Use of otolith microchemistry to study straying and metapopulation dynamics in Norton Sound salmon populations: Pilot study to determine utility

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Abstract

To test the utility of otolith chemical composition as a tool to determine natal stream origin of salmon for the purposes of determining straying and population connectivity, we examined water chemistry and otoliths of juvenile and adult salmon from three watersheds (five rivers) in the Norton Sound region of Alaska. We collected water samples and otoliths of juvenile chum salmon (*Oncorhynchus keta*) and juvenile coho salmon (*O. kisutch*) from the Nome, Niukluk, Fish, North, and Chiroskey rivers within the Nome, Fish, and Unalakleet watersheds. We used laser ablation ICP-MS to quantify element-to-calcium ratios for Mg, Mn, Zn, Sr, and Ba and used multi-collector laser ablation ICP-MS to determine ^{87}Sr:^{86}Sr ratios in otolith regions corresponding to the freshwater residence. Significant differences in both water and otolith elemental composition existed and suggested that otolith composition could be used to discriminate natal origins in both coho and chum salmon but only when ^{87}Sr:^{86}Sr ratios included in the discriminant function analyses. Without ^{87}Sr:^{86}Sr ratios, it was difficult to discriminate among watersheds and rivers. While, further work is needed to evaluate the temporal stability of otolith signals, it does appear feasible to determine natal origins in coho salmon at the regional scale in Norton Sound.

Introduction

Pacific salmon are generally characterized by geographically distinct populations in partial genetic isolation. This structure is a result of the balance between genetic drift within populations and gene flow among populations. Homing or philopatry leads to local breeding populations that are demographically and genetically isolated and adapted to local conditions (Hendry et al. 2004, Utter et al. 2009). Straying or dispersal decreases variance among local breeding populations (Barton and Whitlock 1997) and quantifying the dispersal capabilities and patterns of animals is a critical step in examining both genetic population structure and metapopulation dynamics (Weins 1996; Ims and Yoccos 1997). Although understanding homing and straying or connectivity among populations is an important step in describing the population structure and metapopulation dynamics of populations, little information exists concerning connectivity of salmon populations across much of their range.

There are two general methods for estimating homing and straying in salmon (Slatkin 1987; Hendry et al. 2004). "Indirect" methods rely on accurately identifying genetic differentiation among populations of interest and, then, converting this differentiation to an estimate of gene flow (Slatkin 1987; Hendry et al. 2004). The "direct" method usually involves tagging juveniles in their natal habitat and surveying breeding adults for the presence of tags. Application of direct methods can be constrained by the small sizes of juvenile salmon, which makes it difficult or impossible to apply tags, logistic difficulties of tagging sufficient numbers of juveniles, or tag loss and inability to identify tags in adults. As an alternative, natural tags induced by the environment, such as chemical signatures of otoliths can be used to identify natal stream of origin and, hence, be used to estimate straying or homing (Kennedy et al. 2000; Wells et al. 2003; Brothers and Thresher 2004). Because every individual of the population carries natural tags, use of these tags may resolve the difficulties associated with tagging small individuals and the logistics of tagging sufficient numbers of juveniles to ensure adequate recaptures.

Analysis of otolith chemical composition has been used broadly to examine connectivity among populations of marine fishes (e.g., Thresher 1999; Rooker et al. 2003; Miller et al. 2005). Because otoliths are conservative and metabolically inert, any

elements or compounds incorporated in the calcium carbonate matrix of the otolith are permanently retained, thus acting as an environmental monitor and archive (Campana 1999; Thresher 1999). The composition of elements within the otolith is generally determined by the composition of the ambient water (Campana 1999; Wells et al. 2003; Elsdon and Gillanders 2003) and multi-elemental analyses of otoliths have been used to identify natal origins, habitat associations, and to identify stocks in a variety of fish marine fish species (e.g., Campana et al. 1994; Thorrold et al. 2001; Ruttenberg and Warner 2006). Thorrold et al. (2001) used otolith microchemistry to examine natal homing in weakfish (*Cynoscion regalis*) among estuaries on the east coast of the United States. Because they could identify natal estuary based on otolith microchemistry, they were able to determine the proportion of adults spawning within their natal stream (homing) versus those that were straying from other estuaries. They found that 60 to 81 % of spawning weakfish were homing to spawn in their natal estuary. Using such an approach would be very useful in quantifying stray rates and assessing connectivity among anadromous and freshwater fishes.

While the use of otolith chemical composition as a tool to assess connectivity in freshwater fishes has not been reported as extensively as it has for marine populations, it has been used in a variety of contexts. Strontium isotopes (^{87}Sr:^{86}Sr) have been used to examine movement among tributaries and natal origins (stream of origin) in salmonids (Kennedy et al. 1997, 2000; Ingram and Weber 1999; Barnett-Johnson et al. 2005). For freshwater and anadromous fishes, multi-elemental signatures have been used to determine natal stream of origin (Sohn et al. 2005; Veinott and Porter 2005), examine connectivity and movement among tributaries or areas of lakes (Brazner et al. 2004; Clarke et al. 2007; Marklevitz et al. 2011), and determine the origin of fish stocked or transferred to lakes and streams (Coghlan et al. 2007; Gibson-Reinemer et al. 2009). Milton and Chenery (2001) used otolith composition to examine population structure of the anadromous shad hilsa (*Tenulosa ilisha*). Using eight elements to compare otolith microchemistry among spawning locations, they were able to distinguish among locations but found that movement among locations (straying) was so high that three distinct spawning populations within the Bay of Bengal could be treated as a single breeding population or stock. Wells et al. (2003) quantified molar ratios of magnesium,

manganese, strontium, and barium to calcium in the first summer growth region of westslope cutthroat trout (*O. clarkii*) from the Coeur d'Alene River in Idaho. Using three elements (Mn, Sr, and Ba) individual fish could be classified to streams with an accuracy of 82%. These studies indicate that otolith composition should provide a powerful tool for assessing connectivity among populations of salmon.

Several studies have demonstrated that elemental or isotope composition of otoliths can differ among natal streams used by anadromous salmonids. For example, Veinott and Porter (2005) used four elements to discriminate natal stream of origin for Atlantic salmon (*Salmo salar*) from three streams in Newfoundland, Canada. Similarly, use of ^{87}Sr:^{86}Sr to determine natal stream has been demonstrated for Chinook salmon (*Oncorhynchus tshawytscha*) in the Sacramento – San Joaquin River basin (Ingram and Weber 1999; Barnett-Johnson et al. 2008) and in the Columbia River basin (Barnett-Johnson et al. 2010). Sohn et al. (2005) used eight elements to discriminate chum salmon (*O. keta*) among three rivers in Korea and suggested that multi-elemental analyses of otolith chemical composition could be used to identify natal origins of chum salmon captured at sea.

To determine the utility of using otolith chemical composition to determine natal stream-of-origin among rivers at a regional scale, we examined variability in ambient water chemistry among watersheds and otolith chemical composition in juvenile chum and coho salmon (*O. kisutch*) collected from five rivers within three watersheds in the Norton Sound region of western Alaska. Chum and coho salmon were selected to reflect differences in reliance on freshwater as juveniles. Chum salmon migrate to sea immediately following emergence (Salo 1992), whereas, coho salmon rear in freshwater for up to two years (Sandercock 1992). We hypothesized that wild chum salmon would not reside in natal rivers long enough to deposit sufficient otolith material to allow discrimination among rivers. For three rivers (within two watersheds), we also classified natal stream-of-origin of adult chum and coho salmon, to determine if straying could be estimated based on otolith composition. In addition, we assessed the relative importance of including ^{87}Sr:^{86}Sr isotope ratios with elemental composition in improving discrimination power.

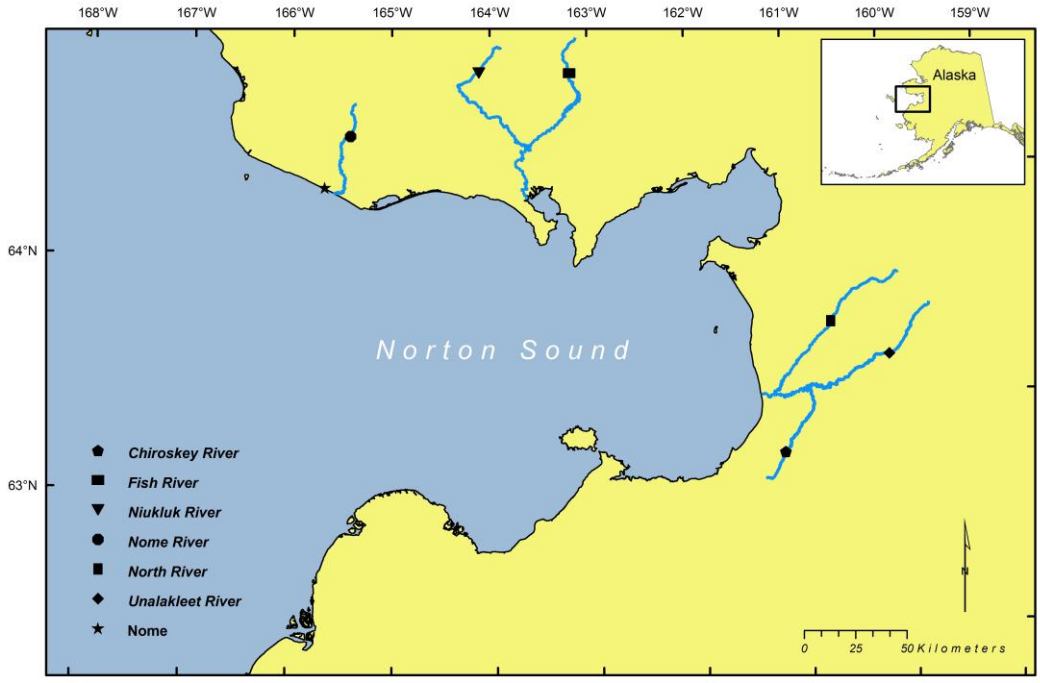

Figure 1. Study rivers, Norton Sound Region, Alaska.

Methods

Study Area

Juvenile chum and coho salmon were collected from watersheds draining to Norton Sound in Western Alaska (Figure 1). Nome River drains a region that contains Precambrian crystalline rocks that were formed between 570 million and 3.6 billion years before present (Figure 1). The Unalakleet River, however, is dominated by Cretaceous rocks formed 65 to 136 million years before present and the Fish River is a mixture of young and old Precambrian rocks (570 million and 3.6 billion years old) and quaternary deposits that were deposited in the last 2 million years. Given this heterogeneity in rock ages and types, we hypothesized that it was likely that the elemental composition of stream water among sites might lead to different elemental and Sr-isotope "signatures" in the freshwater growth region of otoliths.

Juvenile salmon were captured using baited minnow traps and pole seines from multiple sites within each river with a target sample size of 25 individual fish of each species. Fish were frozen at the end of each day and otoliths were removed from frozen fish within one month of capture. Adult chum and coho salmon were collected from subsistence fisheries and carcasses found on the river margins. Otoliths were either removed shortly after capture in gill nets, immediately from carcasses, or heads were frozen for up to one month before removal. All otoliths were stored dry in plastic vials for up to 6 months before preparation and analysis.

Water Chemistry

Water samples were filtered through 0.45-lm membrane filters, then acidified to less than pH 2 with quartz-distilled nitric acid. Samples were diluted from 1 to 6 mL with 1% quartz-distilled nitric acid. We analyzed concentrations of Ca, Mg, Mn, Sr, and Ba in water samples with a Varian Liberty 150 inductively coupled plasma optical emission spectrometer. Concentrations were calculated from the emission intensities and the intensities of standard solutions. Accuracy of the method was verified by running a National Institute of Standards and Technology (NIST) freshwater certified reference material (NIST 1643c).

Otolith Analysis

Otolith preparation followed the methods described by Zimmerman and Reeves (2002) and Donohoe and Zimmerman (2010). One sagittal otolith from each fish was mounted sulcus side down with Crystal Bond 509 on a microscope cover slip attached on one edge to a standard microscope slide. The otolith was ground in the sagittal plane to the level of the nucleus with 2000-grit sandpaper. The mounting medium was heated and the otolith turned sulcus side up. The otolith was then ground with 2000-grit sandpaper in the sagittal plane to the level of the primordia and polished with a slurry of 0.05 µm alumina paste. The cover slip was then cut with a scribe so that several prepared otoliths could be mounted on a single petrographic slide for analysis (Donohoe and Zimmerman 2010).

All otolith analyses were conducted at the Keck Collaboratory for Plasma Mass Spectrometry at Oregon State University. Elemental analyses were conducted using a Thermal Elemental PQ Excell quadropole inductively coupled plasma mass spectrometer

connected to a New Wave DUV 193 nm ArF laser. Analyses were conducted with a 30-µm-diameter spot size and a pulse rate of 15 Hz. All samples were taken from a transect beginning in the core of the otolith and terminating at the edge. Background levels were measured for 30 s prior to otolith ablation and subtracted from those during otolith ablation. Count rates for each analyte isotope (^{24}Mg, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba) were normalized to ^{43}Ca to account for differences in instrument sensitivity and ablation rate (Campana et al. 1997). Each otolith analysis was paired with an analytical transect on a polished sample of NIST 612 glass standard.

Otolith ^{87}Sr:^{86}Sr data were collected using a NuPlasma multi-collector laser ablation-inductively coupled plasma mass spectrometry (MC-LA-ICPMS) and the New Wave DUV193 excimer laser following the methods of Miller and Kent (2009). We followed the general method of Woodhead et al. (2005) to correct for potential Kr and Rb interferences and monitor for Ca argide/dimer formation. Background interferences by Kr isotopes and contributions from any other gas species present within the plasma and sweep gas supplies were corrected by measuring an on-peak baseline prior to ablation of otoliths. Measured backgrounds were subtracted from measured intensities during otolith ablation. Mass biases were corrected by reference to a ^{87}Sr:^{86}Sr ratio of 0.1194 and isobaric interference of ^{87}Rb on ^{87}Sr was corrected for by measuring beam intensity for ^{85}Rb and calculating the contribution of ^{87}Rb. A deep-sea gastropod collected from the Gulf of Mexico was used as an in-house marine carbonate standard.

Data Analysis

To assess the degree of variation in water chemistry among rivers, we analyzed elemental concentrations using one-way analysis of variance (ANOVA). Tukey multiple comparisons were used to assess similarity among rivers and watersheds. Elemental concentrations were natural log transformed to normalize skewness in their distributions prior to statistical analysis.

The chemical composition of otoliths was analyzed using both univariate and multivariate methods. All otolith analytes were natural log transformed to normalize skewness in their distributions prior to statistical analysis. To determine the variability of individual analytes among watersheds, we analyzed element:Ca ratios and ^{87}Sr:^{86}Sr

isotope ratios using one-way ANOVA and Tukey multiple comparisons to assess similarity among watersheds and among rivers.

We used linear discriminant function analysis (DFA) to determine if multi-elemental and Sr isotope signatures could be used to classify fish to watershed or river of origin. Discriminant function models were constructed for both chum and coho salmon at the river and watershed scales. For each species at the watershed level, a discriminant function was constructed using (1) all elemental and isotope data, (2) one was constructed using only the elemental data, and (3) one was constructed using only Sr:Ca ratios and ^{87}Sr:^{86}Sr ratios. Discrimination powers of models were compared using Wilks' Lambda and a cross-validated, leave-one-out approach to classify each fish to their location of origin (Wells et al. 2000; Gibson-Reinemer et al. 2009). The classification accuracy of the discriminant functions were compared to that expected by chance alone under the assumption that random chance will result in correct classifications with a percentage inversely proportional to the number of groups classified (White and Ruttenberg 2007). At the river scale, the discriminant function analysis based on all elements plus ^{87}Sr:^{86}Sr ratios was used to assess classification accuracy. Adult chum and coho salmon captured in the Nome, Niukluk, and Fish rivers were classified based on the discriminant function models constructed using the full data set (i.e, all elemental and isotope data).

Results

Water Chemistry

Concentrations of Mg, Sr, and Ba in water varied significantly among and within watersheds (Figure 2). There was no significant variation in Mn among watersheds (Figure 2b; $F_{4,23} = 1.37$, N.S.). Mean Mg varied significantly among watersheds ($F_{4,23} = 10.44$, $P < 0.001$) and multiple range tests indicated a regional pattern with significant differences between the Unalakleet River tributaries (North and Chiroskey rivers) and the Nome and Niukluk (including Fish River) watersheds (Figure 2a). Similarly, mean Sr varied among watersheds ($F_{4,23} = 15.99$, $P < 0.001$). Based on multiple range tests, mean Sr in the Nome River and the adjacent Niukluk River were not significantly different and both grouped with the distant Chiroskey River (Figure 2c). Within watersheds, mean Sr was not significantly different between the Fish and Niukluk rivers but did differ

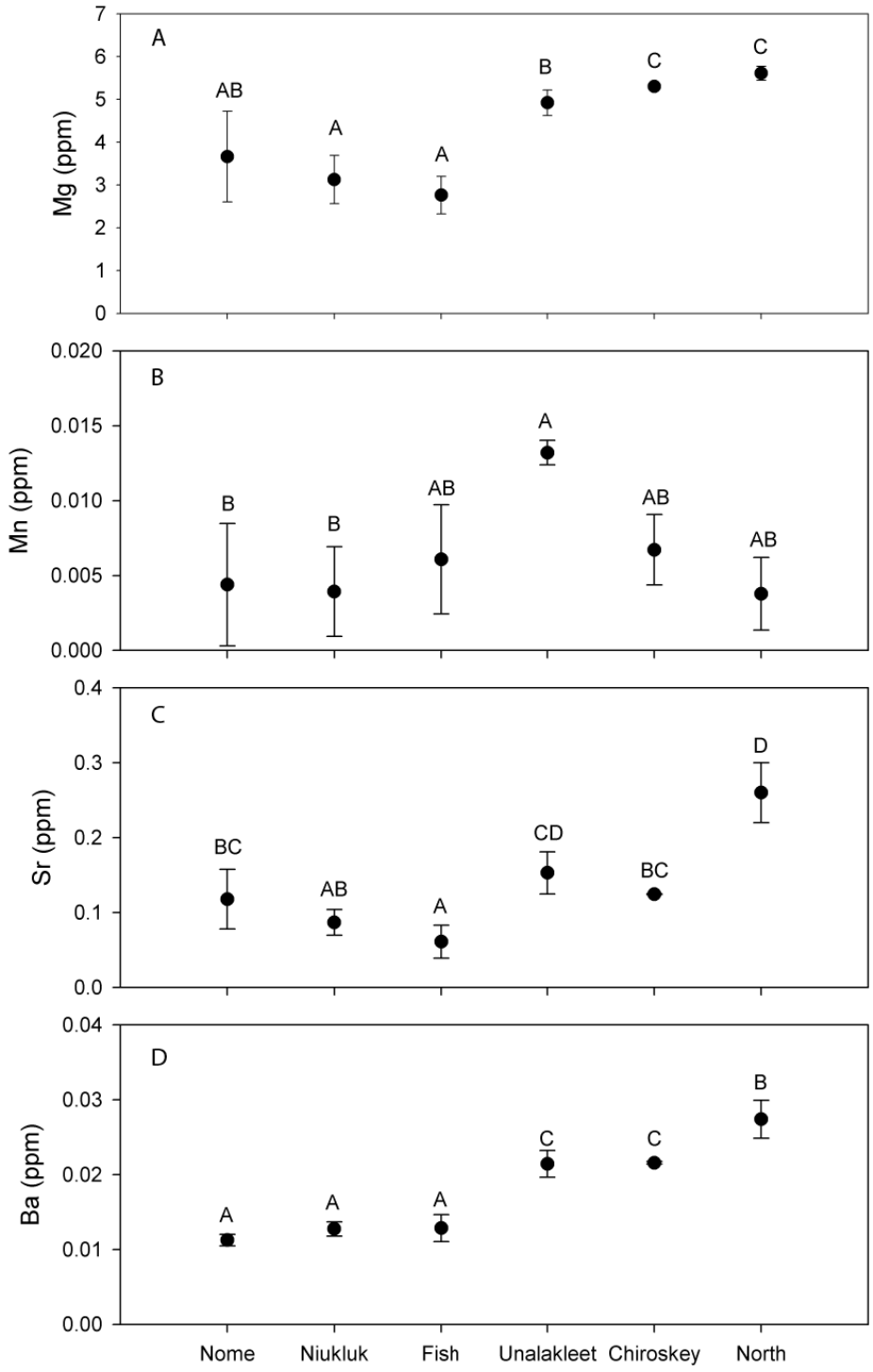

Figure 2. Mean and 95% confidence intervals of water constituents. Letters indicate similarity based on multiple range tests.

significantly between the North and Chiroskey rivers within the Unalakleet River watershed (Figure 2c). Similarly, mean Ba varied among watersheds ($F_{4,23} = 92.7$, $P < 0.001$) and multiple range tests indicated a regional grouping of watersheds with the Nome, Niukluk, and Fish rivers grouping separate from the North and Chiroskey Rivers (Figure 2d).

Composition of Juvenile Otoliths

Otolith composition differed between juvenile chum and coho salmon. For chum salmon, only Sr:Ca and $^{87}Sr:^{86}Sr$ varied significantly among rivers, whereas in coho salmon, Mg:Ca, Zn:Ca, Sr:Ca, Ba:Ca, and $^{87}Sr:^{86}Sr$ varied significantly among rivers (Table 1; Figure 3). Similar to the ambient water chemistry, multiple range tests indicated there were regional patterns and differences among rivers and among watersheds. For example, $^{87}Sr:^{86}Sr$ ratios in both chum and coho salmon were more similar among adjacent sites with the Nome, Niukluk, and Fish rivers forming one group that differed significantly from the North and Chiroskey rivers (Figure 3a). A similar pattern was evident for Sr:Ca in coho salmon but not in chum salmon (Figure 3e).

When constructing discriminant functions for analysis among watersheds, the first two discriminant functions described 100% of the variation for both coho and chum salmon for all combinations of analytes (i.e., (1) all element:Ca ratios plus $^{87}Sr:^{86}Sr$ ratios, (2) all element:Ca ratios, and (3) only Sr:Ca ratios and $^{87}Sr:^{86}Sr$ ratios). For both chum and coho salmon, the full model including all element:Ca ratios and $^{87}Sr:^{86}Sr$ ratios provided the best discrimination among watersheds as indicated by the lowest Wilks' lambda values and overall classification rates (Table 2). For both chum and coho salmon, the discriminant function developed using only Sr:Ca ratios and $^{87}Sr:^{86}Sr$ ratios was only slightly less successful discriminating among watersheds (Table 2) and the discriminant functions constructed using only element:Ca ratios provided these least ability to discriminate among watersheds (Table 2) as indicated by Wilks' lambda and overall classification rates. For coho salmon, the first discriminant function clearly separated Unalakleet watershed coho salmon from those collected in the Fish and Nome rivers (Figure 4). Patterns for chum salmon were similar but less pronounced (Figure 5).

Table 1. Analysis of variance results to test the effect of collection location (river) on otolith elemental composition.

	Coho Salmon			Chum Salmon		
	df	*F*	*p*	*df*	*F*	*p*
Mg:Ca	4, 114	4.507	0.002	4, 114	1.265	NS
Mn:Ca	4, 114	1.905	NS	4, 114	0.785	NS
Zn:Ca	4, 114	3.893	0.005	4, 114	0.711	NS
Sr:Ca	4, 114	132.8	<0.0001	4, 114	17.67	<0.0001
Ba:Ca	4, 114	3.218	0.015	4, 114	2.087	NS
^{87}Sr:^{86}Sr	4, 114	257.8	<0.0001	4, 114	81.47	<0.0001

Table 2. Overall reclassification rates and Wilks' lambda (λ) for discriminant function analyses for chum and coho salmon examined at the watershed level (Nome, Fish, and Unalakleet).

	Chum Salmon		Coho Salmon	
	Classification Rate	Wilks' λ	Classification Rate	Wilks' λ
All elements and Sr/Sr	0.81	0.2008	0.93	0.0510
Elements only	0.65	0.5588	0.83	0.1996
Sr:Ca and Sr/Sr only	0.82	0.2309	0.92	0.0640

At the among river scale, the first two discriminant functions explained 98.9% and 95.9% of the variation for coho salmon and chum salmon, respectively (Figure 6 and 7). The discriminant functions constructed using all element and isotope data had overall jack-knifed classification accuracy of 80% for coho salmon and 68% for chum salmon. Wilks' lamba was 0.0317 for coho salmon and 0.1322 for chum salmon. For coho salmon, misclassifications were typically with the nearest neighbors (Table 3). Nome, Niukluk, and Fish river coho salmon were not misclassified as North or Chiroskey River coho salmon and vice versa (Table 3). This was not the case with chum salmon (Table 4). Nome River chum salmon, for example, were misclassified as North River and Chiroskey River fish were misclassified as Nome River fish (Table 4).

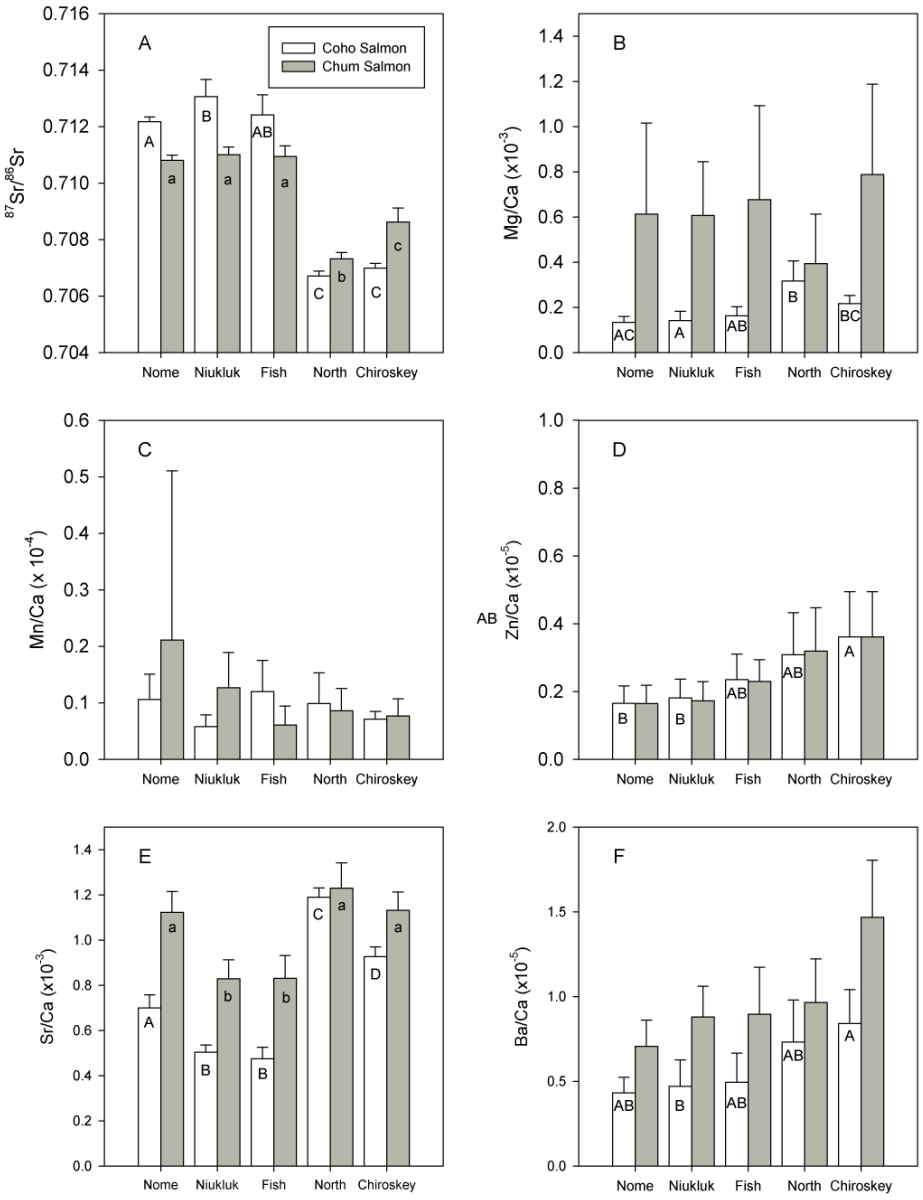

Figure 3. Mean and 95% confidence intervals of analytes measured in the freshwater growth region of juvenile coho and chum salmon otoliths. Capital letters indicate similarity among coho salmon and small letters indicate similarity among chum salmon based on multiple range tests.

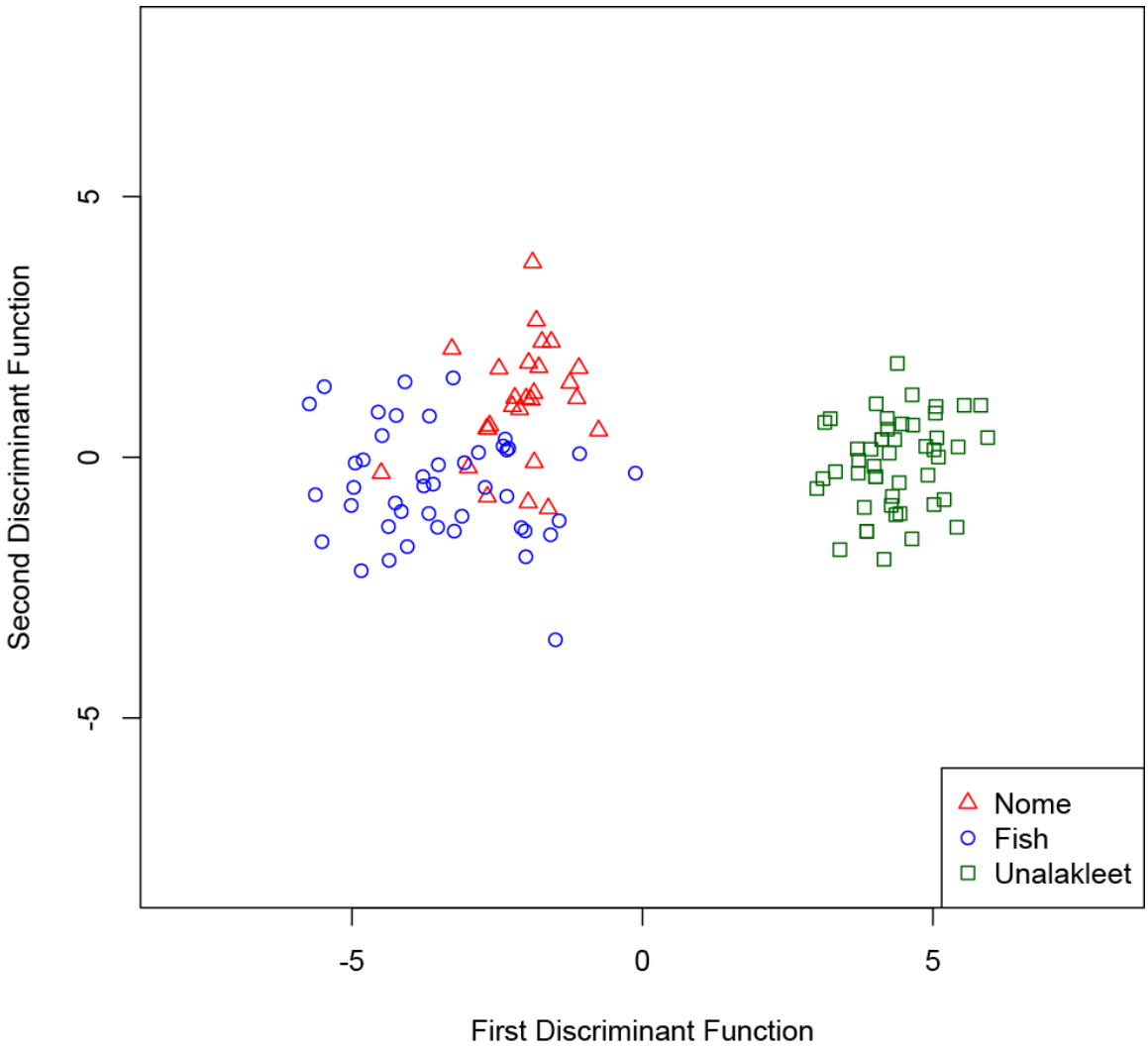

Figure 4. Bivariate plots of the discriminant function scores from the otolith model constructed from all elemental and isotope data at the watershed scale for juvenile coho salmon.

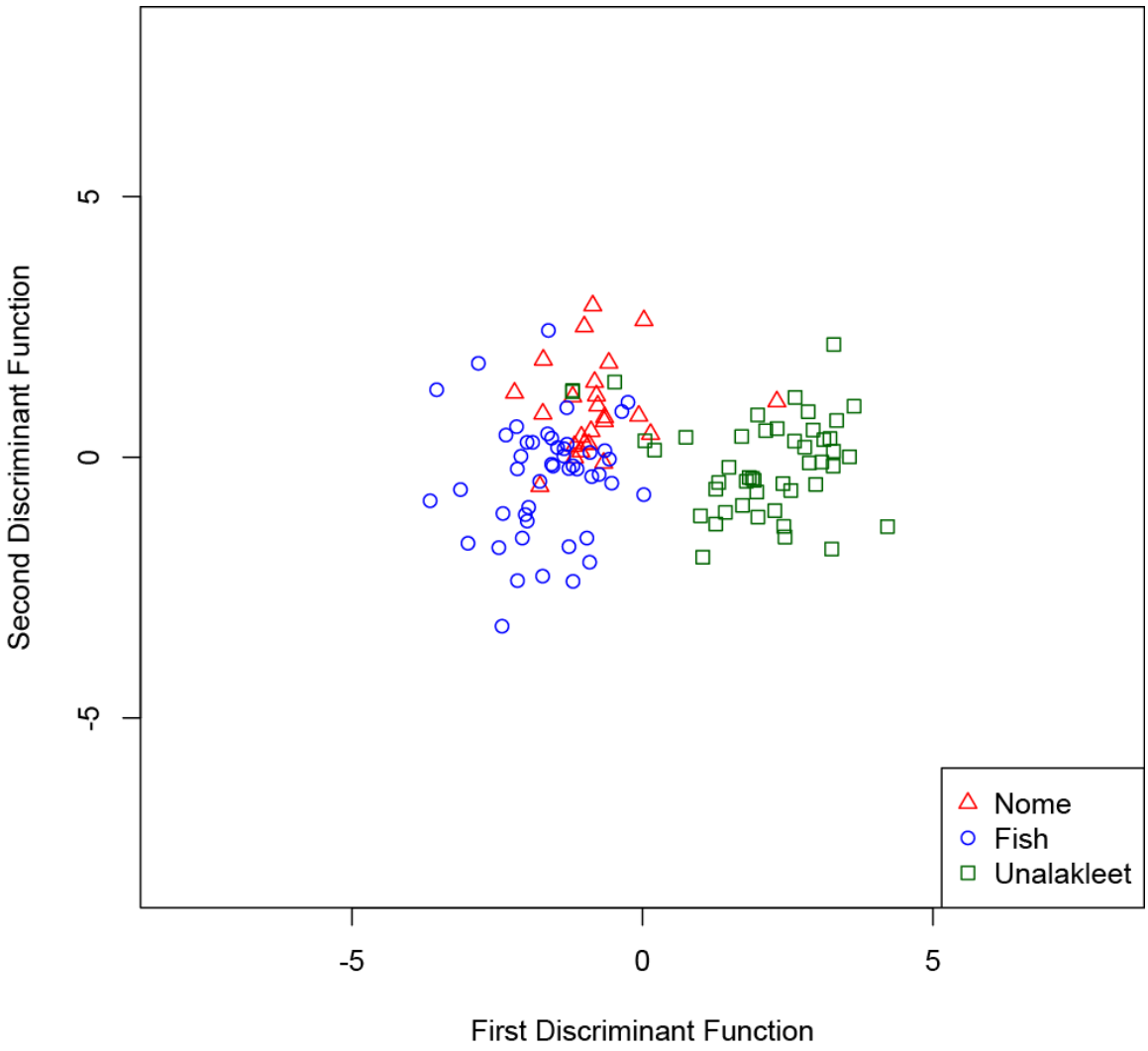

Figure 5. Bivariate plots of the discriminant function scores from the otolith model constructed from all elemental and isotope data at the watershed scale for juvenile chum salmon.

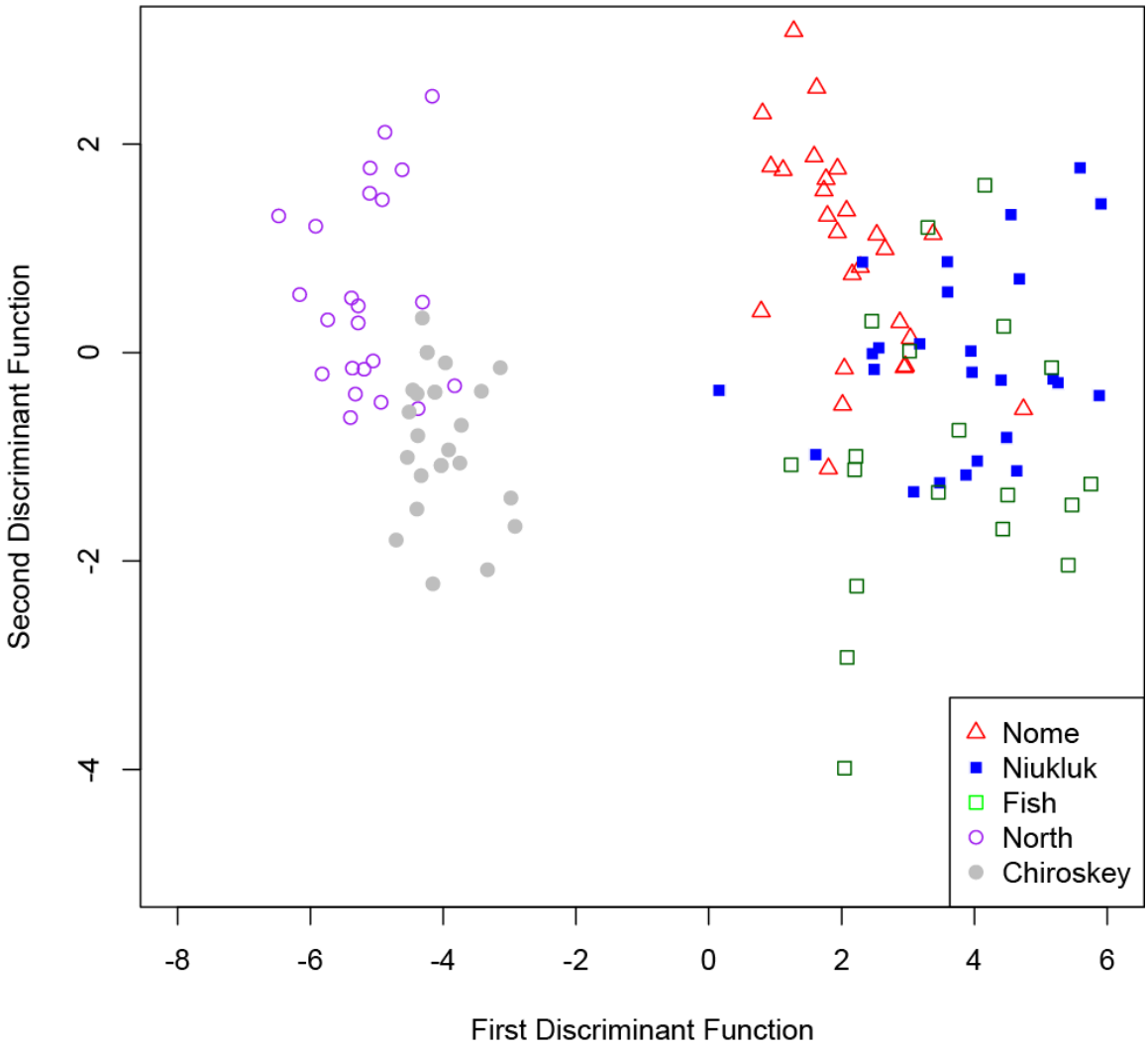

Figure 6. Bivariate plots of the discriminant function scores from the otolith model constructed from all elemental and isotope data at the river scale for juvenile coho salmon.

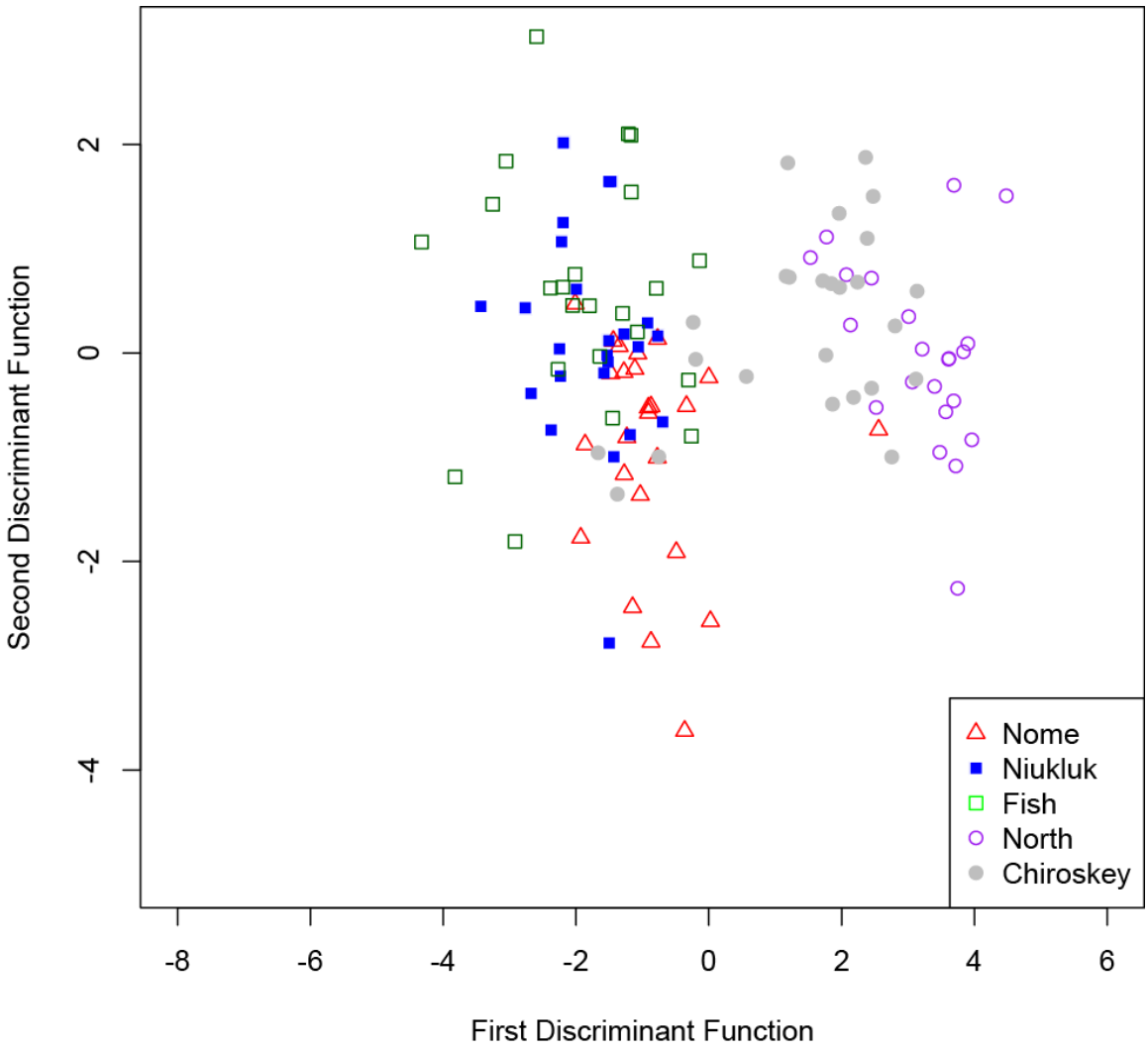

Figure 7. Bivariate plots of the discriminant function scores from the otolith model constructed from all elemental and isotope data at the river scale for juvenile chum salmon.

Table 3. Classification Matrix for coho salmon overall = 0.80 full set

	Actual Origin				
Predicted	Nome	Niukluk	Fish	North	Chiroskey
Nome	**0.81**	0.20	0.16	0	0
Niukluk	0.07	**0.68**	0.26	0	0
Fish	0.11	0.12	**0.58**	0	0
North	0	0	0	**0.87**	0
Chiroskey	0	0	0	0.13	**1.0**

Table 4. Classification matrix for chum salmon overall = 0.68 Full set of data

	Actual Origin				
Predicted	Nome	Niukluk	Fish	North	Chiroskey
Nome	**0.72**	0.17	0.17	0	0.16
Niukluk	0.08	**0.58**	0.22	0	0
Fish	0.16	0.25	**0.57**	0	0
North	0.04	0	0	**0.91**	0.20
Chiroskey	0	0	0.04	0.09	**0.64**

Classification of Adult Otoliths

A total of 23, 24, and 26 adult coho salmon were collected from subsistence fisheries in the Nome, Niukluk, and Fish rivers, respectively and a total of 31, 25, and 24 adult chum salmon were collected from subsistence fisheries in the Nome, Niukluk, and Fish rivers, respectively. At the watershed level, 22% of adult coho salmon collected in the Nome River were classified as originating outside the Nome River watershed and 32% of adult coho salmon captured in the Fish River watershed were classified as originating from outside the Fish River watershed (Table 5). When analyzed at the river level, 22% of adult coho salmon captured in the Nome River were classified as originating outside the Nome River, 45% of adult coho salmon captured in the Niukluk River were classified as originating outside the Niukluk River, and 61% of adult coho salmon captured in the Fish River were classified as originating outside the Fish River (Table 6). At the watershed level, 53% of adult chum salmon collected in the Nome River were classified as originating outside the Nome River watershed and 30% of adult chum salmon captured in the Fish River watershed were classified as originating from outside the Fish River watershed (Table 7). When analyzed at the river level, 52% of adult chum salmon captured in the Nome River were classified as originating outside the Nome River, 44% of adult chum salmon captured in the Niukluk River were classified as originating outside the Niukluk River, and 33% of adult chum salmon captured in the Fish River were classified as originating outside the Fish River (Table 8).

Table 5. Classification matrix for adult coho salmon collected from the Nome and Fish watersheds.

	Predicted Origin		
	Nome	Fish	Unalakleet
Nome	**18**	4	1
Fish	15	**34**	1

Table 6. Classification matrix for adult coho salmon collected from the Nome, Niukluk, and Fish rivers.

	Predicted Origin				
	Nome	Niukluk	Fish	North	Chiroskey
Nome	**18**	4	0	0	1
Niukluk	8	**13**	3	0	0
Fish	8	8	**10**	0	0

Table 7. Classification matrix for adult chum salmon collected from the Nome and Fish watersheds.

	Predicted Origin		
	Nome	Fish	Unalakleet
Nome	**14**	7	10
Fish	15	**34**	0

Table 8. Classification matrix for adult coho salmon collected from the Nome, Niukluk, and Fish rivers.

	Predicted Origin				
	Nome	Niukluk	Fish	North	Chiroskey
Nome	**15**	1	4	6	5
Niukluk	10	**14**	1	0	0
Fish	7	16	**0**	0	1

Discussion

Chemical composition of otoliths provides an opportunity to discriminate natal river or watershed origins (or provenance) of Pacific salmon but life history differences among species may limit the utility of this tool. We were able to distinguish among natal rivers for coho salmon with relatively high confidence because juvenile coho salmon remain in natal rivers for up to two winters before migrating to sea. This time spent rearing in the natal river allows for deposition of sufficient otolith material to provide an unambiguous freshwater region to sample in adult otoliths and allows for sufficient growth beyond any maternal influences. Chum salmon, on the other hand, migrate immediately following emergence from the gravel and, therefore, do not deposit sufficient otolith growth within freshwater that is free from maternal signals and reflective of the natal river or watershed.

Because chum salmon migrate from freshwater immediately following emergence, there is very little otolith growth occurring in their natal river. Further, confounding elemental signals are maternal signals that reflect the marine environment were yolk precursors were deposited (Kalish 1990, Volk et al. 2000, Zimmerman and Reeves 2002). Arai and Hirata (2006) demonstrated differences in Mg, Zn, Sr, and Ba between freshwater and seawater growth regions of chum salmon. Examination of a "typical" profile of Sr as presented by Arai and Hirata (2006) suggests that there were maternal influences throughout the time period identified as freshwater growth (i.e., elevated Sr at the start of the transect and a gradual decline until the fish moved to seawater). Sohn et al. (2005) examined otolith elemental composition of chum salmon juveniles collected from three hatcheries in Korea and found significant differences among sites. Using a discriminant function approach, Sohn et al. (2005) argued that otolith composition could, therefore, be used to identify stocks of chum salmon captured in the ocean. The juvenile salmon examined by Sohn et al. (2005) ranged in mean length from 43 – 82 mm suggesting these fish were held in the hatchery for a longer time than wild chum salmon would rear in freshwater, thus providing more time to deposit a freshwater signal on the otolith. Lengths of chum salmon that we examined ranged from 36 to 43 mm. In another western Alaska River (Kuskokwim River), juvenile chum salmon collected in the estuary showed no indication of freshwater growth (i.e., there was

no drop in otolith Sr:Ca from a maternal signal to a freshwater level) and many fry still had yolk reserves when captured at the mouth of the river (Zimmerman, personal observation; Hillgruber et al. 2007).

Given the confounding issues of maternal signals and short duration of freshwater rearing, we argue that otolith chemical composition is not a robust means of identifying natal stream of origin for wild chum salmon. This is simply an issue related to the life history of the species because chum salmon do not consistently spend sufficient time rearing in freshwater and, therefore, do not deposit enough otolith material corresponding to their natal stream. We suspect the same issue exists for pink salmon (*O. gorbuscha*), which spend even less time in freshwater and frequently spawn just upstream of saltwater.

Inclusion of ^{87}Sr:^{86}Sr ratios in our analyses greatly increased the power to discriminate among watersheds and natal rivers. While facilities with LA-ICP MS instrumentation are becoming relatively common, it is less common to find facilities with the capability to measure isotope ratios (i.e., LA-ICP multi-collector instrumentation). As a result, it would be beneficial if element:Ca ratios alone were capable of discrimination among natal rivers for salmonids. While it has been demonstrated to be feasible in some cases (i.e., Wells et al. 2003; Veinott and Porter 2005), our models based only on element:Ca ratios were not as robust discriminators as those including ^{87}Sr:^{86}Sr ratios. This indicates that the ability to discriminate among sites and the analytes needed to do so likely vary among regions and will vary depending on the question at hand. As a result, we suggest that pilot studies examining water chemistry and otolith elemental and isotopic variability be conducted to determine what tools are needed to achieve the needed results.

In summary, chemical composition of otoliths was sufficiently different among watersheds to allow for classification of natal river at the regional level within Norton Sound for both coho and chum salmon. Distinction among rivers and watersheds of otolith chemical composition was affected both by life history differences of species examined and the ability to discriminate natal origin of chum salmon was hindered because chum salmon do not rear in freshwaters for sufficient time to develop a strong signature that is free from maternal influences. Determination of natal origins for coho

salmon was simplified because coho salmon rear in freshwaters for long enough to provide sufficient otolith material to sample outside of maternal influences. But, although the chemical signals appeared to be sufficient to discriminate among watersheds, geologic patterns may have obscured the ability to distinguish among rivers and watersheds. For example, the Nome and Niukluk rivers share similar geologies, which results in similar otolith signals of salmon from these two rivers. As such, it is not possible to differentiate fish from these rivers. The reclassification rates from this study should not be used to infer straying rates without further study. First, a multi-year study should be conducted to determine the temporal stability of otolith signatures. If they are not stable, it would not be advisable to use juvenile salmon collected in one year as a baseline for adults collected in the same year as we did in this study. It does appear that inclusion of ^{87}Sr:^{86}Sr isotope ratios is required to be able to discriminate among watersheds and rivers. Without ^{87}Sr:^{86}Sr isotope ratios, we would not have been able to discriminate among watersheds.

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