

Multiple pathways of C and N incorporation by consumers across an experimental gradient of salmon carcasses

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Abstract. Numerous studies link resource subsidies to consumers, yet manipulations of subsidy gradients are rare, limiting our ability to predict their effects. We measured natural abundance levels of the stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in consumers representing two taxonomic (invertebrates, fish) and multiple trophic groups (primary, secondary, and tertiary consumers) exposed to a gradient of Pacific salmon carcasses (0–4 kg/m², eight treatment levels) in 16 experimental streams from September to December. We hypothesized (1) that consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values would increase with carcass loading and (2) that these relationships would vary among consumers and trophic levels. Within taxonomic groups, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values increased with carcass loading indicating direct consumption of salmon organic matter ($\delta^{13}\text{C}$ increase) and indirectly by consumption of salmon-enriched prey ($\delta^{15}\text{N}$ increase). The relative importance of these two pathways for invertebrates primary consumers varied with carcass loading, with an abrupt increase in direct consumption above ~0.5 kg/m² salmon carcass wet mass. Both the rates of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ increase and the shape of the response to the carcass gradient varied among invertebrates. The only fish that responded positively to carcasses was a small benthic, secondary consumer, and only for $\delta^{13}\text{C}$. Overall, benthic invertebrate primary consumers were the dominant pathway by which salmon organic matter entered the trophic system, followed by benthic secondary consumers, and then tertiary consumers (juvenile coho salmon and cutthroat trout) that occupy the water column. The attenuating response to subsidies at higher trophic levels was possibly due to a combination of low decomposition and metabolic rates during fall and winter, composition of the subsidy, and habitat use. Our results demonstrate that decomposing salmon carcasses provide variable but potentially important sources of C (range: ~0–60%) and N (range: ~0–35%) supporting benthic consumers during fall and winter, when environmental conditions constrain primary productivity, with some evidence of saturation at carcass loadings >0.1–0.6 kg/m². Furthermore, stable isotope analysis showed that consumers exploited salmon organic matter in different ways, potentially alleviating competition and allowing for a more efficient processing of this resource subsidy.

Key words: assimilation; decomposition; feeding guild; fish; invertebrates; N and C stable isotopes; Pacific salmon carcasses; resource subsidy; salmon organic matter; streams; trophic level.

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INTRODUCTION

The importance of resource subsidies is evident across a range of ecosystem types, from grasslands enriched by excretions from migrating ungulates (Holdo et al. 2007) to freshwater streams with populations of semelparous, spawning anadromous Pacific salmon (*Oncorhynchus* spp.; Schindler et al. 2003). While there have been numerous observational studies on resource subsidy gradients (Hocking and Reimchen 2009), studies where the resource subsidy was manipulated have largely been binary (subsidies present vs. absent; except see Yang 2006, Nowlin et al. 2007, Klemmer and Richardson 2013). For instance, experiments that manipulate adult Pacific salmon carcasses—the focus of our report—as a resource gradient are rare (except see Wipfli et al. 1999), especially in fall/winter when many semelparous salmon populations reproduce (Quinn 2005).

Gradient experiments are important in advancing our understanding of resource subsidies as they provide insight into the form and magnitude of the relationship between the response and independent variable (Cottingham et al. 2005, Klemmer and Richardson 2013). Quantifying the form and magnitude of the ecological response to subsidies can inform on potential conservation or restoration targets, such as population abundance, for species like Pacific salmon (Gende et al. 2002) that deliver subsidies by providing parameter estimates that represent reference points; for example, subsidy amount that leads to a 50% change in a response of interest. Furthermore, the relative importance of energetic pathways from subsidy to consumer—direct vs. indirect consumption via eating enriched prey—may vary with subsidy amount (Richardson and Wipfli 2016), providing insight into key energy pathways supporting consumers.

How resource subsidy gradients influence the recipient food web depends on a variety of factors including biotic characteristics of the consumer (Vanni 2002, Marcarelli et al. 2011, Warry et al. 2016). A consumer's response may depend on trophic level, with effects attenuating at higher trophic levels because higher-level consumers can access multiple resource pools thereby diluting their response to subsidies

(Yang 2006, Marczak et al. 2007, Boecklen et al. 2011). Within a trophic level, variation in mouth size or morphology, feeding mode (Cummins and Klug 1979), and habitat use (Marczak et al. 2007) may influence how consumers respond to resource subsidies. Small primary consumers may be constrained by mouth size to feeding on prey enriched by inorganic nutrients released during decomposition of the subsidy, such as stream biofilm, while carnivores may be large enough to feed on subsidies directly (Bilby et al. 1996). Investigating how different consumers and trophic levels respond to resource subsidy gradients can provide insight into how resources are exploited by recipient food webs and the ecological consequences of this exploitation (Huxel et al. 2002).

Our present understanding of how consumers exploit resource subsidies is partly based on stable isotope analysis (SIA), which measures natural abundance levels of stable isotopes, such as nitrogen ($^{15}\text{N}/^{14}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$), in key resource pools (e.g., salmon carcasses) and focal consumers (Kline et al. 1990, Fry 2006). If key assumptions are addressed, including isotopic equilibrium of consumer and resource pools that are isotopically distinct, this approach delivers a time-integrated measure of all trophic pathways and interactions that lead from resources to consumers providing insight into an organism's isotopic or trophic niche (Bearhop et al. 2004, Layman et al. 2007, Jackson et al. 2011). A consumer's isotopic niche—represented by the multivariate distribution of delta values (e.g., $\delta^{15}\text{N}$, units in ‰ or per mil) with higher values indicating enrichment in the heavier isotope—might also change rapidly to resource availability, thus revealing how consumers exploit changes in subsidy inputs (Warry et al. 2016). Stable isotope analysis can also provide understanding into the different pathways (direct vs. indirect) from resource subsidies to consumers (Kline et al. 2007). In our study system, increases in consumer ^{13}C with adult salmon carcass inputs indicate assimilation by direct consumption of eggs or flesh as consumer carbon stable isotopes change relatively little with each trophic transfer ($\sim 0.5\text{--}1.0\text{‰}$). Whereas increases in only consumer ^{15}N content with carcass biomass would indicate assimilation of salmon organic matter indirectly when carcass nutrients return

to the inorganic state during decomposition and are taken up by primary producers which are then eaten by primary consumers (Kline et al. 2007).

While SIA has been applied extensively to the study of resource subsidies (Hocking and Reimchen 2002), especially subsidies delivered to terrestrial and aquatic systems via inputs of marine organic matter (Chaloner et al. 2002, Spiller et al. 2010), experiments using this approach are rare. This lack of control leads to some uncertainty with regard to interpretation of field studies that measure natural abundance levels of stable isotopes in different media (e.g., soils, plants) because of confounding effects common in observational studies (Gannes et al. 1997, Pináy et al. 2003, Reisinger et al. 2013). As an example, high rates of denitrification in riparian soils can result in ^{15}N signatures in biota that mimic enrichment by spawning populations of Pacific salmon (Pináy et al. 2003).

Although spawning anadromous fish have complex ecological effects on natal streams and adjacent riparian areas, principally as agents of disturbance and resource subsidies (Gende et al. 2002, Schindler et al. 2003, Tiegs et al. 2009), we focus on how adult salmon carcasses are incorporated into stream food webs for the following reasons. First, active enrichment (i.e., inorganic nutrients, commercial fertilizers, salmon carcasses) of nutrient-poor freshwater systems has been used in a management context under the assumption it alleviates food limitation for focal fish populations (Kiffney et al. 2005, Collins et al. 2015). Yet, experiments establishing nutrient/carcass loading–response relationships are rare, though essential to inform on the consequences of active enrichment as a management tool to enhance fish productivity (Shaff and Compton 2009, Richardson and Wipfli 2016). Second, the decomposition phase is proportionally a larger component of total C and N inputs provided by spawning adult salmon relative to inputs from excretion and gametes (Johnston et al. 2004). Third, carcasses may be a particularly significant source of organic matter for consumer in late fall and winter relative to primary producers, whose growth is constrained by abiotic conditions (light, temperature, flow), and detrital inputs, which are less nutrient- and energy-rich than salmon (Volk et al. 2003). Finally, identifying factors

that modify how carcasses are incorporated into recipient food webs is of general interest, as a variety of animals, ranging from insects (Hoekman et al. 2012) to whales (Roman et al. 2014), provide ephemeral, high-quality organic matter subsidizing recipient ecosystems (Parmenter and MacMahon 2009).

We apply SIA to quantify the relationship between a resource gradient represented by decomposing Pacific salmon carcasses and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of multiple consumers representing two taxonomic groups and multiple trophic levels in 16 streamside mesocosms. We predicted that (1) consumer enrichment in ^{13}C and ^{15}N would be positively (asymptotically or linearly) associated with carcass loading because these elements limit production and (2) these relationships would vary with consumer and trophic level because of differences in feeding ecology and habitat use that affect exploitation of resource subsidies. Specifically, we predicted that benthic primary consumers would exhibit the strongest dependence with carcass loading, followed by secondary, and then tertiary consumers (Marczak et al. 2007).

MATERIALS AND METHODS

Experimental set-up and focal consumers

To quantify the assimilation of decomposing salmon carcasses by stream consumers, we conducted a 90-d experiment between September and December 2005 in 16 flow-through mesocosms (~4.8 m long \times 0.3 m wide \times 0.2 m deep) located on a sunlit meadow adjacent to the Cedar River, Washington, USA. Channels were lined with natural river gravel (diameter: ~32–64 mm), while larger cobbles (~diameter: ~64–256 mm) and cinderblocks provided cover for fish. Benthic food webs, consisting of basal resources (algae, bacteria, particulate organic matter) and stream invertebrate assemblages, were established by diverting Cedar River water for 60-d into two head tanks, which then dispersed water to individual channels. Invertebrate colonization also likely occurred by winged aquatic adults; however, these inputs were not measured (Cram et al. 2011 for further details).

Mesocosm invertebrate assemblages at the end of the colonization period were dominated by primary consumers that feed on biofilm and

particulate organic matter accumulating on organic and inorganic substrates including Chironomidae (Diptera), which comprised 60% of total invertebrate biomass, followed by mayflies (Ephemeroptera: Heptageniidae ~20% and Baetidae ~15%). Abundant invertebrate secondary consumers, that largely eat these primary consumers, included two stonefly families (Plecoptera: Perlidae and Perlodidae).

Following the 60-d colonization period, one secondary fish consumer (torrent sculpin, *Cottus rhotheus*, mean length = 77.3 mm) and two tertiary fish consumers (anadromous juvenile coho salmon, *Oncorhynchus kisutch*, mean length = 81.4 mm and resident juvenile coastal cutthroat trout, *Oncorhynchus clarkii clarkii*, mean length = 77.3 mm) were collected from Rock Creek, a nearby tributary. One similar-sized individual of each species was added to each channel to approximate natural densities in Rock Creek (Buehrens et al. 2014, Naman et al. 2014). Fish acclimated to channels for three days before starting the experiment. Appendix S1 provides additional details on the ecology of focal consumers.

While analyzing only one fish per species per channel limited precision and thus our ability to detect a carcass effect, adding more fish would have produced densities at the upper range of natural values, especially for salmonids (Pess et al. 2011, Buehrens et al. 2014), likely increasing competition for resources (Kiffney et al. 2014).

Experimental conditions

The experiment started when carcass material was added to randomly selected channels using a regression design and the following loading densities: 0, 0.001, 0.01, 0.1, 0.5, 1.0, 2.0, and 4.0 kg/m² wet mass ($n = 2$ per treatment). A regression design was used, where replication was sacrificed to increase the range of treatment values, so that we could model the form of the relationship between consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and salmon carcass density, including identifying thresholds, using as few parameters as possible (Cottingham et al. 2005).

Carcass treatment levels were based on salmon spawning densities in the Cedar River above Landsburg Dam between 2003 and 2005, where salmon were excluded for almost 100 yr from historic habitat until construction of a fish ladder

in 2003 (Burton et al. 2013); estimated historic levels returning to coastal streams of the Pacific Northwest (Gresh et al. 2000); and levels reported in other salmon subsidy studies (Bilby et al. 1996, 2001, Johnston et al. 1997, Wipfli et al. 1999, Claeson et al. 2006). The low range of carcass loading (0.001–0.1 kg/m² wet mass) represents values associated with small salmon populations, which are now common across large portions of their range (Gresh et al. 2000). Unfortunately, the functional role of these small populations is poorly understood (Janetski et al. 2009). The highest carcass densities (2 and 4 kg/m²) correspond to large returns of spawning pink salmon in western Washington (*O. gorbuscha*; Michael 1991) and Alaska (Wipfli et al. 1999), and sockeye salmon in British Columbia (Johnston et al. 1997).

Carcass material was added incrementally to the upstream end of treatment channels over the 90-d period to reflect the natural spawn timing (mid-September to December) of sockeye salmon in the Cedar River below the dam with a peak in mid-October (Cram et al. 2011). Daily water temperature during the experiment ranged from 12.5°C in late September to 4.9°C in early December (mean = 8.7°C) while instantaneous flows were maintained at 4 L/s.

Sample collection, isotopic analysis, and key assumptions

Our SIA focused on numerically abundant consumers representing ecologically important components of the Cedar River food web (Cram et al. 2011, Kiffney et al. 2014), as well as different taxonomic, feeding, and trophic groups (Appendix S1: Table S1, Fig. S1). Specifically, immature stages of Chironomidae (chironomids), Baetidae (baetids), and Heptageniidae (heptageniids), and stoneflies were collected opportunistically from each channel in addition to colonization baskets buried within channel substrates (15 cm wide \times 15 cm long \times 5 cm deep; Cram et al. 2011). Replicate samples of primary consumers, each consisting of ~25–50 individuals, were pooled to achieve sufficient biomass for analysis. Because they were larger, each stonefly sample typically consisted of one to three individuals. Invertebrates were placed in clean river water for ~4 h to void their stomachs. Fish were collected by hand using aquaria nets

and euthanized using approved protocols. All organisms were stored in a -20°C freezer before sample processing. Fish dorsal muscle tissue and whole invertebrates were processed for SIA using protocols described by Reichert et al. (2008) and Kiffney et al. (2014).

Consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined using a Costech elemental analyzer attached to a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Cedar River and mesocosm samples were calibrated against internal laboratory standards (aspartic acid and ^{15}N -enriched histidine), which were analyzed at least 10 times throughout each sample set sequence. A National Institute of Standards and Technology Lake Superior Fish Tissue Standard Reference Material (NIST SRM 1946) was also analyzed after every 25 field samples as a control material to determine set-to-set reproducibility. For quality control, all standards and the reference material had standard deviations $<0.3\text{‰}$ for $\delta^{15}\text{N}$ and $<0.2\text{‰}$ for $\delta^{13}\text{C}$.

When using SIA to quantify feeding relationships, two assumptions to assess are that energy sources supporting consumers are isotopically distinct and that consumers are at isotopic equilibrium with these sources. To test for isotopic differences in putative energy sources (biofilm, leaf litter, sockeye carcasses) supporting consumers, biofilm growing on rocks and leaf detritus were collected from each channel along with consumers. However, these samples were either accidentally destroyed or isotopic values were reported as unreliable; therefore, we estimated differences in isotopic composition of biofilm growing on rocks ($n = 21$) and leaf detritus ($n = 9$) collected by hand from riffles in the Cedar River about 500 m upstream of Landsburg Dam and the water intake pipe feeding the mesocosms. Additionally, samples were collected between mid-September and mid-October from 2000 to 2004 (Kiffney et al. 2002), partly overlapping in time with the experiment. These samples were processed for isotopes in the same manner as invertebrates and fish from mesocosms. Stable N and C isotope values for adult sockeye salmon ($n = 4$) were from Johnson and Schindler (2009). Because of their marine origin and trophic position, our isotopic analysis indicates that adult sockeye salmon were highly enriched in ^{13}C and ^{15}N relative to Cedar River leaf litter and biofilm (Appendix S1).

Therefore, we assume these isotopic differences applied to our experimental food web. To estimate consumer isotopic equilibrium, we used a recent empirical model (Thomas and Crowther 2014). Estimates from this model show at the end of the 90-d study our focal consumers replaced 50% of their isotopes with the more enriched sources of N (^{15}N) and C (^{13}C) provided by decomposing salmon carcasses (Appendix S1: Table S1).

Statistical analysis

Univariate response of consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to carcass loading.—We used linear regression and an information-theoretic approach to assess the functional relationship between salmon carcass loading and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at the taxonomic (invertebrates and fish) and individual consumer levels.

At the taxonomic level, we examined the relative support for the full and all possible reduced models. The full model was as follows:

$$Y_{ijk} = \mu + \Delta_i + \alpha_j + \Delta\alpha_{ij} + \varepsilon_{ijk}$$

where μ is the mean intercept (value of the response at 0 kg/m^2 carcass loading), Δ_i is the deviation due to the i th carcass treatment, α_j is deviation from the mean intercept due to the j th consumer (differences in the intercept of the focal species), and $\Delta\alpha_{ij}$ is the deviation due to the interaction of taxonomic identity and carcass loading. Because body size can influence fish stable isotopic composition (Akin and Winemiller 2008), individual weight (g) was also included as a covariate in fish models.

At the level of individual consumer, exploratory plots indicated a positive, but saturating relationship between invertebrate $\delta^{15}\text{N}$ values and carcass loading. Therefore, we evaluated the relative support for both a linear and asymptotic function in predicting the relationship between carcass treatment and invertebrate $\delta^{15}\text{N}$ values; a pseudo-threshold model ($\log(\text{carcass biomass} + 1)$) was used to approximate an asymptotic function (Franklin et al. 2000).

Candidate models were ranked using Akaike's Information Criteria for small sample sizes (AIC_c) and ΔAIC_c ($\Delta_i = \text{AIC}_i - \text{AIC}_{\min}$). Our interpretation focused on models with strong-to-considerable support ($\Delta\text{AIC}_c < 4$; Burnham and Anderson 2002). To assess the strength of

evidence supporting a particular model relative to others, we used Akaike weights (ω_i), which represent the relative likelihood of each model in the model set, and coefficients of determination (R^2 values). For models at the taxonomic level, relative importance of predictor variables was determined by summing the Akaike weights of variables across all models where the variables occur. Model selection was conducted in R version 3.1.3 (R Development Core Team 2013) using the packages Multi-model inference (MuMIn; Barton 2013) and Model selection and multimodel inference based on (Q) AIC(c) (AICcmmodavg; Mazerolle 2015). To compare among consumers and trophic levels, linear regression was used to estimate coefficients and 95% confidence intervals for the relationship between carcass loading and consumer stable isotope values (Appendix S2).

We used the Michaelis–Menten model to represent the nonlinear relationship between invertebrate $\delta^{15}\text{N}$ and carcass loading mechanistically and obtain parameters potentially important in a management context (e.g., half-saturation constant). This model represents a number of biological processes that represent a saturating relationship (Holling 1959) and was parameterized to ease interpretation of the results and allow for a nonzero value of $\delta^{15}\text{N}$ at a loading of 0. The model was of the form:

$$\delta^{15}\text{N}_t = f(L_t) = \frac{N_{\text{inc}}(M - L_{50})L_t}{L_{50}M + (M - 2L_{50})L_t} + N_0$$

Here, N_0 is $\delta^{15}\text{N}$ when loading (L) = 0, N_{inc} is the increase in $\delta^{15}\text{N}$ when moving from a loading of 0 to the max loading ($M = 4.0 \text{ kg/m}^2$), and L_{50} and L_{75} are loadings at which 50% and 75% of this increase are achieved (Appendix S3).

A mass balance model was used to compare the dependence between the proportion of salmon-derived nitrogen and carbon (as % SD-N and SD-C) assimilated by each trophic level and carcass treatment using the following formula from Johnston et al. 1997:

$$\begin{aligned} \text{\%SD-N or C enrichment} \\ = (\delta X_{\text{st}} - \delta X_c) / ((\delta X_s + (\text{TL} \times \delta X_e)) - \delta X_c) \\ \times 100 \end{aligned}$$

where X refers to natural abundances of C or N stable isotopes; δX_{st} is the isotope value of the

organism in the salmon treatment; δX_c is the mean isotope value of the same organism in controls; δX_s is the isotope value for adult sockeye tissue; TL is the trophic level correction factor (one for primary consumers [baetids, heptageniids, and chironomids], two for stoneflies and sculpin, and three for cutthroat and coho); and δX_e is the trophic-level enrichment factor (McCutchan et al. 2003). Consumers in the two lowest carcass loading treatments (0.001 and 0.01 kg/m^2) were excluded from these calculations because their N and C isotopic values overlapped with controls. Simple linear regression was then used to describe the relationship between carcass loading and the percentage of SD-N and C assimilated by each trophic level. If curvature was apparent in this relationship, a quadratic term was also examined in terms of improving model fit; the quadratic term was included in the final model if it improved model fit $>10\%$. A mass balance model was used rather than a mixing model (e.g., SIAR; Parnell et al. 2010), because we were interested in separately quantifying C and N flows from salmon carcasses to consumers as opposed to integrating these elements to estimate contributions of diet sources.

Multivariate changes in consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with carcass loading.—We hypothesized a multivariate response would provide an understanding of energy flow from carcasses to consumers not detected when focusing on one element at a time (Jackson et al. 2011). Thus, we used permutational multivariate analysis of variance (PERMANOVA) to examine the change in primary consumer $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in response to carcass loading simultaneously (Anderson and Walsh 2013). We applied the PERMANOVA to primary consumers because they responded most strongly to carcass subsidies relative to other trophic groups. A similarity matrix based on a Euclidean distance measure was used to obtain P -values by 9999 random permutations of the observations, followed by treatment comparisons (Clarke and Gorley 2015).

Ecological consequences of salmon organic matter.—We used Pearson's correlation coefficient to test the hypothesis that increases in consumer N^{15} and C^{13} were positively associated with consumer biomass in colonization baskets (mg/basket). A positive correlation would indicate a growth

benefit from assimilation of salmon organic matter.

RESULTS

Univariate response of consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to carcass loading

Invertebrates.—The model with the most support predicting whole-body invertebrate $\delta^{13}\text{C}$ values included a positive effect of carcasses and consumer-level differences (Appendix S2: Table S1), with no support for an interaction. Relative variable importance values (0.99) indicated these covariates were of equal weight and together predicted almost 60% of the total variation in $\delta^{13}\text{C}$ invertebrate values.

For individual invertebrate consumers, the top-ranked models included a positive, linear relationship between $\delta^{13}\text{C}$ values and carcass loading for chironomids, heptageniids, and

baetids (Table 1, Fig. 1). Individual slope estimates were all ~ 0.3 , thus indicating comparable rates of C^{13} enrichment with carcass loading despite evidence these taxa relied on different C

Table 1. Top-ranked models ($\Delta\text{AIC}_c < 4$) predicting the relationship between Pacific salmon carcass loading (0–4 kg/m^2 , eight treatments, $n = 2$ per treatment) and invertebrate primary (Baetidae, Heptageniidae, Chironomidae) and secondary (Plecoptera) consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (permil or ‰).

Taxon	Covariates	$\Delta\text{AIC}_c^\dagger$	ω_i^\ddagger	R^2^\S
$\delta^{13}\text{C}$				
Baetidae	$\beta_0 + \beta_{\text{trt}}$	0	0.55	0.14
	β_0	0.4	0.45	
Chironomidae	$\beta_0 + \beta_{\text{trt}}$	0	0.95	0.41
Heptageniidae	$\beta_0 + \beta_{\text{trt}}$	0	0.87	0.32
	β_0	3.8	0.13	
Plecoptera	β_0	0	0.81	
	$\beta_0 + \beta_{\text{trt}}$	3.0	0.19	–0.05
$\delta^{15}\text{N}$				
Baetidae	$\beta_0 + \beta_{\log(\text{trt})}$	0	0.99	0.88
Chironomidae	$\beta_0 + \beta_{\log(\text{trt})}$	0	0.86	0.45
	$\beta_0 + \beta_{\text{trt}}$	3.98	0.12	0.28
Heptageniidae	$\beta_0 + \beta_{\log(\text{trt})}$	0	0.96	0.74
Plecoptera	$\beta_0 + \beta_{\text{trt}}$	0	0.59	0.33
	$\beta_0 + \beta_{\log(\text{trt})}$	1.1	0.35	0.33

Notes: Model covariates include the following: β_{trt} = linear carcass effect and $\beta_{\log(\text{trt})}$ = pseudo-threshold carcass effect ($\delta^{15}\text{N}$ values only). β_0 = intercept-only model. AIC_c , Akaike's Information Criteria for small sample sizes.

† ΔAIC_c represents the difference in AIC_c of a given model relative to the top-ranked model.

‡ ω_i represents Akaike weights or the relative likelihood of the model given the model set.

§ R^2 (adjusted) represents the coefficient of determination or how well the regression line approximates the real data.

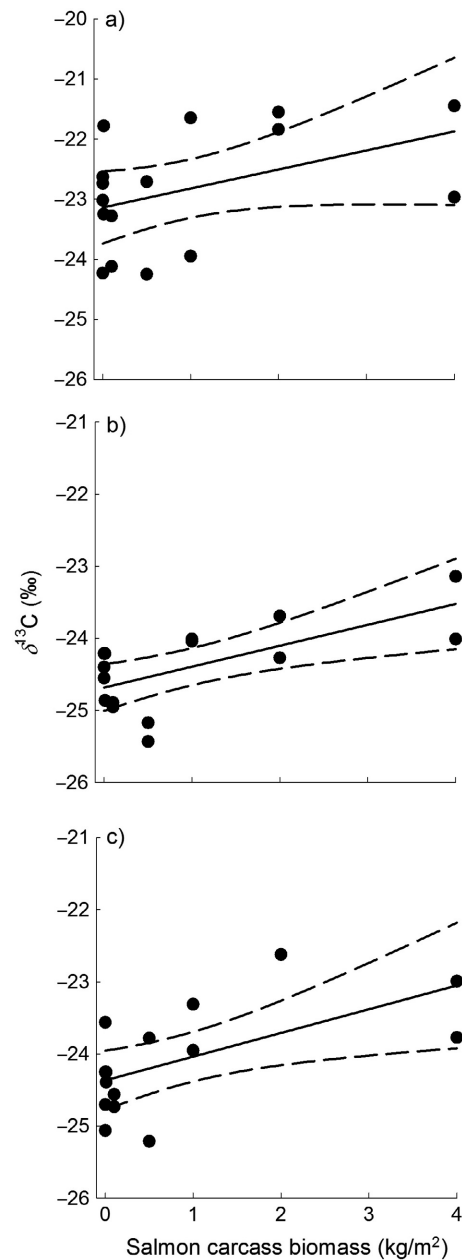


Fig. 1. Scatterplots and best-fit line (dashed lines represent 95% CI) describing the relationship between salmon carcass loading (0–4 kg/m^2 , eight treatments, $n = 2$ per treatment) and $\delta^{13}\text{C}$ values (in permil or ‰) of (a) Baetidae, (b) Chironomidae, and (c) Heptageniidae.

sources (Appendix S2: Table S2). In contrast, there was no evidence for a relationship between $\delta^{13}\text{C}$ values of stoneflies, a secondary consumer, and carcass loading. The fit of the relationship between primary consumer $\delta^{13}\text{C}$ and carcasses loading was weak-to-moderate and two to three times stronger for chironomids ($R^2 = 0.41$) and heptageniids ($R^2 = 0.32$) than for baetids ($R^2 = 0.14$).

The top-ranked model predicting invertebrate $\delta^{15}\text{N}$ values explained 69% of the total variation (Appendix S2: Table S1) and included a positive effect of carcasses, a consumer effect, and an interaction between carcasses and consumer. The interaction term indicates consumers varied in their rate of ^{15}N enrichment. Relative variable importance values showed that carcass loading (1.0), consumer (0.98), and the consumer \times carcass interaction (0.91) were of comparable significance in predicting invertebrate $\delta^{15}\text{N}$ values.

Individual invertebrate $\delta^{15}\text{N}$ values also increased with carcass loading, but the rates and shape of the relationship varied among taxa (Table 1; see Appendix S2: Table S3 for regression coefficients) consistent with the interaction term mentioned previously. Specifically, the top-ranked models indicated a linear increase in stonefly $\delta^{15}\text{N}$ values with carcass loading ($R^2 = 0.33$), while the pseudo-threshold model represented changes for primary consumers. Furthermore, the rate of mayfly ^{15}N enrichment was about 2.8–3 times higher than stoneflies and chironomids. The strength of evidence supporting the top models predicting invertebrate $\delta^{15}\text{N}$ values was demonstrated by their relatively high Akaike weights (0.86–0.99) and coefficients of determination ($R^2 = 0.45$ –0.86).

In support of the linear regression results, the Michaelis–Menten model indicated the relationship between primary consumer invertebrate $\delta^{15}\text{N}$ values and carcass loading was asymptotic for all taxa except stoneflies, with $\delta^{15}\text{N}$ values reaching half of the predicted maximum below loadings of 1.0 kg/m^2 (Figs. 2, 3). Values of $\delta^{15}\text{N}$ were comparable for all taxa at the maximum loading (4 kg/m^2), but Baetidae and Heptageniidae had much lower predicted values at zero loading (N_0) than the other two taxa, resulting in larger increases over the range of loadings, N_{inc} (Fig. 3).

Fish.—The top models predicting fish $\delta^{13}\text{C}$ included species, carcass loading, and fish size explaining between 35% and 50% of total model variation. The best model predicting fish $\delta^{13}\text{C}$ values was about 1.8 times more likely than the second-ranked model, and included a species effect, and positive effects of carcasses and fish size ($R^2 = 0.45$; Appendix S2: Table S1). Relative variable importance values indicated that species (0.99) was 1.3 times more important than carcasses (0.71) and 1.5 times more important than fish size (0.61).

Sculpin was the only fish species with a significant relationship between carcass loading and $\delta^{13}\text{C}$ (Table 2; Appendix S2: Table S2); however, the relatively low coefficient of determination for this model ($R^2 = 0.16$) demonstrated significant unexplained variation.

Table 2. Top-ranked models ($\Delta\text{AIC}_c < 4$) predicting the effects of Pacific salmon carcass loading ($0\text{--}4 \text{ kg/m}^2$, eight treatments, $n = 2$ per treatment) and individual weight (β_{wt} g) on secondary (sculpin) and tertiary (coho and cutthroat) fish consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (permil or ‰).

Taxon	Covariates	$\Delta\text{AIC}_c^\dagger$	ω_i^\ddagger	R^2^\S
$\delta^{13}\text{C}$				
Coho	β_0	0	0.617	
	$\beta_0 + \beta_{\text{trt}}$	3.07	0.133	−0.07
	$\beta_0 + \beta_{\text{wt}}$	3.10	0.131	−0.07
Cutthroat	$\beta_0 + \beta_{\text{wt}}$	0	0.56	0.22
	β_0	1.66	0.24	
Sculpin	$\beta_0 + \beta_{\text{trt}} + \beta_{\text{wt}}$	3.27	0.11	0.19
	$\beta_0 + \beta_{\text{trt}}$	0	0.40	0.16
	β_0	0.47	0.31	
	$\beta_0 + \beta_{\text{wt}}$	2.63	0.11	0
	$\beta_0 + \beta_{\text{trt}} + \beta_{\text{wt}}$	2.82	0.11	0.14
$\delta^{15}\text{N}$				
Coho	β_0	0	0.638	
	$\beta_0 + \beta_{\text{trt}}$	2.50	0.183	0.00
	$\beta_0 + \beta_{\text{wt}}$	3.27	0.120	0.00
Cutthroat	$\beta_0 + \beta_{\text{wt}}$	0	0.78	0.66
	$\beta_0 + \beta_{\text{trt}} + \beta_{\text{wt}}$	2.76	0.20	0.54
Sculpin	β_0	0	0.42	
	$\beta_0 + \beta_{\text{wt}}$	0.55	0.32	0.16
	$\beta_0 + \beta_{\text{trt}}$	2.04	0.15	0.07
	$\beta_0 + \beta_{\text{trt}} + \beta_{\text{wt}}$	3.28	0.08	0.22

Notes: β_0 = intercept-only model. AIC_c , Akaike's Information Criteria for small sample sizes.

† ΔAIC_c represents the difference in AIC_c of a given model relative to the top-ranked model.

‡ ω_i represents Akaike weights or the relative likelihood of the model given the model set.

§ R^2 (adjusted) represents the coefficient of determination or how well the regression line approximates the real data.

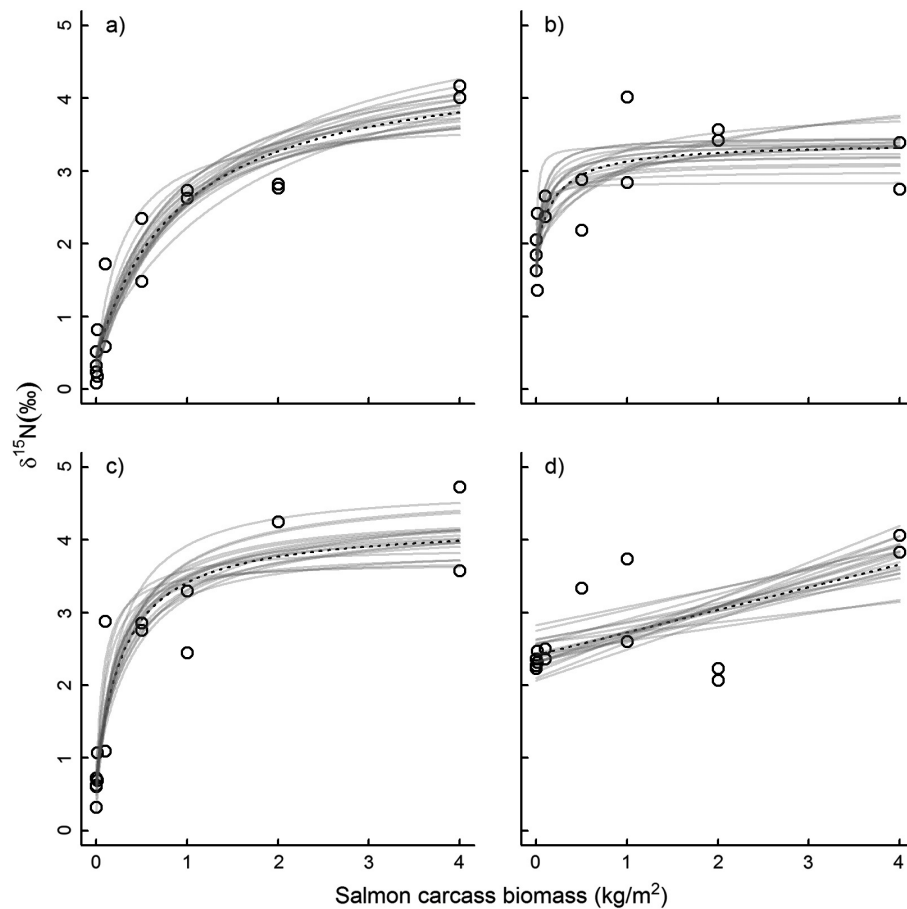


Fig. 2. The relationship between salmon carcass loading (0–4 kg/m², eight treatments, $n = 2$ per treatment) and invertebrate $\delta^{15}\text{N}$ values (in per mil or ‰) modeled using a Michaelis–Menten function (see Appendix S3 for details of model) with 20 curves drawn at random from the bootstrap distribution for (a) Baetidae, (b) Chironomidae, (c) Heptageniidae, and (d) Plecoptera.

There was little evidence that carcass loading influenced fish $\delta^{15}\text{N}$ values; the best model included species and body size as important covariates. This model was 3.7 times more likely than the second-best model while explaining similar amounts of variability (73%), but without a carcass loading term. Relative variable importance values for consumer identity (1.0) and fish weight (0.99) were approximately four times higher than for carcass treatment (0.25).

Body size (+effect) was the only covariate in the top-ranked model predicting cutthroat $\delta^{15}\text{N}$ values ($R^2 = 0.66$). This model was four times more likely than the second-ranked model, which also included body size and a positive effect of carcasses (Table 2; Appendix S2:

Table S2). The null model had the most support explaining coho and sculpin $\delta^{15}\text{N}$ ratios.

Mass balance model

On average, primary consumers (mayflies and chironomids) assimilated from ~2 to 3 times more SD-C (mean, range: ~15%, 0–60%) than secondary (~5%, 0–15%) and tertiary consumers (~7%, 0–30%). Primary consumers also incorporated from two to four times more SD-N (~12%, 0–35%) than both secondary (~6%, range = 0–11%) and tertiary consumers (~3%, 0–18%).

We observed a stronger dependence between carcass loading and the proportion of SD-C and N assimilated by primary consumer tissue

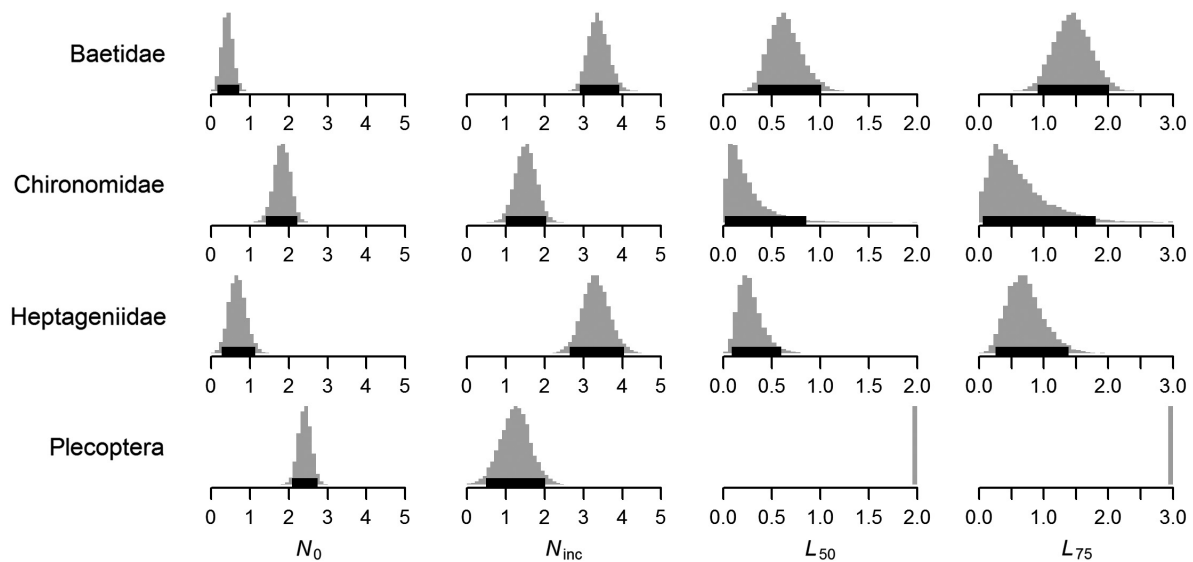


Fig. 3. Bootstrap distributions describing uncertainty in the parameters of the relationship between salmon carcass loading and $\delta^{15}\text{N}$. Black bars represent 95% confidence intervals. Here, N_0 is $\delta^{15}\text{N}$ when loading (L) = 0, N_{inc} is the increase in $\delta^{15}\text{N}$ when moving from a loading of 0 to the max loading ($M = 4.0 \text{ kg/m}^2$), and L_{50} and L_{75} are the loadings at which 50% and 75% of this increase are achieved (Appendix S3: Fig. S1). Note L_{50} and L_{75} values for stoneflies were at 2 and 3 kg/m^2 , indicating no evidence for a saturating relationship between $\delta^{15}\text{N}$ and carcass loading.

relative to higher trophic levels (Fig. 4a–f). Adding a quadratic term increased the coefficient of determination by $\sim 40\%$ for primary consumer SD-C ($R^2 = 0.97$) and 10% for SD-N ($R^2 = 0.98$), indicating a slowing in the rate of assimilation of salmon organic matter at high loadings. There were also positive, but highly variable linear relationships between carcass loading and the percentage of SD-C and N assimilated by secondary consumers and SD-C by tertiary consumers.

Multivariate changes in consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with carcass loading

When examining changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values simultaneously in response to carcass loading, the PERMANOVA revealed two distinct shifts in isotopic composition of the aggregate primary consumer response (pseudo- $F = 4.17$, $P < 0.001$; Fig. 5). The first shift occurred at low-to-moderate carcass levels (≥ 0.01 and ≤ 0.1 and 0.5 kg/m^2) where $\delta^{15}\text{N}$ increased by 100–130%, while $\delta^{13}\text{C}$ declined by about 2%. This first shift implies assimilation of salmon organic matter by primary consumers was predominantly indirect via consumption of enriched prey, because $\delta^{15}\text{N}$

increased without a concurrent increase in $\delta^{13}\text{C}$. In the second shift, occurring at loading densities > 0.1 and 0.5 kg/m^2 treatments, there were increases in both $\delta^{13}\text{C}$ ($\sim 5\text{--}7\%$) and $\delta^{15}\text{N}$ ($\sim 20\text{--}90\%$), indicating consumption of both salmon-enriched prey and salmon tissue directly.

Ecological consequences of salmon organic matter

Pearson's correlation coefficients for the association between $\delta^{13}\text{C}$ values and population biomass (mg/basket) of individual primary consumers were positive ranging from 0.34 to 0.60. Primary consumer biomass in colonization baskets was also positively correlated with whole-body $\delta^{15}\text{N}$ ratios ($r = 0.44\text{--}0.52$).

DISCUSSION

We found significant dependencies between consumer $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values and salmon carcass loading, but these relationships differed markedly within and among taxonomic and trophic groups. Specifically, the SIA showed consumers exploited salmon resource subsidies in dissimilar ways likely due to differences in feeding ecology, metabolic

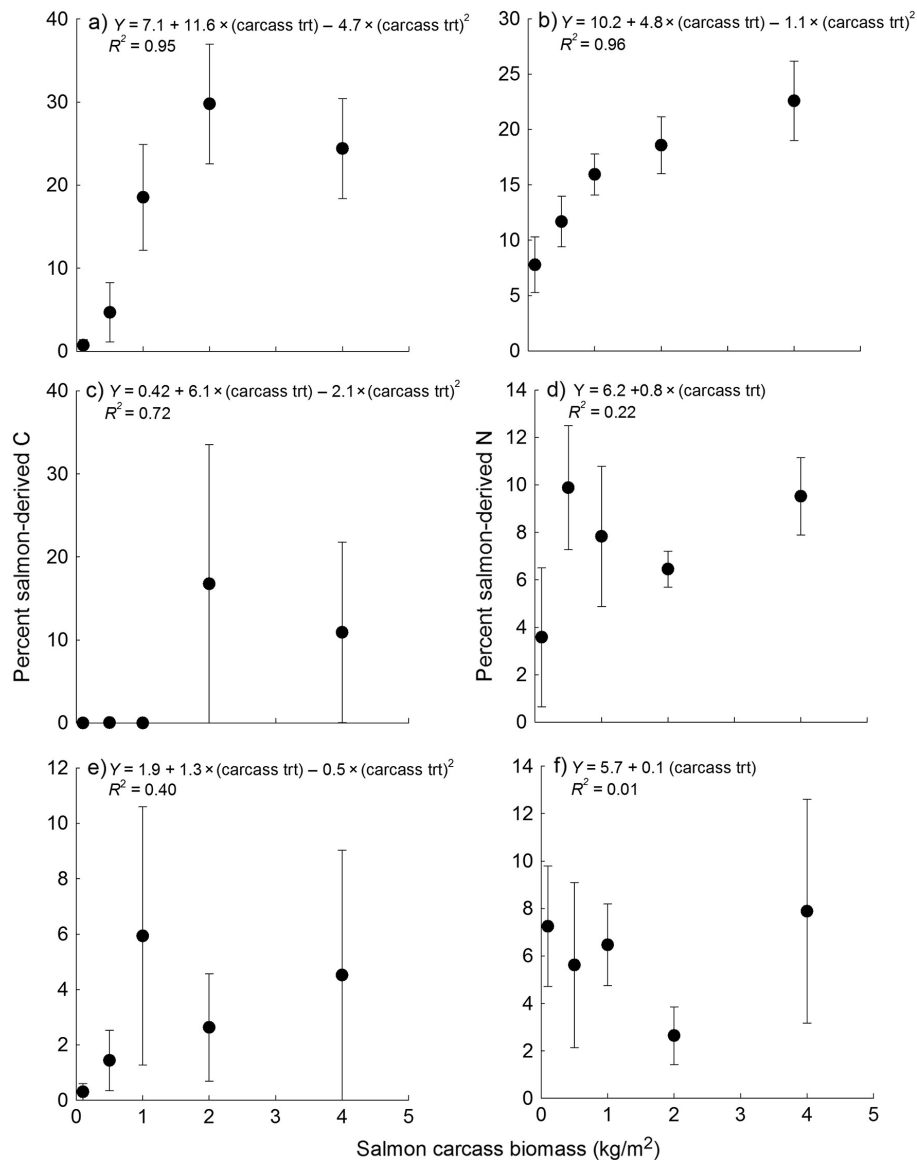


Fig. 4. Mean (± 1 standard error) percent (%) salmon-derived nitrogen (N) and carbon (C) incorporated by primary consumers (Baetidae, Heptageniidae, Chironomidae), panels (a, b); secondary consumers (Plecoptera, sculpin), panels (c, d); and tertiary consumers (juvenile coho and cutthroat trout), panels (e, f) as a function of salmon carcass loading (0.1–4 kg/m², five treatments, $n = 2$ –6 per treatment; treatments 0.001 and 0.01 kg/m² were excluded because their N and C isotopic values overlapped with controls).

demand, habitat use, and carcass density. Our results suggest resource subsidies provided by decomposing salmon carcasses can serve as important sources of C and N supporting stream consumers during fall and winter when other energy-rich food resources are low (stream biofilm) or are abundant but of relatively low nutritional quality (conifer needles; Volk et al. 2003).

Univariate response of consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to carcass loading

Supporting our first hypothesis, stream consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values increased with carcass loading, but the form and magnitude of this relationship varied within and among taxonomic and trophic groups. For example, there was strong support for an asymptotic relationship

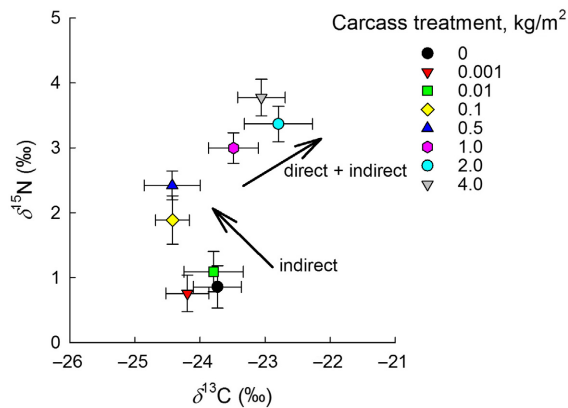


Fig. 5. Biplot (mean \pm 1 standard error) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (in per mil or ‰) for the aggregate primary consumer response (channel-level isotope values pooled across Baetidae, Heptageniidae, and Chironomidae samples) to the Pacific salmon carcass loading gradient. Arrows indicate shifts in isotopic space and assimilation of salmon organic matter via indirect (consuming an enriched food source, such as biofilm) and direct (consumption of salmon tissue) pathways.

between carcass loading and individual primary consumer $\delta^{15}\text{N}$ values (Fig. 2), results consistent with observational studies that applied SIA to examine assimilation of adult salmon inputs by riparian plants (Hocking and Reimchen 2009, Holtgrieve et al. 2010), terrestrial invertebrates (Hocking and Reimchen 2002), fish (Bilby et al. 2001, Swain et al. 2014), and crabs (Harding and Reynolds 2014a). Most of these studies (Bilby et al. 2001) did not explicitly test for a saturating relationship, however.

Based on the Michaelis–Menten model, the carcass loading where primary consumers achieved 50% saturation in ^{15}N ranged sixfold from ~ 0.1 to 0.6 kg/m^2 . A saturating relationship is noteworthy because it indicates a negative feedback between carcass loading and consumer enrichment in ^{15}N . We speculate three factors contributed to this asymptotic relationship: low water temperature, which regulates metabolism of ectothermic consumers as well as the decomposition process; invertebrates achieving dietary requirements with regard to N; and density-dependent processes (Bilby et al. 2001, Hocking and Reimchen 2009, Richardson and Wipfli 2016). Yet, asymptotic

relationships between adult salmon inputs and whole-tissue $\delta^{15}\text{N}$ values are not universal as demonstrated by stoneflies (linear relationship) in our study and select stream invertebrates and fish (linear or random) collected during observational studies that used SIA in Pacific Northwest streams (Johnston et al. 1997, Bilby et al. 2001, Rinella et al. 2013, Harding and Reynolds 2014b). Thus, the relationship between consumer ^{15}N and adult salmon inputs is generally positive; however, this relationship can also be asymptotic or random possibly due to differences in diet and metabolic demand for N.

Also supporting our first hypothesis, primary consumer $\delta^{13}\text{C}$ values increased linearly and at similar rates with carcass loading. A positive, linear relationship between consumer $\delta^{13}\text{C}$ values and carcass loading suggests that salmon-derived C was important in supporting benthic primary consumer production across the range of inputs, possibly because of the scarcity of other higher quality C pools during the study. Despite carbon's fundamental importance in supporting aquatic (Rüegg et al. 2011) and terrestrial food webs (Schmitz et al. 2014), quantifying the influence of resource subsidies on C budgets of aquatic consumer populations using SIA has received less attention than N (Rüegg et al. 2011, Reisinger et al. 2013).

Salmon carcasses potentially represent an important source of energy for stream food webs during fall and winter relative to spring and summer when growth and accumulation rates of stream biofilm in Pacific Northwest streams are higher (Kiffney et al. 2003). Consistent with this hypothesis, invertebrate and fish consumer $\delta^{13}\text{C}$ ratios showed no relationship with salmon density in British Columbia streams with natural salmon runs during summer (Johnston et al. 2004). These authors speculated the lack of consumer dependence on salmon-derived C was the availability of nutritious stream biofilm (Johnston et al. 1997).

Although benthic primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values closely tracked the salmon carcass gradient, there were several lines of evidence they exploited this resource in different ways. First, trophic differences were evident in control streams, where each invertebrate occupied a distinct isotopic niche space as defined by the

bivariate distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Appendix S1: Fig. S1). Second, consumer differences were supported by disparities in slope estimates from the Michaelis–Menten model (Fig. 3), which showed that mayflies, potentially in response to high demand, assimilated ^{15}N at about twice the rate as chironomids. Third, this model also revealed that the carcass loading where primary consumers achieved 50% saturation in ^{15}N ranged sixfold, also potentially indicating large differences in N demand. Fourth, the twofold difference in the strength of the relationship (R^2) between chironomid $\delta^{13}\text{C}$ and carcass loading relative to baetid mayflies suggests chironomids were more effective at directly exploiting salmon organic matter. This apparent preference for direct consumption of salmon organic matter may contribute to the generally positive effects that spawning or dead salmon have on stream Chironomidae populations relative to other invertebrate groups (Lessard et al. 2009, Kiffney et al. 2014). Consumer differences were also evident in the strong relationship between carcass biomass and mayfly $\delta^{15}\text{N}$ values indicating they were primarily dependent on assimilation of salmon organic matter via consumption of salmon-enriched prey.

While primary consumer ^{15}N and ^{13}C content exhibited relatively strong dependencies with salmon carcass loading, these effects weakened with trophic level thereby supporting our second prediction. Specifically, we observed variable effects at the secondary consumer level, with sculpin $\delta^{13}\text{C}$ and stonefly $\delta^{15}\text{N}$ values generally increasing with carcass loading. Both species are restricted to the stream benthos, occupying rocky bottom habitats, potentially facilitating their exposure to and exploitation of salmon organic matter relative to tertiary consumers. Interestingly, consistent with the observed isotopic differences among primary consumers, secondary consumers appeared to exploit salmon carcasses in distinct ways with stoneflies exploiting them indirectly (only $\delta^{15}\text{N}$ increased) and sculpin directly (only $\delta^{13}\text{C}$ increased). We speculate that mouth size may partly explain this difference as sculpin were large enough to consume fragments of salmon flesh directly, while the smaller stoneflies likely focused on consuming the relatively small salmon-enriched mayflies and chironomids.

The dependence between consumer $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values was weakest at the highest trophic level, which we attribute to differences in habitat use (pelagic vs. benthic), composition of resource subsidy, and time of year. Juvenile coho and cutthroat primarily forage in the water column and thus were not exposed to carcass materials deposited on the stream bottom to the same degree as benthic consumers. In terms of subsidy composition, we manipulated carcasses as opposed to also adding eggs or using live adults, which excrete bioavailable nutrients (e.g., ammonium) that can promote benthic productivity and release energy-rich eggs that are consumed by stream-rearing fish (Scheuerell et al. 2007, Swain et al. 2014). Our experiment was also conducted during fall and winter, when low water temperature and shorter days limit decomposition, and fish activity, metabolism, and consumption (Heggenes et al. 1993). Supporting a seasonal effect, juvenile coho muscle tissue contained five times more salmon-derived C (mean ~40%) and eight times more SD-N (mean ~40%) in the same experimental channels receiving 0.6 kg/m² salmon carcass material in summer relative to juvenile coho exposed to similar or higher loadings in the present experiment (Kiffney et al. 2014).

Thus, we predict the magnitude of the response by the recipient food web to anadromous fish is seasonally variable, with larger effects in spring and early summer when warmer water temperatures are within a range that promotes decomposition and positive growth conditions for ectothermic consumers. Moreover, depending on ambient conditions, such as background productivity (Wipfli and Baxter 2010), we expect that higher-level consumers will exhibit stronger responses (e.g., individual growth) to spawning anadromous fish populations that provide excretory products, energy-rich eggs, and decomposition products in addition to acting as agents of disturbance relative to decomposition products alone.

Multivariate changes in consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with carcass loading

One of the more intriguing results from our study was the shift in the relative importance of energetic pathways by which primary consumers assimilated salmon organic matter with carcass loading. Specifically, above ~0.1–0.5 kg/m² of

carcass material, the simultaneous increase in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signifies consumers exploited salmon organic matter by direct consumption and indirectly through eating enriched prey. Below this treatment level, only $\delta^{15}\text{N}$ ratios increased, indicating assimilation of salmon organic matter was indirect.

We hypothesize this shift represented a functional response, where above about 0.5 kg/m^2 the availability and thus exploitation of salmon-enriched C resource pools were greater relative to other pools. For example, carcass-derived organic carbon can form flocs with inorganic materials that blanket the substrate (Rex and Petticrew 2008), adsorb onto benthic biofilms, become trapped within inorganic substrates, or can be assimilated by heterotrophic microorganisms (Bilby et al. 1996, R uegg et al. 2011). We speculate that these abundant, carcass-enriched C pools were then eaten by small primary consumers that dominate many benthic stream invertebrate assemblages (Edwards and Meyer 1990, Hall and Meyer 1998, Kiffney et al. 2003) including the Cedar River (Kiffney et al. 2014). Consistent with our results, density of spawning Pacific salmon had the highest positive effects on the enrichment of both ^{13}C and ^{15}N in sculpin from coastal streams of British Columbia, Canada indicating both direct and indirect consumptive pathways (Swain et al. 2014).

Similar shifts in the relative importance of energetic pathways from resource subsidies to consumers have been noted in other studies, but mostly over time as opposed to over a gradient of resource levels (Claeson et al. 2006, Hoekman et al. 2012, Warry et al. 2016). Variation in the relative importance of direct ingestion of resource subsidies may be of ecological significance to consumers because this pathway is more energetically profitable (Hilderbrand et al. 1999, Minakawa et al. 2002, Marcarelli et al. 2011, Swain et al. 2014).

Ecological consequences of salmon organic matter

While numerous studies have demonstrated that resource subsidies provided by spawning salmon are assimilated by a variety of organisms (Bilby et al. 1996), fewer have established the ecological consequences of this assimilation (except see Swain and Reynolds 2015 as an example with sculpin populations). We observed a positive correlation between primary consumer $\delta^{13}\text{C}$

and $\delta^{15}\text{N}$ values and population biomass in experimental channels. Therefore, our study, in conjunction with others (Rinella et al. 2012), shows that a variety of freshwater and riparian species can assimilate salmon resource subsidies leading to growth benefits in some cases. However, these relationships are often context-dependent, varying with composition and density of the subsidy; biotic characteristics of the recipient consumers; and local environmental conditions, such as time of year or ambient productivity (Bilby et al. 1996, Wipfli et al. 1999, Janetski et al. 2009, Holtgrieve et al. 2010, Swain and Reynolds 2015). Additional studies that address this context dependency will improve our understanding and conservation of resource subsidies, including the species that provide them.

Implications

While this experiment provided a level of control over environmental conditions and salmon carcass loading, our approach had some limitations that may have constrained our ability to detect treatment effects. For example, focal consumers were collected opportunistically from different locations within the channel, which may have led to varying levels of exposure to and exploitation of carcasses. Additionally, our ability to detect treatment effects on fish may have been constrained by sample size.

Despite these limitations, by using native consumers in replicate stream channels, accounting for important confounding variables, such as water temperature and ambient nutrient levels, and testing a natural range of salmon loadings, we suggest our results have relevance to salmon conservation and management. First, we demonstrated the key role of widespread and abundant stream invertebrates in incorporating salmon organic matter into the trophic system. Thus, preserving processes and conditions that contribute to functioning invertebrate populations is likely critical to linking carcasses to higher trophic levels (Yang and Gratton 2014). Second, the range of salmon carcass loading ($\sim 0.1\text{--}0.6 \text{ kg/m}^2$) where primary consumer tissue achieved 50% saturation in ^{15}N corresponds to estimated inflection points for this relationship in plants (Hocking and Reimchen 2009, Holtgrieve et al. 2010) and fish in natural systems (Bilby et al. 2001). These adult salmon input levels are also associated with increases in

individual growth and population size of several invertebrate and vertebrate consumers in other studies (Kohler et al. 2012, Rinella et al. 2012, Kiffney et al. 2014, Swain and Reynolds 2015, Tonra et al. 2015), as well as rates of ecosystem function (Lessard et al. 2009, Holtgrieve and Schindler 2011, Kiffney et al. 2014).

Although our results were largely consistent with these studies, risk assessments should first be conducted to establish whether recipient food webs are resource limited or constrained by other factors (e.g., water quality) before focusing on nutrient enrichment as a restoration strategy (Collins et al. 2015). This assessment should also evaluate the potential consequences of nutrient enrichment on the receiving system, such as unanticipated food web or ecosystem effects (Kiffney et al. 2005, Compton et al. 2006, Collins et al. 2015), as well as the importance of resource inputs that are temporally asynchronous (Schindler et al. 2013). Ultimately, actions that restore a species' population and life history variation to levels where they elicit measurable ecological change, such as changes in energy flow, on the recipient system are likely the most effective approach to their conservation and management (Soulé et al. 2002).

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