

Stable isotope analysis of trophic position and terrestrial vs. marine carbon sources for juvenile Pacific salmonids in nearshore marine habitats

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Abstract Stable isotope analysis was used to determine trophic position and the relative contributions of terrestrial-derived carbon (TDC) and marine-derived carbon for Chinook, *Oncorhynchus tshawytscha* (Walbaum), pink, *Oncorhynchus gorbusha* (Walbaum), and chum, *Oncorhynchus keta* (Walbaum) salmon fry in near-shore marine habitats. Chum fry were enriched in $\delta^{13}\text{C}$ relative to pink fry, and enriched in $\delta^{15}\text{N}$ relative to both Chinook and pink fry. Between 5.5 and 39.7% of the carbon in the three species was TDC. The TDC was higher in chum fry ($28.7 \pm 4\%$ SD) than in pink fry ($24.9 \pm 4.4\%$ SD), but TDC did not differ between Chinook fry ($27.8 \pm 9.5\%$ SD) and either chum or pink fry. The fry of these three species of Pacific salmon may form a trophic hierarchy with chum fry occupying the highest trophic position and the three species may also partition resources according to carbon source.

KEYWORDS: salmonids, stable isotope analysis, supra-littoral, terrestrial-derived carbon, trophic hierarchy.

Introduction

Welch & Parsons (1993) suggested that adult Pacific salmon, *Oncorhynchus* spp. may form a trophic hierarchy by partitioning food resources to reduce inter-specific competition during their marine phase, when they co-occur in regions where prey resources may be limited. They concluded that Chinook, *Oncorhynchus tshawytscha* (Walbaum), occupies the highest trophic position, followed by coho, *Oncorhynchus kisutch* (Walbaum), sockeye, *Oncorhynchus nerka* (Walbaum), and pink, *Oncorhynchus gorbusha* (Walbaum). Adult chum, *Oncorhynchus keta* (Walbaum), were found to occupy a different branch of the food web. By contrast, Welch & Parsons (1993) found two distinct feeding groups for juvenile salmon, one with coho and Chinook salmon, and one with chum, pink, and sockeye salmon. Their results suggested that immature

chum salmon at sea feed on prey higher in the food chain relative to the other species. This study was conducted to provide similar information on trophic position for chum, pink, and Chinook salmon fry (hereafter chum, pink, and Chinook) in nearshore marine habitats and to assess the relative contributions of terrestrial-derived carbon (TDC) and marine-derived carbon (MDC) in the tissue for each species.

The foreshore areas of enclosed coastal seas such as the Strait of Georgia are an important habitat for Pacific salmon fry (Healey 1982). In this region, chum, Chinook, and pink are often found in shallow water, often less than 10 cm deep, adjacent to the supra-littoral zone during high tide (Grout, Levings, Nidle, Piercey & Marsden 1998) where they feed on a variety of terrestrial and aquatic prey (Levy & Levings 1978; Grout *et al.* 1998). It is unknown, however, whether supra-littoral habitats constitute an important food

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source for salmon fry; in particular, whether supra-littoral vegetation is an important supplier of allochthonous prey compared with autochthonous prey from the adjacent intertidal zone. Romanuk & Levings (2003) showed that insects dropping from vegetated supra-littoral habitats may constitute a highly abundant source of prey items for juvenile salmon. However, little quantitative information exists on the role of TDC derived from primary producers in the supra-littoral and subsequently incorporated into juvenile salmon.

Stable isotope analysis (SIA) of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) from Chinook, pink, and chum salmon was used to determine whether muscle tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggest trophic hierarchies and the relative contributions of TDC and MDC using a two-source mixing model with bases of common marine algae and terrestrial supra-littoral vegetation (Romanuk & Levings 2003). As variations in isotope ratios in consumers result from the trophic position at which they feed as well as from spatial and temporal changes in the isotopic compositions at the base of the food web (Peterson & Fry 1987), the isotopic composition of salmon fry can be used to infer whether salmon are feeding on terrestrial or marine prey.

Methods

Chum, pink and Chinook salmon were collected by beach seining at high tide using a 3×1 m beach seine with a 6-mm mesh size. Tides in British Columbia are of the mixed, semi-diurnal form and the amplitude of the higher high tide in the study area from March to July averaged 4.17 m above chart datum. Seining was conducted when the tide was higher than 3.05 m. Four sites were located on Bowen Island ($49^{\circ}23' \text{N}$ $123^{\circ}23' \text{W}$) on the south-east shore of the Strait of Georgia at the mouth of Howe Sound. Two additional sites were located at Furry Creek ($49^{\circ}38' \text{N}$ $123^{\circ}12' \text{W}$), located ~ 30 km to the north of Bowen Island, on the east side of Howe Sound. The beaches were classified as rocky or gravel shorelines (Levings, Foreman & Tunnicliffe 1983), as they were not characterised by intertidal estuarine vegetation such as sedges (*Carex* spp.) and rushes (*Scirpus* spp.).

Seventeen Chinook, 284 chum and 91 pink salmon were collected for SIA from March 2002 to June 2002. Live tissue samples of 13 taxa of supra-littoral (terrestrial) vascular plants and six taxa of marine intertidal algae were collected in June 2002 from Furry Creek. Leaves (terrestrial plants) or algal fronds (marine intertidal algae) were collected by hand.

Salmon were kept in a cooler in the field and then frozen in the laboratory at -20°C . Samples of veget-

ation and algae were washed with distilled water to remove attached particulate matter and then frozen for storage at -20°C . Wet mass (g) and fork length (mm) were obtained for individual salmon. Flank muscle tissue was then removed from each fish for SIA. All samples were oven dried at 60°C to constant weight. Samples were ground into powder, oxidised, and the produced CO_2 and N_2 were analysed with a continuous flow-isotope ratio mass spectrometer (Finnigan-Mat Delta Plus and NC2500 elemental analyser). Ratios of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) were expressed as the relative per mL (‰) difference between the sample and conventional standards (Pee Dee Belemite carbonate and N_2 in air) according to:

$$\Delta X = [R_{\text{sample}}/R_{\text{standard}} - 1] \times 1000(\text{‰}),$$

where $X = ^{13}\text{C}$ or ^{15}N , and $R = ^{13}\text{C} : ^{12}\text{C}$ or $^{15}\text{N} : ^{14}\text{N}$.

Stable isotope analysis was performed on 17 individual Chinook, 86 samples of chum consisting of 1, 2 or 3 individuals (total 284 individual fish), and 30 pink samples consisting of between one and five individuals (total 91 individual fish). Samples for chum and pink salmon were combined for two reasons. First, it was not possible to obtain enough tissue from every fish caught for analysis because of the small size of some of the chum and pink salmon. Secondly, the purpose of using SIA was to determine average carbon dependence and trophic information. Therefore, it was decided to increase the number of fish that were analysed rather than trying to extract results from fewer individual fish. Insufficient Chinook salmon were captured so the Chinook samples were not combined to ensure sufficient statistical power. However, pooling samples also reduces intra-specific sample variance. To determine differences in (i) intra-sample variance between combined and individual fish samples SIA values and (ii) relationships between fish length and SIA values, the SIA values were also analysed from individual fish for chum ($n = 13$) and pink ($n = 5$) salmon. For all three species, and for both combined and individual fish samples, each sample totalled ~ 1.5 g wet weight yielding ~ 0.2 mg of dry tissue weight.

Contributions of TDC and MDC to the assimilated carbon in salmon fry were determined using a mixing model:

$$PA = (\delta^{13}\text{C}_{\text{consumer}} - f - \delta^{13}\text{C}_{\text{sourceB}}) / (\delta^{13}\text{C}_{\text{sourceA}} - \delta^{13}\text{C}_{\text{sourceB}}),$$

where PA is the proportion of source A in the salmon fry consumers and f is the isotopic fractionation (‰). An isotopic fractionation of 1 was used (see Peterson & Fry 1987). The mixing model calculated the contribution of

each primary source assuming that only two sources are contributing to the isotopic signatures of the consumers. Source A was calculated as the average $\delta^{13}\text{C}$ of terrestrial vascular vegetation and source B was calculated as the average $\delta^{13}\text{C}$ of marine macroalgae.

Other sources of organic matter such as eelgrass, *Zostera marina* (L.), marine phytoplankton, riverine seston, or benthic microalgae may contribute carbon with a wide range of $\delta^{13}\text{C}$ values in near-shore habitats. However, only terrestrial vascular plants and marine macroalgae were used as the endpoints in the mixing model to determine the proportions of TDC and MDC for salmon fry for a number of reasons. First, eelgrass is relatively scarce in Howe Sound because there is little intertidal and shallow subtidal habitat on the east side of this steep-sided fjord (Levings & McDaniel 1976). Secondly, riverine particulate organic carbon (POC) has SIA values that reflect contributions from terrestrial organic matter. Canuel, Cloern, Ringelberg, Guckert & Rau (1995) showed that riverine POC in San Francisco Bay had elevated C:N ratios, $\delta^{13}\text{C}$ values ranging from -24 to -27‰ , and contained sterols, which all suggest strong associations with terrestrial organic matter. Thirdly, benthic microalgae have $\delta^{13}\text{C}$ values that are similar to macroalgae (Jones, Cifuentes & Kaldy 2003; Kang, Kim, Lee, Kim, Lee & Hong 2003). Fourthly, it has proved very difficult to obtain marine phytoplankton samples in estuaries that are free of other forms of POC, such as terrigenous carbon and resuspended detritus (Parsons & Lee Chen 1995; Fry 1996). Furthermore, $\delta^{13}\text{C}$ values for marine phytoplankton are strongly affected by environmental factors and species characteristics (Goericke & Fry 1994; Burkhardt, Riebesell & Zondervan 1999) making interpretation of $\delta^{13}\text{C}$ in marine phytoplankton difficult because of high variability in $\delta^{13}\text{C}$. However, as food webs leading to juvenile salmon in nearshore estuaries are predominantly detritus based (Healey 1979; Naiman & Sibert 1979; Simenstad & Wissmar 1985), leaving marine phytoplankton out of the mixing models should have minimal effects on the results. Lastly, fresh terrestrial vascular plant tissue and marine macroalgae was used to determine the basal sources rather than their detrital forms. To determine whether the detrital forms of terrestrial vascular plants and marine macroalgae differed from fresh material, beach wrack [which represented a mixture of macroalgae species but was dominated by *Fucus gardneri* (L.)] was collected at all six sites and the terrestrial and marine components were separated for SIA analysis.

Trophic position was determined by calculating fish sample $\delta^{15}\text{N}$ relative to a basal $\delta^{15}\text{N}$ marine

macroalgae signature calculated from the mean $\delta^{15}\text{N}$ signature for six species of macroalgae. Trophic position was calculated for each species using,

$$\text{trophic position} = [(fish \delta^{15}\text{N} - algal \delta^{15}\text{N})/3.4]2,$$

where 3.4 represents a trophic position increment in $\delta^{15}\text{N}$ (Vander Zanden, Cabana & Rasmussen 1997).

Analysis of variance (ANOVA) was used to determine differences in SIA values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, trophic position, % terrestrial proportion) for chum, pink, and Chinook salmon. *Post hoc* Tukey tests were used to assess significant differences in means between species. Proportions were arcsine transformed prior to analysis. Pearson correlation coefficients were used to determine the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Linear regression was used to test for a relationship between fork length and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. *F*-tests were used to determine significant differences in variances. All analyses were performed using Statistica v6.0 (Statsoft Inc., Tulsa, USA).

Results

Differences in carbon between fresh plant tissue and marine macroalgae and their detrital forms

There were no significant differences in $\delta^{13}\text{C}$ between fresh terrestrial vascular plant tissue (see Table 2) and the terrestrial plant component of detritus collected from beach wrack ($P = 0.089$, fresh terrestrial plant material mean $\delta^{13}\text{C} -28.33 \pm 2.43$ SD, $n = 13$; terrestrial wrack detritus mean $\delta^{13}\text{C} -26.9 \pm 1.04$ SD, $n = 6$) nor between fresh *F. gardneri* and the marine macroalgae component of detritus collected from beach wrack ($P = 0.23$, *F. gardneri* mean $\delta^{13}\text{C} -19.84 \pm 1.13$ SD, $n = 6$; macroalgae wrack detritus mean $\delta^{13}\text{C} -18.85 \pm 1.49$ SD, $n = 6$). However, $\delta^{13}\text{C}$ values for *F. gardneri* were depleted in comparison with the other species of marine macroalgae collected (see Table 2). Thus, the mean $\delta^{13}\text{C}$ values of six species of marine macroalgae were used as the marine carbon endpoint to reflect more accurately MDC.

Effects of pooling samples on $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ for chum and pink salmon

There were no differences in mean $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ between individual and combined fish samples for chum ($\delta^{13}\text{C}$ $P = 0.82$, $\delta^{15}\text{N}$ $P = 0.12$), however, the variance in $\delta^{13}\text{C}$ was greater in the individual fish samples (2.13) than in the combined fish samples (1.0, $P = 0.026$). For pink salmon there was no difference in

Table 1. Mean, minimum, maximum and standard deviations (SD) for fork length (mm), wet mass (g), $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, %N and C:N ratio for chum, pink and Chinook salmon. Fork length and wet mass values are shown for fish used in the individual fish stable isotope analysis samples only

	Chum				Pink				Chinook			
	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
Length (mm)	38.06	29	52	3.98	31.2	27	39	2.64	54.9	42	72	8.7
Mass (g)	0.519	0.14	1.43	0.215	0.3	0.1	0.7	0.1	2.08	0.9	4.66	1.13
$\delta^{13}\text{C}$	-19.35	-23.59	-17.41	1.074	-20.29	-22.59	-17.95	1.184	-19.53	-25.45	-16.37	2.531
$\delta^{15}\text{N}$	13.49	10.4	15.99	1.169	12.5	11.19	14.59	0.902	12.52	9.92	14.18	1.284
%C	45.7	41.84	52.57	2.185	45.8	41.91	49.79	1.957	44.22	42.18	46.7	1.36
%N	13.29	11.65	15.79	0.71	13.48	11.92	14.29	0.58	13.65	12.77	14.62	0.513
C/N	3.45	3.18	3.89	0.171	3.4	3.18	3.73	0.133	3.24	3.13	3.44	0.071

Table 2. Observed values, mean and standard deviations for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C, %N, and C:N ratio for 13 species of supra-littoral vegetation and six species of marine algae used to calculate the basal resources for the two-source mixing model

Source	Common name	Latin name	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	%C	%N	C:N
Vegetation	Red Alder	<i>Alnus rubra</i> (Bong)	-28.78	-0.72	51.60	2.62	19.71
Vegetation	Salmonberry	<i>Rubus spectabilis</i> (Pursh)	-30.44	-0.50	47.07	2.10	22.38
Vegetation	Nootka Rose	<i>Rosa nutkana</i> (K. Presl)	-27.77	-0.09	49.42	3.01	16.40
Vegetation	Grass	Poaceae	-30.04	0.64	47.84	2.41	19.87
Vegetation	Beach Pea	<i>Lathyrus japonicus</i> (Willd)	-28.23	-0.44	48.06	6.62	7.25
Vegetation	Bracket Fungus	<i>Basidiomycetes</i> sp.	-22.71	-4.20	49.23	0.88	55.83
Vegetation	Western Red Cedar	<i>Thuja plicata</i> (Donn)	-26.28	-3.53	50.71	1.04	48.76
Vegetation	Salal	<i>Gaultheria shallon</i> (Pursh)	-28.46	-3.16	48.29	1.12	42.94
Vegetation	Sitka Spruce	<i>Picea sitchensis</i> (Bong)	-28.21	-1.81	51.42	1.41	36.53
Vegetation	Hairy Cat's Ear	<i>Hypochaeris radicata</i> (L.)	-31.69	-0.97	44.61	2.82	15.80
Vegetation	Black Twinberry	<i>Lonicera involucrata</i> (Banks ex. Spreng)	-27.37	-2.84	50.07	2.69	18.65
Vegetation	Blueberry	<i>Vaccinium</i> spp. (L.)	-31.81	-4.27	51.29	1.69	30.30
Vegetation	Moss	Bryophyta	-26.62	-0.34	13.40	0.46	29.20
		Mean	-28.34	-1.71	46.39	2.22	27.97
		SD	2.34	1.62	9.71	1.50	13.80
Algae	Japanese Weed	<i>Sargassum muticum</i> (Fensholt)	-14.64	2.83	42.37	3.22	13.17
Algae	Bleach Weed	<i>Prionitis lanceolatus</i> (Harvey)	-16.88	6.39	36.16	2.94	12.32
Algae	Black Tassel	<i>Pterosiphonia bipinnata</i> ((Postels & Rupr.) Falken b)	-19.49	4.67	40.34	4.82	8.38
Algae	Tangle	<i>Laminaria</i> spp. (L.)	-10.83	6.84	35.07	1.51	23.25
Algae	Green Tuft	<i>Cladophora microcladioides</i> (Collins)	-18.05	5.06	32.16	3.22	9.99
Algae	Rock Weed	<i>Fucus gardneri</i> (L.)	-16.12	4.47	45.10	1.56	28.82
		Mean	-16.00	5.04	38.53	2.88	15.99
		SD	3.025	1.44	4.89	1.23	8.16

$\delta^{15}\text{N}$ between individual and combined fish samples ($P = 0.527$), while $\delta^{13}\text{C}$ was more enriched in individual fish samples (-19.24) than in combined fish samples (-20.5, $F_{1,29} = 5.463$, $P = 0.027$). There were no differences in the variance for pink salmon between individual and combined fish samples for either $\delta^{13}\text{C}$ ($P = 0.485$) or $\delta^{15}\text{N}$ ($P = 0.098$).

SIA values of salmon fry

Fork length (mm) and wet mass (g) were greatest in Chinook salmon followed by chum and pink salmon (Table 1). Fork length and wet mass were significantly

higher in Chinook than in chum or pink salmon ($P > 0.001$). There were no differences in fork length or wet mass between pink and chum salmon ($P < 0.05$). Across all three species, fork length was unrelated to $\delta^{13}\text{C}$ ($r = -0.13$, $P = 0.497$) and only marginally related to $\delta^{15}\text{N}$ ($r = -0.332$, $P = 0.052$) for SIA analysis of individual fish. There was no significant relationship between either $\delta^{13}\text{C}$ ($P = 0.305$, $n = 5$) or $\delta^{15}\text{N}$ ($P = 0.862$, $n = 5$) and fork length for pink salmon (Fig. 1a, b). Similarly, there was no significant relationship between either $\delta^{13}\text{C}$ ($P = 0.473$, $n = 17$) or $\delta^{15}\text{N}$ ($P = 0.167$, $n = 17$) and fork length for Chinook salmon (Fig. 1a, b). For chum salmon, there

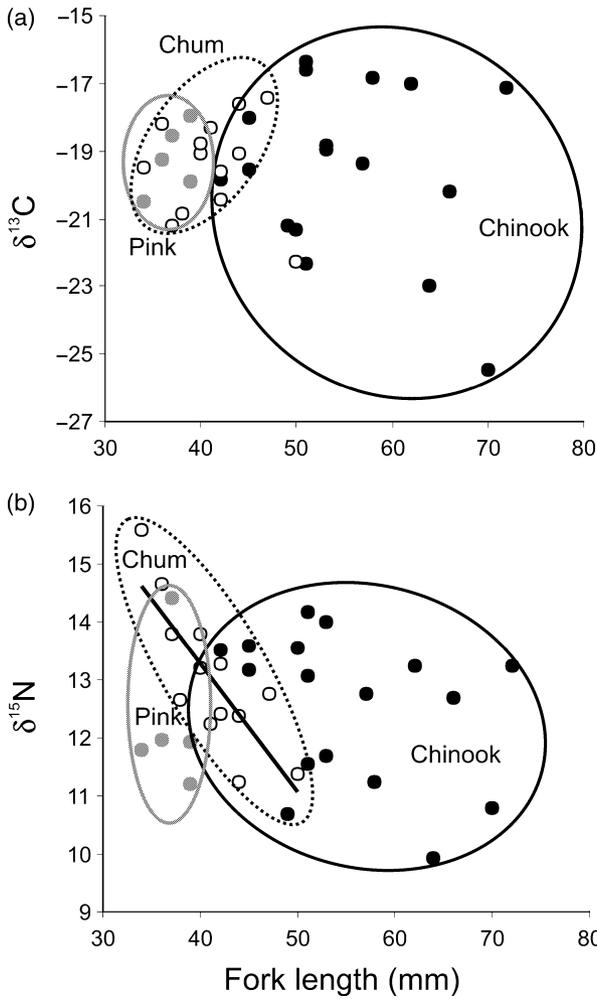


Figure 1. Relationship between fork length (mm) and (A) $\delta^{13}\text{C}$ and (B) $\delta^{15}\text{N}$ for (a) chum (white circles and hatched line showing the slope of the regression in panel B), (b) pink (grey circles), and (c) Chinook (black circles).

was no significant relationship between $\delta^{13}\text{C}$ and fork length ($P = 0.881$, $n = 13$; Fig. 1a), and a significant negative relationship between $\delta^{15}\text{N}$ ($r = -0.81$, $P < 0.001$, $n = 13$; Fig. 1b).

There was a significant difference in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for the three species of salmon fry ($\delta^{13}\text{C}$ $F_{2,130} = 5.31$, $P = 0.006$; $\delta^{15}\text{N}$ $F_{2,130} = 11.52$, $P < 0.001$; Table 1). *Post hoc* analyses showed that $\delta^{13}\text{C}$ was only significantly more enriched in chum than in pink salmon ($P = 0.003$) and that chum salmon had higher $\delta^{15}\text{N}$ values than both Chinook ($P = 0.003$) and pink salmon ($P < 0.001$). Across all the species there was a positive correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($r = 0.41$, $P < 0.001$; Fig. 2). There was no significant correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for pink salmon ($r = 0.131$, $P = 0.491$; Fig. 3). By contrast, there was a

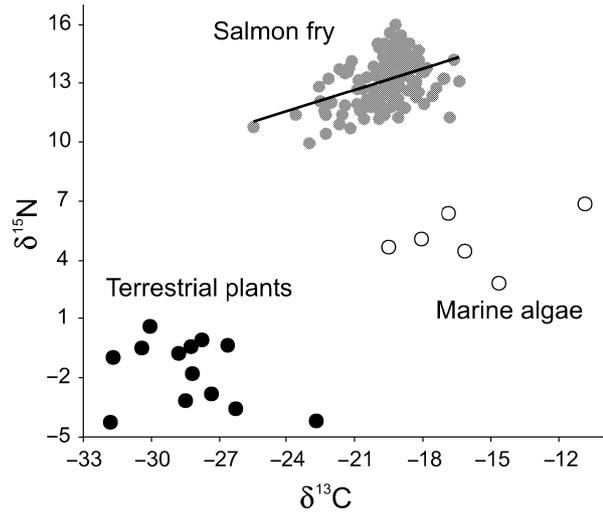


Figure 2. Correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across all three species of salmon fry (grey circles), for six species of marine algae (open circles), and 13 species of terrestrial supra-littoral vegetation (black circles). The regression line is for all three species of salmon fry.

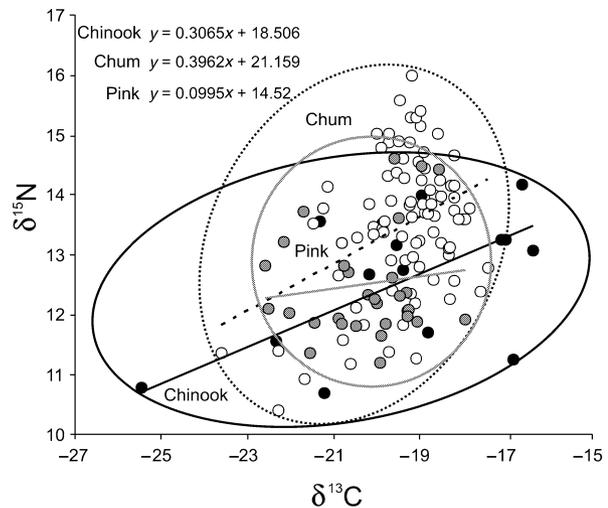


Figure 3. Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for (a) chum salmon (white circles), (b) pink salmon (grey circles) and (c) Chinook salmon (black circles).

weak positive correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for chum salmon ($r = 0.364$, $P = 0.001$; Fig. 3) and a stronger positive correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for Chinook salmon ($r = 0.604$, $P = 0.01$; Fig. 3).

Trophic position of salmon fry

Chum salmon had a significantly higher mean trophic position (mean 3.48 ± 0.344 SD) than either Chinook (mean 3.20 ± 0.377 SD, $P < 0.003$) or pink salmon

(mean 3.19 ± 0.326 SD, $P < 0.001$; Fig. 3). There was no significant difference in variance in trophic position between chum and either pink ($P = 0.06$) or Chinook salmon ($P = 0.276$). However, Chinook had a higher variance in trophic position than pink salmon ($P = 0.047$).

TDC and MDC in salmon fry

Algae exhibited higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values relative to terrestrial vegetation (Table 2) with no overlap in either $\delta^{13}\text{C}$ (mean -28.34 ± 2.43 SD) or $\delta^{15}\text{N}$ (mean -16.0 ± 3.02 SD; Fig. 2).

Chum salmon had significantly higher mean proportion of TDC in their tissue (mean $28.5 \pm 4\%$ SD) than pink salmon (mean $24.9 \pm 4.4\%$ SD, $P = 0.003$) but not Chinook salmon (mean $27.8 \pm 9.5\%$ SD, $P = 0.879$; Fig. 4). There was no difference in mean TDC between Chinook and pink salmon ($P = 0.154$). Chum salmon had higher variance in TDC relative to pink

salmon ($P = 0.007$). There was no difference in the variance in TDC between chum and Chinook salmon ($P = 0.757$) or between Chinook and pink salmon ($P = 0.159$). Carbon in chum salmon was strongly skewed (skewness = 1.315) towards TDC relative to Chinook salmon (skewness = 0.751) or pink (skewness = 0.318).

Discussion

Chum, Chinook, and pink salmon had similar nitrogen and carbon sources, as shown by the overlap in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between the three species, but the relative importance of terrestrial carbon was greater for chum salmon than the other two species. Previous SIA studies on adult Pacific salmonids living offshore in the northeast Pacific indicated two distinct feeding groups with a similarity in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between coho and Chinook salmon, and between pink, sockeye, and chum salmon (Welch & Parsons 1993). In this study, SIA results for chum, Chinook, and pink salmon had high overlap in the sources of carbon and nitrogen, but despite this overlap, the three species showed differences in trophic position.

Stable isotope studies of marine food webs, including fish, suggest that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ should increase at each trophic position at a ratio of approximately 3.4:1 (Rau 1980; Wada, Terazaki, Kabaya & Nemoto 1987). Satterfield & Finney (2002) suggested that slopes different from 3.4:1 indicated resource partitioning. In this study, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were correlated across all three species, but the slope of the regression line was 0.417 ± 0.08 , which is lower than reported by Welch & Parsons (1993) for all five species of Pacific salmon rearing offshore in the northeast Pacific (0.83 ± 0.29), and lower than reported by Satterfield & Finney (2002) for mature Pacific salmon re-entering fresh water to spawn (1.14 ± 0.08). Satterfield & Finney (2002) suggested that for mature salmon re-entering fresh water to spawn, slopes lower than 3.4:1 may have been due to either feeding at different water depths or in different regions. However, for salmon fry caught adjacent to supra-littoral beach habitat at high tide, differences in slope are unlikely to be due to feeding at different water depths or regions because the fry are adapted to use shallow nearshore habitats, usually < 1 m deep, where they apparently are able to maximise food intake and minimise predation risk (Levings 1994). The lower slope of the $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ relationships observed in this study was probably because of differences in the relative proportions of TDC and MDC across species as the slope of the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was not consistent with a trophic response.

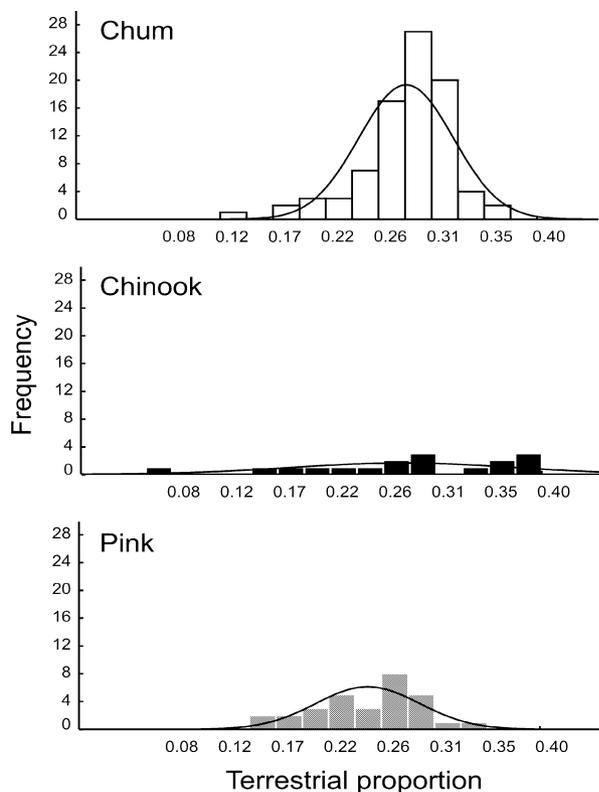


Figure 4. Histograms showing the proportions of terrestrial-derived carbon (TDC) for: (a) chum salmon (open bars); (b) Chinook salmon (black bars) and (c) pink salmon (grey bars). The lines are fitted normal distributions. The y -axis represents the number of occurrences of the value of TDC for individual fish. The x -axis represents the actual proportions of TDC.

Trophic position was significantly higher for chum than for both Chinook and pink salmon. The high trophic position of chum corroborates findings inferred from SIA by Welch & Parsons (1993) that immature chum caught in the ocean feed on prey higher in the food web, such as carnivorous, gelatinous zooplankton, than coho, sockeye, or pink salmon. In this study the high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for chum, pink, and Chinook salmon suggest that salmon fry from inshore waters may also be feeding on carnivorous prey. However, a wider range in $\delta^{15}\text{N}$ values for chum salmon was observed than for juvenile salmon offshore, implying that in inshore waters chum salmon are feeding on species at both lower and higher trophic positions, whereas in the ocean, chum salmon specialise in prey fairly high in trophic position (Welch & Parsons 1993).

Stable isotope analysis has previously been used to assess the relative proportions of allochthonous and autochthonous inputs to juvenile salmonids in streams (e.g. Doucett, Power, Barton, Drimmie & Cunjak 1996; Perry, Bradford & Grout 2003). Juvenile salmon in streams derive a large proportion of their carbon from allochthonous sources, in many cases >50% of their energy intake is from terrestrial sources (Hunt 1975; Manson & MacDonald 1982). Allochthonous inputs from insects and spiders dropping into streams have been shown to exceed production of *in situ* aquatic insects (Polis, Anderson & Holt 1997). For example, Perry *et al.* (2003) reported that juvenile Chinook salmon derived between 59 and 100% of the carbon from terrestrial sources. Wipfli (1997) showed that terrestrial invertebrates constituted ~50% of the diet of coho salmon, *O. kisutch*, cutthroat trout, *Oncorhynchus clarki* (Richardson), and dolly varden, *Salvelinus malma* (Jordan & Evermann), in streams in Alaska.

While it is possible that some of the TDC in salmon fry observed in this study was a result of freshwater stream production, it is likely that most of the TDC was derived from marine supra-littoral vegetation rather than freshwater output. First, Romanuk & Levings (2003) showed that terrestrial invertebrate drop into marine near-shore habitats, such as Furry Creek, are likely derived from supra-littoral vegetation not freshwater sources. Secondly, T.N. Romanuk & C.D. Levings (unpublished data) showed that the proportion of TDC in salmon fry does not change predictably along the freshwater to marine gradient in estuaries in this region.

Histogram analysis of TDC for chum, Chinook and pink salmon showed that while on average there is no significant difference between chum and Chinook

salmon for TDC, TDC is more frequently higher for chum salmon with a distribution skewed towards TDC. By contrast, Chinook salmon appear to be the most opportunistic feeders of the three species with a wide range in TDC values and pink appear to rely on a diet with higher proportions of MDC (Fig. 4).

Differences in carbon source partitioning and trophic position for Chinook, chum and pink salmon can also be seen by considering differences between species in the variance of carbon and nitrogen values. Differences in the variances of TDC and trophic position between species suggested that chum salmon fed on prey with a wider range of carbon sources than pink salmon, and that Chinook salmon feed on prey with a greater range in trophic position than pink salmon. To determine whether these differences were due in part to using combined fish samples for SIA for chum and pink salmon but not for Chinook salmon, the variances of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between individual and combined fish samples for chum and pink salmon were also investigated. Variance in $\delta^{15}\text{N}$ was not different for either chum or pink salmon between individual fish and combined fish samples, thus using individual and combined fish samples should not have affected the relative trophic position. Variance in $\delta^{13}\text{C}$ was higher in the individual samples for chum salmon but not pink salmon, however, there was no difference in mean TDC between combined ($28.6 \pm 3.8\%$ SD, $n = 73$) and individual ($28.3 \pm 5.5\%$ SD, $n = 13$) fish samples, suggesting that while mean TDC was likely unaffected by combining samples, the range of TDC for chum salmon may have been slightly underestimated in estuaries and near-shore habitats.

Terrestrial organisms constituted a significant proportion of the diet of juvenile salmonids in estuaries and nearshore marine habitats (e.g. Healey 1980; Sobocinski, Cordell & Simenstad 2004). Some authors suggested that the allochthonous components of the diet originated from wind-borne dispersal and riverine drift sources (Busby & Barnhart 1995). By contrast, Healey (1980) reported that adult insects accounted for 11–15% of the gut contents of pink and 7–8% of chum salmon (by volume) for fry caught in a fully marine site in the Strait of Georgia which was not influenced by the Fraser plume or any other estuary; these insects were also likely wind-borne from supra-littoral habitats. Naiman & Sibert (1979) suggested that invertebrates from allochthonous sources are eaten by juvenile salmonids in estuaries. However, as in this study, the ultimate source of these insects is unknown. More recently, Sobocinski *et al.* (2004) showed that in the gut contents of juvenile Chinook salmon caught in marine near-shore habitats in Puget Sound, Washing-

ton, terrestrial or riparian insects were the dominant prey items numerically, constituting ~50% of individuals and ~15% of the volume, and Sobocinski (2003) showed that these insects originated from vegetated near-shore habitats.

These results could have important implications for management of salmon populations in near-shore marine habitats. Evidence of carbon source partitioning suggests that the diet of the three species is composed of differing proportions of MDC and TDC. Romanuk & Levings (2003) previously showed that removal of supra-littoral vegetation has numerous effects on both terrestrial and marine supra-littoral arthropod assemblages in marine near-shore habitats. In particular, sites without vegetation had significantly fewer species and substantially lower abundances of both terrestrial and aquatic arthropods. Removal of supra-littoral vegetation could have cascading effects on juvenile salmon by altering the proportions of allochthonous and autochthonous carbon inputs and the total available carbon. However, the amount of TDC from supra-littoral vegetation relative to TDC provided by rivers and estuaries connected with a particular water body needs to be determined before the significance of the effect can be assessed. Further comprehensive data on feeding habits of juvenile salmon in the coastal zone, including detailed identification of their insect prey, are required to answer these questions.

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