

## Linking Juvenile Chum Salmon (*Oncorhynchus keta*) Nutritional Condition and Trophic Status to Genetic-Based Stock Assignments

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**Keywords:** salmon, condition, trophic, stable isotopes, stock origin, marine ecosystems

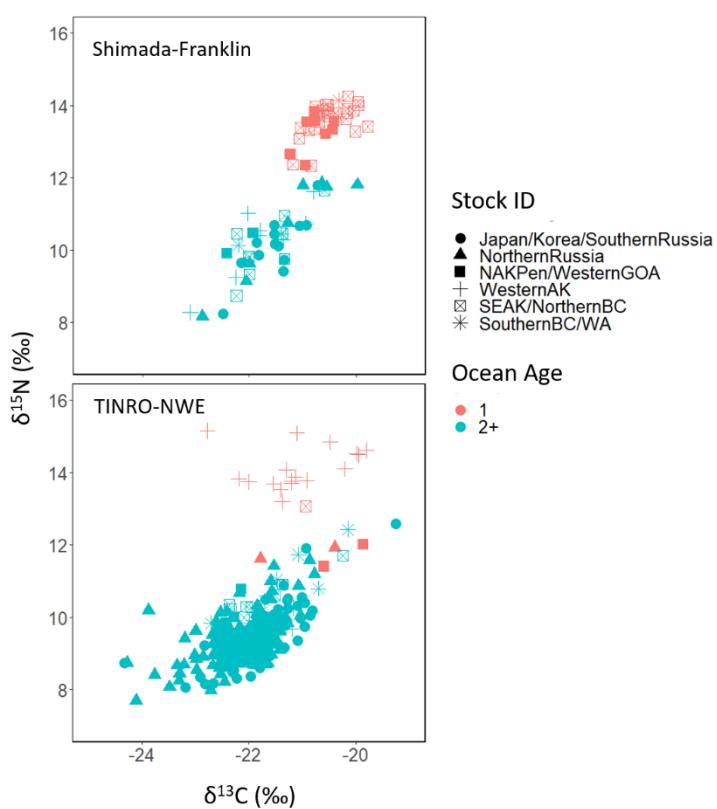
In the high seas, Pacific salmon (*Oncorhynchus* spp.) stocks exhibit considerable mixing across the North Pacific, which increases the potential for inter- and intra-species resource competition. Stock origin and potential stock-specific phenotypic differences in feeding may therefore be an important factor in understanding density dependence and other drivers of nutritional quality. Evidence of stock-specific differences in feeding of north Pacific salmon have been shown from stable isotope analysis of sockeye salmon returns (Welch and Parsons 1993; Johnson and Schindler 2012), and stock-specific growth rates (Tucker et al. 2009). Here we assess stock specific differences in stable isotopes and measures of nutritional condition (lipid and protein) in chum salmon (*O. keta*) collected from the North Pacific during the 2022 International Year of the Salmon Pan Pacific Winter Survey. We discuss the importance of incorporating genetic stock identification, life history stage and growth when applying biochemical measures to understanding trophic processes from winter months in high latitude systems.

Chum salmon were collected in 2022 from the R/V *TINRO* (2–20 March), R/V *Bell M. Shimada* (1 February–7 March), CCGS *Sir John Franklin* (19 February–21 March), and F/V *Northwest Explorer* (3–17 April) trawl surveys. Fish were measured onboard for length (fork length, FL) and weight, and a muscle plug was collected, frozen and shipped to the lab for the analysis of stable isotopes, lipid, and protein; fin clips were collected for genetic stock identification (GSI) from the same fish. In the lab, muscle plugs were dried and homogenized to a fine powder and subsequently sampled and weighed for stable isotope analysis (SIA), lipid and protein. Stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were measured by a ThermoScientific FlashSmart Elemental Analyzer (EA) coupled to a ThermoScientific Delta V Isotope Ratio Mass Spectrometer (0.3 and  $0.5 \pm \text{SD}$ , respectively). Carbon isotopes values were not corrected for lipids due to the total carbon nitrogen (C:N) ratio being  $< 3.5$  (Post et al. 2007). Measurement of percent lipids was performed using a sulfo-phospho-vanillin (SPV) reaction method (Pinger et al. 2022) as a percent of the wet mass, and percent protein was measured from tissues by combustion through a Thermo Flashsmart EA, with percent nitrogen values being multiplied by a protein scaling factor (6.25 for marine animal tissues) to determine total dry protein content. All biochemical measures were completed at the NOAA Auke Bay Laboratories (Juneau, Alaska). Genetic stock identification (GSI) was performed at the Alaska Department of Fish and Game Gene Conservation Laboratory (Anchorage, Alaska).

Samples were collected and analyzed from a total of 397 chum salmon representing six stock groups among the four vessels. To maximize the between-stock comparisons, we grouped samples from the R/V *TINRO* and F/V *Northwest Explorer* (TINRO-NWE), and the R/V *Shimada* and CCGS *Franklin* (Shimada-Franklin), representing the western and eastern regions of the pan-Pacific expedition, respectively. Initial stable isotope analysis results showed a strong isotopic separation that was related to body size, with smaller fish ( $< 27.3$  cm FL) having distinctly higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values relative to larger individuals. As a result, we included ocean age as a grouping variable in our analyses. Ocean age was measured from scales at the Alaska Department of Fish and Game (ADF&G) Mark, Tag and Age Laboratory (Juneau, AK) following Frank and Reynolds (2016). When ocean age values were unavailable, we used the size frequency distribution of the catch to delineate ocean ages 1, 2 and 3+ where information on scale age was unavailable.

For the analysis of stable isotopes, we used the R packages SIBER v2.1.6 (Jackson et al. 2011) and nichROVER (Lysy et al. 2014; Swanson et al. 2015) to estimate isotopic niche area and overlap between stocks within a vessel group. For SIBER niche area estimates, we specified 20,000 iterations and credible intervals of 95%. For the analysis of percent lipid and protein, we compared stocks by the linear trends of lipid and protein with size (weight, kg), with a positive slope indicating a trajectory that is nutritionally replete, with flat or negative linear trends with size being indicative of lower nutritional condition (flat) or starvation (negative) state (Castellini and Rea 1992). Due to limited sample sizes across stocks and size range, statistical inference on the regression of percent lipids with size was limited to the following stocks based on sample size for the TINRO-NWE (Stock assignments: WesternAK, SEAK/NBC, Russia, Japan/Korea/SRussia) and Shimada-Franklin (SEAK/NBC) vessel groups.

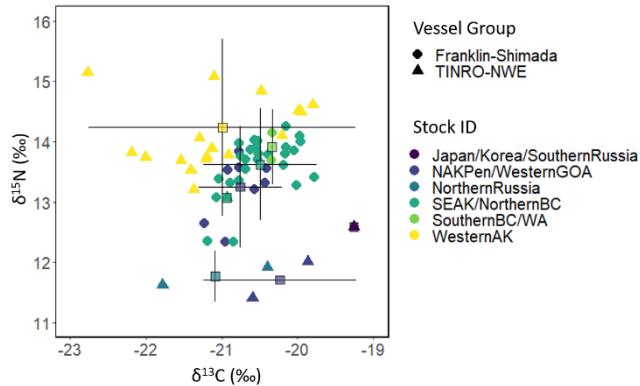
An initial assessment of the isotope results showed ocean age 1 fish from all four vessels were significantly different in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from the age 2+ fish ( $p = 0.0001$ ; Fig. 1). Relatively high  $\delta^{15}\text{N}$  ( $> 13.0\text{\textperthousand}$ ) and  $\delta^{13}\text{C}$  ( $> -20.0\text{\textperthousand}$ ) values denote a strong coastal signature that is almost certainly a carryover of last summer's feeding and growth, where isotopic signatures are predominantly incorporated from added tissue mass (Hesslein et al. 1993; Miller et al. 2006; Weidel et al. 2011; Hertz et al. 2016). In the late fall and winter months, reduced or near cessation of growth would have minimized isotopic turnover rates, resulting in a lingering summer signal.



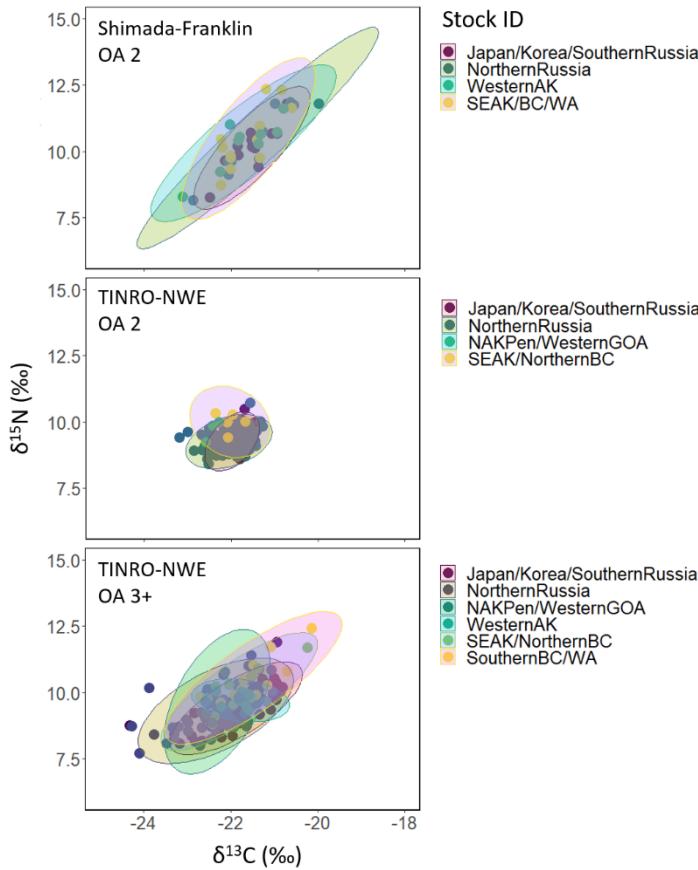
**Fig. 1.** Stable isotope  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plots of chum salmon by stock origin and ocean age collected from the 2022 winter expedition vessels *R/V Shimada* and *CCGS Franklin* (Shimada-Franklin) and *R/V TINRO* and *F/V Northwest Explorer* (TINRO-NWE).

The comparison of stocks within ocean ages showed one chum from the WesternAK having the highest  $\delta^{15}\text{N}$ , followed by the SEAK/NorthernBC and Southern BC/WA stocks (Fig. 2). This is consistent with the ability of juvenile chum to feed on higher trophic level (relatively higher  $\delta^{15}\text{N}$ ) larval-juvenile fish and euphausiids (Auburn and Ignell 2000; Brodeur et al. 2007), but also in the case of Western AK, the persistence of a highly elevated  $\delta^{15}\text{N}$  baseline in the northern Bering Sea (NBS) (Hertz et al. 2018; Schell et al. 1998). A comparison of juvenile chum  $\delta^{15}\text{N}$  values collected from the NOAA/AFSC 2022 NBS survey showed similarly elevated  $\delta^{15}\text{N}$  values (mean  $15.8 \pm 1.3$ , unpublished data) to the WesternAK stock (mean  $14.3 \pm 0.8$ ), confirming that the high values in the age 1 fish were from summer feeding and growth. Stock groups from the western Pacific (Japan/Korea/SouthernRussia and NorthernRussia) showed the lowest  $\delta^{15}\text{N}$  values, which is more consistent with an offshore high seas isotopic

signature. Given the distance from their natal origin to where they were collected and their more high seas isotopic signatures, suggests the western Pacific stocks likely initiated offshore feeding earlier in the previous year's summer growth period.



**Fig. 2.** Stable isotope  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plots of ocean age 1 chum salmon by stock origin from the 2022 winter expedition vessels R/V *Shimada* and CCGS *Franklin* (Shimada-Franklin) and R/V *TINRO* and F/V *Northwest Explorer* (TINRO-NWE).

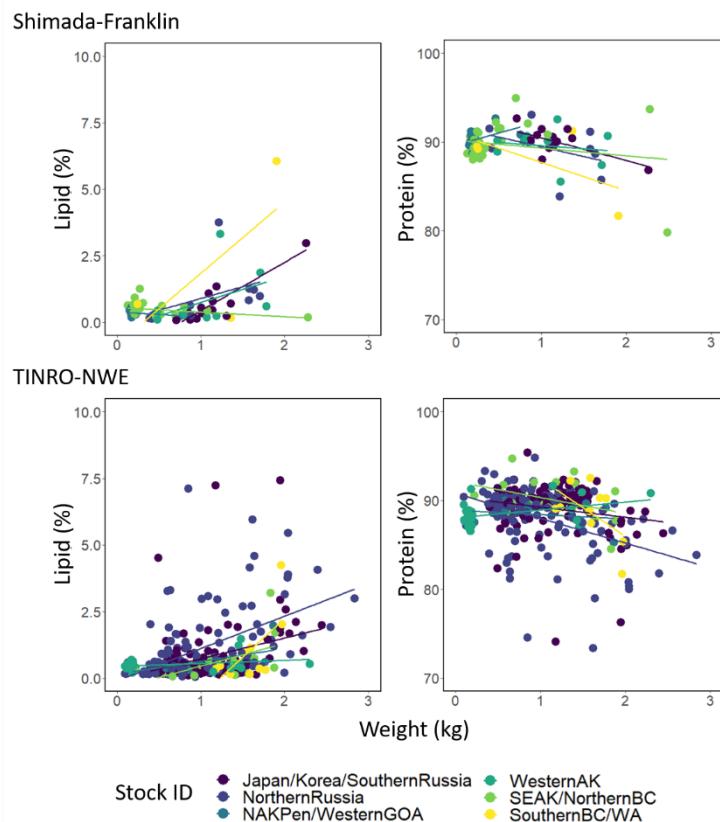


**Fig. 3.** Stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) Bayesian bivariate ellipses representing the 95% isotopic niche space of chum salmon genetic stock identification assignments by ocean age (OA) from the Shimada-Franklin and TINRO-NWE vessels.

Results from the isotope niche overlap of stocks showed Bayesian ellipses of age 2+ chum having a high percent overlap among all stocks (Fig. 3). The average stock niche overlap for age 2 chum was 74 and 77% for the TINRO-NWE and Shimada-Franklin, respectively. For ocean age 3+ (TINRO-NWE), niche overlap was slightly lower at 66%, which may be due to the larger size range of fish and concomitant range in trophic level feeding in this age category. Overall, the low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  open ocean isotopic signature and the high degree of isotopic niche

overlap indicates age 2 and 3+ chum salmon are feeding on the same high-seas resource. However, as with the age 1 fish, the isotopic signatures in age 2+ fish also likely represent a high degree of summer feeding and growth, and a more temporally protracted measure relative to smaller fish (age 1) as a larger body mass requires more time to isotopically integrate with diet. This summer growth signature brings into question the utility of stable isotope analysis in high latitude studies in the winter months when growth and subsequently isotopic turnover is minimal in fish and other ectotherms. We used muscle plugs for our analysis, a tissue that is commonly used in stable isotope analysis of fish as a time-averaged measure of trophic level feeding, but it is also highly dependent on added tissue mass in driving the rate of isotopic shift. Alternative tissues with higher metabolic demands driving isotopic turnover, such as liver may be used in tracking winter feeding; however as a metabolizing tissue the isotopic signature may reflect in-part endogenous reserves (Perga and Gerdeaux 2005), particularly in winter months when food resources are low.

Results from nutritional measures showed lipid was very low, with ocean age 1 and 2 mean lipid values among vessel groups of 0.5 and 0.6%, respectively (Table 1). Regression results from five stocks that had adequate sample sizes showed four with a moderate to highly significant ( $p = 0.06$  to  $< 0.0001$ ) increase in lipid with size; this general trend was observed across the other stocks analyzed (Fig. 4). The low percentage of lipids shows the utilization of lipid reserves over the fall-winter months; however, the increase in lipids with size suggests that, at the stock level, the chum salmon analyzed were not in a starving phase. The total percent protein among all stocks and the nearly flat or slight reduction in percent protein with size (Fig. 4), suggests that catabolism at the level of starvation is not occurring. It is important to note that our measures are from muscle tissue, and that the low percent lipid values observed here do not account for energy reserves in the liver and other tissues, such as subcutaneous and visceral lipids. However, our analyses provides an assessment on the general trends between stocks, which show a similar response in their over-winter existence. This observation coincides with our results from stable isotopes that the stocks measured here are feeding from a shared high-seas resource.



**Fig. 4.** Scatterplots displaying the stock-specific relationship between percent lipid and protein by size (weight) of chum salmon sampled during winter 2022.

**Table 1.** Summary of stock specific stable isotope, lipid and protein values of chum salmon (*O. keta*) by ocean age and vessel group collected from the 2022 International Year of the Salmon (IYS) pan Pacific winter survey. Stocks denote regions or combined regions of natal origin, with embedded abbreviations as follows: NAKPen (Alaska Peninsula), GOA (Gulf of Alaska), BC (British Columbia), WA (Washington), and SEAK (Southeast Alaska).

Vessel Group/Ocean Age/Stock	n	Mean (SD)			
		$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Lipid (%)	Protein (%)
<b>Shimada-Franklin</b>					
1 NAKPen/WesternGOA	8	13.3 (0.6)	-20.8 (0.3)	0.4 (0.2)	90.1 (1.0)
SEAK/NorthernBC	26	13.7 (0.5)	-20.5 (0.4)	0.6 (0.3)	89.5 (0.9)
SouthernBC/WA	2	14 (0.4)	-20.4 (0.1)	0.7	89.4 (0.2)
2 Japan/Korea/SouthernRussia	12	10 (0.7)	-21.6 (0.5)	0.6 (0.4)	90.4 (1.2)
NAKPen/WesternGOA	2	10.2 (0.4)	-22.2 (0.4)	0.3 (0.1)	91.8 (1.3)
NorthernRussia	9	10.5 (1.5)	-21.4 (1)	1.1 (1.2)	89.2 (2.9)
SEAK/NorthernBC	6	9.9 (0.8)	-21.9 (0.4)	0.4 (0.2)	92.1 (1.7)
SouthernBC/WA	1	10.4	-21.5	0.2	91.3
WesternAK	8	10.6 (0.7)	-21.6 (0.6)	0.9 (1.2)	89.5 (2.2)
3+ Japan/Korea/SouthernRussia	1	11.8	-20.8	3.0	86.9
SEAK/NorthernBC	3	10.7 (1.0)	-21.4 (0.9)	0.4 (0.2)	88.1 (7.4)
SouthernBC/WA	1	10.1	-22.3	6.1	81.7
WesternAK	1	8.3	-23.2	0.3	90.1
<b>TINRO-NEW</b>					
1 Japan/Korea/SouthernRussia	1	12.6	-19.3	0.4	89.3
NAKPen/WesternGOA	2	11.8 (0.5)	-20.3 (0.6)	0.5 (0.2)	87.7 (0.2)
NorthernRussia	2	11.8 (0.3)	-21.1 (1.0)	0.4 (0.3)	88.8 (2.3)
SEAK/NorthernBC	1	13.1	-21.0	0.3	90.6
WesternAK	18	14.3 (0.8)	-21.0 (0.9)	0.5 (0.2)	88.2 (1.2)
2 Japan/Korea/SouthernRussia	33	9.3 (0.5)	-22.0 (0.3)	0.6 (0.8)	89.2 (1.7)
NAKPen/WesternGOA	3	9.7 (0.5)	-22.5 (0.2)	0.4 (0.2)	89.3 (0.7)
NorthernRussia	86	9.4 (0.6)	-22.1 (0.5)	0.6 (0.6)	89.2 (2.1)
SEAK/NorthernBC	5	10.1 (0.4)	-22.1 (0.3)	0.3 (0.1)	89.8 (1.3)
3+ Japan/Korea/SouthernRussia	72	9.4 (0.7)	-21.9 (0.6)	0.9 (1.6)	89.1 (3.2)
NAKPen/WesternGOA	5	9.6 (0.7)	-22.3 (0.3)	0.8 (0.4)	88.3 (1.9)
NorthernRussia	58	9.3 (0.8)	-22.3 (0.7)	1.8 (3.9)	86.8 (4.2)
SEAK/NorthernBC	12	10.0 (0.8)	-21.8 (0.6)	0.7 (0.7)	89.9 (2.8)
SouthernBC/WA	11	10.6 (0.9)	-21.5 (0.7)	1.0 (0.8)	90.2 (3.1)
WesternAK	8	9.7 (0.3)	-21.8 (0.4)	0.6 (1.5)	89.4 (1.9)

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