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ARTICLE

Species and Life History Affect the Utility of Otolith Chemical Composition for Determining Natal Stream of Origin for Pacific Salmon

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Abstract

To test the utility of otolith chemical composition as a tool for determining the natal stream of origin for salmon, we examined water chemistry and otoliths of juvenile and adult Chum Salmon *Oncorhynchus keta* and Coho Salmon *O. kisutch* from three watersheds (five rivers) in the Norton Sound region of Alaska. The two species are characterized by different life histories: Coho Salmon rear in freshwater for up to 3 years, whereas Chum Salmon emigrate from freshwater shortly after emergence. We used laser ablation (LA) inductively coupled plasma (ICP) mass spectrometry (MS) to quantify element: Ca ratios for Mg, Mn, Zn, Sr, and Ba, and we used multicollector LA-ICP-MS to determine ^{87}Sr:^{86}Sr ratios in otolith regions corresponding to the period of freshwater residence. Significant differences existed in both water and otolith elemental composition, suggesting that otolith composition could be used to discriminate the natal origin of Coho Salmon and Chum Salmon but only when ^{87}Sr:^{86}Sr ratios were included in the discriminant function analyses. The best discriminant model included ^{87}Sr:^{86}Sr ratios, and without ^{87}Sr:^{86}Sr ratios it was difficult to discriminate among watersheds and rivers. Classification accuracy was 80% for Coho Salmon and 68% for Chum Salmon, indicating that this method does not provide sufficient sensitivity to estimate straying rates of Pacific salmon at the scale we studied.

Identification of the natal origins of fish is fundamental to understanding population dynamics and population structure (Secor 2010). In the study of population dynamics, natal origin is the critical piece of information that allows for the matching of fishery catch with production (Cadrin and Secor 2009). For geographically structured populations such as Pacific salmon, identification of natal origin is needed in studies of straying and homing. Pacific salmon are typically structured as geographically distinct populations in partial genetic isolation. This structure reflects a balance between genetic drift within populations and gene flow among populations. Homing of adult spawners to their natal habitat (i.e., philopatry) results in breeding populations that are (1) adapted to local conditions and (2) demographically and genetically isolated from one another (Hendry et al. 2004; Utter et al. 2009). Straying or dispersal decreases the variance among local breeding populations (Barton and Whitlock 1997), and the quantification of dispersal capabilities and patterns is a critical step in examining both genetic structure and

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metapopulation dynamics in animal populations (Wiens 1996; Ims and Yoccoz 1997).

Chemical composition of otoliths has been used to examine natal origin and connectivity among populations of marine fishes (e.g., Thresher 1999; Rooker et al. 2003; Miller et al. 2005). Because otoliths grow throughout the life of the fish and are both conservative and metabolically inert, elements or compounds that are incorporated into the calcium carbonate matrix are permanently retained and thus act as an environmental monitor and archive (Campana 1999; Thresher 1999). Composition of elements within otoliths is generally determined by the composition of ambient water (Campana 1999; Elsdon and Gillanders 2003; Wells et al. 2003; Zimmerman 2005), and when coupled with the chronologically resolved structure of otoliths, the otolith chemical composition can be used to indicate environmental conditions or residency during specific life stages, specific years, or both (Campana 1999).

Multi-elemental analyses of otoliths have been used to identify natal origins, habitat associations, and stock structure in a variety of 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. Those authors found that 60–81% of spawning Weakfish were spawning in their natal estuary. Such an approach would be very useful for quantification of straying rates and assessment of connectivity among metapopulations of anadromous and freshwater fishes.

Although the use of otolith chemical composition as a tool to assess connectivity has not been reported as extensively for freshwater fishes as for marine fishes, it has been used in a variety of contexts. Isotopes of strontium (^{87}Sr and ^{86}Sr) have been used to examine salmonid movement among tributaries and natal origin (stream of origin; Kennedy et al. 1997, 2000; Ingram and Weber 1999; Barnett-Johnson et al. 2005). For a variety of freshwater and anadromous fishes, multi-elemental signatures have been used to determine natal stream of origin (Sohn et al. 2005; Veinott and Porter 2005; Veinott et al. 2012), connectivity or movement among tributaries or lake habitats (Brazner et al. 2004; Clarke et al. 2007; Marklevitz et al. 2011), and origin of fish that are stocked in or transferred to lakes and streams (Coghlan et al. 2007; Gibson-Reinemer et al. 2009). Milton and Chenery (2001) used otolith composition and genetic analyses to examine population structure of an anadromous shad, the Hilsa *Tenualosa ilisha*. Using eight elements to compare otolith microchemistry among Hilsa spawning locations, Milton and Chenery (2001) were able to distinguish among locations, but they 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 Mg, Mn, Sr, and Ba to Ca in the first summer growth region of otoliths in Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* from the Coeur d'Alene River, Idaho. Based on the use of three elements (Mn, Sr, and Ba), individual fish could be classified to streams with an accuracy of 82%. These studies indicate that otolith composition could provide a powerful tool for assessing connectivity among populations of anadromous Pacific salmon.

Several studies have demonstrated that elemental or isotope composition of otoliths can differ among the natal streams used by anadromous salmonids. For example, Veinott and Porter (2005) compared elemental signatures using four elements and determined that otolith signatures of Atlantic Salmon *Salmo salar* from three streams in Newfoundland, Canada, differed sufficiently to permit determination of the natal stream of origin. Similarly, the utility of ^{87}Sr:^{86}Sr for determining natal stream has been demonstrated for Chinook Salmon *O. tshawytscha* in the Sacramento River–San Joaquin River basin, California (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 among Chum Salmon *O. keta* from three rivers in Korea and suggested that multi-elemental analyses of otolith chemical composition could be used to identify the natal origin of Chum Salmon captured at sea; however, this has not been investigated for wild Chum Salmon.

To further investigate whether otolith chemical composition can be used to discriminate among natal streams of origin and to estimate straying in Pacific salmon species, we examined variability in ambient water chemistry, chemical composition of otoliths in juvenile salmon, and chemical composition of juvenile growth zones in otoliths of adult salmon from several rivers in the Norton Sound region of western Alaska. Rivers and watersheds were selected to represent the greatest possible degree of geologic variability (which should be reflected in water chemistry and otolith composition). These watersheds are underlain by differing geology, which is an important prerequisite for otolith microchemical studies (Barnett-Johnson et al. 2008; Elsdon et al. 2008). The Nome River watershed (Figure 1) drains a region containing Precambrian crystalline rocks that were formed between 570×10^6 and 3.6×10^9 years before present. The Unalakleet River watershed is dominated by younger Cretaceous rocks that were formed 65×10^6 to 136×10^6 years before present. The Fish River watershed is a mixture of young and old Precambrian rocks (between 570×10^6 and 3.6×10^9 years old) and quaternary deposits that were formed within the last 2×10^6 years. Given this heterogeneity in rock ages and types, we thought it likely that elemental composition of stream water would differ among sites and that this would be reflected in the elemental and Sr isotope signatures within the freshwater growth region of otoliths. Chum Salmon and Coho Salmon *O. kisutch* were selected as contrasting species to illustrate how reliance on freshwater as juveniles may affect the utility of otolith-based methods of discriminating natal origin; Chum Salmon typically migrate to sea immediately after emergence (Salo 1991), whereas Coho Salmon rear in freshwater for up to 3 years (Sandercock 1991). In the Norton Sound region of Alaska, Coho Salmon spawning populations typically consist

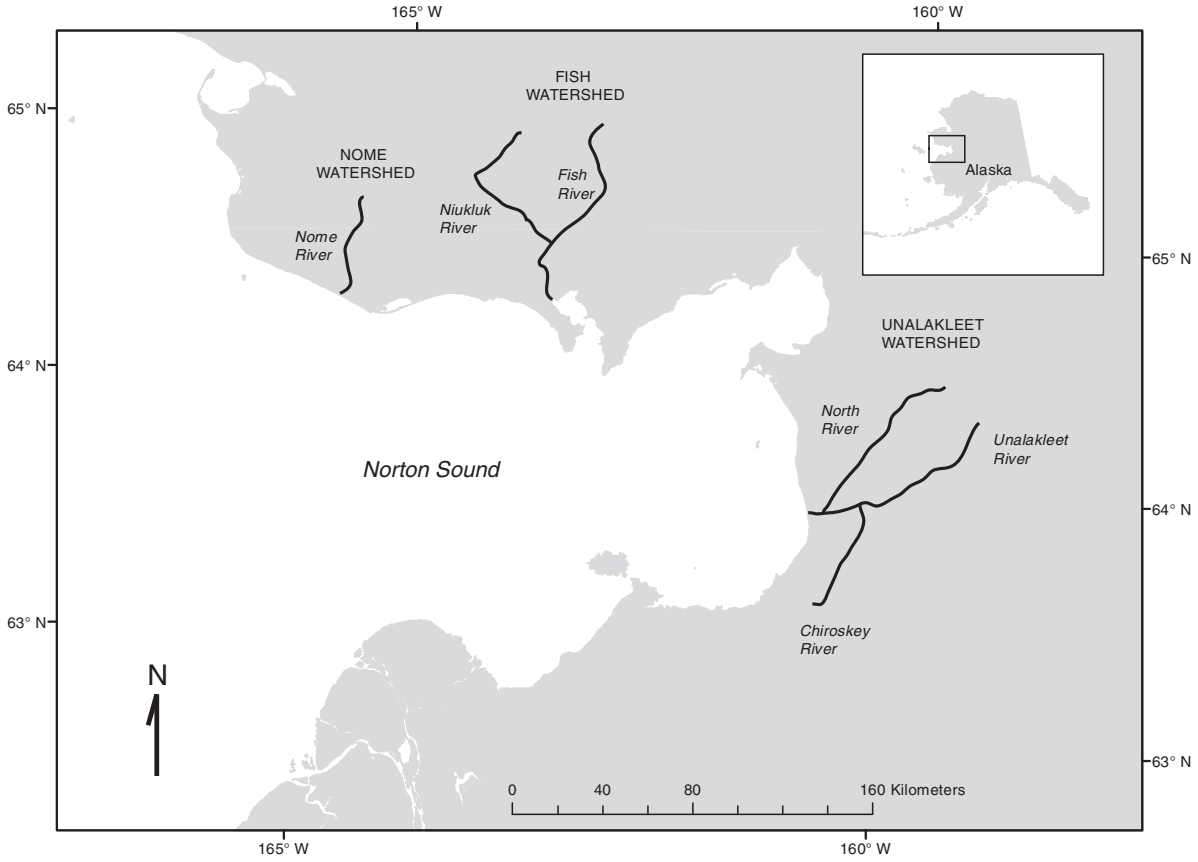

FIGURE 1. Map of the study area in Norton Sound, Alaska.

of three year-classes (ages 1.1, 2.1, and 3.1), and Chum Salmon spawning populations consist of up to four year-classes (ages 0.2, 0.3, 0.4, and 0.5; Kent et al. 2008). We hypothesized that otolith-based discrimination of the stream of origin is possible for Coho Salmon but that short freshwater residence times and lingering maternal signals in Chum Salmon would prevent the adequate accrual of otolith material beyond any maternal influences (such as those described by Kalish 1990), thereby hindering accurate discrimination. Finally, we assessed whether the addition of ^{87}Sr:^{86}Sr isotope ratios to standard otolith elemental composition analyses would improve discriminatory power.

METHODS

Study area.—Juvenile Chum Salmon and Coho Salmon were collected from five rivers in three watersheds (Nome, Fish, and Unalakleet River watersheds) that drain to Norton Sound in western Alaska (Figure 1). The hydrology of rivers in this region is dominated by snowmelt and summer rainfall, with peak flows occurring between June and September. Juvenile salmon were captured in June 2005 by using baited minnow traps and pole seines at multiple sites within each river. Our target sample size was 25 fish of each species in each river. At the time of sampling, juvenile Coho Salmon had spent at least 1 year in freshwater, whereas Chum Salmon had emerged within the preceding weeks. Fish were frozen at the end of each day in the field, and otoliths were removed from thawed fish within 1 month of capture. Adult Chum Salmon and Coho Salmon were collected in August and September 2005 from either subsistence fisheries or as carcasses found on river margins, and our target sample was 25 adult fish per species from the Nome, Fish, and Niukluk rivers. Given the pilot nature of this study and the lack of sampling opportunities in all rivers, we only collected adult samples from three rivers to test the utility of the method. All otoliths were stored dry in plastic vials for up to 6 months before preparation and analysis.

Water chemistry.—Three to five surface water samples (30 mL) were collected from each river at the same times and locations as juvenile fish sampling. Water samples were filtered through 0.45-μm membrane filters before being acidified to pH less than 2 with quartz-distilled nitric acid. In the laboratory, samples were diluted from 1 to 6 mL with 1% quartz-distilled nitric acid and then were analyzed for Ca, Mg, Mn, Sr, and Ba with a Varian Liberty 150 inductively coupled plasma (ICP) optical emission spectrometer at the Keck Collaboratory for Plasma Spectrometry, Oregon State University. Concentrations were calculated from emission intensities and the intensities of standard solutions. Accuracy of the method was verified by

running a National Institute of Standards and Technology (NIST) certified freshwater reference material (NIST 1643c) at the start and end of the analysis session and after every five analyses.

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 2,000-grit sandpaper. The mounting medium was heated, and the otolith was turned sulcus side up. The otolith was then ground with 2,000-grit sandpaper in the sagittal plane into the nucleus and was 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).

Analyses of otolith chemistry were conducted at the Keck Collaboratory for Plasma Spectrometry. Elemental analyses were conducted using a Thermal Elemental PQ Excell quadropole ICP mass spectrometer connected to a New Wave Research deep ultraviolet (DUV) 193-nm argon fluoride 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 that began in the core of the otolith and terminated at the otolith's edge. Background levels were measured for 30 s prior to otolith ablation and were subtracted from the measurements obtained during otolith ablation. Count rates for each analyte isotope (^{24}Mg, ^{55}Mn, ^{66}Zn, ^{88}Sr, and ^{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 to allow for calculations of element concentrations. Mean elemental data corresponding to otolith growth deposited during the first summer (and outside of the nucleus) were used to characterize each fish.

Otolith ^{87}Sr:^{86}Sr data were collected on a second otolith transect via the methods of Miller and Kent (2009). Multicollector (MC) laser ablation (LA) ICP mass spectrometry (MS) instrumentation included the New Wave Research DUV 193-nm excimer laser (see above) and a NuPlasma MC-ICP mass spectrometer. We followed the general method of Woodhead et al. (2005) to correct for potential krypton and rubidium (Rb) interferences and to monitor calcium argide or dimer formation. Background interferences by krypton 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 the ablation of otoliths. Measured backgrounds were subtracted from the intensities measured during otolith ablation. Mass biases were corrected by reference to an ^{87}Sr:^{86}Sr ratio of 0.1194, and we corrected for isobaric interference of ^{87}Rb on ^{87}Sr 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. Mean ^{87}Sr:^{86}Sr corresponding to otolith growth deposited during the first summer (and outside of the nucleus) was used to characterize each fish.

Data analysis.—Statistical analyses were conducted in R version 2.14.1 (R Development Core Team 2011) and the Statistical Analysis Systems version 9.1.3 (SAS 2003). To assess the degree of variation in water chemistry at our study sites, we compared elemental concentrations of Ba, Mg, Mn, and Sr among rivers and among watersheds by using multivariate ANOVA (MANOVA) followed by both multivariate and univariate pairwise contrasts. Analyses were performed on $\log_e$-transformed data to meet homogeneity of variance and normality assumptions. For MANOVAs and multivariate pairwise contrasts, we report results of Pillai's trace, which is the most robust test (in comparison with Wilks' lambda, the Hotelling–Lawley trace, and Roy's greatest root) when assumptions are not met (Gotelli and Ellison 2004). Univariate pairwise comparisons were achieved with Tukey's test.

Analyses of otolith constituents were performed on $\log_e$-transformed data to meet the assumptions of homogeneity of variance and normality. Chemical composition of otoliths (Sr isotope ratios and element: Ca ratios) for the otolith growth region corresponding to the first summer (and outside of the nucleus) was compared among rivers by using MANOVAs and pairwise contrasts. This was done for each species, and data were transformed as described above. We then used linear discriminant function analysis to determine whether multi-elemental and Sr isotope signatures could be used to classify fish to the watershed or river of origin. Discriminant function models were constructed for both Chum Salmon and Coho Salmon at the river scale and watershed scale by using the juvenile otolith data. For juveniles of each species and hierarchical grouping (watershed versus river), a discriminant function was constructed using (1) all elemental and isotope data; (2) only elemental data; and (3) only Sr:Ca and ^{87}Sr:^{86}Sr ratios. Discriminatory power was compared among models by using Wilks' lambda and a cross-validated leave-one-out approach to classify each fish to its location of origin (Wells et al. 2000; Gibson-Reinemer et al. 2009). Accuracy of the classifications determined by discriminant functions was compared with that expected by chance alone under the assumption that random chance will result in correct classifications with a percentage that is inversely proportional to the number of groups classified (White and Ruttenberg 2007). Otoliths from adult salmon were classified using the baseline discriminant function constructed with the juvenile otolith data, and classifications were compared with the capture locations.

RESULTS

Water Chemistry

Water chemistry (defined here as Mg, Mn, Sr, and Ba concentrations) varied significantly among watersheds and among rivers (MANOVAs: $F_{>8,\,52} > 6.83$, $P < 0.0001$; Figure 2).

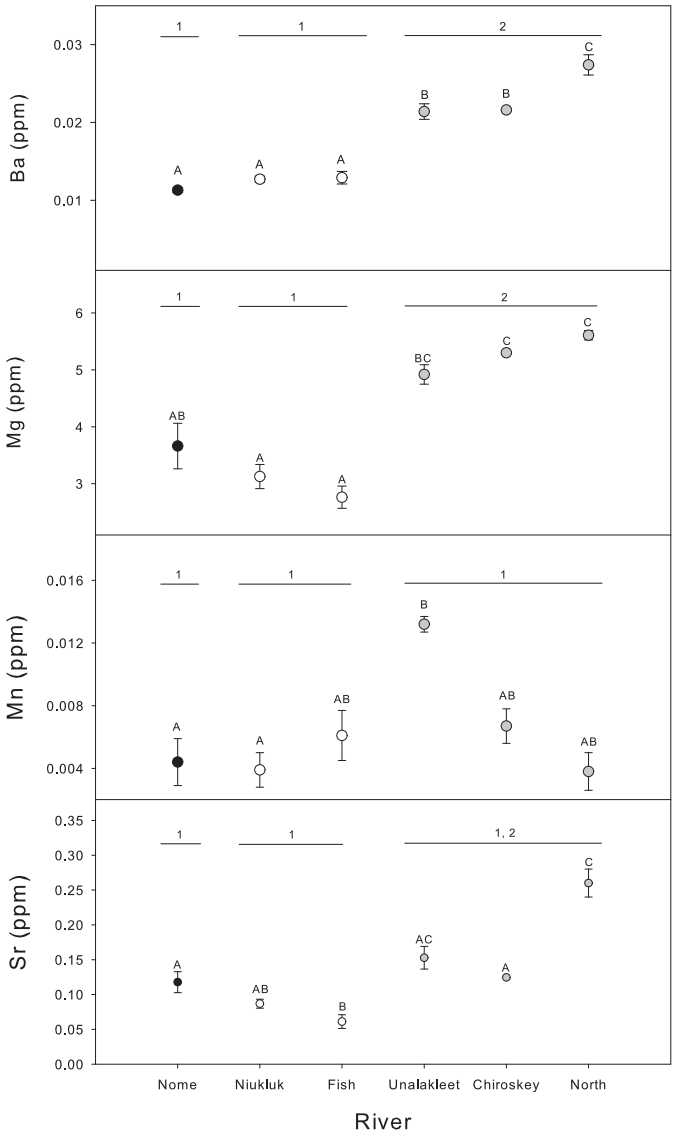

FIGURE 2. Mean (±SE) concentrations (ppm) of water constituents in six Alaskan rivers. Letters indicate significant univariate pairwise differences among rivers, whereas numbers indicate significant univariate pairwise differences among watersheds (Tukey's tests: $P < 0.05$). Rivers grouped within watersheds are indicated by the horizontal lines at the top of the plot (Nome River, Fish River, and Unalakleet River watersheds, respectively).

Univariate pairwise comparisons showed that differences among watersheds were driven by variability in Ba, Mg, and Sr concentrations (Tukey's tests: $P < 0.05$); there were no significant differences in Mn (Tukey's test: $P > 0.05$; Figure 2).

When all elements were considered together for among-river comparisons, the adjacent Nome and Niukluk rivers had similar water chemistry ($F_{4, 22} = 4.45$, $P = 0.09$), despite being located in different watersheds. Pairwise differences between all other rivers were significant ($F_{4, 22} > 4.5$, $P < 0.01$; Figure 2). Once again, these differences were largely driven by variations in Ba, Mg, and Sr (Figure 2), although Mn concentrations were significantly higher in the Unalakleet River than in the North River or Niukluk River (Tukey's tests: $P < 0.05$). Overall, it appeared that water chemistry reflected regional variations in geology (as described above), and we therefore expected that analyses of otolith composition among rivers and among watersheds would be informative.

Composition and Discrimination of Otoliths from Juveniles

Otolith composition within the first-summer growth region varied significantly among watersheds and among rivers for both juvenile Chum Salmon and juvenile Coho Salmon (MANOVAs: $F_{>5, 112} = 9.21$, $P < 0.0001$; Figure 3). For both species, differences among watersheds reflected variability in ^{87}Sr:^{86}Sr and Sr:Ca (Tukey's tests: $P < 0.05$), whereas for Coho Salmon there was also significant among-watershed variability in Mg:Ca (Tukey's tests: $P < 0.05$; Figure 3).

Similar to the observations for water chemistry, Chum Salmon in the Nome and Niukluk rivers had similar otolith composition (i.e., with all elements considered together; $F_{5, 108} = 1.30$, $P = 0.27$), but all other pairwise differences were significant ($F_{5, 108} > 4.66$, $P < 0.0007$). In contrast, the composition of otoliths in Coho Salmon differed significantly among all rivers ($F_{5, 110} > 3.03$, $P = 0.0133$). For both species, differences among rivers reflected variability in ^{87}Sr:^{86}Sr and Sr:Ca (Tukey's tests: $P < 0.05$; Figure 3). For Coho Salmon, there was additional among-river variability in Mg:Ca and Ba:Ca (Tukey's tests: $P < 0.05$; Figure 3).

When constructing discriminant functions for analysis among watersheds, we found that the first two discriminant functions described 100% of the variation in both Coho Salmon and Chum Salmon for all combinations of analytes: (1) all element: Ca ratios and the ^{87}Sr:^{86}Sr ratio; (2) all element: Ca ratios; and (3) only Sr:Ca and ^{87}Sr:^{86}Sr ratios. For juveniles of both Chum Salmon 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 (Table 1) and by the overall classification rates. For both Chum Salmon and Coho Salmon, the discriminant function that was developed by using only Sr:Ca and ^{87}Sr:^{86}Sr ratios was only slightly less successful at discriminating among watersheds (Table 1), whereas discriminant functions that were constructed using only the element: Ca ratios provided the least ability to discriminate among watersheds (Table 1). For Coho Salmon, the first discriminant function, which was constructed based on all element: Ca ratios and ^{87}Sr:^{86}Sr ratios, clearly separated fish that were captured in the Unalakleet River watershed (Chiroskey and North rivers) from fish that were collected in the Fish River and Nome River watersheds (Figure 4a). Patterns for Chum Salmon were similar but less pronounced (Figure 4b).

At the among-river scale, the first two discriminant functions explained 98.9% of the variation for Coho Salmon and 95.9% of the variation for Chum Salmon (Figure 5a, b). For the discriminant functions using all element and isotope data, the

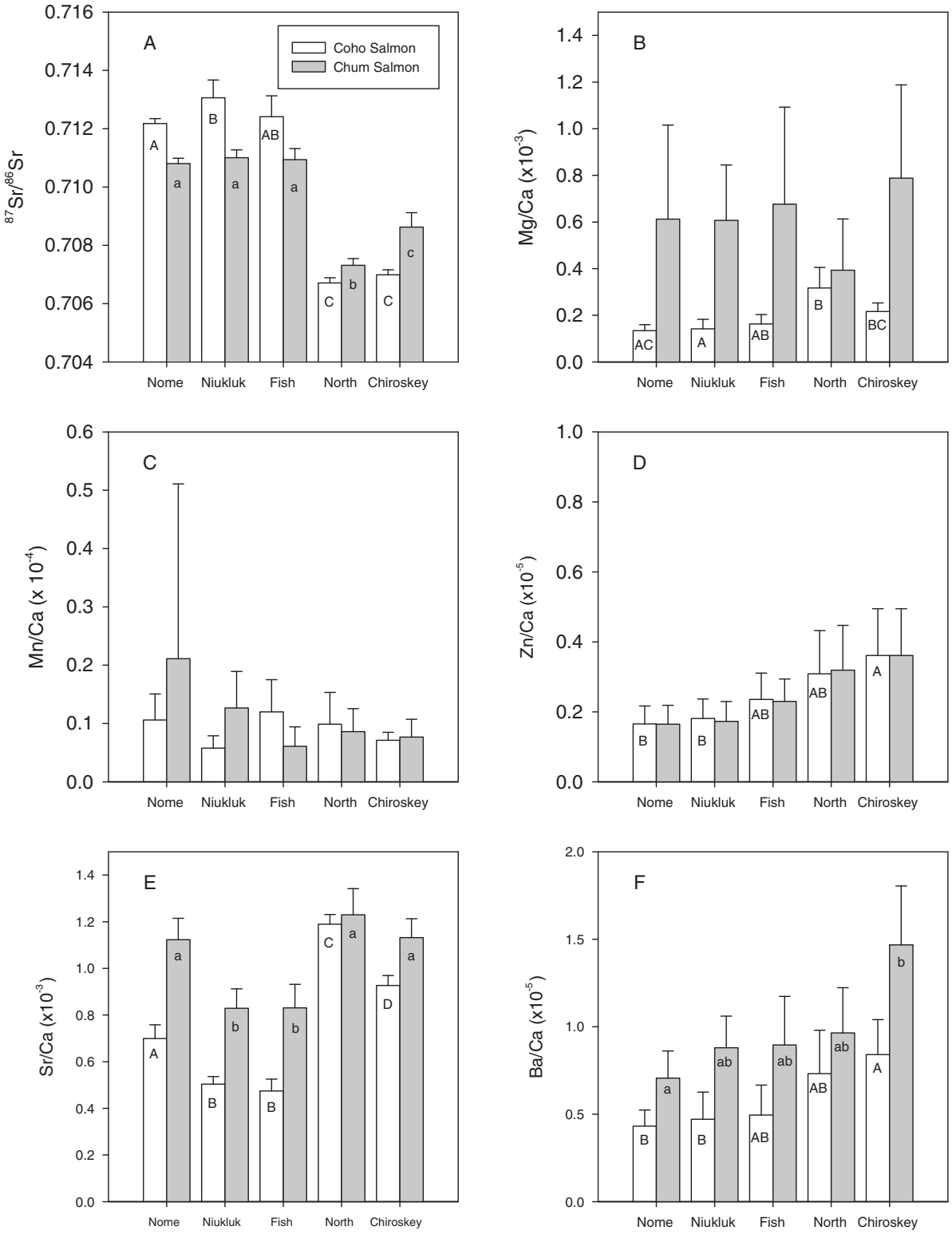

FIGURE 3. Mean ratios (+95% confidence interval) of analytes measured in the freshwater growth region of otoliths from juvenile Coho Salmon and Chum Salmon. Capital letters indicate significant univariate pairwise differences between rivers for Coho Salmon, and lowercase letters indicate significant pairwise differences between rivers for Chum Salmon (Tukey's tests: $P < 0.05$).

TABLE 1. Overall classification rates and Wilks' lambda (λ) for discriminant function analyses of otolith chemical composition in juvenile Coho Salmon and Chum Salmon examined at the watershed level (Nome, Fish, and Unalakleet River watersheds, Alaska).

	Juvenile Coho Salmon		Juvenile Chum Salmon	
Analysis	Classification rate	Wilks' λ	Classification rate	Wilks' λ
All element: Ca ratios and the ^{87}Sr:^{86}Sr ratio	0.93	0.0510	0.81	0.2008
Element: Ca ratios only	0.83	0.1996	0.65	0.5588
Sr:Ca and ^{87}Sr:^{86}Sr ratios only	0.92	0.0640	0.82	0.2309

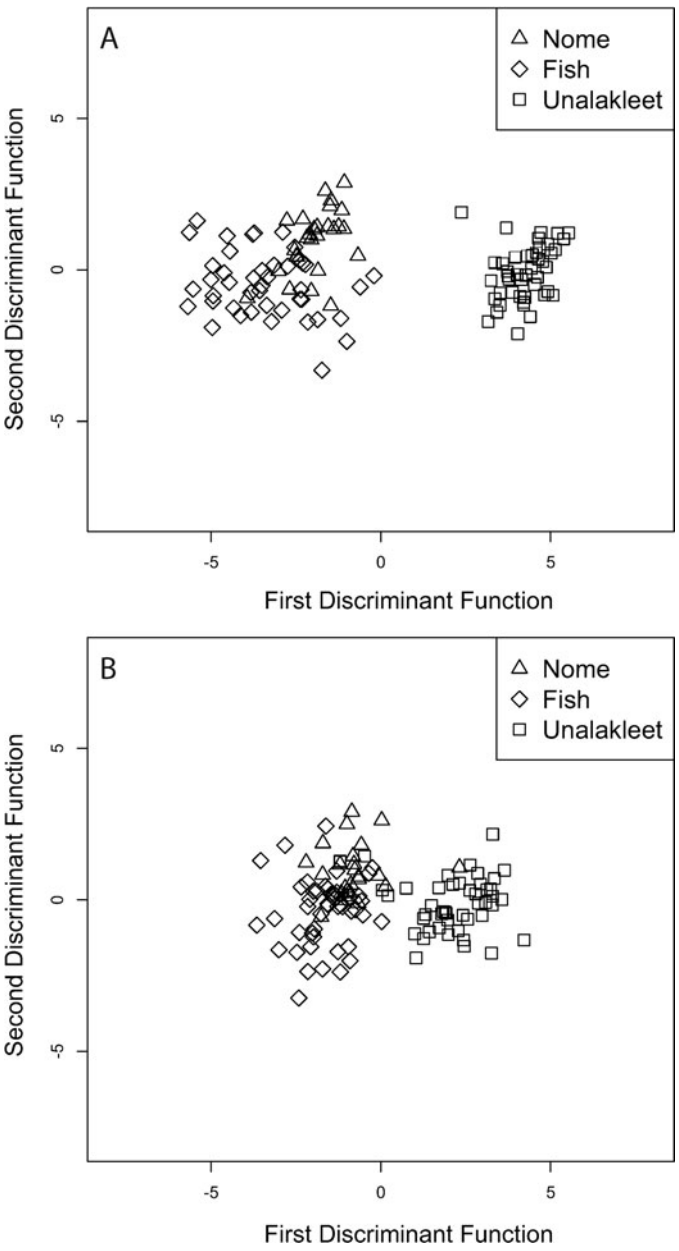

FIGURE 4. Bivariate plots of discriminant function scores from the otolith model constructed based on all elemental and isotope data at the watershed scale for **(A)** juvenile Coho Salmon and **(B)** juvenile Chum Salmon (triangles = Nome River watershed; diamonds = Fish River watershed; squares = Unalakleet River watershed).

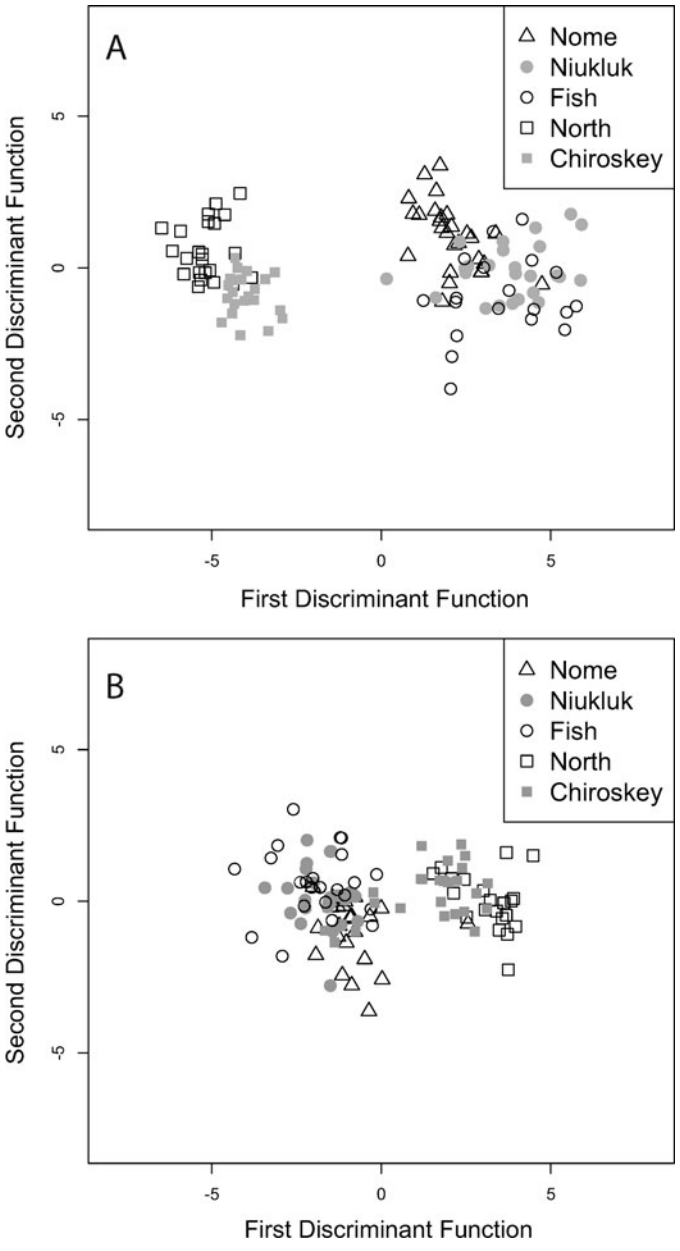

FIGURE 5. Bivariate plots of discriminant function scores from the otolith model constructed based on all elemental and isotope data at the river scale for **(A)** juvenile Coho Salmon and **(B)** juvenile Chum Salmon (triangles = Nome River; solid gray circles = Niukluk River; open circles = Fish River; open squares = North River; solid gray squares = Chiroskey River).

TABLE 2. Classification matrix (river scale) based on otolith chemical composition in juvenile Coho Salmon and Chum Salmon (overall classification rates for the full data set = 0.80 for Coho Salmon and 0.68 for Chum Salmon). Values in bold italics represent correct classification to the river of origin.

Predicted river of origin	Actual river of origin				
	Nome	Niukluk	Fish	North	Chiroskey
Juvenile Coho Salmon					
Nome	***0.81***	0.20	0.16	0.00	0.00
Niukluk	0.07	***0.68***	0.26	0.00	0.00
Fish	0.11	0.12	***0.58***	0.00	0.00
North	0.00	0.00	0.00	***0.87***	0.00
Chiroskey	0.00	0.00	0.00	0.13	***1.00***
Juvenile Chum Salmon					
Nome	***0.72***	0.17	0.17	0.00	0.16
Niukluk	0.08	***0.58***	0.22	0.00	0.00
Fish	0.16	0.25	***0.57***	0.00	0.00
North	0.04	0.00	0.00	***0.91***	0.20
Chiroskey	0.00	0.00	0.04	0.09	***0.64***

TABLE 3. Classification matrix (watershed scale) based on otolith chemical composition in adult Coho Salmon and Chum Salmon collected from the Nome and Fish River watersheds (adults were not captured in the Unalakleet River watershed). Values in bold italics represent correct classification to the watershed of capture.

Predicted watershed of origin	Watershed of capture	
	Nome River	Fish River
Adult Coho Salmon		
Nome River	***0.78***	0.30
Fish River	0.18	***0.68***
Unalakleet River	0.04	0.02
Adult Chum Salmon		
Nome River	***0.45***	0.31
Fish River	0.23	***0.69***
Unalakleet River	0.32	0.00

overall jack-knifed classification accuracy was 80% for Coho Salmon and 68% for Chum Salmon. Proportion of misclassified fish varied from 9% (North River) to 43% (Fish River) for juvenile Coho Salmon and from 0% (Chiroskey River) to 42% (Fish River) for juvenile Chum Salmon. For Coho Salmon, misclassifications were typically with nearest neighbors (Table 2). That is, Coho Salmon from the Nome, Niukluk, and Fish rivers were not misclassified as originating from the North River or Chiroskey River and vice versa (Figure 1; Table 2). This was not the case with Chum Salmon, as there were misclassifications between samples from the two furthest watersheds (Table 2). For example, 4% of Nome River Chum Salmon were misclassified as North River fish, and 20% of Chiroskey River Chum Salmon were misclassified as Nome River fish (see Figure 1 for location reference; Table 2).

Classification of Otoliths from Adults

In total, 23, 24, and 26 adult Coho Salmon and 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 watershed were classified as originating from outside of that watershed, and 32% of adult Coho Salmon captured in the Fish River watershed were classified as originating from outside of that watershed (Table 3). When analyzed at the river level, 22% of adult Coho Salmon that were captured in the Nome River were classified as originating outside of the Nome River, 45% of adult Coho Salmon captured in the Niukluk River were classified as originating outside of that river, and 61% of adult Coho Salmon captured in the Fish River were classified as originating outside of that river (Table 4). At the watershed level, 53% of adult Chum Salmon collected in the Nome River watershed were classified as originating from outside of that watershed, and 30% of adult Chum Salmon captured in the Fish River watershed were classified as originating from outside of that watershed (Table 3). When analyzed at the river level, 52% of adult Chum Salmon captured in the Nome River were classified as originating outside of that river, 44% of adult Chum Salmon captured in the Niukluk River were classified as originating outside of that river, and 33% of adult Chum Salmon captured in the Fish River were classified as originating outside of that river (Table 4).

TABLE 4. Classification matrix (river scale) based on otolith chemical composition in adult Coho Salmon and Chum Salmon that were collected from the Nome, Niukluk, and Fish rivers. Values in bold italics represent correct classification to the river of capture.

Predicted river of origin	River of capture		
	Nome	Niukluk	Fish
Adult Coho Salmon			
Nome	***0.78***	0.33	0.31
Niukluk	0.18	***0.54***	0.31
Fish	0.00	0.13	***0.38***
North	0.00	0.00	0.00
Chiroskey	0.04	0.00	0.00
Adult Chum Salmon			
Nome	***0.48***	0.40	0.29
Niukluk	0.03	***0.56***	0.67
Fish	0.13	0.04	***0.00***
North	0.20	0.00	0.00
Chiroskey	0.16	0.00	0.04

DISCUSSION

Results from our study suggest that otolith "tags" may be a useful tool for determining the natal river or watershed of origin (i.e., provenance) of Pacific salmon, but several caveats must be carefully considered before applying this technique (Wells et al. 2003; Elsdon et al. 2008). First, the accuracy of discrimination will depend on variability in water chemistry among the watersheds or rivers of interest, and such variability is ultimately driven by variability in the underlying geology. For this reason, we found that the proportion of misclassifications was lower among watersheds than among rivers (because rivers within the same watershed have similar underlying geology and water chemistry), and we suggest that underlying geology should be considered and analyses of water chemistry should be conducted before analyzing otolith composition. This is particularly important in regions of relatively homogeneous geology.

Life history traits may also 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 their natal rivers for up to three winters before migrating to sea. This allows for substantial accrual of freshwater-derived otolith material, providing an unambiguous freshwater region to sample in the otoliths of adults. A relatively protracted juvenile rearing period in freshwater allows for the sampling of otolith freshwater growth regions that are unlikely to be affected by maternal material. Chum Salmon, on the other hand, migrate to sea immediately after emergence from the gravel. Our results (relatively poor discrimination among rivers or among watersheds and a high proportion of misclassifications) indicate that while in freshwater, Chum Salmon do not deposit enough maternally independent otolith material to enable use of otolith tags for determination of natal origin.

Although Chum Salmon migrate from freshwater immediately after emergence and the possibility of confounding maternal material being present is high (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 in the otoliths of Chum Salmon. However, examination of a "typical" profile of Sr, as presented by Arai and Hirata (2006), suggests that there were in fact 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 in Chum Salmon juveniles collected from three hatcheries in Korea and found significant differences among sites. Using a discriminant function approach similar to the one used in our study, Sohn et al. (2005) argued that otolith composition could 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 to 82 mm, whereas the lengths of Chum Salmon that we examined ranged from 36 to 43 mm. This suggests that the juvenile Chum Salmon studied by Sohn et al. (2005) were held in hatcheries for a longer period of time than Chum Salmon would rear in freshwater in the wild. This artificially long rearing period would allow for greater accrual of freshwater-derived otolith material. In another western Alaska river (Kuskokwim River), juvenile Chum Salmon that were collected in the estuary showed no indication of freshwater growth (i.e., there was no decline in otolith Sr:Ca from a maternal signal to a freshwater level), and many fry still had yolk reserves when captured at the river mouth (Hillgruber et al. 2007; C. E. Zimmerman, personal observation). Given the confounding issues of maternal signals and a 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. We suspect that the same issue exists for Pink Salmon *O. gorbuscha*, which spend even less time in freshwater and frequently spawn just upstream of salt water (Heard 1991).

Inclusion of ^{87}Sr:^{86}Sr ratios in the analyses greatly increased our ability to discriminate among watersheds and among natal rivers. Although facilities with LA-ICP-MS instrumentation are becoming relatively common, it is less common to find facilities with the capability of measuring isotope ratios (i.e., MC-LA-ICP-MS instrumentation). As a result, it would be beneficial if element: Ca ratios alone were capable of facilitating discrimination among natal rivers for salmonids. Although the use of element: Ca ratios has been demonstrated to be feasible in some cases (e.g., Wells et al. 2003; Veinott and Porter 2005; Veinott et al. 2012), our models based only on element: Ca ratios were not as robust as the models that included ^{87}Sr:^{86}Sr ratios. This indicates that the analytes required for discrimination among sites likely vary among regions and will differ depending on the question at hand. We suggest that pilot studies examining water chemistry and otolith elemental and isotopic variability be incorporated into study designs; this will improve both scientific and economic efficiencies.

Although we found that otolith composition was sufficiently different among watersheds and rivers to allow classification of natal origin, this method was not robust enough to allow for estimation of straying rates among rivers in the Norton Sound region. Based on classifications of the otoliths from adults, we estimated that 22–32% of adult Coho Salmon had provenance outside of the watershed from which they were collected (i.e., they were strays). These values are relatively high when compared with those of other wild populations. Labelle (1992), for example, reported overall straying rates of approximately 4.7% for Coho Salmon on Vancouver Island, British Columbia. In that study, straying rates were typically greater than 2% but did range as high as 40% in one case. The misclassification rates for juvenile Coho Salmon captured in the Nome, Niukluk, and Fish rivers were similarly high (Table 2), indicating that our estimates of straying could simply be misclassifications of adults that actually originated from the same streams in which they were captured. Although our classification rates for juvenile salmon (our baseline) are similar to those reported in other studies (Wells et al. 2000; Brazner et al. 2004; Gibson-Reinemer

et al. 2009), they may not be sufficiently precise to allow for the examination of straying. However, this does not preclude the use of otolith chemical composition for examining straying at other locations. For example, Veinott and Porter (2005) and Veinott et al. (2012) reported classification accuracies of 83–100% (with most near 100%) for populations of Atlantic Salmon and Brown Trout *Salmo trutta* from streams at the same spatial scales we examined. In our study, adjacent rivers did not have sufficient geological differences and resulting water chemistry differences to allow for a meaningful analysis of straying at that spatial scale.

In summary, for both Coho Salmon and Chum Salmon, the chemical composition of otoliths was sufficiently different among watersheds to allow for reasonable classification of natal river at the watershed level within Norton Sound. Patterns in geology were not distinct enough to allow for robust discrimination among rivers, however. At the scale of geologic diversity in this study, it appears that the inclusion of ^{87}Sr:^{86}Sr ratios is necessary for discriminating among watersheds and among rivers; without the ^{87}Sr:^{86}Sr ratios, we would not have been able to discriminate among watersheds. Differentiation and classification among rivers were also affected by life history. Our ability to discriminate the natal origin of Chum Salmon was hindered by the fact that these fish do not rear in freshwater for a sufficient period to develop a strong freshwater otolith signature that is free from maternal influences. As such, it is not possible to differentiate Chum Salmon from these rivers. Misclassification proportions from this study should not be used to infer straying rates without further investigation. First, a multiyear study should be conducted to determine the temporal stability of otolith signatures. If there is significant temporal variability, it would be inadvisable to use juvenile salmon collected in a single year as a baseline for adults collected in the same year (as was done in this study). Genetic analyses should also be used as a complementary approach to estimate straying.

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