and an overlap in otolith chemistry in Rainbow Trout from different streams and possibly, watersheds.

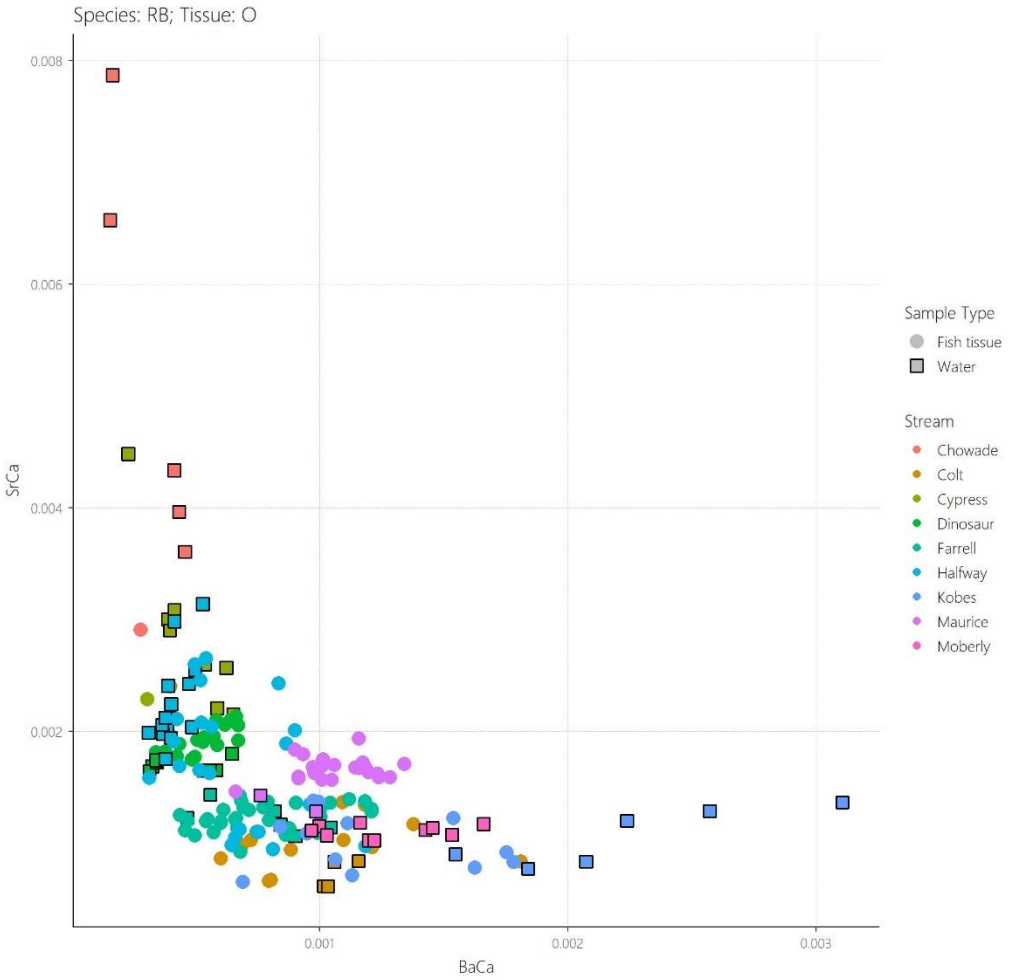

Figure 57. Water and tissue data used to develop and train the Rainbow Trout otolith model.

Table 24. Prediction Accuracy (% correctly classified stream and watershed) and sample sizes for Rainbow Trout otoliths in training data, validation data.

Observed Occupancy		Training Data						Validation Data				
		Water			Tissue			Natal		1st Summer		
		% correct		n	% correct		n	% correct		% correct		n
Watershed	Stream	Stream	Watershed		Stream	Watershed		Stream	Watershed	Stream	Watershed	
Maurice	Maurice			0	93	93	42	0	0	0	0	1
Farrell	Farrell	83	83	6	97	97	35	74	74	83	83	23
Dinosaur	Dinosaur	89	89	9	100	100	20					0
Halfway	Halfway	62	62	13	27	36	11					0
	Chowade	100	100	5	0	100	1					0
	Colt	100	100	4	55	64	11	17	17	0	0	6
	Cypress	71	86	7	0	100	2					0
	Kobes	100	100	1	33	58	12	0	40	20	20	5
	Overall	80	82	45	79	56	134	51	57	57	57	35

Notes: Grey cells give sample sizes for water and tissue training data, and validation data.

Table 25. Confusion matrix for Rainbow Trout otoliths (correct prediction in gray cell) – capture locations shown in columns, predicted occupancy shown in rows. Number outside bracket is predicted natal, and number inside bracket is predicted first summer.

Predicted Location		Capture Location			
Watershed	Stream	Maurice Ck.	Farrell Ck.	Halfway R.	
				Colt Ck.	Kobes Ck.
Dinosaur R.			2 (2)	1 (0)	
Maurice Ck.			2 (2)		
Farrell Ck.		0 (1)	17 (19)	4 (6)	3 (4)
Halfway R.	Colt Ck.			1 (0)	2 (0)
	Kobes Ck.	1 (0)			0 (1)
	Chowade R.		1 (0)		
	Mainstem		1 (0)		
	Total	1	23	6	5

There are subtle differences in Sr:Ca and Ba:Ca ratios between streams and rivers that may only be differentiated by a visual assessment of the life-history graphs at this time. For example, RB #610 was

captured from Farrell Creek, was predicted by the model to have recruited from Farrell Creek (Figure 58), and its entire life history chemistry is within the Farrell Creek region (pink ellipse). In comparison, RB #896 from Kobes Creek (Figure 59) actually looks like it could be from Colt Creek (due to large overlap in ellipses). Looking at other life histories of fish from Colt Creek, such as RB #1337, they typically have lower Ba:Ca ratios compared to Kobes Creek fish and slightly lower Sr:Ca ratios compared to Farrell Creek fish (Figure 60).

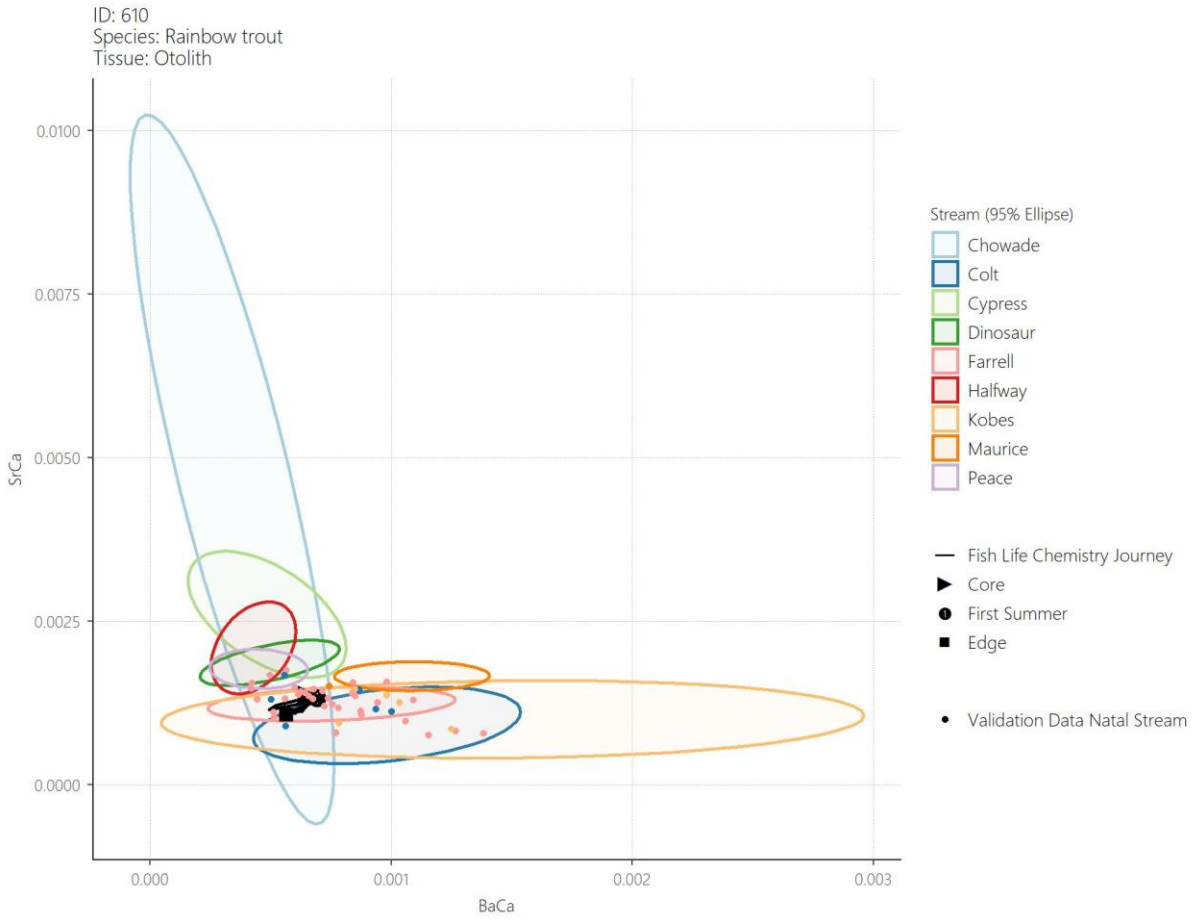

Figure 58. Representative life-history chemistry for Farrell Creek - RB #610 (102 mm) characterized by life history within Farrell Creek region (pink ellipse), with slightly higher Sr:Ca than Colt region (dark blue ellipse), and with lower Ba:Ca than most of the Kobes Creek region (large light orange ellipse).

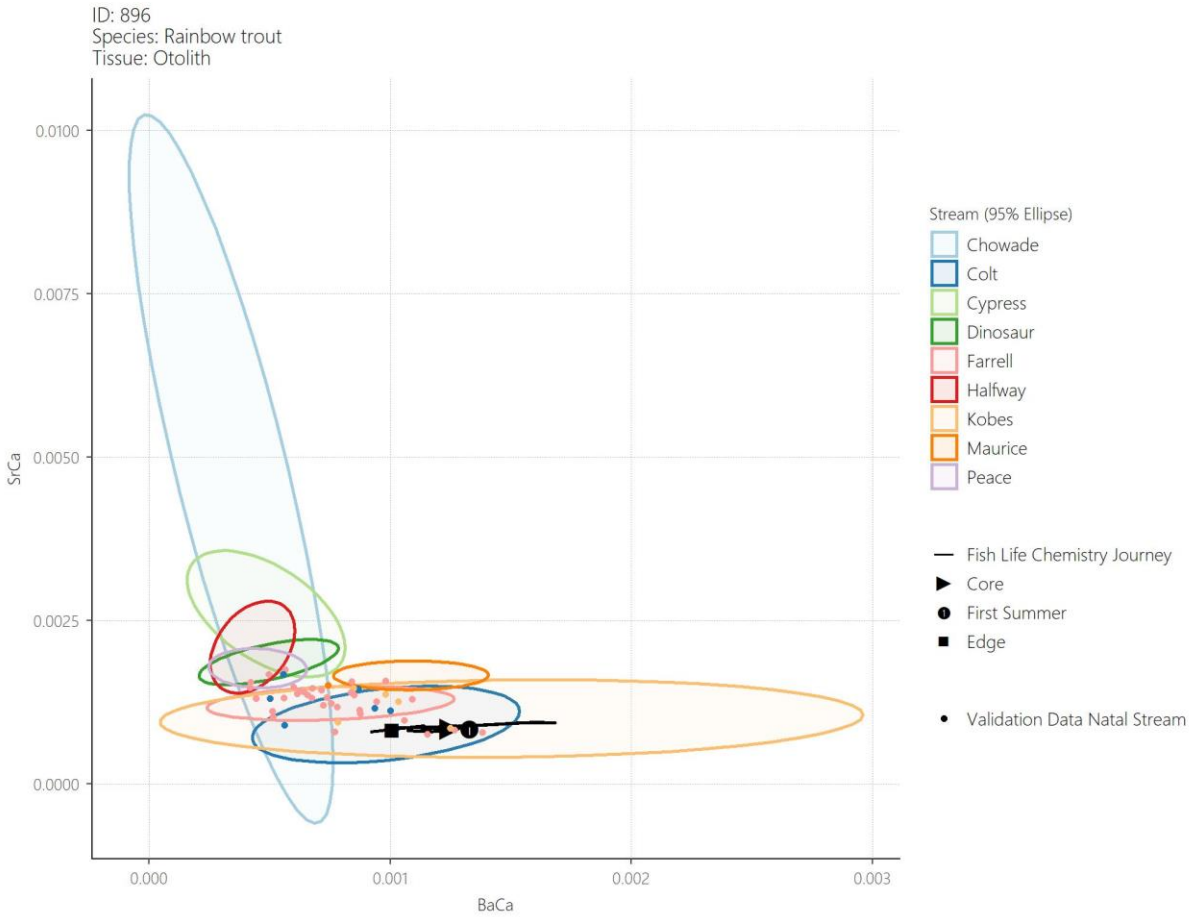

Figure 59. Life-history chemistries for RB #896 (99 mm) captured in Kobes Creek, and predicted recruitment as Kobes Creek. It is difficult to differentiate between Kobes and Colt creeks in this particular case. Kobes Creek chemistry is characterized by higher Ba:Ca than Colt and Farrell Creeks, and lower Sr:Ca than Farrell and Maurice creeks.

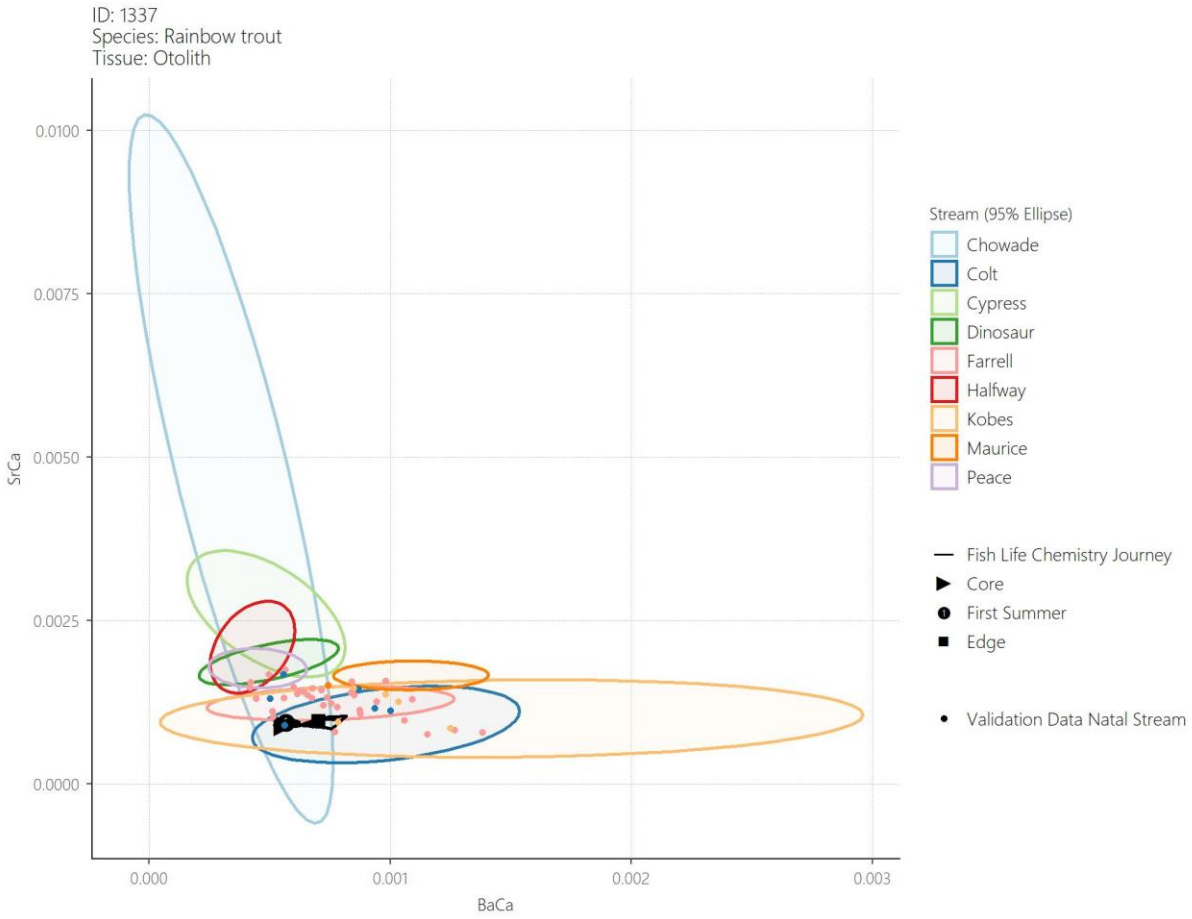

Figure 60. Life-history chemistry for RB #1337 (75 mm) from Colt Creek characterized by lower Ba:Ca than Kobes Creek, and slightly lower Sr:Ca than Farrell Creek.

4.4.1.2. Prediction of Stream Occupancy in Test Data

Eleven of the 15 unknown-origin Rainbow Trout from which otoliths were collected were caught in Farrell Creek, and the other four were captured in the Halfway River (two from Colt Creek and one each from Kobes Creek and Chowade River) (Table 26). The Chowade River captured fish (RB #1426) was predicted to have originated from Cypress Creek, but likely remained in Chowade River its entire life based on life-history chemistry (Figure 61). The Kobes Creek captured fish (RB #951) and one of the Colt

Creek captured fish (RB #1037) were predicted to have originated from Colt Creek (Figure 62). Rainbow Trout individual, RB #1100 (captured in Colt Creek), was predicted to have recruited from Farrell Creek; however, based on its life-history graph it more likely spent its life until capture in Colt Creek (Figure 63). RB #606 was predicted to have recruited from Colt Creek in the Halfway River watershed; however, the life-history graph suggested this fish never left Farrell Creek where it was captured (i.e., the life-history path never falls within the Peace River ellipse; Figure 64). RB #603, captured in Farrell Creek was predicted to have recruited from Dinosaur Reservoir and life history supports this prediction (Figure 65).

Table 26. Model predicted Rainbow Trout recruitment sources based on otolith structures. Only capture locations (along the top) and streams included in model (rows) are presented in the table.

Predicted Location		Capture Location				
Watershed	Stream	Farrell Cr.	Halfway R.			Total
			Colt Ck.	Kobes Ck.	Chowade R.	
Dinosaur R.		1				1
Maurice Ck.			1			1
Farrell Ck.		4				4
Halfway R.	Colt Ck.	4	1	1		6
	Kobes Ck.	2				2
	Cypress Ck.				1	1
Total		11	2	1	1	15

With the support from the life history figures, the model suggests that all four Rainbow Trout from the Halfway River watershed remained within the watershed for their entire lives until capture. Of the 11 Rainbow Trout captured in Farrell Creek, almost half recruited from Farrell Creek, another half from Colt or Kobes creeks in the Halfway River watershed, and one from Dinosaur Reservoir.

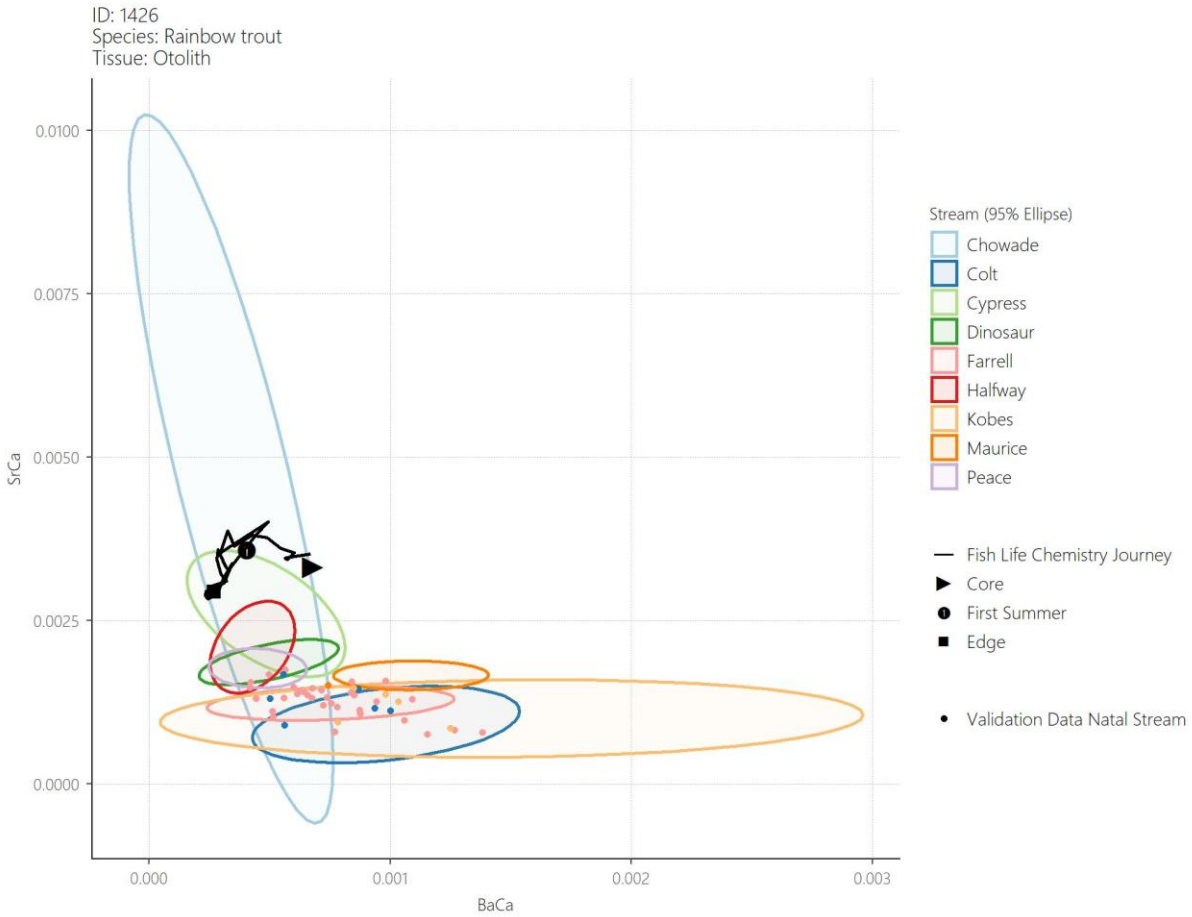

Figure 61. Life-history chemistry for RB #1426 (139 mm) which was predicted to have recruited from Cypress Creek, but likely remained in Chowade River from hatching until capture.

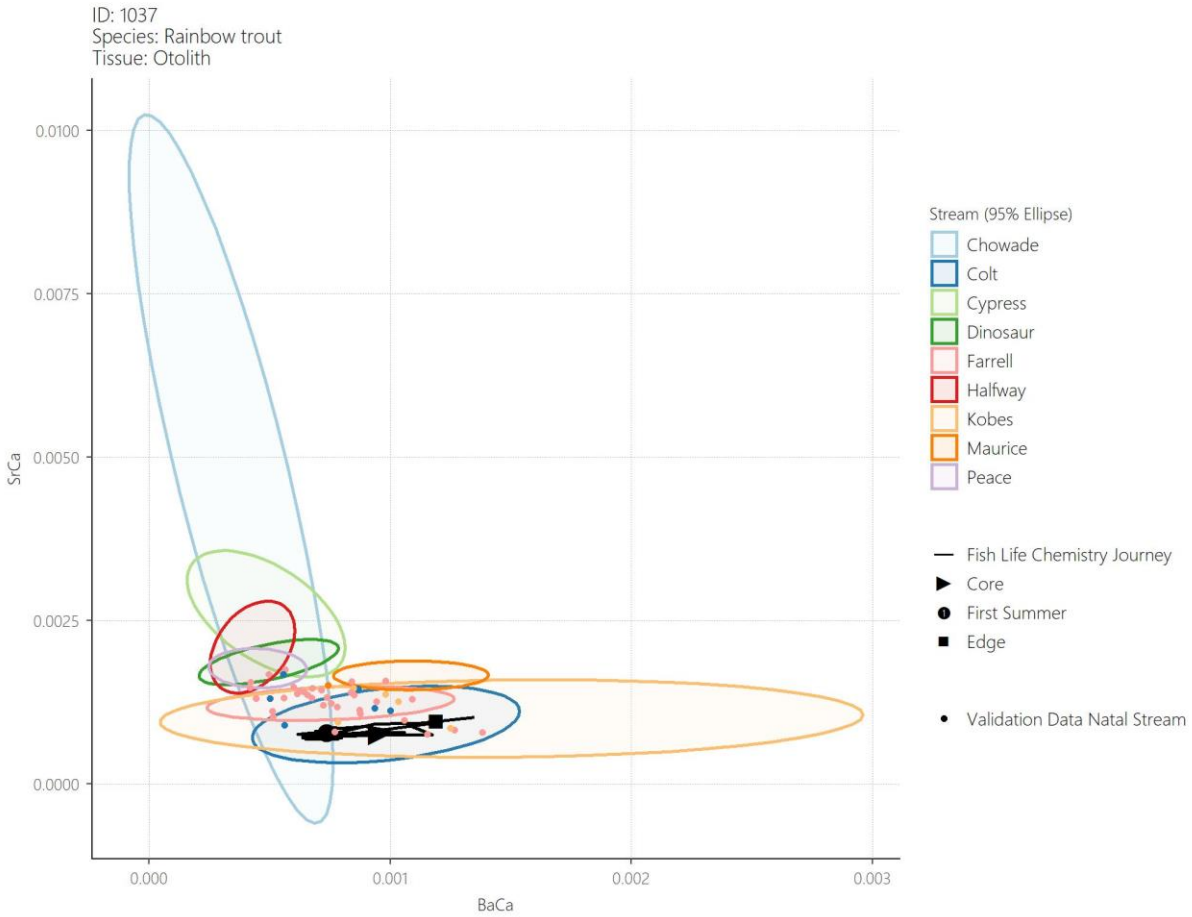

Figure 62. Life-history chemistry for RB #1037 (168 mm) which remained in Colt Creek from hatching to capture.

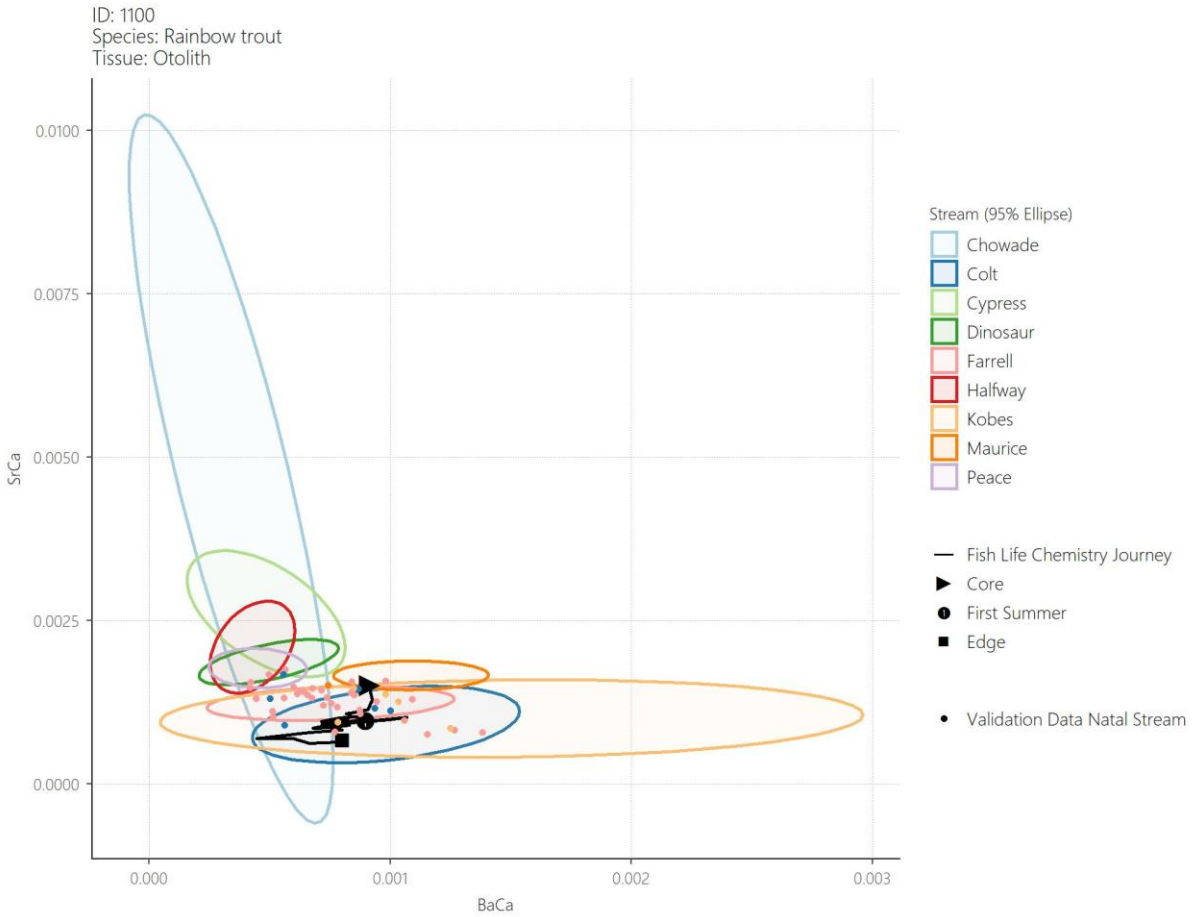

Figure 63. Life-history chemistry for RB #1100 (172 mm), which was predicted to have recruited from Farrell Creek, but likely remained in Colt Creek its entire life until capture based on little variability in chemistry and no evidence of entering the Peace River.

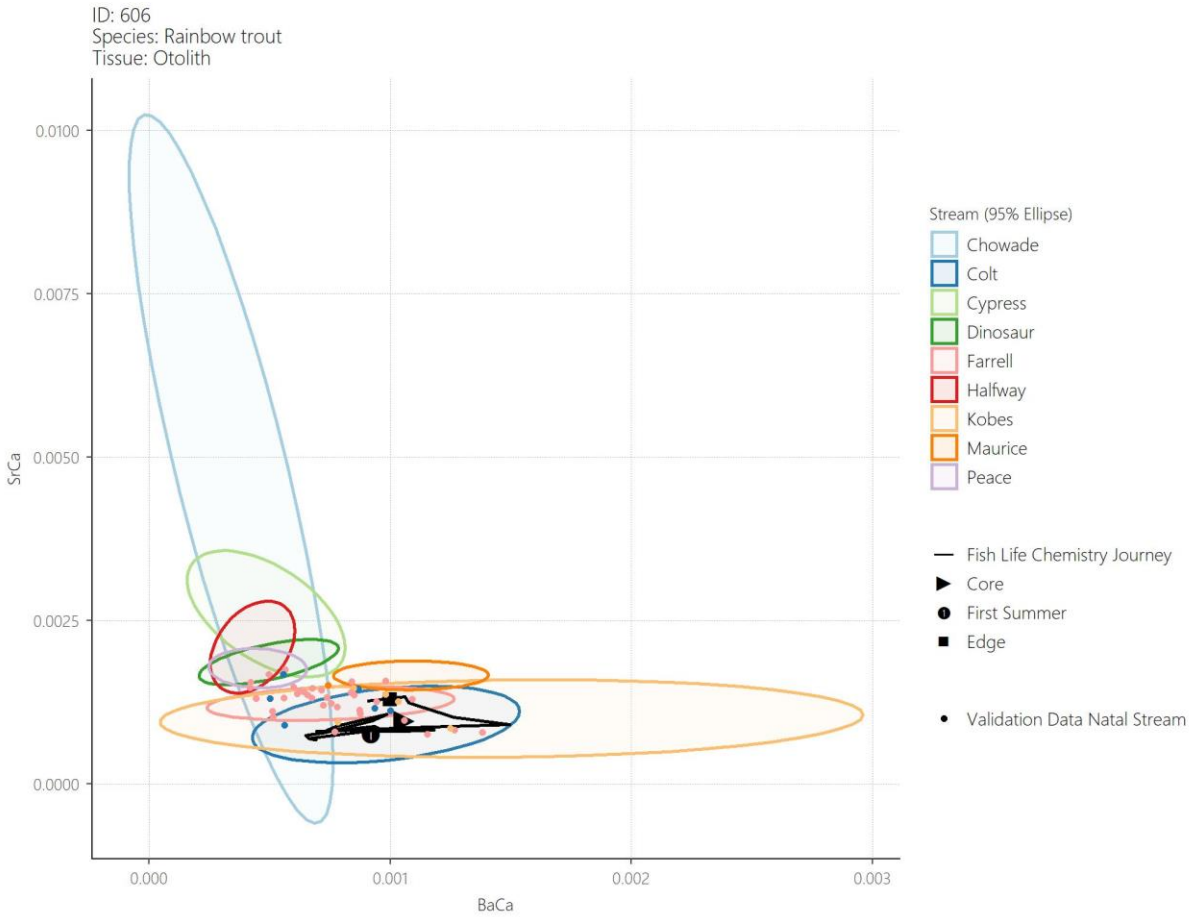

Figure 64. Life-history chemistry for RB #606 (126 mm), predicted to have recruited from Colt Creek; however, there is no chemical evidence it left Farrell Creek where it was captured.

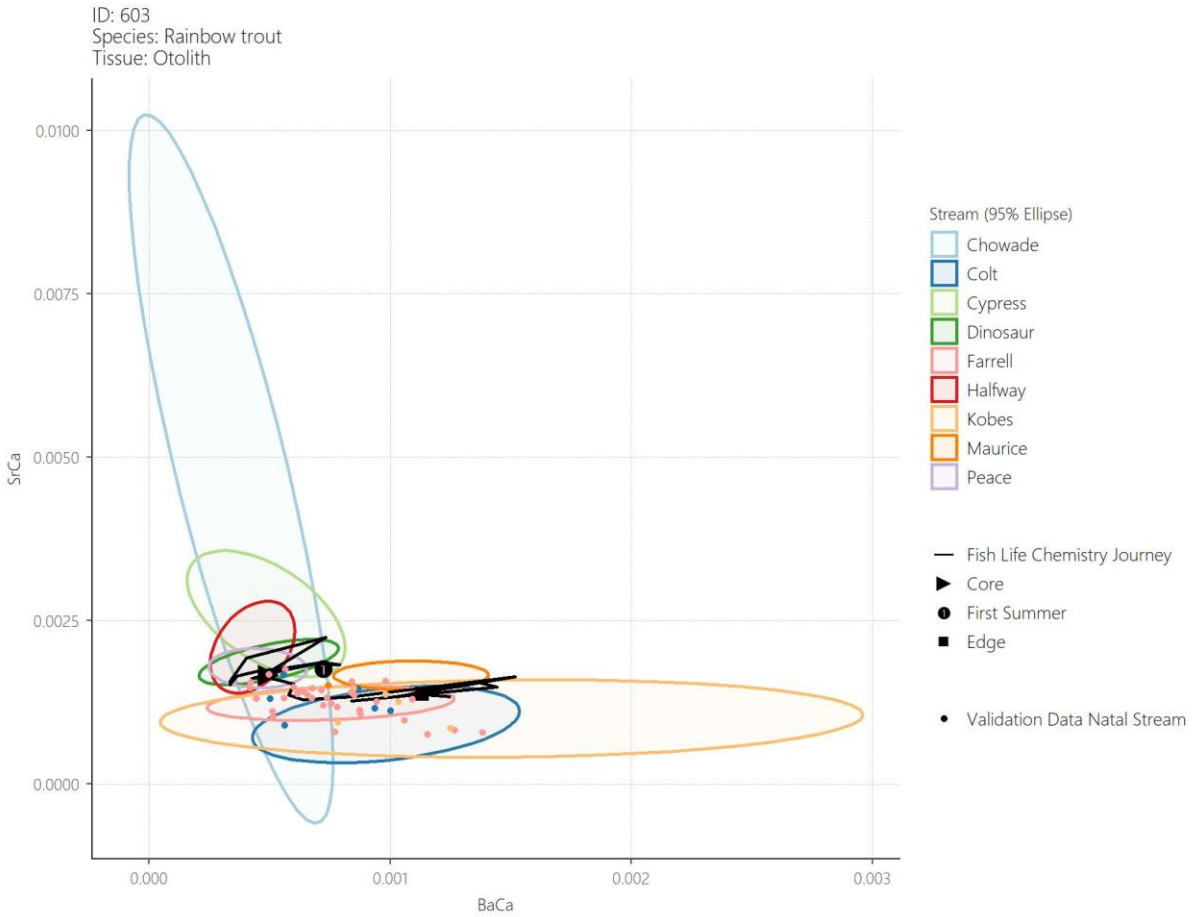

Figure 65. Life-history chemistry for RB #603 (126 mm) was predicted to have recruited from Dinosaur Reservoir (confirmed by life history) and then migrated to Farrell Creek until capture.

4.4.2. Fin Rays

In total, 23 Rainbow Trout fin rays were collected in 2019 and 2020 (Table 27 and Figure 66):

Table 27. Number and fork length of Rainbow Trout fish captured with fin ray samples.

Capture Watershed	Capture Site	Number of Fin Rays Collected	Fork Length (mm) range
Halfway River	Colt Creek	6	65 - 172
	Kobes Creek	4	83 - 156
Farrell Creek	Farrell Creek mainstem	9	72 - 151
Maurice Creek	Maurice Creek mainstem	1	90
Peace River	Upstream of Project	2	246 - 444
	Downstream of Project	1	198

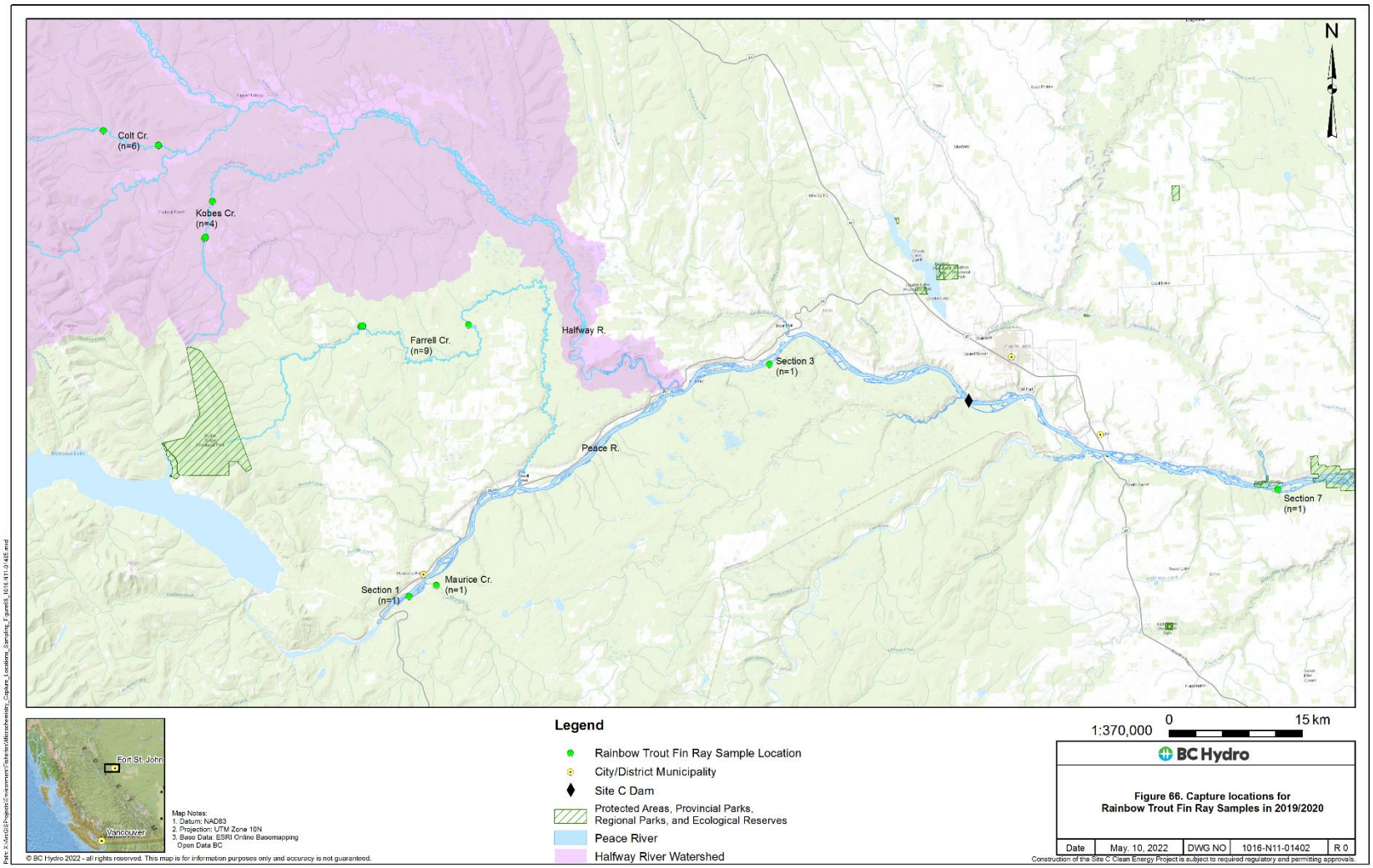
N
Colt Cr.
(n=6)
Kobes Cr.
(n=4)
Farrell Cr.
(n=9)
Halfway R.
Section 3
(n=1)
Peace R.
Section 7
(n=1)
Maurice Cr.
(n=1)
Section 1
(n=1)
Fort St. John
Vancouver
Map Notes:
1. Datum: NAD83
2. Projection: UTM Zone 10N
3. Base Data: ESRI Online Basemapping
Open Data BC
© BC Hydro 2022 - all rights reserved. This map is for information purposes only and accuracy is not guaranteed.
Legend
Rainbow Trout Fin Ray Sample Location
City/District Municipality
Site C Dam
Protected Areas, Provincial Parks,
Regional Parks, and Ecological Reserves
Peace River
Halfway River Watershed
1:370,000
0
15 km
BC Hydro
Figure 66. Capture locations for
Rainbow Trout Fin Ray Samples in 2019/2020
Date
May. 10, 2022
DWG NO
1016-N11-01402
R 0
Construction of the Site C Clean Energy Project is subject to required regulatory and permitting approvals.

Figure 66.

4.4.2.1. Rainbow Trout Fin Ray Model Performance

Water chemistry results (n=50) from seven waterbodies, and fin ray capture chemistries (n=19) from four waterbodies, for a total of 69 chemistries from eight waterbodies (i.e., prior locations), were used to build and train the model (Figure 67). Further, the sub-set of 15 RB otoliths used for validation purposes were input into the model to test prediction accuracy, and then visually assessed using the life-history graphs.

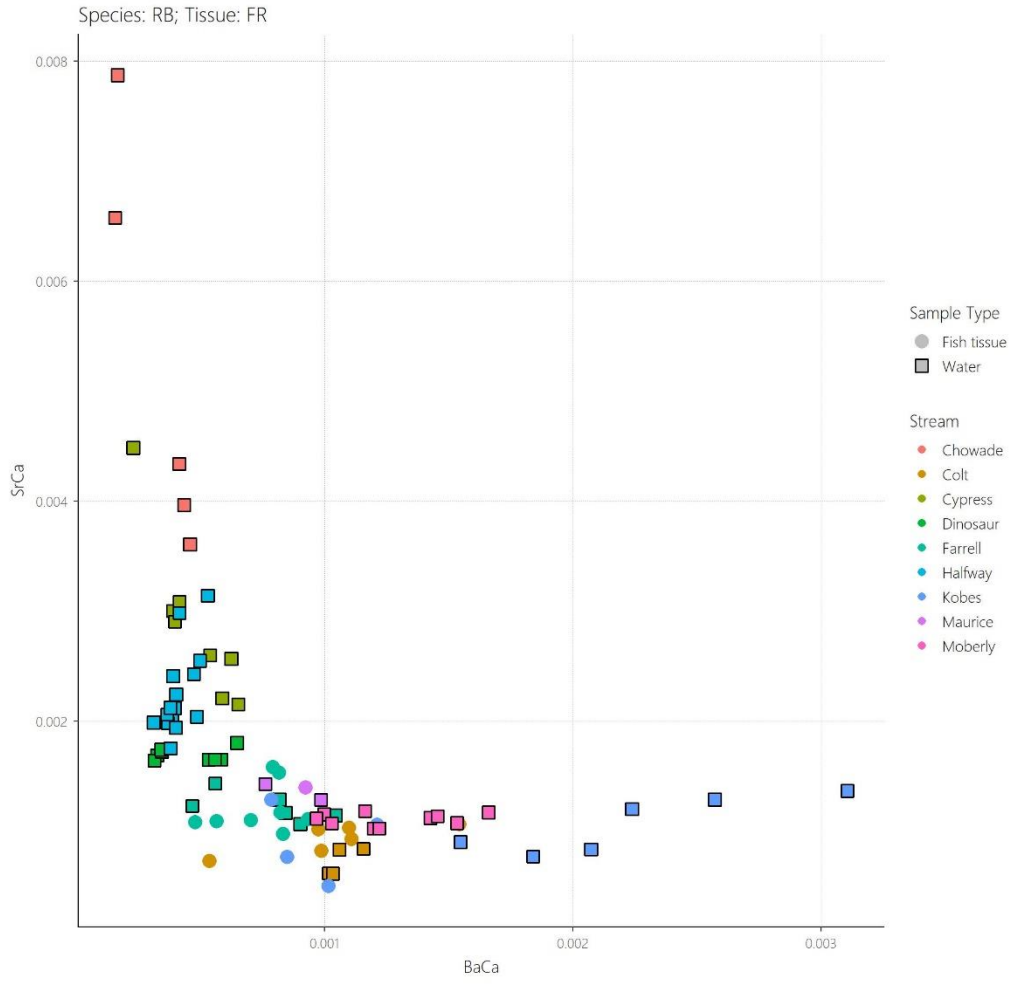

Figure 67. Water and tissue data used to train the Rainbow Trout fin ray model.

3.2.2.2 Rainbow Trout Fin Ray Model Performance

The Rainbow Trout fin ray model provided improved prediction accuracies on training data compared to its otolith counterpart, particularly for water samples and for watershed-level predictions (Table 28); stream-level predictions exceeded 75% at most sites and for both sample types. The only exceptions were at Maurice and Kobes creeks where none of the (respectively) two and four fin rays were correctly classified. Further, Maurice Creek aside, watershed-level predictions of training data exceeded 75% for all sites and sample types. Misclassification of Maurice Creek samples arose due to the low sample size for this site (n=2), which was insufficient to model; therefore, visual assessment of life-history graphs is currently the only way to predict recruitment from this location.

Table 28. Prediction Accuracy (% correctly classified stream and watershed) and sample sizes for Rainbow Trout fin rays in training data, validation data.

Observed Occupancy		Training Data						Validation Data				
		Water			Tissue			Natal		1st Summer		
		% correct		n	% correct		n	% correct		% correct		n
Watershed	Stream	Stream	Watershed		Stream	Watershed		Stream	Watershed	Stream	Watershed	
Maurice	Maurice			0	0	0	2	0	0	0	0	1
Farrell	Farrell	100	100	6	75	75	8	43	43	43	43	7
Dinosaur	Dinosaur	89	89	9			0					0
Halfway	Halfway	85	92	13			0					0
	Chowade	100	100	5			0					0
	Colt	100	100	4	80	80	5	75	100	75	100	4
	Cypress	100	100	7			0					0
	Kobes	83	100	6	0	75	4	67	100	67	100	3
	Overall	92	96	50	53	68	19	53	67	60	67	15

Note: Grey cells give sample sizes for water and tissue training data, and validation data.

Among validation data, prediction accuracy was high at both stream and watershed levels for all Halfway River tributaries, and were within the ranges seen for training data, indicating minimal overfitting (albeit on only seven samples and only for the streams represented). Representative life-history chemistries from fish in Colt and Kobes creeks are provided in Figure 68 and Figure 69 respectively. At Farrell Creek, three of seven validation samples (43%) were correctly classified (e.g., Figure 70); the remaining four were often misclassified as recruiting from Halfway River tributaries, specifically Colt or Kobes creeks. Validation prediction rates for this site were lower than obtained for

training data, implying overfitting for this site. The Maurice Creek-captured fish (RB #1659) was erroneously predicted to have recruited from Colt Creek; the life-history chemistry from this fish does not help differentiate Maurice Creek from Farrell, Colt or Kobes creeks (Figure 71).

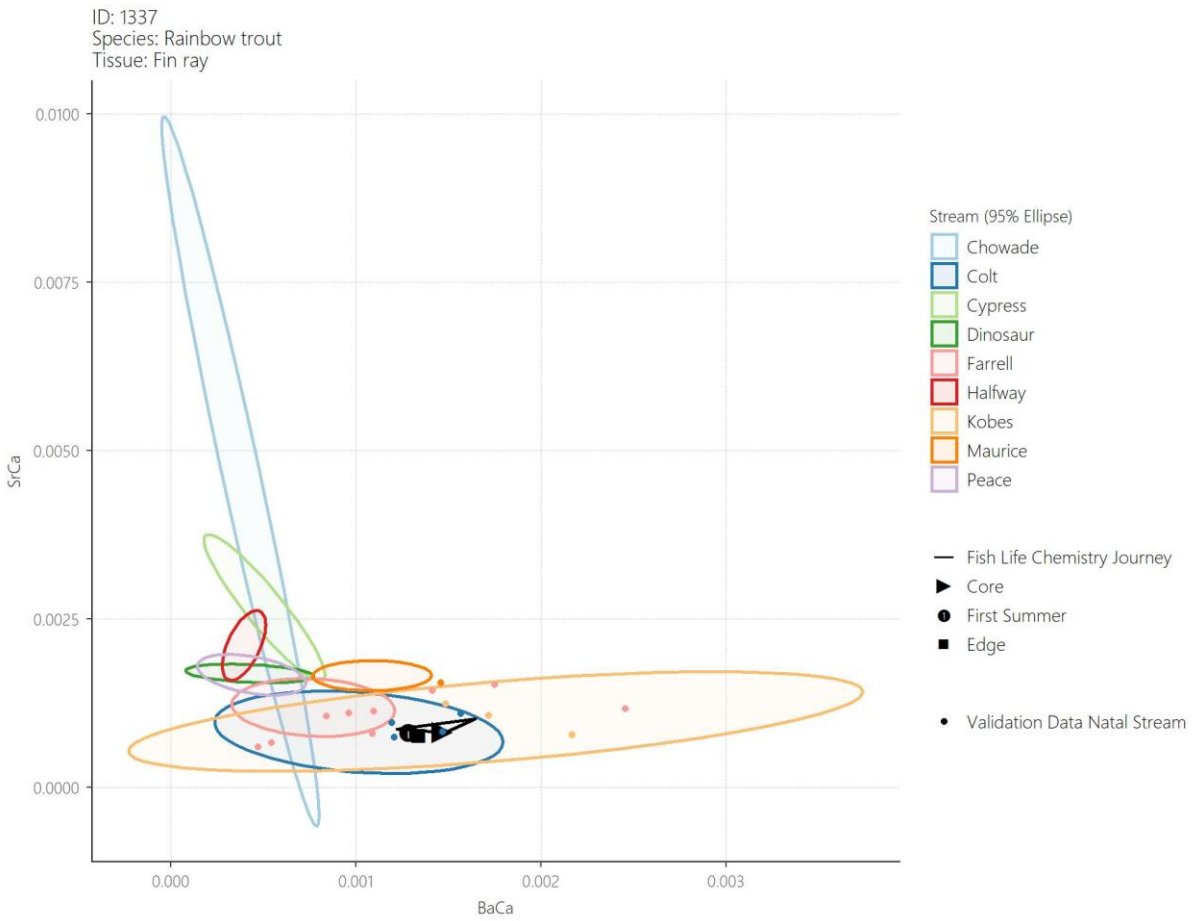

Figure 68. Representative life-history chemistry for Colt Creek - RB #1337 (75 mm).

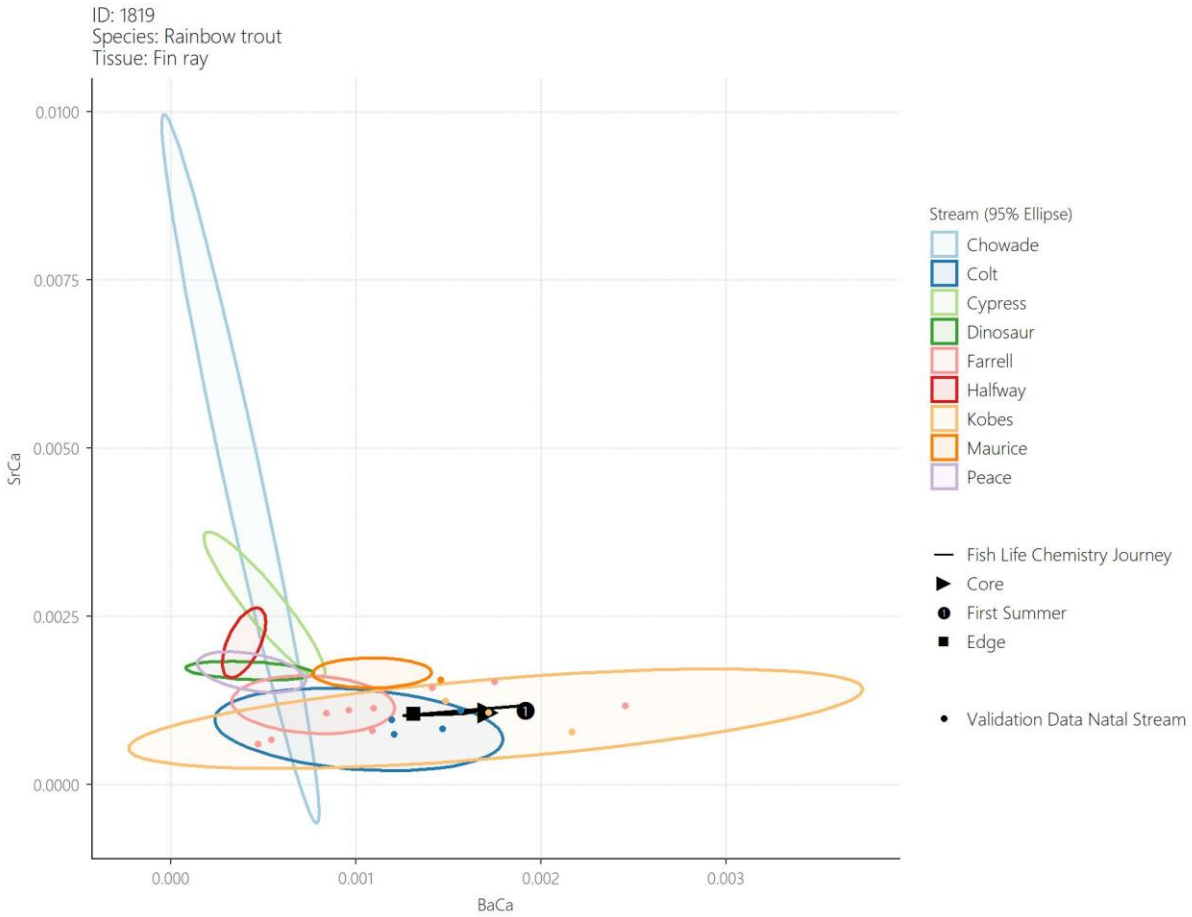

Figure 69. Representative life-history chemistry for RB #1819 (83 mm) from Kobes Creek.

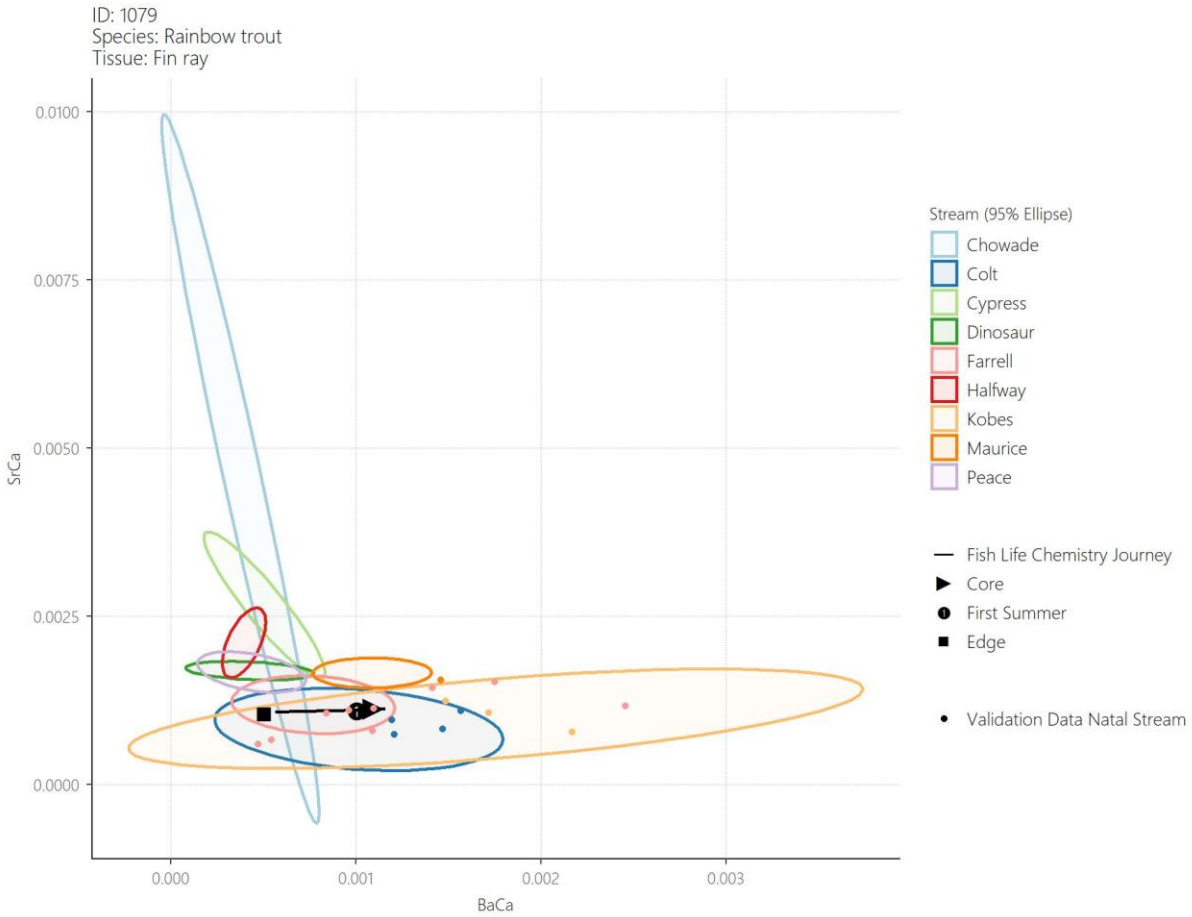

Figure 70. Representative life-history chemistry for RB #1079 (75 mm) from Farrell Creek.

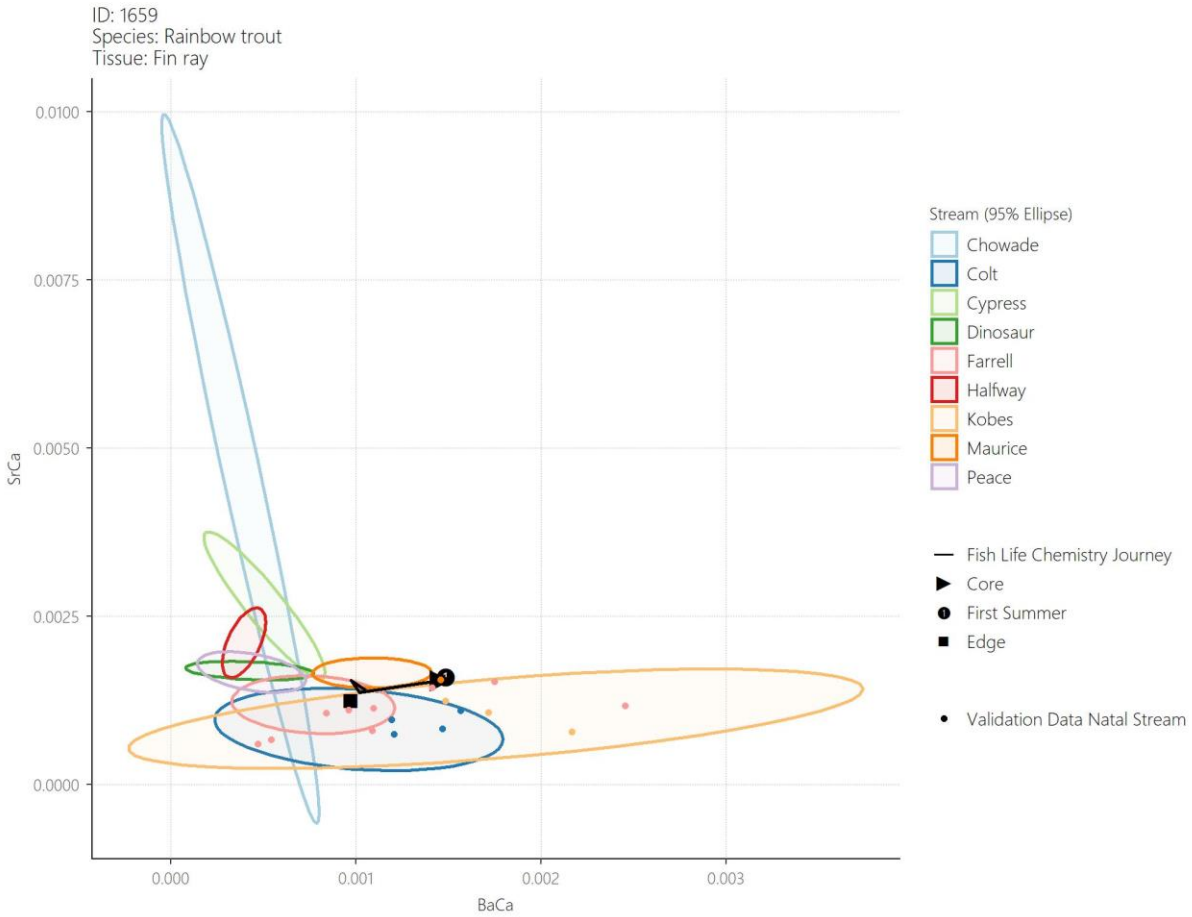

Figure 71. Representative life-history chemistries for RB #1659 (90 mm) from Maurice Creek. However, patterns are difficult to differentiate from Colt, Kobes or Farrell creeks' chemistries.

4.4.2.2. Prediction of Stream Occupancy in Test Data

Eight fin rays were collected from Rainbow Trout with unknown natal origin (Table 29), and the model predicted that five (63%) recruited from Colt Creek from the Halfway River watershed. However, there are some discrepancies between the model predictions and the life-history chemistries of some of the trout. The Kobes Creek-caught Rainbow Trout (RB #951) was predicted by the model to have recruited from Colt Creek, but given the chemical similarity between Colt and Kobes creeks, this fish likely spent its life in Kobes Creek until capture (Figure 72). Two fish were captured in Colt Creek, with one predicted to have originated from Colt Creek (Figure 73), and the other to have recruited from Farrell Creek; however, this latter fish (RB #1100, where the otolith prediction was also misclassified) likely recruited from Colt Creek as well, based on the low variability in chemical signature over time and no evidence in the life-history graph that it ever left its natal watershed (Figure 74). Two Rainbow Trout were captured in Farrell Creek, where both were predicted by the model to have recruited from Colt Creek; however, both RB #1019 and RB #1020 most likely recruited from Farrell, as—again—there is no chemical evidence in the life-history graphs of these fish leaving their natal habitat prior to capture (Figure 75 and Figure 76).

Table 29. Model predicted Rainbow Trout recruitment sources based on fin ray structures. Only capture locations (along the top) and streams included in model (rows) are presented in the table.

Predicted Location		Capture Location					
Watershed	Stream	Farrell Ck.	Halfway R.		Peace R.		Total
			Colt Ck.	Kobes Ck.	Upstream	Downstream	
Maurice Ck.					1		1
Farrell Ck.			1			1	2
Halfway R.	Colt Ck.	2	1	1	1		5
	Total	2	2	1	2	1	8

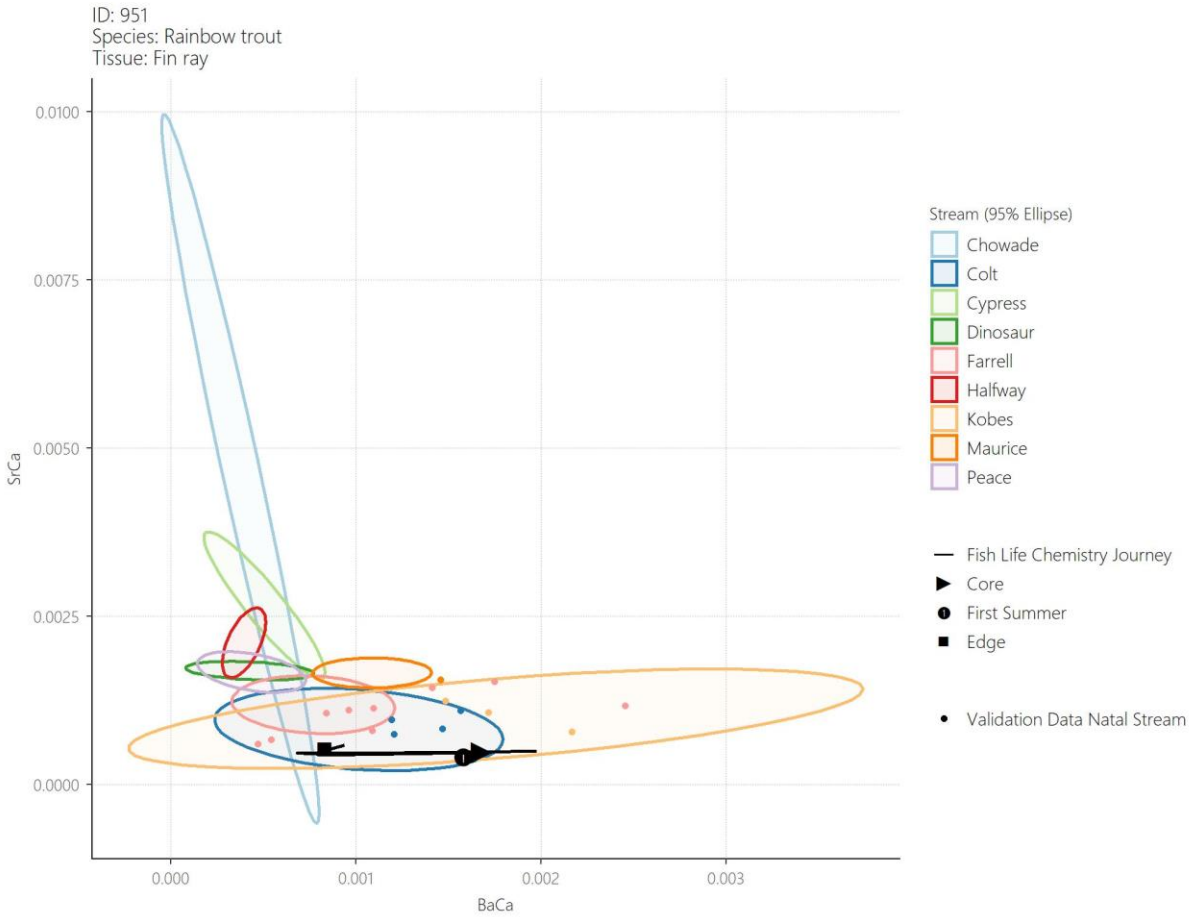

Figure 72. RB#951 (captured in Kobes Creek) was predicted to have recruited from Colt Creek, but due to similarity in chemistry between Colt and Kobes creeks, this fish likely recruited from Kobes Creek.

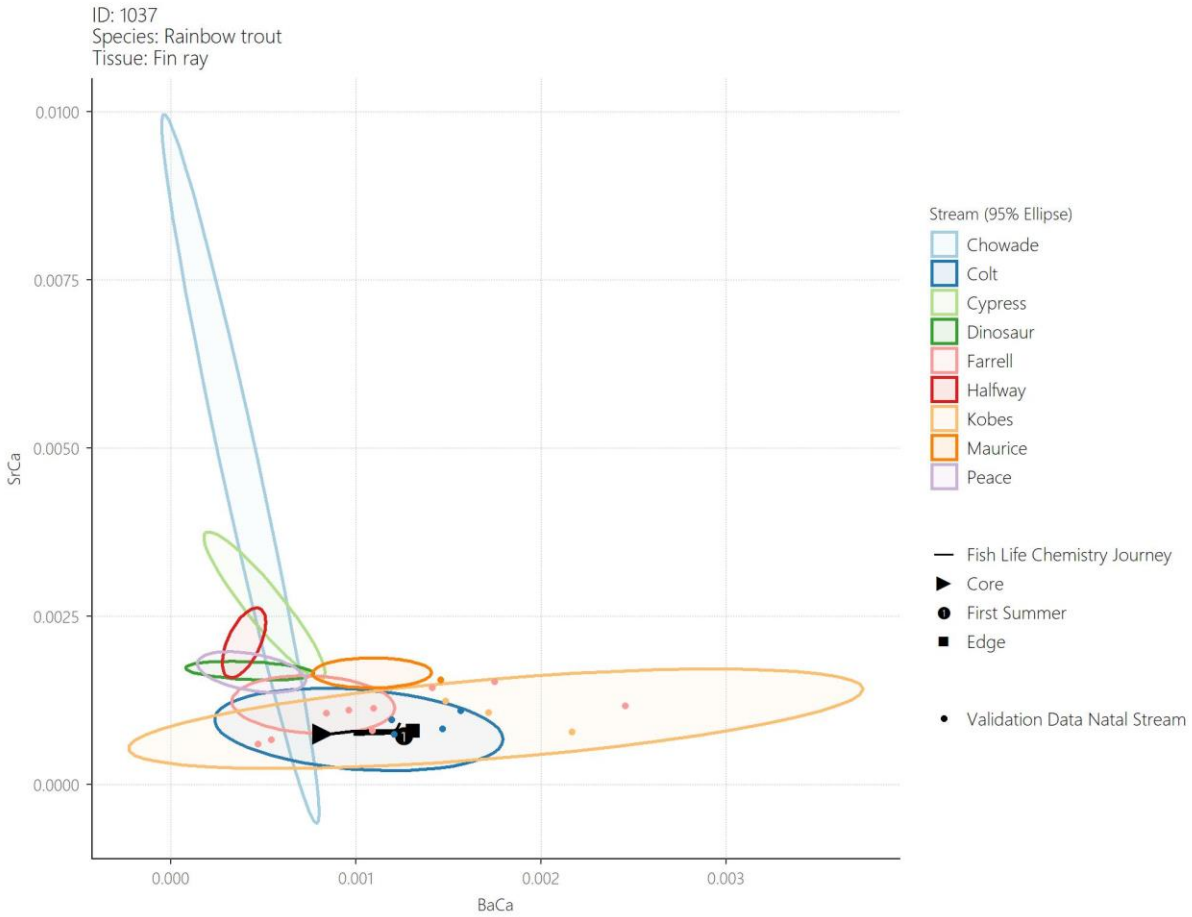

Figure 73. Life-history chemistries for RB #1037 (168 mm) was captured in Colt Creek where it was predicted to have it natal origins.

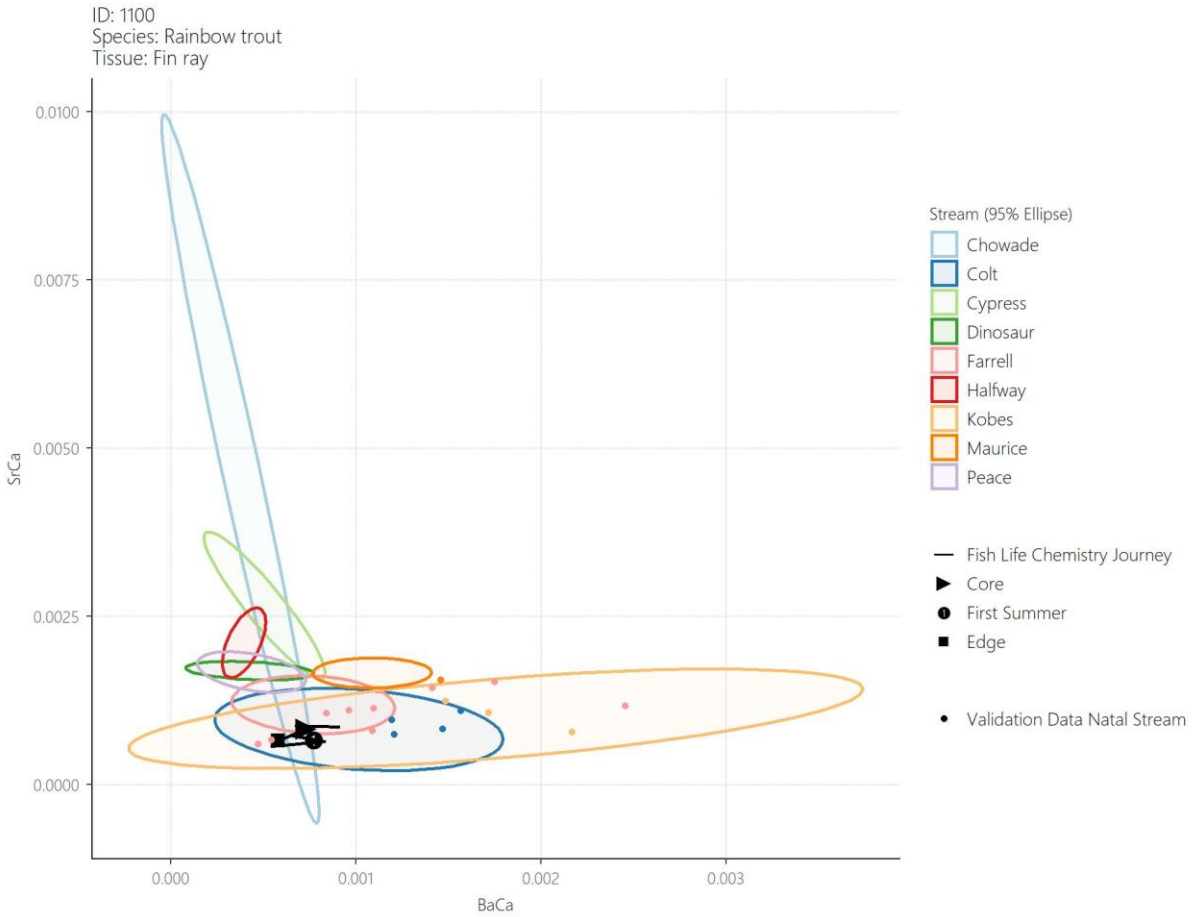

Figure 74. RB#1100 (172 mm, captured in Colt Creek) was predicted to have recruited from Farrell Creek, but due to lack of variability in fin ray chemistry over time and no apparent migration from Halfway River watershed through the Peace River, this fish likely recruited from Colt Creek.

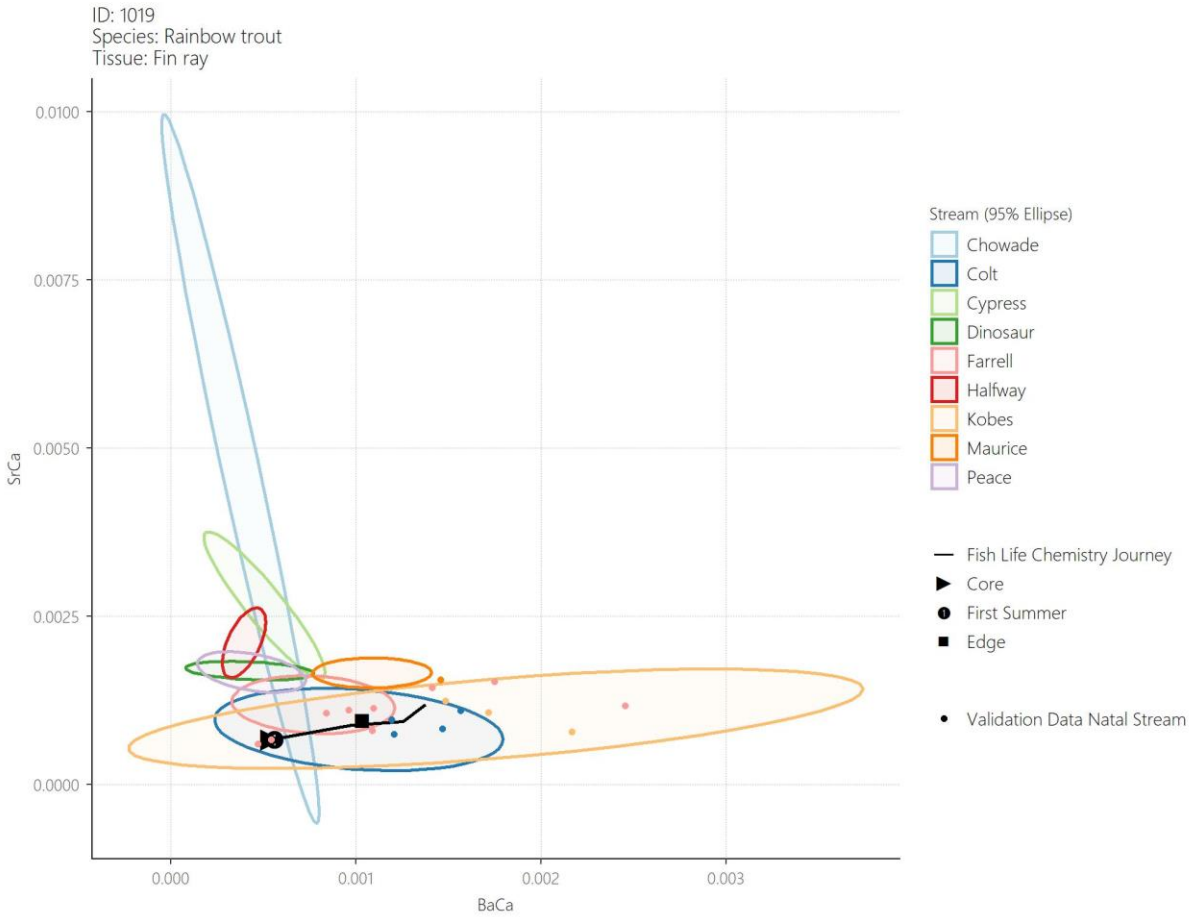

Figure 75. RB#1019 (146 mm, captured in Farrell Creek) was predicted to have recruited from Colt Creek, but due to lack of variability in fin ray chemistry over time and no apparent migration from Farrell Creek to Halfway River watershed through the Peace River, this fish likely recruited from Farrell Creek.

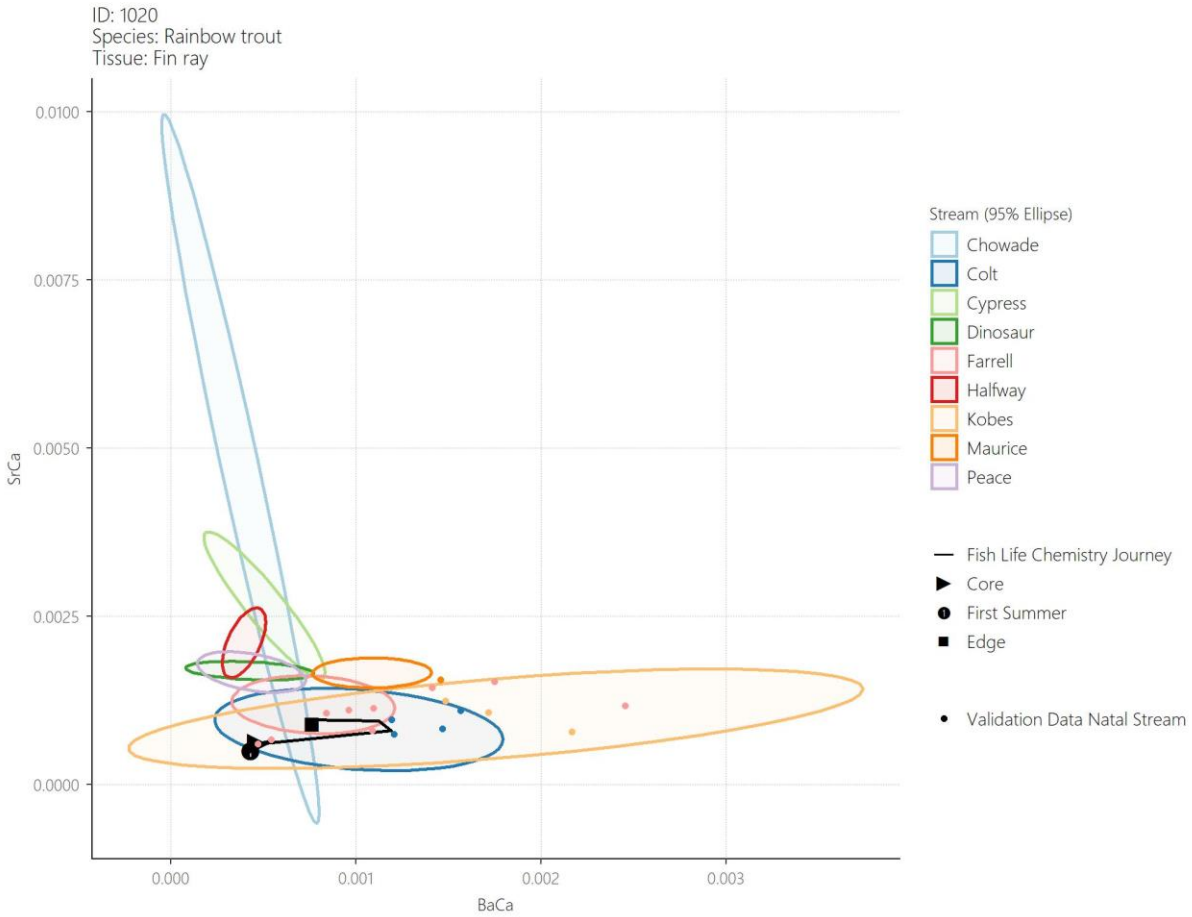

Figure 76. RB#1020 (151 mm, captured in Farrell Creek) was predicted to have recruited from Colt Creek, but due to lack of variability in fin ray chemistry over time and no apparent migration from Farrell Creek to Halfway River watershed through the Peace River, this fish likely recruited from Farrell Creek.

Three Rainbow Trout were captured in the Peace River: two upstream and one downstream of the Project site. The upstream-captured trout were predicted to have originated from Maurice Creek (RB #1685; Figure 77), and Colt Creek (#447; Figure 78). The downstream-captured trout (RB #4572) was predicted to have originated from Farrell Creek; however, the life history suggests this fish may have originated from Dinosaur Reservoir or the Halfway River mainstem due to its lack of variability and similarity in chemistry to the Peace River (Figure 79).

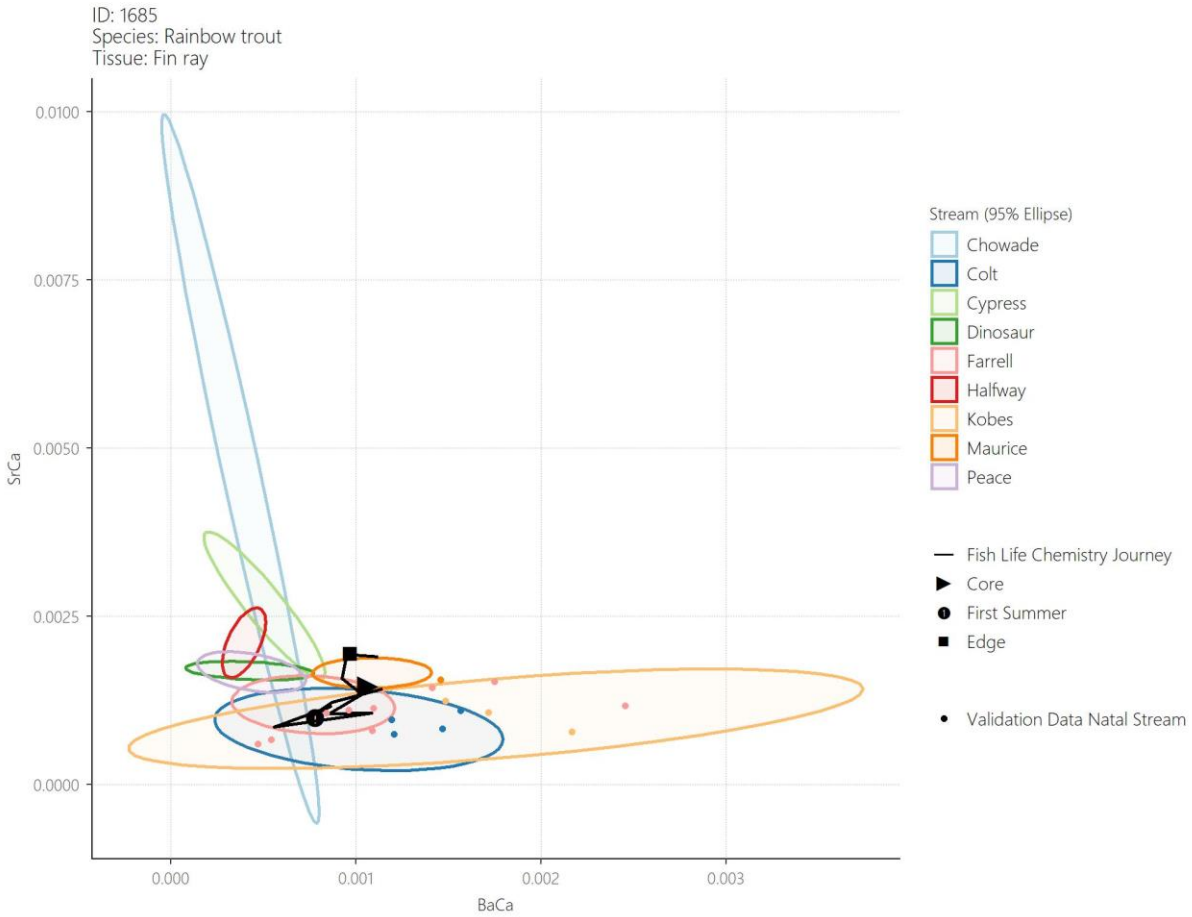

Figure 77. RB#1685 (246 mm, captured in Peace River upstream) was predicted to have recruited from Maurice Creek.

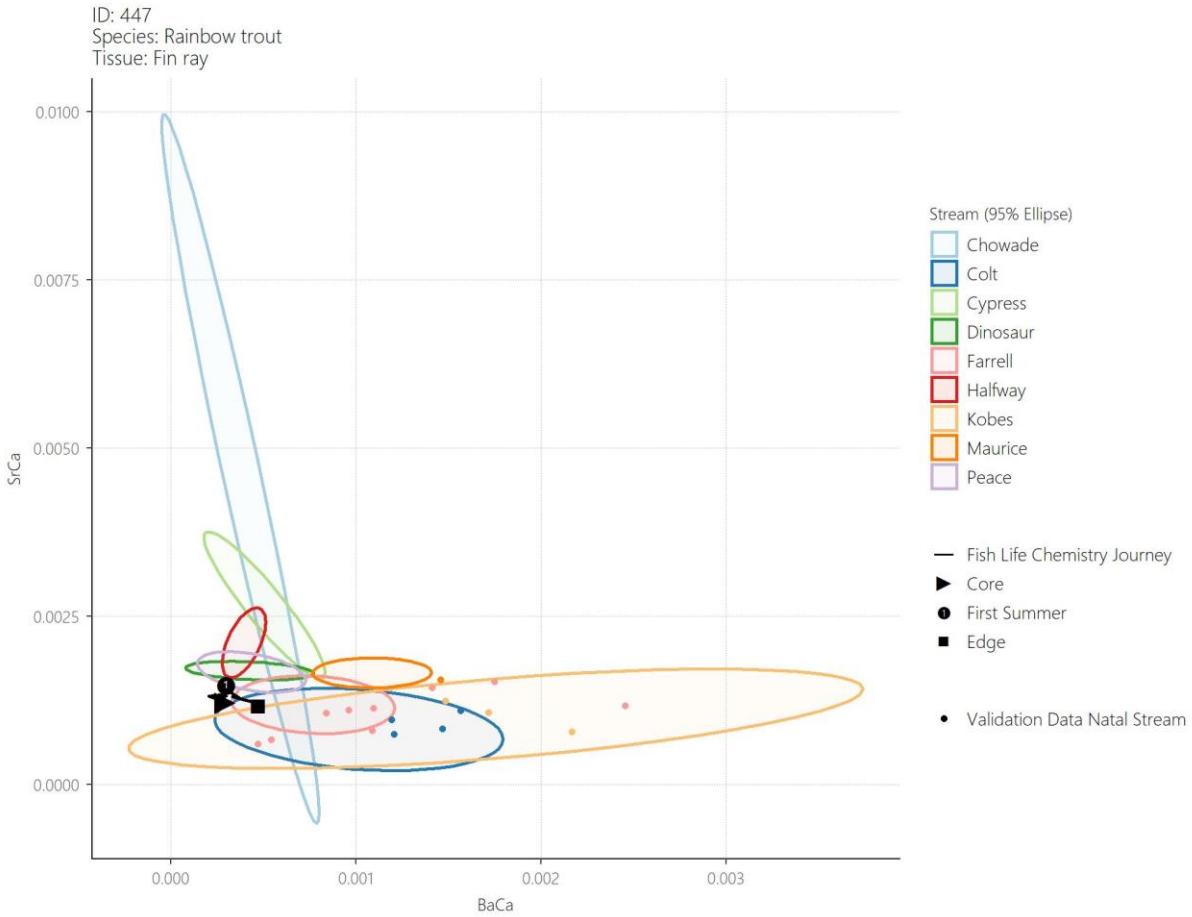

Figure 78. RB#447 (444 mm, captured in Peace River upstream) was predicted to have recruited from Colt Creek.

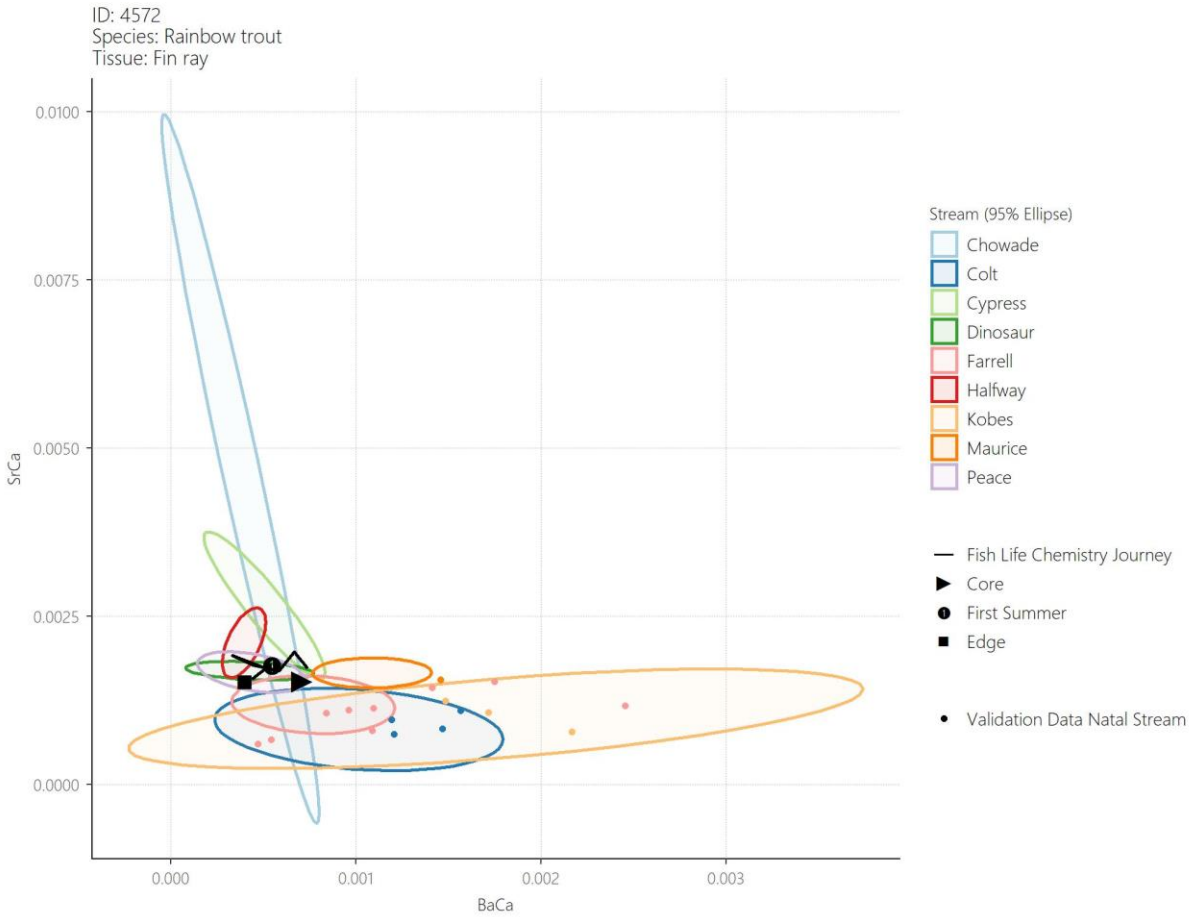

Figure 79. RB#4572 (198 mm, captured in Peace River downstream) was predicted to have recruited from Farrell Creek, but life-history chemistry suggests possibly a recruitment from Dinosaur Reservoir or Halfway River mainstem.

Evidence from the nQDA model predictions and life-history chemistries, suggests that Rainbow Trout captured in Peace River tributaries likely recruit from those same tributaries. Rainbow Trout captured in the Peace River have varied recruitment potential from possibly Maurice Creek, Dinosaur Reservoir or Halfway River watershed, albeit this generalization is based on a very small sample size (n=3), and there is little chemical differentiation among locations.

4.4.3. Summary of Recruitment Sources for Rainbow Trout

The Rainbow Trout otolith and fin ray models have some potential issues with prediction accuracy due to small sample sizes for many locations and significant overlap (i.e., lack of differentiation) among some

of the recruitment sources. The use of the life-history chemistries of individuals provided additional support toward the predictions. Very few Rainbow Trout were sampled with unknown recruitment sources (only three fin ray samples would be considered "unknown" and they were from the Peace River – all other fin ray and otolith samples had known recruitment sources). The Rainbow Trout from the Halfway River watershed remained within the watershed for their entire lives until capture. The Rainbow Trout captured in Farrell Creek, either recruited from Farrell Creek (and remained there for their entire lives) or from other upstream sources, such as Halfway River watershed (Colt or Kobes creeks) and possibly, Dinosaur Reservoir. The Peace River-captured Rainbow Trout were all predicted to have recruited from upstream sources: one from Maurice Creek, one from Colt Creek (in Halfway River watershed), and one from Farrell Creek.

There were clear similarities between these recruitment sources for Rainbow Trout and those reported previously, albeit this report is based on relatively low sample sizes. Earthtone and Mainstem (2013) predicted dominant recruitment from Farrell Creek, Halfway River watershed (only Cameron River and mainstem predicted), Gething Creek, and Maurice Creek. Smaller sources were also upstream: Johnson Creek (<1 %) and Lynx Creek (< 1%), with 6% unknown sources (Earthtone and Mainstem 2013). Only 15 Rainbow Trout were analyzed by TrichAnalytics (2020) with only two of those from the Peace River. The rainbows captured in Halfway River tributaries (Colts, Kobes, and Cypress creeks) were all predicted to have recruited from their natal streams (TrichAnalytics 2020). The Rainbow Trout captured in Farrell Creek came from an unknown source. Both Peace River-captured trout were predicted to have recruited from the Halfway River watershed and, hence, had no apparent downstream sources.

5. SUMMARY AND CONCLUSIONS

The objective of the Fish Otolith and Fin Ray Microchemistry Study was to determine Arctic Grayling, Bull Trout, and Rainbow Trout recruitment sources upstream and downstream of the Project, using otoliths and fin ray microchemistry. The study was successfully carried out by adjusting the modeling approach used in past studies in several ways with the intention of improving the accuracy of the predictions obtained. Revisions to the approach used in this study included:

1) a total of 352 otolith and 141 fin ray capture chemistries from past studies dating back to 2010 were used to help improve the calculation of incorporation coefficients for each species and tissue combination, where possible;

2) a nested quadratic discriminant analysis (nQDA) statistical model was used, as opposed to the linear discriminant function analysis used previously by Earthtone and Mainstream (2013) and TrichAnalytics (2020), which accommodated inter-site differences in chemistry variability and allowed prediction of watershed followed by stream/tributary within the watershed (two-tiered approach);
3) the models were validated using fish captured in 2019/2020 with known recruitment locations (based on fork length at capture) to help identify any issues with model overfitting (e.g., differences between water, capture and natal chemistries); and
4) life-history graphs were used to help refine recruitment predictions from the model output.

Arctic Graylings captured in the Peace River recruited from the Moberly, Halfway and Beatton River watersheds, which is similar to what has been previously reported for this study area. These conclusions are predominantly based on the fin ray analysis and model due to the higher sample size and higher accuracy.

For Bull Trout, fin rays were also used to derive overall predictions for recruitment sources, as more fin rays were available. Streams in the Halfway River watershed (mainly Chowade River and Cypress Creek) were the dominant recruitment sources for both upstream and downstream Peace River captures. Moberly River offered a small upstream source and the Pine River was predicted as the only potential downstream recruitment source. These conclusions echo those reported previously for Bull Trout.

There were clear similarities between these recruitment sources for Rainbow Trout and those reported previously. The Rainbow Trout captured in the Halfway River watershed also originated from there. The Rainbow Trout captured in Farrell Creek, either recruited from Farrell Creek or recruited from other upstream sources, such as Halfway River watershed and possibly, Dinosaur Reservoir. The Peace River-captured Rainbow Trout were all predicted to have recruited from upstream sources: one from Maurice Creek, one from Colt Creek (in Halfway River watershed), and one from Farrell Creek.

Overall, there is consistency in potential recruitment sources for each of the three species, with some variability in which recruitment source dominates among years or across studies. This nuance could reflect real variability over time in fish population dynamics, or reflect differences among studies, such as the composition of samples analysed.

The microchemistry of fish tissues has been an ongoing investigation for BC Hydro as part of the Site C Project, and has been evolving with each new monitoring program as more knowledge and data become available. As such, there are opportunities to continue improvement of modeling and prediction accuracy for future programs using fish tissue microchemistry.

The recruitment models for otoliths and fin rays provided within this study have varying accuracy depending on species, tissue, and location. The addition of life-history chemistry graphs, in combination with the model predictions and capture locations, helped clarify some potential misclassifications predicted with the model alone. Through examination of the training data (i.e., data used to build the model), validation data (i.e., samples with known recruitment sources), prediction data (i.e., samples with unknown recruitment sources), and life-history chemistry for all samples (e.g., capture location chemistry relative to natal chemistry, etc.), potential paths forward for model improvement are provided for each species below.

Arctic Grayling:

- collect water samples from Bratland and Laprise creeks (there are currently none), which could support improvement in the calculation of incorporation coefficients and increase the number of samples used to train/develop the model;
- where possible, capture Arctic Grayling (preferably <1 year old) from Colt Creek, Chowade River, Cypress Creek, Pine River and Beatton River, which could help increase sample numbers for both training data and validation of the model; and
- if available, analyze the fin rays from the Moberly River captured grayling from the 2019/2020 program (only one from the eight captures was analyzed).

Bull Trout:

- collect water samples from Maurice Creek (there are currently only two), which could support improvement in the calculation of incorporation coefficients and increase the number of samples used to train/develop the model for this location;
- where possible, capture Bull Trout (preferably <1 year old) from Halfway River tributaries, especially the mainstem, Needham Creek and Colt Creek, in addition to Maurice Creek, Moberly River and Pine River, which could help increase sample numbers for both training data and validation of the model.

Rainbow Trout:

- collect water samples from Maurice Creek and Kobes Creek, which could support improvement in the calculation of incorporation coefficients and increase the number of samples used to train/develop the model for this location;
- collect water samples from other locations that could be added to the model (e.g., Lynx Creek; those sites included in Earthtone and Mainstem (2013) that were not modeled in this study due to small sample size); and
- where possible, capture Rainbow Trout (preferably <1 year old) from Halfway River tributaries (particularly the mainstem, Chowade River, and Cypress Creek), in addition to Maurice Creek, and Dinosaur Reservoir, which could help increase sample numbers for both training data and validation of the model.

All species:

- include the current validation data (i.e., natal regions) with the future training data (i.e., currently only water and capture chemistries) to possibly improve any model overfitting (where natal region has different chemistry from the capture region even though they are from the same stream/river);
- evaluate the reasons why for some species (particularly Bull Trout) the natal/core region chemistry is different from the capture/edge tissue chemistry if from the same stream/river causing model overfitting (e.g., is there a chemistry gradient of Ba:Ca or Sr:Ca from upstream to downstream, where natal habitat is in a different location than where the capture or water sampling event occurred);
- pending the above, possibly collecting water samples along a stream gradient for some locations (e.g., Fiddes Creek) to support more accurate model development;
- integrate (at least some) past natal recruitment predictions into model development as "known" locations (assuming natal predictions have high confidence), which may increase sample numbers for some locations.
- use fin rays only for recruitment predictions to maintain a non-lethal monitoring approach
- increase the number of Rainbow Trout sampled to improve model sample numbers, as well as upstream versus downstream recruitment conclusions for this species.

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