Integrating Multiple Intrinsic Markers to Infer Habitat Use of Sockeye Salmon Stocks (*Oncorhynchus nerka*) in the North Pacific Ocean

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Pacific salmon (*Oncorhynchus* spp.) are renowned for their high mobility, complex population structure, and homing to natal spawning grounds. Following a year or more in freshwater and estuarine habitats, sockeye salmon (*O. nerka*) in British Columbia, for example, are thought to move north and north-west along the coast during their first summer and winter at sea before migrating offshore into the North Pacific Ocean. During their marine phase, they have the potential to occupy a vast range between the Aleutian Islands and the Washington/Oregon coasts before returning to their home rivers and spawning grounds 2–6 years later (Tucker et al. 2009; Farley et al. 2018). Recent studies on salmonids have greatly advanced our understanding of the timing of ocean entry, natal origins, and habitat use during the first year of life (e.g., Barnett-Johnson et al. 2008; Miller et al. 2010; Volk et al. 2010; Stocks et al. 2014; Campbell et al. 2015). However, much less is known about their habitat use, distribution, and migration patterns after their first autumn at sea—a period that comprises the majority of their lives. The great obstacle to resolving ocean habitat use is the logistical challenge of capturing/tracking salmon on the high seas. Yet, tracing movement pathways through the North Pacific is essential to understanding how salmon populations are and will be impacted by regionally dynamic changes in ocean conditions. To advance techniques for tracing complex oceanic movements of salmon, we evaluate the utility of integrated intrinsic genetic, chemical, and microstructural markers to infer habitat use, movement patterns, and their relationship with relative growth rates of *O. nerka* during their marine phase.

Fig. 1. Sampling locations for returning sockeye salmon (*Oncorhynchus nerka*) along central and southern British Columbia, Canada, July–September 2015.

Archived otoliths collected from returning *O. nerka* captured in fisheries and research surveys between July–September 2015 (Fig. 1) were used in our analyses. These samples were opportunistically selected because genetic stock identifications and stable isotopic analyses of muscle tissue had been completed previously for specimens represented in the otolith archive. Stock identifications were determined from analyses of fourteen microsatellites following Beacham et al. (2014) using an ABI 3730 capillary DNA sequencer. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analyses of dorsal muscle tissue were performed using a PDZ Europa Hydra 20-20 continuous-flow isotope ratio mass spectrometer to assess dietary variation that may reflect foraging and occupancy in different food webs. A lipid correction factor was applied to $\delta^{13}\text{C}$ values (Hoffman and Sutton 2010). Otoliths were thin-sectioned, imaged, and aged prior to elemental analyses and seasonal increment widths were measured to evaluate variation in summer growth rates. Otolith microchemistry was assayed using laser ablation inductively coupled plasma mass spectrometry. Otoliths were ablated from edge to core (spot size: 47 μm ; scan speed: 5 $\mu\text{m s}^{-1}$) to capture elemental variation across the entire life of each fish. Element-to-calcium ratios for lithium (^7Li), magnesium (^{24}Mg), strontium (^{88}Sr), barium (^{138}Ba), and lead (^{208}Pb) were collected to characterize potential

spatial/environmental differences among fish during their summers and final winter at sea. Elemental data were averaged from each mid-point of summer (50 μm) and final winter (15 μm) growth bands to align and standardize comparisons across individuals and cohorts. We applied hierarchical cluster analyses with Ward's linkage method and Euclidean distance to objectively identify groups and patterns within temporally-aligned otolith elemental data. The optimum number of groups was determined from cubic clustering criteria. Differences in otolith chemistry among group classifications and the influence of age, sex, and genetic stock were subsequently tested with non-parametric multi-response permutation procedures (MRPP). Relative differences in growth among groups were examined with analysis of variance (ANOVA) and Tukey's Honestly Significant Difference tests were conducted to determine which groups accounted for any observed variation in growth.

Fig. 2. Diagrammatic representation of the results of hierarchical cluster analyses based on otolith chemical composition across the lifetime of sockeye salmon examined in this study by season and year, 2010–2015. Circles represent individual group designations resulting from cluster analysis. S = summer, W = winter, Ba = barium, Ca = calcium, Li = lithium, Mg = magnesium, Sr = strontium, and Pb = lead. Sample size (n) and the relative contribution of select element-to-calcium ratios are reported. Arrows between groups indicate the direction of change, if any, in group membership across years/seasons. With the exception of summer 2010, all group classifications reflect mixed cohort analyses.

Otoliths were processed and assayed from a total of 50 *O. nerka* that were comprised of Rivers Inlet ($n = 16$) and Fraser River (Chilko ($n = 17$), Chilko South ($n = 11$), Harrison ($n = 2$), Great Central ($n = 1$), Middle ($n = 1$), and Raft ($n = 3$)) stocks. Samples were comprised of four age classes that included sockeye from 2009–2012 cohorts. Profiles of otolith chemistry across the lifetime of individual *O. nerka* distinguished early versus late marine migrants. Distinctive groups were revealed by variation in otolith chemistry. Mean mid-summer elemental composition indicated that sockeye experienced both shared and disparate environmental conditions or locations during their time at sea. Cluster analyses of the 2010 cohort found significant differences in otolith chemistry during their first summer prior to marine residence that aligned with genetic stock identifications and spatial differences at large and fine spatial scales (Fig. 2; MRPP: $T = -12.3$, $A = 0.48$, $p < 0.01$). Three separate groups were identified from intrinsic chemical markers during the summer of 2011. Location or conditions were found to be highly similar among individuals in 2012 (Fig. 2). In the summers of 2013 and 2014, variation in otolith chemistry separated five distinct groups. Variation in otolith Sr/Ca composition was a consistently important determinant of differences among groups. Otolith Sr/Ca ratios increase significantly with salinity in sockeye salmon and can serve as powerful markers of habitat use (Zimmerman et al. 2005). Two age 1 Chilko sockeye, for example, exhibited characteristics of freshwater residence in the summer of 2013 based on low otolith Sr/Ca and Mg/Ca ratios and higher Li/Ca and Ba/Ca ratios in comparison to other individuals in the study (Fig. 2). In the winter of 2015, otolith chemistry was homogenous among our samples, implying that a shared location or set of environmental conditions were again encountered during migration. Similarly, stable C and N isotopic analyses indicated extensive dietary overlap and that the fish were feeding within the same food web and likely occurred in the same region during the spring and summer prior to spawning. Groups designated through cluster analyses were comprised of multiple stocks,

suggesting a high degree of mixing during migration. Otolith chemical composition did not differ by sex or stock (MRPP), supporting the premise that the elements considered in this study reflected underlying spatial/environmental differences among fish that could be used to reconstruct migratory pathways. Variation in otolith chemistry corresponded with differences in growth rates among some groups (Fig. 3; ANOVA (2014): $F = 2.75$; $p = 0.04$; ANOVA (2013): $F = 4.22$; $p = 0.02$).

Fig. 3. Box plots depicting relative growth (otolith increment width) among designated groups based on differences in otolith chemistry using hierarchical cluster analyses for summers 2014 and 2013. Asterisks (*) denote those groups found to differ significantly by analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (THSD) tests.

Our results demonstrate that otolith chemistry provides valuable information on the habitat use and environmental history of salmon during the marine as well as early life stages. Integrating microstructural growth increment information with chemical chronologies and genetic stock identifications has the potential to generate new insight into the consequences of variation in migratory pathways and identify patterns of movement, distribution, and habitat use at multi-regional scales. Distinct inshore and offshore oceanographic gradients encountered during salmon migrations in the North Pacific are likely to be recorded within salmon otoliths. Likelihood probability distributions estimated from combined oceanographic and chemical data across potential migration routes or a chemical atlas based on otolith edge chemistry of the same species collected in different locations could offer a foundation for reconstructing migratory pathways from otolith chemistry. We recommend the inclusion of otolith stable oxygen isotope analyses in future investigations to provide a seasonal water temperature proxy that may complement and strengthen inferences on movement patterns and habitat use.

REFERENCES

- Barnett-Johnson, R. T.E. Pearson, F.C. Ramos, C.B. Grimes, and R.B. MacFarlane. 2008. Tracking natal origins of salmon using isotopes, otoliths, and landscape geology. *Limnol. Oceanogr.* 53(4): 1633–1642.
- Beacham, T.D., R.J. Beamish, J.R. Candy, C. Wallace, and S. Tucker. 2014. Stock-specific migration pathways of juvenile sockeye salmon in British Columbia waters and in the Gulf of Alaska. *Trans. Am. Fish. Soc.* 143: 1386–1403.
- Campbell, L.A., D.L. Bottom, E.C. Volk, and I.A. Fleming. 2015. Correspondence between scale morphometrics and scale and otolith chemistry for interpreting juvenile salmon life histories. *Trans. Am. Fish. Soc.* 144: 55–67.
- Farley Jr., E.V., T.D. Beacham, and A.V. Bugaev. 2018. Ocean ecology of sockeye salmon. *In* The Ocean Ecology of Pacific Salmon and Trout. Edited by R.J. Beamish. Am. Fish. Soc., Bethesda, MD. pp. 319–389.

- Hoffman, J.C., and Sutton, T.T. 2010. Lipid correction factor for carbon stable isotope analysis of deep-sea fishes. *Deep Sea Res. I* 57(8): 956–964.
- Miller, J.A., M.R. Bellinger, J.T. Golden, L. Fujishin, and M.A. Banks. 2010. Integration of natural and artificial markers in a mixed stock analysis of Chinook salmon (*Oncorhynchus tshawytscha*). *Fish. Res.* 102: 152–159.
- Stocks, A.P., E.A. Pakhomov, and B.P.V. Hunt. 2014. A simple method to assess the marine environment residence duration of juvenile sockeye salmon (*Oncorhynchus nerka*) using laser ablation. *Can. J. Fish. Aquat. Sci.* 71(10): 1437–1446.
- Tucker, S., M. Trudel, D.W. Welch, J.R. Candy, J.F.T. Morris, M.E. Thiess, C. Wallace, D.J. Teel, W. Crawford, E.V. Farley Jr., and T.D. Beacham. 2009. Seasonal stock-specific migrations of juvenile sockeye salmon along the west coast of North America: implications for growth. *Trans. Am. Fish. Soc.* 138: 1458–1480.
- Volk, E.C., D.L. Bottom, K.K. Jones, and C.A. Simenstad. 2010. Reconstructing juvenile Chinook salmon life history in the Salmon River Estuary, Oregon, using otolith microchemistry and microstructure. *Trans. Am. Fish. Soc.* 139: 533–549.
- Zimmerman, C.E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. *Can. J. Fish. Aquat. Sci.* 62(1): 88–97.