Analyzing the chemical tracers in fin rays: a non-lethal approach to infer the migratory patterns of bull trout

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Abstract

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Effective management and conservation of migratory species requires identifying the habitats that individuals within and among populations use throughout their lives. As a diversity of life history strategies can buffer individuals and populations from changing environmental conditions, it is crucial to identify and conserve the habitats that maintain different life histories that may be present in and among populations. Bull trout (*Salvelinus confluentus*), native to western North America, were listed under the United States Endangered Species Act (ESA) over 30 years ago due to extensive declines in abundance and distribution. Bull trout can exhibit a variety of different life history strategies both within and among populations. To effectively prioritize bull trout conservation and recovery efforts, it is important to identify the diversity of life history strategies and habitats used by individuals within populations. Certain chemical tracers (e.g., strontium) recorded in fish fin rays reflect the ambient water chemistries a fish encounters throughout its life, therefore offering a non-lethal opportunity to reconstruct life histories and habitat use. In this thesis, I developed a non-lethal technique and chemical baseline using pectoral fin rays, and subsequently applied this technique and baseline to infer migratory patterns of bull trout in the White River, a large tributary to the Puyallup River in Washington state, USA. In Chapter 1, I used the chemical signatures recorded in non-migratory sculpin species and adult bull trout to assess the reliability of fin rays in establishing a geochemical baseline across the Puyallup River basin. I compared the chemical signatures in sculpin fin rays to corresponding water samples from sampling locations and otoliths, bony structures which have widely been used to reconstruct life histories but require lethal sampling. I show that the Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios in sculpin fin rays are similar to corresponding otoliths as these tracers incorporated into fin rays linearly with surrounding water chemistries, and the combination of these tracers allows for discrimination of the Puyallup River basin into its three sub-basins: the White River, the Puyallup River, and the Carbon River. Additionally, I found that the laser ablation measurements of ^{87}Sr/^{86}Sr ratios in bull trout fin rays resulted in coarser temporal resolution data and higher measured values compared to corresponding otoliths. In Chapter 2, I used the Sr/Ca and Ba/Ca ratios in White River bull trout fin rays to reconstruct their migratory patterns. I show that White River bull trout use habitats distributed across and outside of the entire Puyallup basin, including the marine environment, with predominant habitat use in the White River sub-basin. Defining the range of migration strategies among individuals within this population using a non-lethal approach will allow resource managers to develop land and fisheries management plans that maintain expression of the diversity of life history strategies that characterize this population.

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Chapter 1. Are fin rays a reliable alternative to otoliths for establishing geochemical baselines and assessing habitat use by riverine fishes?

1.1 Abstract

The naturally occurring chemical constituents recorded in calcified structures (e.g., otoliths and fin rays) of fishes have been widely used to identify natal sources, establish chemical baselines, and reconstruct migratory patterns. Pectoral fin rays offer an alternative structure to otoliths for inferring habitat use by freshwater fishes based on microchemical composition of these calcified structures. We established a baseline of chemical tracers across the Puyallup River basin in Washington state using resident sculpin fin rays. Our results indicated that Sr/Ca, Ba/Ca, and $^{87}Sr/^{86}Sr$ ratios all behaved conservatively in sculpin fin rays and reflected the surrounding water environments in which fish were captured. Moreover, linear regressions showed that the relationships between water chemistry and fin ray chemistry mirrored otoliths for Sr/Ca, Ba/Ca, and $^{87}Sr/^{86}Sr$ ratios; however, the incorporation rates varied among tracers. The Puyallup River basin could be reliably partitioned into its three sub-basins, the White River, the Puyallup River, and the Carbon River, based on Sr/Ca, Ba/Ca, and $^{87}Sr/^{86}Sr$ ratios. Laser ablation measurements of $^{87}Sr/^{86}Sr$ ratios in fin rays resulted in coarser temporal resolution data compared to corresponding otoliths. Additionally, the laser ablation measurements of $^{87}Sr/^{86}Sr$ ratios in fin rays were consistently higher than the $^{87}Sr/^{86}Sr$ ratios measured in corresponding otoliths. Our results indicate some limitations of $^{87}Sr/^{86}Sr$ ratios measured in fin rays using laser ablation, and suggest that the dissolved Sr/Ca, Ba/Ca and $^{87}Sr/^{86}Sr$ ratios in sculpin fin rays are reliable, non-lethal alternatives to otoliths for establishing chemical baselines.

1.2 Introduction

The chemical constituents archived in fish otoliths have been widely used to reconstruct environmental life histories. Otoliths, paired calcium carbonate ($CaCO_3$) structures used in hearing and balance, are metabolically inert and provide information for a fish's entire life. A chronological chemical record is stored in fish otoliths as elements are incorporated into the $CaCO_3$ matrix over time (Campana 1999; Campana and Thorrold 2001; Pracheil et al. 2014). Certain elements (e.g., strontium, barium) in otoliths reflect the chemistry of the water a fish resides in and offer the ability to trace the environments a fish has occupied over its life (Bath et al. 2000; Walther and Thorrold 2006; Doubleday et al. 2014; Eldson et al. 2008). This tool has been particularly important for classifying natal sources (Barnett-Johnson et al. 2008; Strohm et al. 2017), reconstructing habitat use and movement patterns (Wells et al. 2003; Muhlfeld et al. 2012; Walsworth et al. 2015), stock identification and production (Brennan et al. 2019), and identifying anadromy (Brenkman et al. 2007; Austin et al. 2019).

While the geochemical signatures archived in fish otoliths have accurately established spatial baselines and reconstructed life-history patterns, removing otoliths is lethal and undesirable for species that cannot be sacrificed (e.g., those that are federally protected under the ESA). Teleost fin rays offer a non-lethal alternative to otoliths. Fin rays, primarily composed of calcium phosphate, grow additively and incrementally as new layers encapsulate old layers (Tzadik et al. 2017). Thus, a fin ray cross-section contains annuli with the oldest layers at the center representing early life history and new layers at the edge. As fin ray growth resembles otoliths, these structures have been used to estimate age and growth (Beamish 1981; Zymonas and McMahon 2009; Murie et al. 2009; Griffin et al. 2017; Rude et al. 2013). Furthermore, fin rays incorporate strontium (Sr) proportionally to its relative concentration in ambient waters and can therefore identify natal origins of fish (Phelps et al. 2012; Linley et al. 2016) and assess movement patterns (Clarke et al. 2007).

Strontium isotope ratios ($^{87}Sr/^{86}Sr$) and concentrations are the most reliable and well-known geochemical signatures used to identify natal origins and reconstruct migratory patterns of freshwater and anadromous fishes as Sr substitutes for Ca in calcified structures (Campana 1999; Campana et al. 2000; Kennedy et al. 2000; Brennan et al. 2015a; Miller et al. 2011; Wells et al. 2003). The natural variation of $^{87}Sr/^{86}Sr$ in river waters is determined by underlying bedrock age and composition as the ^{87}Sr isotope forms by the radioactive decay of ^{87}Rb while ^{86}Sr is stable. Therefore, higher $^{87}Sr/^{86}Sr$ ratios in river waters are often a result of older rock types or those with higher Rb/Sr ratios (Capo et al. 1998). $^{87}Sr/^{86}Sr$ ratios provide a sensitive framework to identify natal origins and quantify freshwater migratory patterns in fishes due to the lack of significant biological fractionation during uptake and deposition, resulting in otolith $^{87}Sr/^{86}Sr$ ratios that are a direct reflection of ambient waters (Capo et al. 1998; Kennedy et al. 2002; Barnett-Johnson et al. 2005; Brennan et al. 2015b). While $^{87}Sr/^{86}Sr$ ratios in otoliths have been the primary tool to reconstruct freshwater migratory patterns, this method requires substantial geologic heterogeneity across a watershed to provide sufficient $^{87}Sr/^{86}Sr$ variation to distinguish habitats. In the instance of limited geologic heterogeneity, the use of multiple tracers may strengthen the ability to accurately identify habits.

Elemental concentrations of Sr, barium (Ba), magnesium (Mg), and manganese (Mn) expressed as ratios relative to calcium (Ca) have been used to reconstruct environmental life histories of freshwater and anadromous fishes due to the predictable relationship between the chemical composition of water and calcified structures (Wells et al. 2003; Zimmerman and Reeves 2002; Walsworth et al. 2015). However, biological fractionation can alter this relationship due to physiological processes, diet, and/or genetics, limiting its reliability (Sturrock et al. 2015; Jaecks et al. 2016; Barnes and Gillanders 2013). Thus, the combination of $^{87}Sr/^{86}Sr$ ratios and elemental concentrations may provide a more robust approach to reconstructing the freshwater environments of fishes. However, this approach is complicated when applied to fin rays as an alternative to otoliths. Polyatomic interferences that occur during laser ablation of biological apatite (i.e., calcium phosphate fin rays) lead to elevated and inaccurate $^{87}Sr/^{86}Sr$ ratios (Horstwood et al. 2008; Willmes et al. 2016; Lewis et al. 2014). Little research has been completed to correct for this interference and how it varies across individual samples and machine runs. While the combined elemental and isotopic signatures in fin rays have the potential to establish spatial baselines and reconstruct movement patterns, their reliability across multiple tracers and measurement methods remains poorly understood.

To accurately reconstruct the movement patterns of migratory fishes, it is necessary to first establish a spatial baseline of geochemical tracers and identify the scale at which habitats can be characterized. Sculpin species have successfully indicated provenance and established geochemical baselines due to their sedentary life history pattern and long-term site fidelity (Brennan et al. 2015b; Prichard et al. 2018). Resident sculpin species are convenient candidates to establish a geochemical baseline as individual fish will integrate their site's chemical signature across seasons and years, thus capturing the temporal variation that occurs in river water chemistry in a manner that a single water sampling effort cannot (Brennan et al. 2015b). This sentinel family has been used for many site-specific studies due to their sedentary behavior, high abundance, and wide distributions (Gray et al. 2004; Gray et al. 2005). Limited home ranges for slimy sculpin, (*Cottus cognatus*) and mottled sculpin (*C. bairdii*) have been identified in previous tagging studies (Morgan and Ringer 1992; Breen et al. 2009). Despite small differences in different species' chemical signatures, sculpin species have accurately classified natal habitats of other migratory species according to their microchemical signatures at coarse spatial resolutions (Prichard et al. 2018).

Here, we assessed our ability to establish a geochemical baseline across the Puyallup River basin in Washington state using water and resident sculpin sampling efforts. The Puyallup River basin supports local populations of several salmonid species, and spans habitats from Mount Rainier National Park to Puget Sound. Identifying the spatial variation in chemical tracers across the Puyallup River basin provides the opportunity for future studies to quantify the movement patterns of migratory species. There is specific interest in establishing a baseline with sculpin fin ray signatures as an alternative to otoliths for future application to research on the migratory patterns of ESA-listed bull trout *(Salvelinus confluentus)*. Using resident sculpin species distributed across the Puyallup River basin, we identified which chemical tracers act conservatively in pectoral fin rays. Moreover, we identified the spatial resolution at which these tracers vary across the basin. In addition to establishing the baseline, we assessed the reliability of chemical constituents stored in fin rays compared to otoliths using both dilution and laser ablation methods in paired samples. First, we compared structural differences in resident sculpin fin rays and otoliths among tracers using dilution methods. Then, we analyzed the variation in ^{87}Sr/^{86}Sr ratios measured using laser ablation in paired otoliths and fin rays of adult bull trout. Characterizing the scale at which geochemical signatures vary across the Puyallup River basin and validating the use of fin rays are necessary efforts for future analyses using tracers to reconstruct life history patterns of endangered species that cannot be lethally sampled.

1.3 Methods

1.3.1 *Study area*

The Puyallup River basin drains ~ 2500 km^2 with major tributaries (White River and Carbon River) originating from glaciers of Mount Rainier in western Washington. The largest contributing tributary, the White River, drains ~1200 km^2 area and flows east roughly 120 km from Emmons glacier of Mount Rainier to its confluence with the Puyallup which eventually flows to Puget Sound. Several salmonid species are native to the basin, including Chinook salmon (*Oncorhynchus tshawytscha*), coho *(O. kisutch)*, chum *(O. keta)*, pink *(O. gorbuscha)*, steelhead/rainbow *(O. mykiss)*, cutthroat *(O. clarki)*, bull trout, and mountain whitefish (*Prosopium williamsoni*). The major tributaries of the Puyallup vary on a coarse scale in geologic age and composition, as the White River is predominantly andesite, while the Puyallup and Carbon Rivers are primarily alluvium rock types (Fiske et al. 1963). The heterogeneity of the geology across the basin indicates the potential for differences in geochemical tracers in river waters.

1.3.2 *Water, fin ray, and otolith collection and preparation*

Water and resident sculpin samples were collected from nine mainstem and tributary sties distributed across the Puyallup River basin in 2018 and 2019 (Figure 1.1). Three torrent (*C. rhotheus*), riffle (*C. gulosus*), or shorthead (*C. confusus*) sculpins (*n*=27) were captured at each site using electrofishing and euthanized using tricaine methanesulfonate (MS-222). Sagittal otoliths and ventral portions of the second leading pectoral fin ray were extracted from individual sculpins in the lab and cleaned with deionized water to remove tissue. Cleaned structures were stored dry in polypropylene tubes. At each sculpin sampling location, a river water sample was collected using a syringe filtration technique. River water samples (*n*=15) were collected in 250 mL acid-washed low-density polyethylene (LDPE) bottles upstream of the collector to prevent contamination. Water samples were filtered through a 0.45 µm polypropylene membrane Luer-lock syringe filter using a 60 mL polypropylene syringe into 60 mL acid-washed LDPE bottles. Within 3 days of collection, filtered water samples were sent to the University of Utah, Department of Geology and Geophysics, inductively coupled plasma mass spectrometry (ICPMS) Laboratory, and acidified with 2mL ultra-pure concentrated HNO_3 within 7 days. Triplicate samples and blanks were collected at three sites (one per sampling day) to assess collection methods.

1.3.3 *Isotopic and elemental dilution analysis of water, otoliths, and fin rays*

Isotopic and elemental analyses of water, otolith, and fin ray samples were completed at the University of Utah, Department of Geology and Geophysics, ICPMS Laboratory. $^{87}Sr/^{86}Sr$ ratios and concentrations of ^{88}Sr, ^{137}Ba, ^{44}Ca, ^{24}Mg, and ^{55}Mn in water, otolith, and fin ray samples were quantified using solution based ICPMS. Water, otolith, and fin ray samples were digested by refluxing in 1 mL of HNO_3 (trace metal grade) in a 7 mL PTFE vial at 120^{o} C for 1 hour. For trace element concentrations and $^{87}Sr/^{86}Sr$ ratios, an aliquot of 20 uL of the digest was diluted and tested for Sr, Ba, Ca, Mg and Mn concentrations in a quadrupole ICPM (Agilent 7500ce). Additionally, a second aliquot containing 300 ng of Sr was diluted with water to reach an acid concentration of 6 M HNO_3 and purified using Sr-spec resin (Eichrom) in an automated system (PrepFAST MC, Elemental Scientific). The Sr fraction was obtained in water, acidified to 5% HNO_3 and run for $^{87}Sr/^{86}Sr$ in a multicollector ICPMS (Neptune, Thermo Scientific).

Standard reference material SRM 987 (National Institute of Standards and Technology) was used to check the quality of the method. First, a solution of SRM 987 in 6 M HNO_3 was purified in the same way as samples and run as a sample. Second, a solution of SRM 987 in 5% HNO_3 was run as a standard with the samples (1 standard every 3 samples). The SRM 987-certified

$^{87}Sr/^{86}Sr$ value is 0.71034 ± 2.6×10^{-4}. The long-term precision average and SD measured in the University of Utah's lab is 0.710285 ± 1.5×10^{-5}. The lab reports the average and SD measured during each sample run, with a typical precision below 1.0×10^{-5} (SD). Runs were rejected if: 1) average standard value was outside 2 SDs of long-term precision; or 2) the SD of the standards run in the same run was larger than 0.000015.

1.3.4 *Comparison of paired fin ray and otolith $^{87}Sr/^{86}Sr$ ratios in bull trout*

Laser ablation multi-collector ICPMS of bioapatite (i.e., calcium phosphate) produces inaccurate and elevated $^{87}Sr/^{86}Sr$ ratios caused by polyatomic interference. We calculated the offset produced by this interference by measuring $^{87}Sr/^{86}Sr$ ratios along the growth axes of a fin ray and otolith from the same fish and subsequently quantifying the differences between the $^{87}Sr/^{86}Sr$ ratios across the two transects. From ten adult bull trout that had died in the Puyallup River basin and were opportunistically collected, one sagittal otolith and the ventral portion of the second leading pectoral fin ray were extracted per fish. Extracted fin rays were cleaned with warm, deionized water and scrubbed with a toothbrush to remove tissue. Fin rays were embedded in epoxy resin using methods similar to those outlined in Koch and Quist (2007). A 0.36 mm thick section was cut distal to the articulating process of each fin ray using a Buehler Isomet low-speed saw (Koch et al. 2008) (Figure 1.2). Fin ray cross-sections were individually mounted on microscope cover slips. Cover slips were attached to standard microscope slides with Crystal Bond 509 adhering the sample to one side. Fin rays were polished with 1 µm alumina powder wetted with water on a polishing cloth, dried, and mounted onto petrographic slides for laser ablation analysis.

Similar to fin ray samples, sagittal otoliths were individually mounted sulcus side down on microscope cover slips glued to standard microscope slides. Otoliths were ground to the point of core exposure with 1200 and 2000 silicon carbide grit paper and polished with similarly to fin rays. Following polishing, cover slips were removed from their respective microscope slides, and all otoliths were re-mounted on a single petrographic slide that contained multiple prepared otoliths. Prior to elemental and isotopic analyses, fin rays and otoliths were sonicated for two minutes in MilliQ water and dried in a laminar flow bench overnight.

Laser ablation (LA) transects measured $^{87}Sr/^{86}Sr$ ratios along the longest growth axes of fin ray cross sections and corresponding otoliths using multicollector ICPMS (Neptune, Thermo Scientific). LA transects measuring $^{87}Sr/^{86}Sr$ ratios were implemented with a 65 µm diameter spot size and scan speed of 2 µm/s. Analytical accuracy was assessed by measuring $^{87}Sr/^{86}Sr$ ratios of a marine shell prior to, after, and in between every six samples. Two paired fin ray/otolith samples were measured in July 2019. The remaining eight samples were made available and subsequently measured in July 2020.

1.3.5 *Statistical analyses*

We analyzed the reliability of the microchemical signatures in sculpin fin rays using a series of statistical tests that quantified chemical variation across the Puyallup River basin and structural differences between otolith and fin ray microchemistries. All statistical analyses were conducted in the R Statistical Environment (R Core Team, 2017). We analyzed differences in the chemical compositions of sculpin structures across sub-basins (i.e., Puyallup, White, Carbon) using univariate analysis of variance (ANOVA) tests (a=0.05) and Tukey's honestly significant difference (HSD). Linear regression was used to determine the relationship between water chemistry and sculpin structure chemistry for Sr/Ca, Ba/Ca, Mg/Ca, Mn/Ca, and $^{87}Sr/^{86}Sr$ ratios. Mg/Ca ratios were log transformed prior to statistical analyses to reduce departures from normality and homoscedasticity of model errors. A linear discriminant analysis (LDA) was used to verify the natal sites of individual sculpin and identify the spatial resolution of chemical variation in the basin based on digested fin ray Sr/Ca, Ba/Ca and $^{87}Sr/^{86}Sr$ ratios. This classification method uses the combination of all three chemical tracers to assess the potential discriminatory power of sites across the basin. Digested sculpin fin ray Sr/Ca, Ba/Ca and $^{87}Sr/^{86}Sr$ ratio data were log transformed and grouped based on sub-basin sampling location (Puyallup, White, Carbon). Prior probabilities in the LDA were set to be equal across all three sub-basins.

The $^{87}Sr/^{86}Sr$ laser ablation offset in fin rays was quantified by calculating the difference between $^{87}Sr/^{86}Sr$ ratios along the growth axes of fin rays and corresponding otoliths. While fin rays and otoliths both grow somewhat synchronously, these structures vary in size and subsequently have growth axes of different lengths. Here, all otolith transects were longer than their corresponding fin ray transect lengths. Thus, a comparison along the growth axes of these two structures required scaling of the data. To match each otolith distance increment and corresponding $^{87}Sr/^{86}Sr$ ratio to the equivalent fin ray distance increment and its $^{87}Sr/^{86}Sr$ ratio, each otolith and fin ray transect were normalized to have a distance profile that ranged from 0 to 1. Once the distance values were scaled from 0 to 1, the range of distance increments in the otolith transect that contained each distance value in the fin ray transect were identified. For the range of otolith distance increments that corresponded to a single fin ray distance increment, the average otolith $^{87}Sr/^{86}Sr$ ratio was calculated to produce a single $^{87}Sr/^{86}Sr$ ratio for the otolith distance value corresponding to the single fin ray distance increment. Once each fish had matching distance profiles for otolith and fin ray $^{87}Sr/^{86}Sr$ ratios, the offset was calculated by subtracting the otolith $^{87}Sr/^{86}Sr$ ratio from the fin ray $^{87}Sr/^{86}Sr$ ratio. This same process was completed for the ^{88}Sr signal intensity, which is a good representation of Sr concentration, along the growth axes of each fin ray and otolith pair. However, for the ^{88}Sr signal, the difference was calculated by subtracting the fin ray ^{88}Sr signal intensity from the otolith ^{88}Sr signal intensity as otolith ^{88}Sr intensities were consistently higher than corresponding fin ray intensities. The median ^{87}Sr/^{86}Sr ratio offset for each fish was calculated and correlated to fin ray mean ^{88}Sr intensity to explore a potential influence of ^{88}Sr intensity on the calcium phosphate interference.

^{87}Sr/^{86}Sr ratio laser ablation data were fit to generalized additive models (GAMs) to analyze variation in ^{87}Sr/^{86}Sr ratios along the growth axes of bull trout fin rays and otoliths. GAMs take the form of a generalized linear model (GLM) but include a smooth function that allows for a non-linear relationship between the response variable and covariates (Wood 2017). As the parametric assumption required by the GLMs is relaxed, the data itself estimate the shape of the fitted trend. The GAM model structure is:

$$y_i = \mathrm{f}(x_i) + \varepsilon$$

where y is the response variable, x is the covariate (distance along the growth axis), $\mathrm{f}(x_i)$ is the smooth function, and ε is an error term. GAMs were fit to ^{87}Sr/^{86}Sr ratio laser ablation data with thin plate regression spline functions using the ‘mgcv’ package in R.

1.4 Results

1.4.1 *Geochemical baseline*

Sculpin microchemistry data varied among chemical tracers and sub-basins. Based on ANOVAs and Tukey’s HSD tests, we found significant differences between the White, Puyallup, and Carbon sub-basins for Sr/Ca, Ba/Ca, and ^{87}Sr/^{86}Sr ratios in sculpin fin rays (Table 1.1). White River sculpin fin ray Mn/Ca ratios were significantly different from both the Puyallup and Carbon Rivers; however, the Puyallup and Carbon Rivers did not vary significantly in their Mn/Ca signatures. All sites were statistically indistinguishable according to Mg/Ca signatures in sculpin fin rays. Average fin ray chemistry per sampling site across the basin ranged from 0.49 – 1.61 mmol/mol for Sr/Ca, 11.53 – 65.39 µmol/mol for Ba/Ca, 0.70392 – 0.70618 for $^{87}Sr/^{86}Sr$ ratios, 23.0 – 30.9 mmol/mol for Mg/Ca and 0.058 – 0.41 mmol/mol for Mn/Ca (Supplemental Table 1.1).

Significant positive relationships were quantified between water and structure chemistries for some chemical tracers but not all (Figure 1.3). Sculpin fin ray and water chemistries were significantly positively correlated for Sr/Ca (coefficient = 0.33, CI = 0.28, 0.38; adjusted R^2 = 0.87; $p < 0.001$), Ba/Ca (coefficient = 0.09, CI = 0.07, 0.11; adjusted R^2 = 0.79; $p < 0.001$), and $^{87}Sr/^{86}Sr$ ratios (coefficient = 1.01, CI = 0.93, 1.09; adjusted R^2 = 0.96; $p < 0.001$). No apparent relationship existed for Mg/Ca (coefficient = 0.02, CI = -0.05, 0.09; adjusted R^2 = -0.02; $p = 0.53$) and Mn/Ca (coefficient = 0.048, CI = -0.02, 0.12; adjusted R^2 = 0.06; $p = 0.17$) between fin ray chemistry and water chemistry. Similar to their corresponding fin rays, sculpin otolith and water chemistries were significantly correlated for Sr/Ca (coefficient = 0.32, CI = 0.26, 0.39; adjusted R^2 = 0.79; $p < 0.001$), Ba/Ca (coefficient = 0.026, CI = 0.021, 0.030; adjusted R^2 = 0.82; $p < 0.001$), and $^{87}Sr/^{86}Sr$ ratios (coefficient = 0.98, CI = 0.91, 1.05; adjusted R^2 = 0.97; $p < 0.001$). While there was a statistically significant relationship between otolith chemistry and water chemistry for Mg/Ca (coefficient = -0.29, CI = -0.47, -0.11; adjusted R^2 = 0.28; $p = 0.03$), the variation in river water Mg/Ca was not reflected in the otoliths. There was no significant relationship between otolith chemistry and water chemistry for Mn/Ca (coefficient = 0.001, CI = -0.003, 0.005; adjusted R^2 = -0.04; $p = 0.6$).

The Sr/Ca, Ba/Ca and $^{87}Sr/^{86}Sr$ ratios in sculpin fin rays were used in the LDA as these tracers showed significant positive correlations between water and structure chemistries (Figure 1.3, A – I). Mg/Ca and Mn/Ca were not included in the LDA because the ratios recorded in sculpin fin rays did not track variation in water chemistry (Figure 1.3, J – O) The LDA shows sub-basin scale divergence in multidimensional space as a function of Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios (Figure 1.4). Sites from each sub-basin clustered together due to their similar geochemical signatures. Sculpin fin rays from sites in and tributaries to the Carbon River and Puyallup River have higher Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios, and separate in multidimensional space from White River sculpins. Pearson's correlation coefficients indicate that Sr/Ca and Ba/Ca contribute similar and greatest discriminatory power to the LDA with values of -0.969 and -0.929, respectively. (Table 1.2). Furthermore, the LDA accurately classified 96% of resident sculpins to their capture location (Table 1.3).

1.4.1 *Comparison of bull trout fin ray and otolith ^{87}Sr/^{86}Sr ratios*

The ^{87}Sr/^{86}Sr ratio offset in fin rays varied across individuals and machine run years. The median ^{87}Sr/^{86}Sr ratio offset value between fin rays and otoliths for all ten bull trout samples ranged from 0.00022 to 0.0013 ($\mu = 0.00058 \pm 0.00039$ 1SD) with fin rays having slightly higher ^{87}Sr/^{86}Sr ratios. Two otolith/fin ray pairs were analyzed in 2019 and had median offset values of 0.00121 and 0.0013 ($\mu = 0.00126 \pm 0.00006$ 1SD). Eight additional otolith/fin ray pairs were analyzed in 2020 and had median offset values ranging from 0.00022 to 0.00063 ($\mu = 0.00041 \pm 0.00018$ 1SD). The mean fin ray ^{88}Sr intensity for all ten samples ranged from 0.35 to 1.04 volts. No apparent relationship between fin ray ^{88}Sr instrument intensity and corresponding ^{87}Sr/^{86}Sr ratio offset existed for these samples (Figure 1.5).

We identified various patterns in the LA transects of bull trout fin rays and their corresponding otoliths. Slight changes apparent in the otolith were not always captured in the fin ray. The same laser spot size was used for both structures; however, fin ray cross-sections were substantially smaller than corresponding otoliths. The mean laser ablation transect length of fin rays was 417.7 µm ± 162.4 µm (1 SD), while mean otolith transect length was 1446.9 µm ± 274.5 µm (1 SD). The large spot size required to acquire sufficient ^{88}Sr signal in fin rays resulted in noisier and blurred ^{87}Sr/^{86}Sr ratios as the laser spot integrated over more fin ray annuli compared to the corresponding otolith (Figure 1.6). However, if changes in Sr are large, the fin ray is capable of capturing those changes, though the resolution is lower than that of the otolith. Peaks in Sr were wider and flatter in the fin ray compared to the otolith as a result of the spot size integration (Figure 1.7).

1.5 Discussion

Our data indicate that sculpin fin rays, similar to their corresponding otoliths, incorporate Sr, Ba and ^{87}Sr/^{86}Sr ratios proportionally to surrounding river water environments. These results generally agree with previous work assessing the reliability of fin ray chemistry where Sr, Ba and ^{87}Sr/^{86}Sr ratios in fin rays were strongly correlated with water chemistry (Clarke et al. 2007; Linley et al. 2016; Wolff et al. 2013). Furthermore, our results show different incorporation rates between otoliths and fin rays for Sr/Ca and Ba/Ca. This may be due to the physiological pathways in which elements are accreted into calcified structures. While otoliths precipitate ions from their surrounding endolymph, fin rays incorporate ions directly from the blood to the bone (Campana 1999; Mahamid et al. 2010). Because of the lack of significant biological fractionation in ^{87}Sr/^{86}Sr ratios, these signatures in fin rays circumvent the physiological influences present in elemental incorporation and provide reliable correlations between water ^{87}Sr/^{86}Sr ratios and digested fin ray ^{87}Sr/^{86}Sr ratios. Our regression results suggest that the archived Sr/Ca, Ba/Ca, and ^{87}Sr/^{86}Sr ratios in sculpin fin rays provide reliable environmental history information in the Puyallup River basin.

Our data also showed the lack of reliability of Mg and Mn in sculpin fin rays due to the poor correlations between structure chemistry and water chemistry. The relationships between water and structure chemistries for Mg and Mn are variable as some studies have shown significant linear relationships (Ramsay et al. 2011), while others have shown similar results to ours that lack this relationship (Strohm et al. 2017, Linley et al. 2016; Gibson-Reinemer et al. 2009). The use of Mg and Mn in the reconstruction of environmental life histories requires the validation of the assumption that structure chemistry reflects surrounding water chemistry.

The $^{87}Sr/^{86}Sr$ ratios archived in fish fin rays reflect the water chemistry fish inhabit. However, the measurements of $^{87}Sr/^{86}Sr$ ratios are altered when using laser ablation methods. Here, we showed that the $^{87}Sr/^{86}Sr$ ratios measured in bull trout fin rays were higher than corresponding otoliths, and the magnitude of this offset varies across individuals and machine run times. Our results identify that this offset can range from 0.0002 to 0.001. Moreover, we show the temporal resolution loss that accompanies the laser ablation measurements of $^{87}Sr/^{86}Sr$ ratios in fin rays. Peaks in fin ray $^{87}Sr/^{86}Sr$ ratios were wider and flatter than peaks in corresponding otoliths, and slight changes in otolith $^{87}Sr/^{86}Sr$ ratios were not reflected in fin rays. There are tradeoffs to measuring $^{87}Sr/^{86}Sr$ ratios in fin rays as an alternative to otoliths as fin ray $^{87}Sr/^{86}Sr$ ratios will integrate with coarser temporal resolution.

We hypothesized that ^{88}Sr signal intensity, which is a good proxy for Sr concentration, interacts with the calcium phosphate interference that results in elevated $^{87}Sr/^{86}Sr$ ratios; however, our results do not suggest that this correlation exists within our samples. Previous efforts reduced machine oxide levels to correct for the calcium phosphate interference that results in inaccurate $^{87}Sr/^{86}Sr$ ratio measurements, however this method simultaneously decreases ^{88}Sr signal intensity (Willmes et al. 2016). Therefore, the use of this method requires samples collected from Sr-rich environments (i.e., high ^{88}Sr signal intensities) and is not applicable in our case as our samples had low ^{88}Sr signal intensities averaging from 0.35 to 1.04 V. Future efforts to calibrate the ^{87}Sr/^{86}Sr ratio interference that occurs during laser ablation of calcium phosphate materials should include large sample sizes with varying ^{88}Sr signal intensities and machine run times, and should involve analysis of the relationship between fin ray phosphorous concentrations and measured offset in the ^{87}Sr/^{86}Sr ratios.

The LDA used to discriminate among tributaries of the Puyallup River included Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios in sculpin fin rays and resulted in 96% classification accuracy at the sub-basin scale. Furthermore, Sr/Ca and Ba/Ca contributed similarly and the most to the first linear discriminant that accounted for 95% of the variance. This is particularly important for future studies reconstructing movement patterns in the Puyallup River basin using fin rays as these efforts will require the use of only Sr/Ca and Ba/Ca ratios as the ^{87}Sr/^{86}Sr ratio laser ablation data will likely be inaccurate. However, future studies using otoliths should include all three tracers to accurately map individuals back to habitats at the sub-basin scale. Leveraging the combined variation of Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios across the Puyallup River basin allows for the discrimination of sampling locations at the sub-basin scale.

The Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios archived in resident sculpin fin rays can be used to establish a spatial baseline that partitions the Puyallup River basin at the sub-basin scale. The application of this baseline to other migratory species in the basin assumes similar elemental incorporation across species and will require some flexibility in order to provide accurate reconstructions for additional species. Given the limited chemical variation across the basin and the need to expand the values constraining the current sculpin baseline so that it can apply to other species, future reconstructions of migratory species in the Puyallup basin may be limited to coarser geographic resolutions than identified here. The elevated laser ablation $^{87}Sr/^{86}Sr$ ratios in fin rays, compared to their paired otoliths, limit the reliability of this tool. This is particularly problematic when working in low-Sr environments or with limited isotopic variation. In our case, the magnitude of calcium phosphate interference is as large as our differences in river water $^{87}Sr/^{86}Sr$ ratios across the Puyallup basin. However, if sufficient geologic heterogeneity allows for large differences in river water $^{87}Sr/^{86}Sr$ ratios, the interference in fin rays may be small enough to distinguish habitats.

Establishing spatial chemical baselines in river waters is imperative for studies aiming to use chemical tracers to identify migratory patterns and natal origins of freshwater fishes. Extending this method from multiple water sampling efforts to the use of resident fish allows us to capture the temporal variation that occurs in river water chemistry. Moreover, the use of fin rays as an alternative to otoliths provides a non-lethal sampling opportunity that is particularly important for species of conservation concern. While there are tradeoffs to the use of fin rays instead of otoliths (e.g., resolution loss in laser ablation $^{87}Sr/^{86}Sr$ ratios), we show that multiple tracers behave conservatively in sculpin fin rays and can be used to establish a chemical baseline across a diverse river network. The use of conservative tracers in fish fin rays has broad applications for ESA-listed species recoveries as this tool provides an opportunity to identify the natal origins and habitat networks necessary for species to complete their life-histories using a non-lethal method. Moreover, as conservative tracers recorded in fin rays capture life history variation on an individual basis, we can examine the diversity of life history strategies expressed in a given population. As we identify the habitat networks and migratory corridors required by ESA-listed species for the entirety of their lives, resource managers can prioritize effective restoration and conservation strategies for species and their necessary habitats.

1.6 Figures

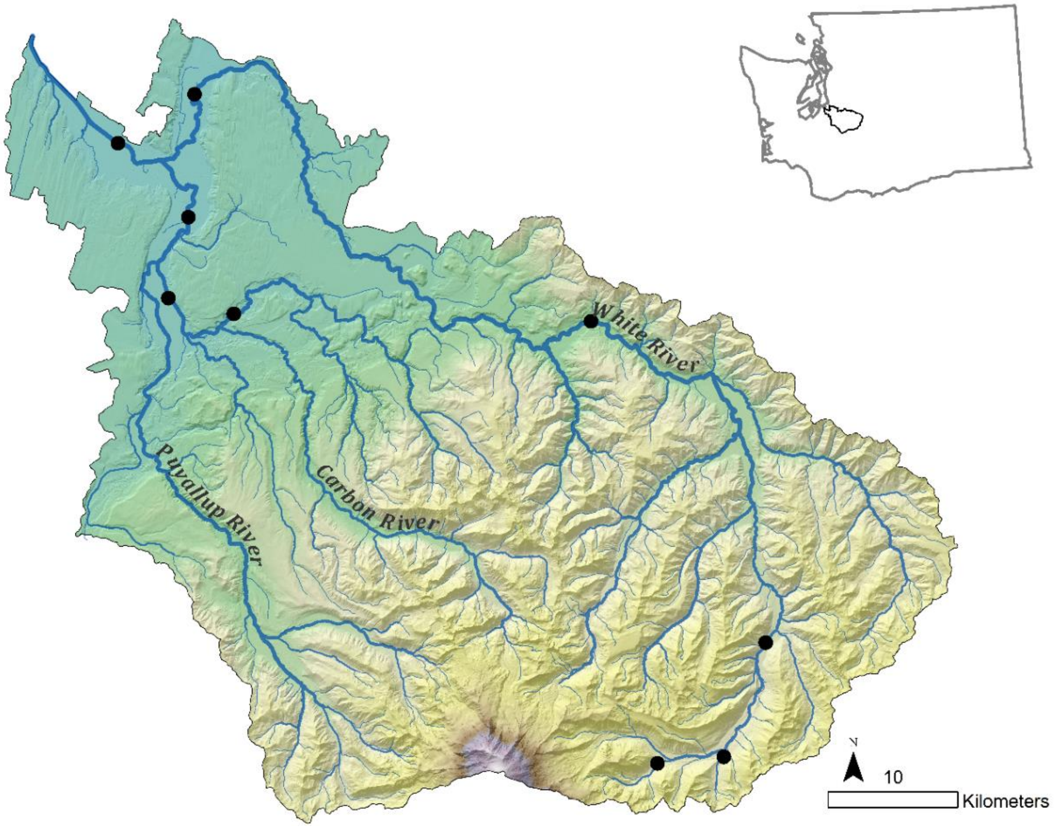

Figure 1.1. Map of the Puyallup River basin. Sculpin sampling locations are indicated by black dots.

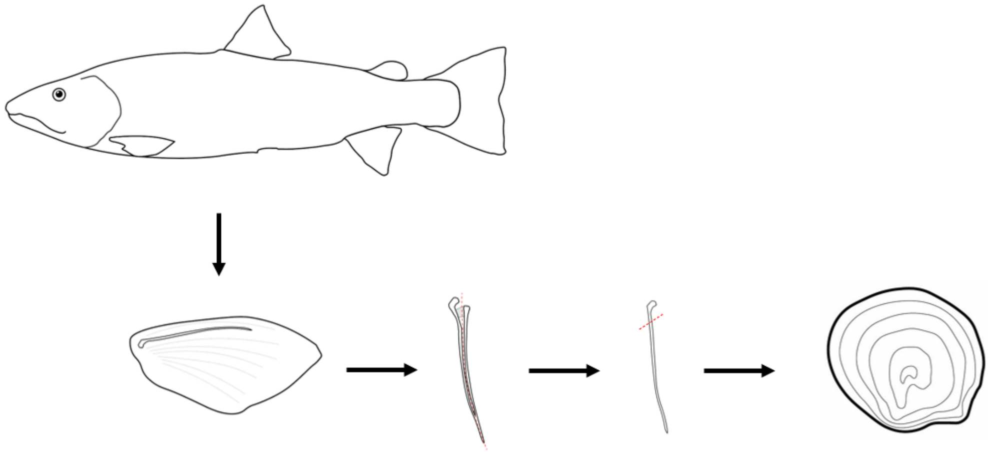

Figure 1.2. Fin ray extraction diagram. The ventral portion of the second leading pectoral fin ray was extracted from bull trout in the field. In the lab, a ~360 µm thick cross section was cut distal to the articulating process of each fin ray.

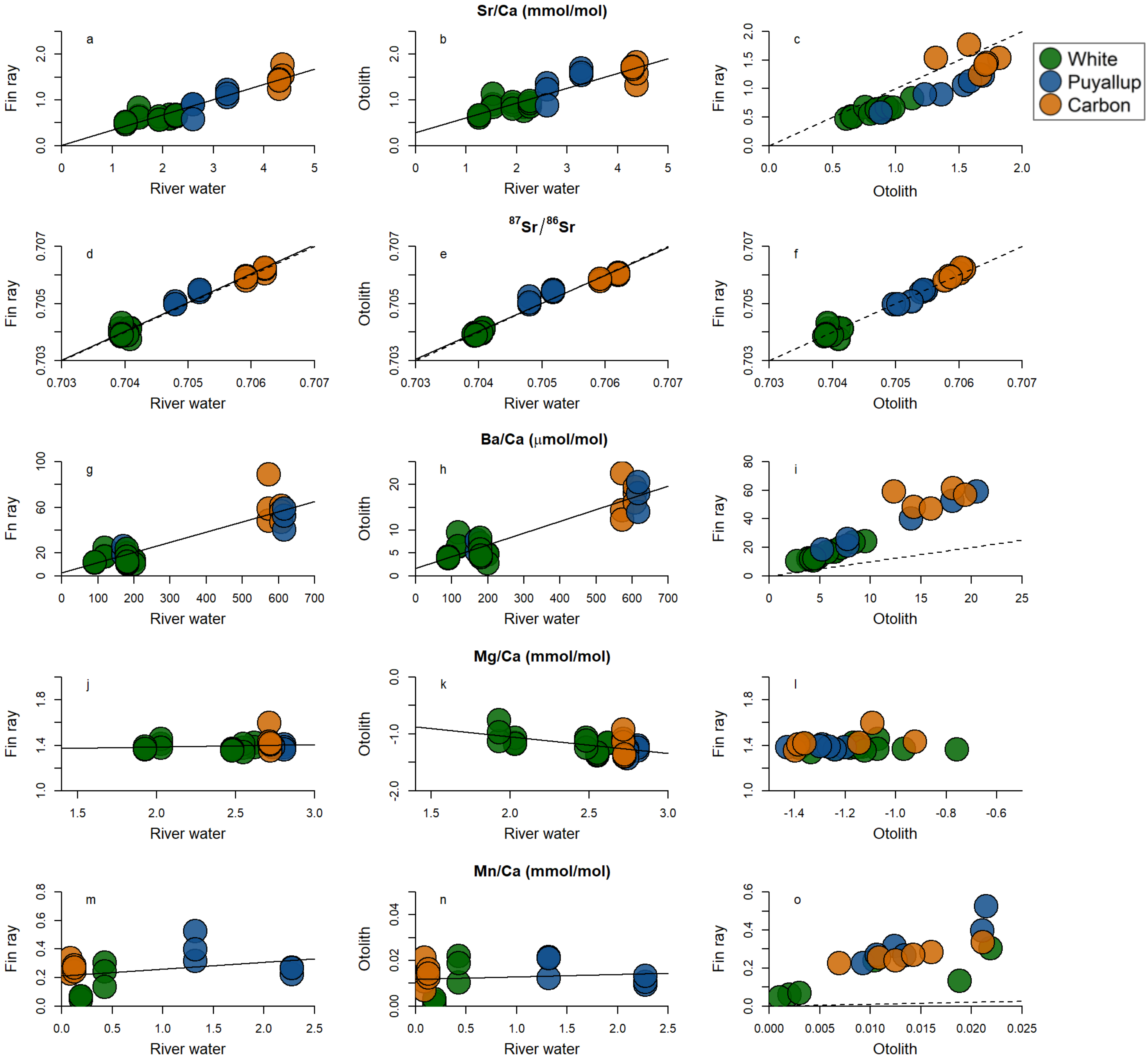

Figure 1.3. Linear regressions of river water to fin ray (column 1) and river water to otolith (column 2). Column 3 includes correlation plots between otoliths and fin rays. Rows are distinguished by the tracer measured in each structure and corresponding water samples. Dashed lines are the 1:1 line and best-fit linear regressions are indicated by the solid lines.

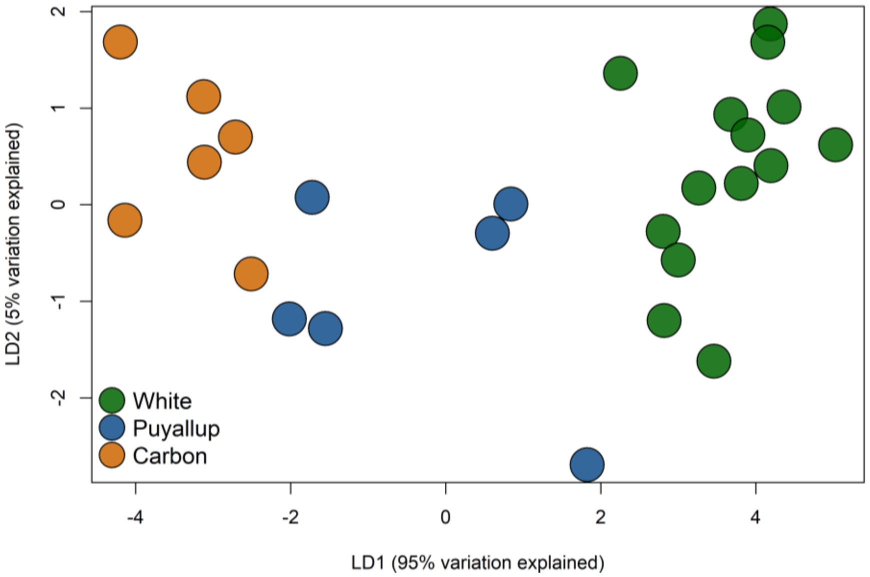

Figure 1.4. Canonical plot from the linear discriminant analysis of Sr/Ca, Ba/Ca, and $^{87}Sr/^{86}Sr$ ratios in resident sculpin fin rays. Sampling locations are grouped at the sub-basin scale and represented by green circles for the White River, blue circles for the Puyallup River, and orange circles for the Carbon River. The first linear discriminant accounts for 95% of the total variation.

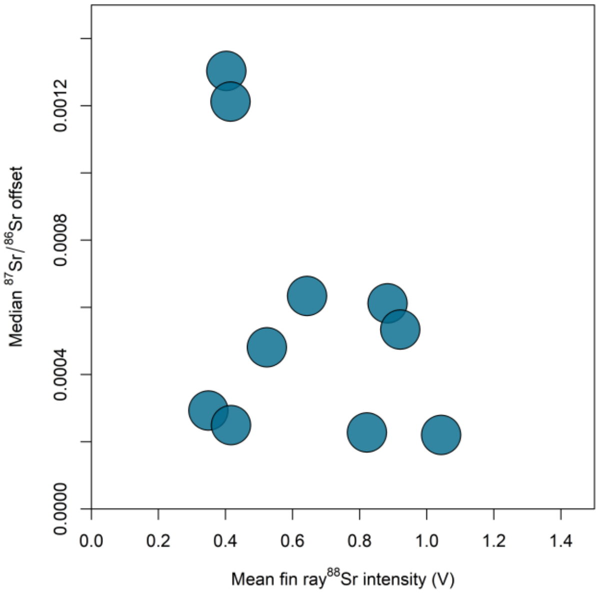

Figure 1.5. Correlation plot of mean fin ray ^{88}Sr intensities, which are the average ^{88}Sr intensities across the laser ablation transect for each bull trout fin ray (n=10), and the corresponding median $^{87}Sr/^{86}Sr$ ratio offsets, which is the median difference between the $^{87}Sr/^{86}Sr$ ratio in the bull trout fin ray and the $^{87}Sr/^{86}Sr$ ratio in the bull trout otolith.

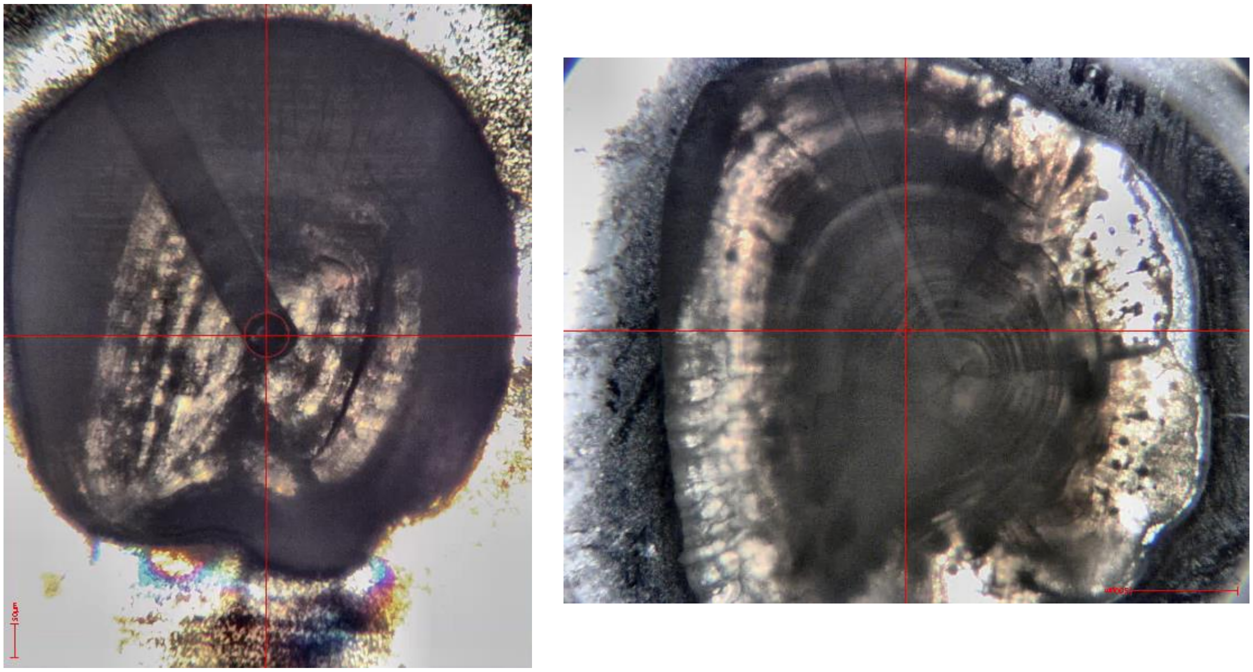

Figure 1.6. Images of a bull trout pectoral fin ray (left) and corresponding otolith (right). Lines across the longest growth axis represent the laser ablation transect using a 65µm spot size at a speed of 2 µm/s.

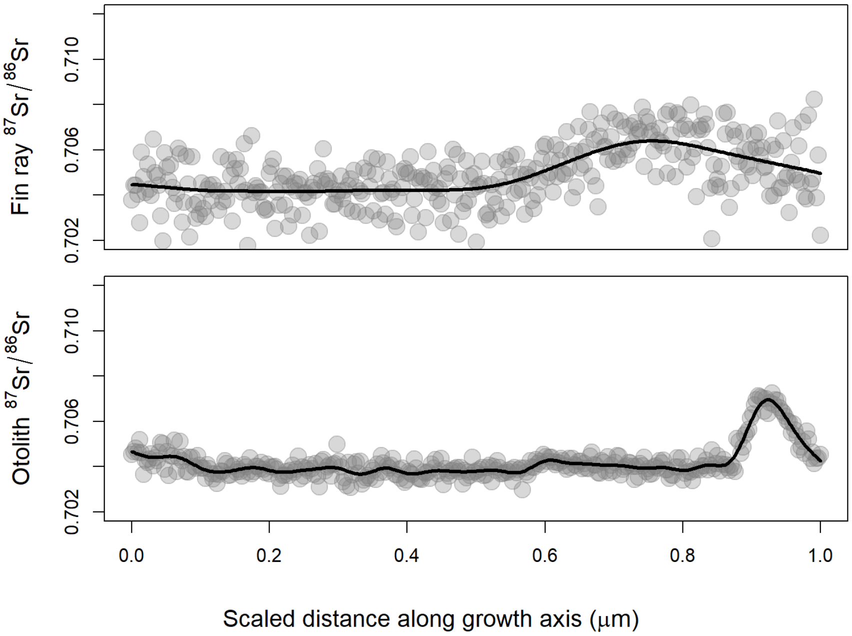

Figure 1.7. Example laser ablation ^{87}Sr/^{86}Sr ratios in paired bull trout fin ray/otolith transects from bull trout. Grey points are raw ^{87}Sr/^{86}Sr ratios, and the black line is a GAM fit to the laser ablation data. Growth axes have been scaled from 0 to 1 to account for differences in structure size.

1.7 Tables

Table 1.1. ANOVA predicted digested sculpin fin ray means and standard errors for each tracer among sub-basins and p-values from Tukey's HSD post hoc to evaluate sub-basin scale differences. Significant differences indicated with *.

Tracer	Sub-basin comparison (predicted mean and standard error)	p-value
Sr/Ca	Puyallup (0.97 ± 0.06 mmol/mol) and Carbon (1.49 ± 0.06 mmol/mol)	<0.001*
	White (0.61 ± 0.04 mmol/mol) and Carbon (1.49 ± 0.06 mmol/mol)	<0.001*
	White (0.61 ± 0.04 mmol/mol) and Puyallup (0.97 ± 0.06 mmol/mol)	<0.001*
^{87}Sr/^{86}Sr	Puyallup (0.70523 ± 7.25e-05 mmol/mol) and Carbon (0.70604 ± 7.25e-05 mmol/mol)	<0.001*
	White (0.70399 ± 4.75e-05 mmol/mol) and Carbon (0.70604 ± 7.25e-05 mmol/mol)	0.002*
	White (0.70399 ± 4.75e-05 mmol/mol) and Puyallup (0.70523 ± 7.25e-05 mmol/mol)	0.001*
Ba/Ca	Puyallup (36.16 ± 4.47 mmol/mol) and Carbon (60.21 ± 4.47 mmol/mol)	0.002*
	White (15.02 ± 2.83 mmol/mol) and Carbon (60.21 ± 4.47 mmol/mol)	<0.001*
	White (15.02 ± 2.83 mmol/mol) and Puyallup (36.16 ± 4.47 mmol/mol)	0.001*
Mg/Ca	Puyallup (24.35 ± 1.25 mmol/mol) and Carbon (27.81 ± 1.25 mmol/mol)	0.14
	White (24.55 ± 0.79 mmol/mol) and Carbon (27.81 ± 1.25 mmol/mol)	0.09
	White (24.55 ± 0.79 mmol/mol) and Puyallup (24.35 ± 1.25 mmol/mol)	0.99
Mn/Ca	Puyallup (0.33 ± 0.03 mmol/mol) and Carbon (0.26 ± 0.03 mmol/mol	0.32
	White (0.12 ± 0.02 mmol/mol) and Carbon (0.26 ± 0.03 mmol/mol)	0.001*
	White (0.12 ± 0.02 mmol/mol) and Puyallup (0.33 ± 0.03 mmol/mol)	<0.001*

Table 1.2. Pearson coefficients by tracer for each linear discriminant and the variance explained by each linear discriminant in total.

Tracer	LD1	LD2
Sr/Ca	-0.969	0.159
^{87}Sr/^{86}Sr	-0.717	-0.567
Ba/Ca	-0.929	0.086
Variance explained	0.95	0.05

Table 1.3. LDA classification accuracy according to the sub-basin scale.

Sub-basin of origin	Assigned sub-basin		
	Carbon	**Puyallup**	**White**
Carbon	6	0	0
Puyallup	1	5	0
White	0	0	14

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Chapter 2. Migratory patterns within a coastal bull trout population as inferred from fin ray microchemistry

2.1 Abstract

A major challenge in the conservation of migratory fishes is identifying and protecting the habitats that different populations, and their life history variants, need to complete their life cycles. Traditional tagging techniques can be limited in their ability to accurately reconstruct these habitat networks as they typically do not integrate over the entirety of a fish's life. By contrast, chemical tracers (e.g., Sr) archived in fin rays, which can be sampled non-lethally, reflect the water chemistry a fish encounters at various points during its life and enables the reconstruction of migration patterns and life history strategies throughout complex river networks. Bull trout (*Salvelinus confluentus*), native to western North America, rely on connectivity in rivers that span habitat from alpine headwaters to the coastal plain. Here we identify how bull trout in the White River, Washington, USA, exploit habitat networks across this riverscape using the Sr/Ca and Ba/Ca signatures recorded in the fin rays of individuals (n=68) sampled from the White River. Our results suggest that this bull trout population uses habitats distributed across the entire Puyallup River basin, of which the White River is a tributary, and the marine environment. The majority of individuals (72%) resided in the White River sub-basin for their entire lives prior to capture, while a smaller fraction (24%) performed inter-tributary migrations earlier in life. The remaining 4% of individuals exhibited marine migrations to Puget Sound. Defining the relevant riverscape, and range of migration strategies among individuals within this population of an ESA-listed species, will allow resource managers

to develop land and fisheries management plans that maintain expression of the diversity of migratory and life history strategies that characterize this population.

2.2 Introduction

Many freshwater and anadromous fish species display highly migratory patterns in which a variety of habitats are used to complete their life cycle (Dodson et al. 2013; Chapman et al. 2012). One major advance in our understanding of the ecology of these species is the growing appreciation for the vast amount of habitat that fishes use over the course of their lives in river basins. While historical fish ecology has emphasized long distance movements as a behavioral strategy mostly used for adult fish during their spawning migrations, we now know that most fishes use extensive networks of habitat for spawning, rearing, feeding, and coping with changing environmental conditions over their entire lives (Schlosser 1991; Fausch et al. 2002; Chapman et al. 2012, Bentley et al. 2015; Dunham et al. 2008).

Further complicating our understanding of migratory behaviors of different species, it remains unclear if all individuals of a population follow the same migratory program, or whether there is a diversity of strategies expressed among the individuals that constitute a population. Partial migration is a phenomenon widely expressed across taxa where populations include both migratory and resident individuals (Chapman et al. 2011). The expression of partial migration and a diversity of life history strategies within a population can provide stability in response to environmental perturbations as individuals exploit patchily distributed resources in different habitats (Chapman et al. 2012; Bentley et al. 2015; Walsworth et al. 2015). However, a current major challenge in the conservation of populations that include both migratory and non-migratory individuals is quantifying the extent and locations of the habitat networks required given the variation in movement behaviors within the population.

For many highly migratory species, traditional tagging techniques (e.g., radio tags, passive integrated transponders) are often unable to reconstruct lifelong movement patterns as they are typically limited to a specific life-stage. By contrast, the natural variation of strontium (Sr) in river waters, determined by geologic heterogeneity, is reflected and stored in calcified structures of fish, and has reliably reconstructed lifelong movement patterns (Capo et al. 1998; Campana 1999; Kennedy et al. 2000; Barnet-Johnson et al. 2008; Brennan et al. 2015a). Sr is the most widely used tracer to identify fish life history patterns, as Sr readily substitutes for calcium (Ca) in calcified structures and can distinguish environments given Sr variation in surrounding water bodies (Campana 1999; Campana et al. 2000; Kennedy et al. 2000; Brennan et al. 2015b; Miller et al. 2011; Wells et al. 2003). Other trace elements, including barium (Ba) and magnesium (Mg), have been used to reconstruct movement patterns and identify natal origins due to their predictable relationships between water and calcified structures (Bath et al. 2000; Walther and Thorrold 2006; Doubleday et al. 2014; Eldson et al. 2008). Currently, the microchemical signatures in otoliths (calcium carbonate ear stones in fishes) are predominantly used to reconstruct life history patterns as otoliths are metabolically inert and can provide information for the entire fish's life (Campana 1999; Campana and Thorrold 2001; Pracheil et al. 2014). However, otolith extraction is lethal and thus an undesirable technique for threatened and endangered species such as those listed under the US Endangered Species Act (ESA).

Microchemistry studies have typically relied on analysis of otoliths from dead specimens or lethal sampling; however, there is interest in moving toward non-lethal methods for research on protected species (Clarke et al. 2007; Wolff et al. 2013). Teleost fin rays offer a non-lethal alternative to otoliths. Fin rays, primarily composed of calcium phosphate, grow additively and incrementally as new layers encapsulate old layers (Tzadik et al. 2017). Thus, a cross-section of a fin ray contains annuli with the oldest layers at the center representing early life history and the newest layers at the edge representing recent life history. As fin ray growth has some of the same physical properties of otoliths, these structures have been used to estimate age and growth (Beamish 1981; Zymonas and McMahon 2009; Murie et al. 2009; Griffin et al. 2017; Rude et al. 2013). Furthermore, fin rays incorporate Sr proportionally to its relative concentration in ambient waters (Chapter 1) and can therefore identify natal origins of fish (Phelps et al. 2012; Linley et al. 2016) and assess movement patterns (Clarke et al. 2007). The ability to circumvent mortality through fin ray extractions is particularly important for endangered species with small population sizes.

Life history variation has been well-documented in salmonids, including bull trout. Bull trout, native to the northwestern region of North America, were listed as threatened under the US ESA in 1999 due to extensive declines in abundance and distribution primarily resulting from habitat fragmentation (USFWS 2012). This salmonid species can exhibit multiple life history forms within a given population (Hogen and Scarnecchia 2006; Watry and Scharnecchia 2008; Austin et al. 2019; Brenkman et al. 2007). Bull trout populations have been documented expressing fluvial potadromous (Swanberg 1997; Hogen and Scarnecchia 2006; Strarcevich et al. 2012), adfluvial potadromous (Fraley and Shepard 1989; Watry and Scharnecchia 2008), resident (Jakober et al. 1998), and anadromous (Brenkman et al. 2007; Hayes et al. 2011; Austin et al. 2019) life history forms. Given the important role of life history diversity in stabilizing population dynamics of salmonids (Schindler et al. 2010; Moore et al. 2104), it is important to understand how bull trout exhibit such diversity within a population, and if so, how these forms partition the riverscape to inform conservation strategies.

Migratory species depend on networks of habitats to complete their life-cycles, and are particularly vulnerable to impacts such as urbanization, timber harvest, and hydropower development, all of which occur in the Puyallup River basin in Washington state. This river basin supports local populations of bull trout and Pacific salmon, and spans habitats from Mount Rainier National Park to Puget Sound. To prioritize restoration strategies, it is imperative to quantify the extent of habitat networks used by bull trout in this riverscape. Here, we used the Sr/Ca and Ba/Ca signatures recorded in bull trout fin rays to reconstruct the migratory patterns expressed in a population native to the White River, a major tributary to the Puyallup River. We sought to identify the habitat networks necessary for bull trout to complete their life-cycles using a non-lethal method. Identifying the habitats used by bull trout in the White River, as part of the larger Puyallup River basin, will inform fisheries and land management decisions that seek to maintain the expression of life history diversity and maintain these populations.

2.3 Methods

2.3.1 *Study area*

The Puyallup River basin drains ~ 2500 km^2 with major tributaries (White River and Carbon River) originating from glaciers of Mount Rainier in western Washington. The largest contributing tributary, the White River, drains ~1200 km^2 area and flows east roughly 120 km from Emmons glacier of Mount Rainier to its confluence with the Puyallup River, which in turn flows to Puget Sound. Many salmonid species are native to the basin, including Chinook salmon (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), chum (*O. keta*), pink (*O. gorbuscha*), steelhead/rainbow (*O. mykiss*), cutthroat (*O. clarki*), bull trout, and mountain whitefish (*Prosopium williamsoni*). The major tributaries of the Puyallup vary on a coarse scale in geologic age and composition, as the White River is predominantly andesite, while the Puyallup and Carbon Rivers are primarily alluvium rock types (Fiske et al. 1963). The heterogeneity of the geology throughout the basin indicates the potential for differences in Sr in river waters.

2.3.2 *Fin ray collection and preparation*

Bull trout fin ray samples (*n*=68) were collected at the U.S. Army Corps of Engineers' fish passage facility in the White River during the summers of 2016, 2017, and 2018. Fish were dip-netted from the facility's fish trap into a tank and anesthetized with tricaine methanesulfonate (MS-222). The ventral portion of the second leading pectoral fin ray was extracted using a scalpel and stored dry in a polypropylene centrifuge tube until preparation and elemental analysis. Once the extraction was complete, fish were placed in a dip net and quickly moved to a tanker truck to be transported back into the White River upstream of Mud Mountain Dam. Fin rays were extracted under the FWS recovery sub permit TE-034104-105, FWSWFWS-15, Amendment 15.

Extracted fin rays were cleaned with warm, deionized water and scrubbed with a toothbrush to remove tissue. Fin rays were embedded in epoxy resin using methods similar to those outlined in Koch and Quist (2007). A 0.36 mm thick section was cut distal to the articulating process of each fin ray using a Buehler Isomet low-speed saw (Koch et al. 2008) (Chapter 1). Fin ray cross-sections were individually mounted on microscope cover slips. Cover slips were attached to standard microscope slides with Crystal Bond 509 adhering the sample to one side. Fin rays were polished with 1 µm alumina powder wetted with water on a polishing cloth, dried, and mounted onto petrographic slides for laser ablation analysis. Prior to elemental and isotopic analyses, fin rays were sonicated for two minutes in MilliQ water and dried in a laminar flow bench overnight.

2.3.3 *Fin ray elemental analysis*

Elemental analyses of fin ray samples were completed at the University of Utah, Department of Geology and Geophysics, inductively coupled plasma mass spectrometry (ICPMS) Laboratory. Elemental laser ablation (LA) transects measured ^{88}Sr, ^{137}Ba, and ^{44}Ca isotopes from the center to the edge of each fin ray cross section using an Agilent quadrapole ICP-MS (7500ce). LA transects were conducted with a 40 µm diameter spot size, scan speed of 2 µm/s, and a laser energy at 75% attenuation. Background levels were measured for 30s prior to fin ray ablation and removed during fin ray ablation data reduction. Count rates for each isotope measured were normalized to 43Ca. Using the same approach, the U.S. Geological Survey MACS-3 standard was analyzed prior to, after, and in between every ten fin ray samples to calculate elemental concentrations. Raw isotope counts were converted into molar ratios of Element/Ca (mmol/mol).

2.3.4 C*hemical baseline*

To accurately reconstruct the migratory patterns of bull trout according to their microchemical signatures, it was necessary to first establish a spatial baseline of chemical tracers across the Puyallup River basin. Previous research showed that the Sr/Ca, Ba/Ca and ^{87}Sr/^{86}Sr ratios in dissolved resident sculpin fin rays reflect ambient waters and can partition the Puyallup basin into three areas: the White River basin, the lower Puyallup River, and the lower Carbon River (Chapter 1). The baseline used here includes nine mainstem and tributary sites distributed across the Puyallup basin (Figure 2.2). Each site was characterized with Sr/Ca and Ba/Ca ratios from three resident sculpins of the same species and grouped by sub-basin (i.e., White, Puyallup, Carbon). The baseline was established by averaging all sculpin fin ray microchemistry data from sampling locations in the White River and subsequently aggregating and averaging all sculpin fin ray microchemistry data from sampling locations in both the Puyallup and Carbon rivers. The incorporation rate of elements varies across species and may require assignments at broader geographic scales when using differing species to establish baselines (Prichard et al. 2018). As the lower Puyallup and lower Carbon river sampling locations showed some overlap in their combined chemical signatures, we merged the Puyallup and Carbon rivers as one location to make more conservative estimates and avoid misclassifications. In order to include the marine environment as a location possibility, we estimated sculpin fin ray signatures using reduced major axis linear regression equations and existing otolith microchemistry data from Pacific staghorn sculpins *(Leptocottus armatus)* collected in the Puyallup River's estuary, Commencement Bay. The mean ± 2SD of Sr/Ca and Ba/Ca ratios in White River sculpins, lower Puyallup and lower Carbon River sculpins, and Commencement Bay estimates were used to establish our chemical baseline with three location options: the White River, the lower Puyallup/Carbon Rivers, and the marine environment (Table 2.1). Greater spatial resolution was not possible because of the limited amount of chemical variation across the basin.

2.3.5 *Statistical analysis*

Generalized additive models (GAMs) were fit to laser ablation data to analyze variation in Sr/Ca and Ba/Ca along the growth axes of fish fin rays. GAMs take the form of a generalized linear model (GLM) but include a smooth function that allows for a non-linear relationship between the response variable and covariates (Wood 2017). As the parametric assumption required by GLMs is relaxed, the data itself estimate the shape of the fitted trend. The GAM model structure is:

$$y_i = \mathrm{f}(x_i) + \varepsilon$$

where y is the response variable, x is the covariate (distance along the growth axis), $\mathrm{f}(x_i)$ is the smooth function, and ε is an error term. GAMs were fit to Sr/Ca and Ba/Ca laser ablation data with thin plate regression spline functions using the 'mgcv' package in R. To avoid over-fitting or over-smoothing the GAMs, the basis dimension, k, (the maximum effective degrees of freedom allowable) was scaled with the number of data points (n) in each laser ablation transect with the following equation: $k = 10 * n^{2/9}$, where the effective degrees of freedom are related to the number of splines fit to each GAM (Brennan et al. 2015b).

Chronological clustering was used to identify changes in Sr/Ca and Ba/Ca GAMs along the laser ablation path. Chronological clustering classifies multivariate data into groups while preserving the temporal structure of the data. Each fish's combined Sr/Ca and Ba/Ca signatures were classified into 6 distinct clusters using the CONISS method (Grimm, 1987) based on a Euclidean distance matrix in the 'rioja' package in R (Juggins, 2017). The number of clusters were defined using a broken stick model where the variance explained by a certain number of clusters is greater than what would be expected from a random distribution (Bennett, 1996). Using Bayes' theorem and the spatial baseline defined by resident sculpin Sr/Ca and Ba/Ca ratios, each data point in a time series was classified to the White River, Puyallup/Carbon Rivers, or marine environment. In a given cluster, the posterior probabilities of a data point originating from the three location options were averaged across all data points. Subsequently, each cluster was assigned the most probable location. A proportion of samples (*n* = 17) had contaminated Ba concentrations. These samples were clustered and assigned most probable locations based only on Sr/Ca ratios. All statistical analyses were conducted in the R Statistical Environment (R Core Team, 2017).

2.4 Results

Total lengths of bull trout sampled ranged from 278 mm to 613 mm (μ = 467 mm). According to the Sr/Ca and Ba/Ca ratios recorded in pectoral fin rays, 72% of individuals (*n* = 49) sampled remained in the White River for their entire lives until capture (Figure 2.3). The median average Sr/Ca ratio for all data points classified to the White River was 0.53 mmol/mol. Laser ablation transects for fish classified to the White River showed consistently low Sr/Ca (~0.5 mmol/mol) ratios with little variability across individuals (Figure 2.4). Sixteen of the 68 bull trout sampled (24%) moved outside of the White River to the Puyallup or Carbon sub-basins at some point in their lives (Figure 2.5). The median average Sr/Ca ratio for data points classified to the Puyallup/Carbon Rivers was 0.91 mmol/mol. The individuals that migrated outside of the White River had total lengths ranging from 312 mm to 590 mm (μ = 464 mm) at capture. Migratory individuals appeared to only have a single movement prior to capture, however the change in Sr/Ca varied across individuals (Figure 2.6). The remaining 4% of bull trout (*n* = 3) sampled appeared to use the marine environment. These individuals exhibited a single migration to salt water prior to capture and showed simultaneously high Sr/Ca and low Ba/Ca ratios (Figure 2.7).

We assumed that microchemical signatures at the end of the time series should be classified to the White River, as fish were captured in the White River. This assumption was met for most individuals except for two which were not classified to the White River at the end of their laser ablation transects. We presume that these classifications were anomalies compared to the rest of our samples and their subsequent classifications. All individuals were clustered to the White River for early life history (first 100 µm) except one, which was classified to the Puyallup/Carbon basins for the first half of its life until capture (0-300 µm out of 600 µm total).

2.5 Discussion

Characterizing the habitats necessary for migratory individuals to complete their life cycles is fundamental to identifying the life history patterns within a population, and to designing effective conservation plans that will enable the expression of diversity in life history strategies. Using the microchemical signatures recorded in pectoral fin rays, we reconstructed the migratory patterns of White River bull trout and identified three life history strategies expressed within the population. Our results show that White River bull trout use habitats distributed across and outside of the entire Puyallup basin, including the marine environment, with predominant habitat use in the White River sub-basin. The habitats used by White River bull trout encompass heterogenous landscapes that include urban, agricultural, and federally protected areas. The use of fin ray cross-sections as an alternative to otoliths allowed us to simultaneously fill a substantial knowledge gap and avoid sacrificing individuals that are listed under the ESA.

Our results demonstrated that 72% of bull trout sampled used habitats solely within the White River basin during their lives prior to capture. The White River sub-basin encompasses a large amount of area relative to the entire Puyallup River basin as it drains roughly half of the area in the basin. Moreover, the land use practices throughout the White River are diverse as the upper basin is federally managed under the National Park Service and U.S. Forest Service while the middle and lower sections of the White River are subject to timber harvest, flood control and diversion dams, agricultural practices, and heavily developed areas. Despite the vast heterogeneity of land use practices in the White River, it is apparent that bull trout may use habitats throughout the entire sub-basin, as our capture location was located in the middle section of the river and individuals were sampled during their upstream migration in summer months. We assume these upstream movements were spawning or foraging migrations; however, it is possible that these movements are due to other environmental conditions (e.g., warming temperatures in the lower basin in summer months). Based on capture information alone, we can assume that individuals are using habitats both up and down stream of impassible barriers in the White River. The microchemistry data presented here supports this assumption as we identify the predominant use of the White River basin for the entirety of individuals' lives prior to capture. The movements of freshwater fishes are primarily influenced by the availability of resources and environmental conditions that will optimize growth, reproduction, and survival (Fausch et al. 2002; Chapman et al. 2012). However, large migrations involve tradeoffs between the energetic cost to move and growth potential in different habitats (Chapman et al. 2012). Here, we can infer that large-scale movements outside of the White River basin are not common to all White River bull trout to successfully grow and reproduce.

Although less common, large-scale movements outside of the White River are an important behavioral strategy expressed in this bull trout population. Roughly a quarter (24%) of individuals sampled displayed migrations outside of the White River at some point in their lives, prior to capture. The expression of partial migration in this population may be influenced by genetics or environmental conditions (Chapman et al. 2012). Bull trout express a wide range of life history patterns among and within populations (Hogen and Scarnecchia 2006; Watry and Scarnecchia 2008), including facultative migratory strategies where individuals can change migratory strategies to exploit heterogeneously distributed resources and habitats that vary in space and time (Austin et al. 2019). Here, a proportion of individuals performed long migrations presumably to capitalize on resources and environmental conditions outside of the White River basin. As the decline of migratory forms in other populations of bull trout have been associated with habitat fragmentation (Nelson et al. 2002), it is becoming increasingly apparent that the maintenance of life history strategies hinges on connectivity among complementary habitats. Fragmentation of the Puyallup River basin could prove to be an impediment towards maintenance of the migratory component of this population (Rieman and McIntyre 1993; Morita et al. 2009; Rolls 2011).

We identified anadromous migrations in three individuals sampled in the White River, however, the classification of these movements can change depending on the marine threshold values used to identify anadromy. It is often assumed that an increase in Sr/Ca in calcified structures reflects a marine migration, however, high Sr/Ca ratios in river waters can lead to confounding results (Kraus and Secor 2004; Brown and Severin 2009). In our case, digested sculpin fin ray Sr/Ca ratios from the lower Carbon River (μ= 1.61 mmol/mol ± 1.4, 1 SD), a tributary to the Puyallup River, are greater than the Sr/Ca value in otoliths often used to identify anadromy (1.5 mmol/mol). Here, rather than using a single value to identify anadromy, we used existing otolith microchemistry data from Commencement Bay sculpins to estimate a distribution of possible Sr/Ca and Ba/Ca fin ray values in the marine environment.

Our results classify anadromous movements in a considerably small (4%) proportion compared to other populations in Washington state. Using the Sr/Ca and Ba/Ca signatures recorded in adult bull trout otoliths, Austin et al. (2019) estimated that 41% of individuals sampled in the Skagit River basin appeared to use the marine environment. Moreover, 23% of samples had microchemical signatures indicating maternal marine migrations. Additionally, Brenkman et al. (2007) identified anadromous movements from 85% of bull trout sampled in the Hoh River, with 75% of those individuals making multiple migrations to the Pacific Ocean. However, these studies identified marine migrations with peaks over 1.5 mmol/mol or generally steep peaks in Sr/Ca ratios. As marine residence time effects the amount of Sr/Ca incorporated in calcified structures, anadromy identification rules can be subjective and variable across species and studies. As we applied a more conservative approach, our results contrast with the current knowledge of bull trout populations in Washington state with access to the marine environment as our analysis of microchemical signatures in bull trout fin rays suggests that this population has little reliance on marine resources outside of the Puyallup basin. This may be a result of a significantly modified estuary as Commencement Bay has been substantially altered to create several man-made industrial waterways. If the Puyallup basin provides sufficient resources for individuals to successfully grow, the risk to emigrate to the marine environment may not provide enough benefits relative to the freshwater resources available.

Diverse life history strategies can buffer individuals and populations from environmental perturbations (Schindler et al. 2015). While use of the White River basin is the current dominant life history strategy expressed in this population, changing environmental conditions may result in a shift in the population's dominant life history strategy. As a diversity of strategies support the stability and persistence of populations, it is crucial to identify and maintain connectivity among the habitats that enable the expression of these different life histories. In this study, we used a non-lethal method to identify the diversity of life history strategies in White River bull trout. These life history forms use habitats distributed within and outside of the White River, including the marine environment. The use of pectoral fin ray sections as an alternative to otoliths presents a unique and novel opportunity to gain information for species of low population sizes that cannot be sacrificed for lethal sampling methods. Although we were limited to a coarser spatiotemporal resolution compared to otolith microchemistry data, this method was still capable of identifying three different life history strategies present in the population. As conservative tracers recorded in fin rays capture life history variation on an individual basis, this tool has broad applications for threatened and endangered species as we can examine the diversity of life history strategies expressed in a given population using a non-lethal approach. Defining the relevant riverscapes for bull trout populations across their range will allow resource managers to develop land and fisheries management plans that maintain the expression of all life history strategies and evaluate stressors in river basins relative to the habitats used by bull trout throughout their lives.

2.6 Figures

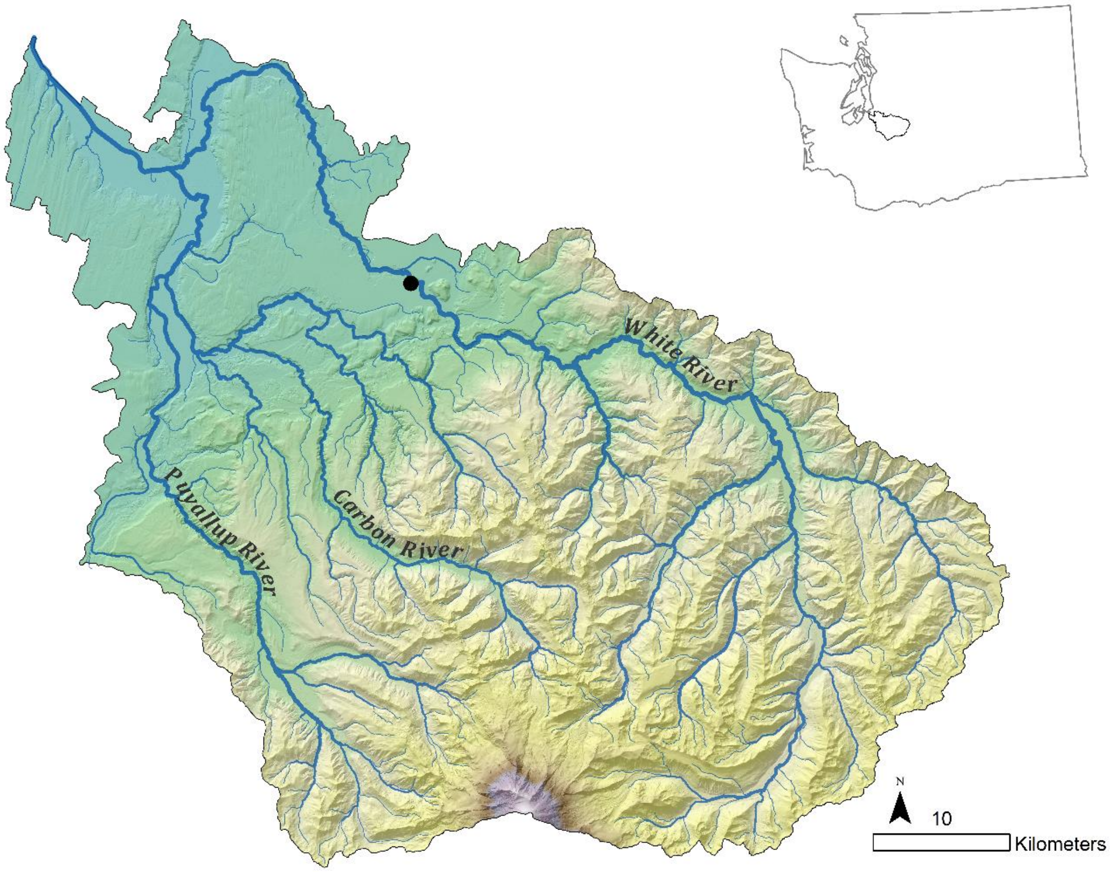

Figure 2.1. Map of the Puyallup River basin. The black dot indicates the U.S. Army Corps of Engineers' fish passage facility in the White River where bull trout fin ray samples were collected.

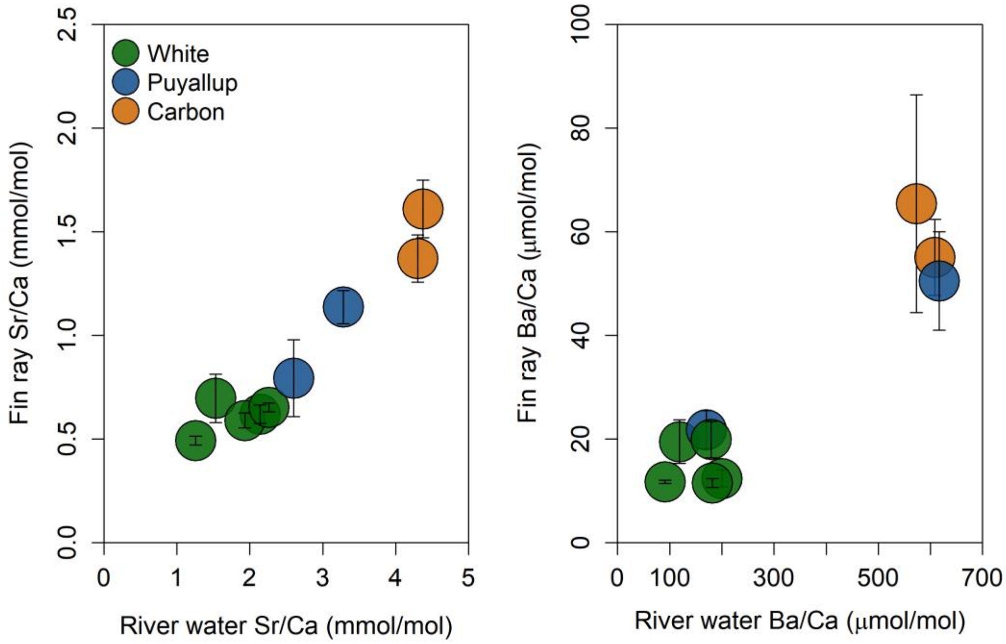

Figure 2.2. Puyallup River basin baseline values for water and average dissolved resident fin ray Sr/Ca and Ba/Ca ratios ±1SD.

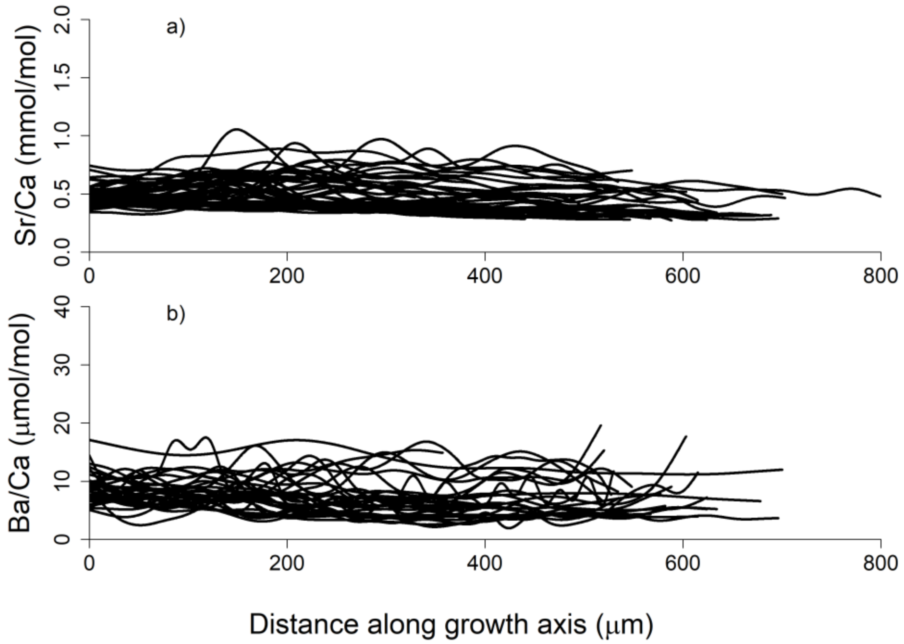

Figure 2.3. Sr/Ca (a) and Ba/Ca (b) GAMs for all individuals with classifications solely to the White River.

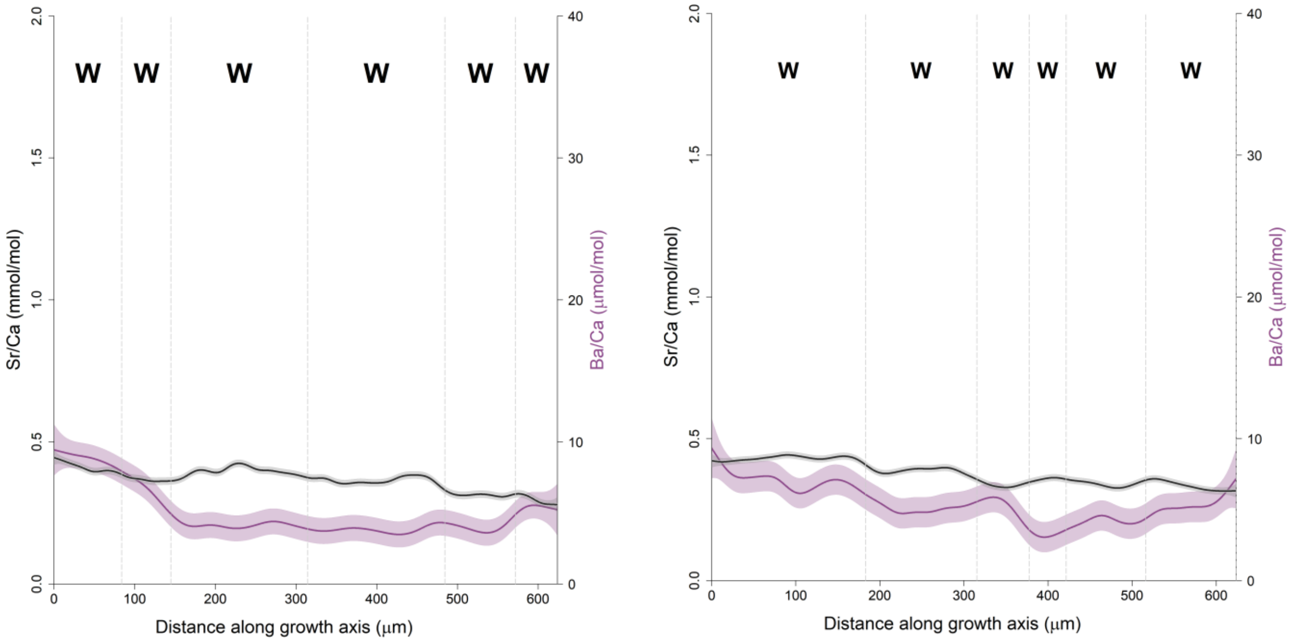

Figure 2.4. Sr/Ca and Ba/Ca GAMs for two individual bull trout classified to the White River for their entire lives. Grey dashed lines indicate the cluster breaks in the transect identified by the chronological cluster model. Habitat assignments are indicated at the top of each cluster where W indicates the White River.

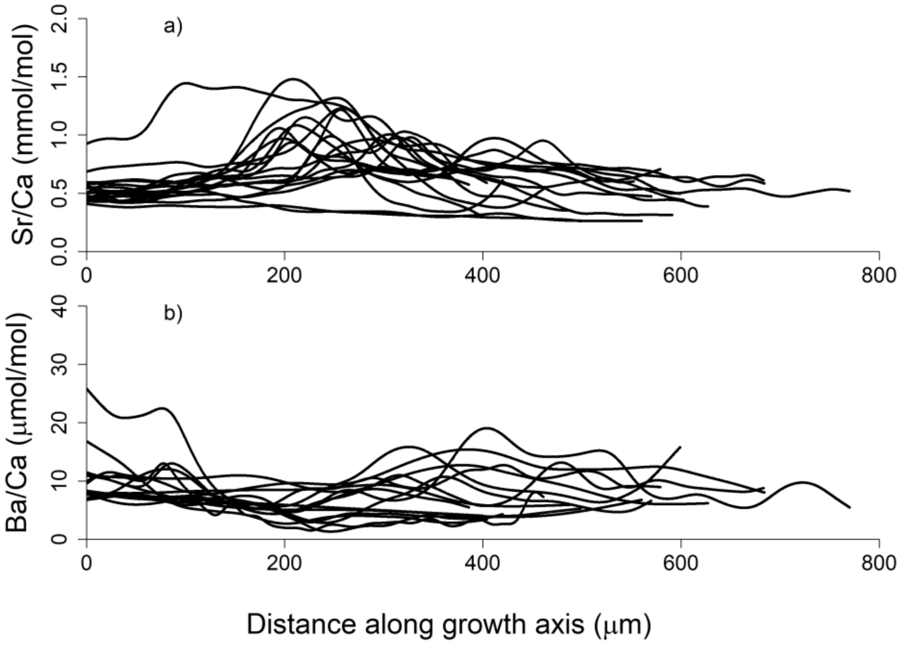

Figure 2.5. Sr/Ca (a) and Ba/Ca (b) GAMs for all individuals with classifications to habitats outside of the White River.

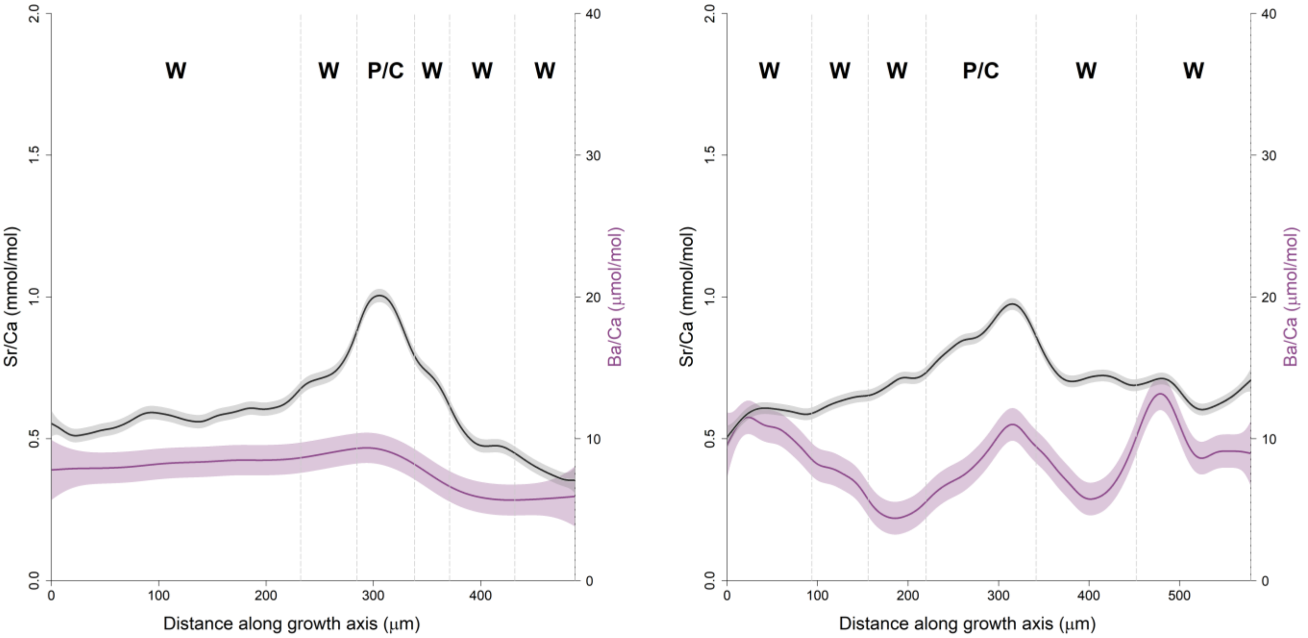

Figure 2.6. Sr/Ca and Ba/Ca GAMs for two bull trout with freshwater movements outside of the White River. Grey dashed lines indicate the cluster breaks in the transect identified by the chronological cluster model. Habitat assignments are indicated at the top of each cluster where W indicates the White River and P/C indicates the Puyallup and Carbon rivers.

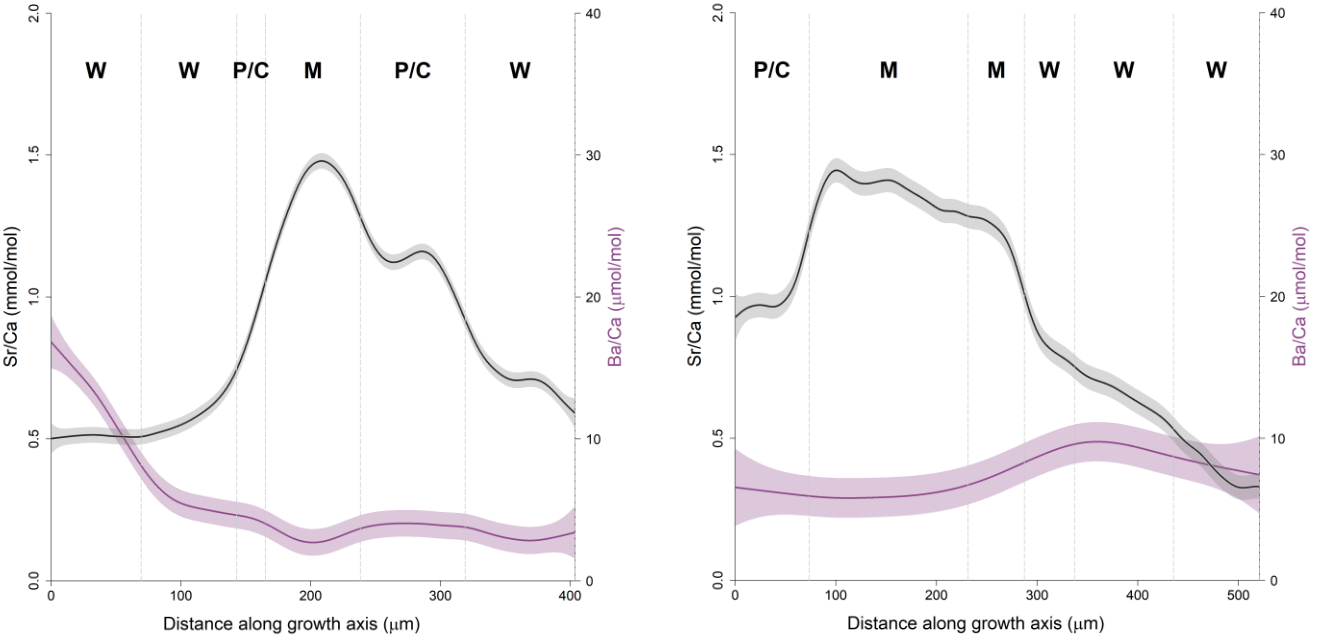

Figure 2.7. Sr/Ca and Ba/Ca GAMs for two bull trout with anadromous migrations. Grey dashed lines indicate the cluster breaks in the transect identified by the chronological cluster model. Habitat assignments are indicated at the top of each cluster where W indicates the White River, P/C indicates the Puyallup and Carbon rivers, and M indicates the marine environment.

2.7 Tables

Table 2.1. Baseline values from digested sculpin fin rays. Means include 3 sculpins per site. Estuary Sr/Ca and Ba/Ca ratio means and standard deviations were estimated using reduced major axis linear regression equations and otolith microchemistry data from sculpins collected in Commencement Bay.

Site	Sub-basin	Site type	Sr/Ca mean	Sr/Ca SD	Ba/Ca mean	Ba/Ca SD
Fryingpan Creek	White	Tributary	0.70	0.12	19.49	4.21
Klickitat Creek	White	Tributary	0.49	0.02	11.76	0.33
Upper White River	White	Mainstem	0.65	0.02	11.53	0.87
Middle White River	White	Mainstem	0.59	0.04	19.91	3.48
Lower White River	White	Mainstem	0.62	0.04	12.41	1.68
Lower Puyallup River	Puyallup	Mainstem	0.79	0.18	21.82	3.67
Middle Puyallup River	Puyallup	Mainstem	1.14	0.08	50.5	9.48
Lower Carbon River	Carbon	Mainstem	1.61	0.14	65.39	20.96
South Prairie Creek	Carbon	Tributary	1.37	0.11	55.03	7.33
Estuary	Estuary	Estuary	2.47	0.31	14.85	10.26

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Supplemental Information for Chapter 1

Supplemental Table 1.1. Summary statistics of digested sculpin fin ray chemistries for all sampling locations. NAs indicate samples below the detection limit.

Sr/Ca (mmol/mol)

Site	Sub-basin	Site type	Fin ray mean	Fin ray SD	Otolith mean	Otolith SD	Water
Fryingpan Creek	White	Tributary	0.70	0.12	0.96	0.15	1.53
Klickitat Creek	White	Tributary	0.49	0.02	0.64	0.03	1.26
Upper White River	White	Mainstem	0.65	0.02	0.92	0.07	2.26
Middle White River	White	Mainstem	0.59	0.04	0.88	0.08	1.93
Lower White River	White	Mainstem	0.62	0.04	0.82	0.05	2.14
Lower Puyallup River	Puyallup	Mainstem	0.79	0.18	1.16	0.25	2.6
Middle Puyallup River	Puyallup	Mainstem	1.14	0.08	1.61	0.08	3.28
Lower Carbon River	Carbon	Mainstem	1.61	0.14	1.57	0.25	4.37
South Prairie Creek	Carbon	Tributary	1.37	0.11	1.7	0.03	4.3

^{87}Sr/^{86}Sr ratios

Site	Sub-basin	Site type	Fin ray mean	Fin ray SD	Otolith mean	Otolith SD	Water
Fryingpan Creek	White	Tributary	0.70392	5.6e-05	0.70392	4.7e-05	0.70394
Klickitat Creek	White	Tributary	0.70392	4.2e-05	0.70392	1.1e-05	0.70392
Upper White River	White	Mainstem	0.70405	0.0002	0.70391	1.1e-05	0.70395
Middle White River	White	Mainstem	0.70393	4.0e-05	0.70396	2.5e-05	0.70404
Lower White River	White	Mainstem	0.7040	0.0002	0.70412	2.9e-05	0.70408
Lower Puyallup River	Puyallup	Mainstem	0.70501	6.1e-05	0.70509	0.0001	0.7048
Middle Puyallup River	Puyallup	Mainstem	0.70545	3.2e-05	0.70544	4e-05	0.70518
Lower Carbon River	Carbon	Mainstem	0.70618	0.00011	0.70604	3.5e-05	0.70621
South Prairie Creek	Carbon	Tributary	0.7059	8.7e-05	0.70583	4.7e-05	0.70592

Ba/Ca (μmol/mol)

Site	Sub-basin	Site type	Fin ray mean	Fin ray SD	Otolith mean	Otolith SD	Water
Fryingpan Creek	White	Tributary	19.49	4.21	7.42	1.78	118.56
Klickitat Creek	White	Tributary	11.76	0.33	3.99	0.16	90.95
Upper White River	White	Mainstem	11.53	0.87	4.31	0.19	181.29
Middle White River	White	Mainstem	19.91	3.48	7.05	1.30	179.51
Lower White River	White	Mainstem	12.41	1.68	4.12	1.16	199.71
Lower Puyallup River	Puyallup	Mainstem	21.82	3.67	6.91	1.45	170.24
Middle Puyallup River	Puyallup	Mainstem	50.5	9.48	17.55	3.29	616.4
Lower Carbon River	Carbon	Mainstem	65.39	20.96	16.35	5.34	572.98
South Prairie Creek	Carbon	Tributary	55.03	7.33	17.85	1.71	607.96

Mg/Ca (mmol/mol)

Site	Sub-basin	Site type	Fin ray mean	Fin ray SD	Otolith mean	Otolith SD	Water
Fryingpan Creek	White	Tributary	26.15	2.27	0.07	0.01	106.73
Klickitat Creek	White	Tributary	23.60	0.82	0.11	0.05	84.66
Upper White River	White	Mainstem	23.0	0.47	0.07	0.01	301.59
Middle White River	White	Mainstem	23.82	1.95	0.04	0.003	355.05
Lower White River	White	Mainstem	26.17	1.86	NA	NA	415.3
Lower Puyallup River	Puyallup	Mainstem	24.43	0.33	0.05	0.009	547.53
Middle Puyallup River	Puyallup	Mainstem	24.27	1.29	0.06	0.006	640.58
Lower Carbon River	Carbon	Mainstem	30.9	7.22	0.09	0.03	519.03
South Prairie Creek	Carbon	Tributary	24.73	1.87	0.04	0.002	526.89

Mn/Ca (mmol/mol)

Site	Sub-basin	Site type	Fin ray mean	Fin ray SD	Otolith mean	Otolith SD	Water
Fryingpan Creek	White	Tributary	0.058	0.0125	0.002	0.001	118.56
Klickitat Creek	White	Tributary	0.074	0.013	0.002	0.0006	90.95
Upper White River	White	Mainstem	0.116	0.024	0.004	0.0004	181.29
Middle White River	White	Mainstem	0.113	0.038	0.003	0.001	179.51
Lower White River	White	Mainstem	0.226	0.086	0.02	0.006	199.71
Lower Puyallup River	Puyallup	Mainstem	0.253	0.024	0.01	0.002	170.24
Middle Puyallup River	Puyallup	Mainstem	0.41	0.105	0.02	0.005	616.4
Lower Carbon River	Carbon	Mainstem	0.272	0.057	0.01	0.007	572.98
South Prairie Creek	Carbon	Tributary	0.265	0.022	0.01	0.002	607.96