Marine carbon and nitrogen in southeastern Alaska stream food webs: evidence from artificial and natural streams

Dominic T. Chaloner, Kristine M. Martin, Mark S. Wipfli, Peggy H. Ostrom, and Gary A. Lamberti

Abstract: Incorporation of marine-derived nutrients (MDN) into freshwater food webs of southeastern Alaska was studied by measuring the natural abundance of nitrogen and carbon stable isotopes in biota from artificial and natural streams. Biofilm, aquatic macroinvertebrates (detritivores, shredders, and predators), and fish (coho salmon, *Oncorhynchus kisutch*, and cutthroat trout, *Oncorhynchus clarki*) were sampled from streams in which Pacific salmon (*Oncorhynchus* spp.) carcasses had been artificially placed or were present naturally. In the presence of carcasses, all trophic levels incorporated marine-derived nitrogen (range, 22–73% of total N) and carbon (range, 7–52% of total C). In general, chironomid midges assimilated more marine-derived nitrogen and carbon than did other consumers. The assimilation of MDN by aquatic organisms and subsequent isotopic enrichment (5–6‰ for ^{15}N, 3–4‰ for ^{13}C) were similar in experimentally and naturally carcass-enriched streams. For specific taxa, however, percent assimilation for marine nitrogen and carbon were often dissimilar, possibly because of fractionation or transfer inefficiencies. These results suggest that pathways of MDN incorporation into stream food webs include both consumption of salmon material by macroinvertebrates and fish and uptake of mineralized MDN by biofilm. Incorporation of MDN into multiple trophic levels demonstrates the ecological significance of annual returns of anadromous fishes for sustaining the productivity of freshwater food webs.

Résumé : La mesure de l'abondance naturelle des isotopes stables d'azote et de carbone chez les organismes vivants de cours d'eau naturels et artificiels a permis d'étudier l'incorporation des nutriments d'origine marine (MDN) dans les réseaux alimentaires d'eau douce dans le sud-est de l'Alaska. Nous avons prélevé des échantillons de biofilm, de macroinvertébrés aquatiques (détrivivores, déchiqueteurs et prédateurs) et de poissons (saumons coho, *Oncorhynchus kisutch*, et truites fardées, *Oncorhynchus clarki*) dans des cours d'eau où des carcasses de saumons du Pacifique (*Oncorhynchus* spp.) se retrouvaient naturellement ou avaient été ajoutées expérimentalement. En présence des carcasses, les organismes de tous les niveaux trophiques incorporent de l'azote (étendue, 22–73 % de N total) et du carbone (étendue, 7–52 % de C total) d'origine marine. En général, les chironomides assimilent plus d'azote et de carbone d'origine marine que ne le font les autres invertébrés. L'assimilation de MDN par les organismes aquatiques et l'enrichissement subséquent en isotopes (5–6 ‰ pour ^{15}N, 3–4 ‰ pour ^{13}C) sont semblables dans les cours d'eau enrichis naturellement ou artificiellement avec les carcasses de saumons. Cependant, les pourcentages d'assimilation d'azote et de carbone d'origine marine par des taxons particuliers peuvent souvent varier, probablement à cause de l'inefficacité du fractionnement ou du transfert. Nos résultats laissent croire que les voies d'entrée du MDN dans le réseau alimentaire des cours d'eau incluent la consommation de tissus de saumons par les macroinvertébrés et les poissons, ainsi que l'absorption du MDN minéralisé par le biofilm. L'incorporation de MDN à de multiples niveaux trophiques souligne l'importance écologique du retour annuel des poissons anadromes pour le maintien de la productivité des réseaux alimentaires d'eau douce.

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Introduction

Pacific salmon (*Oncorhynchus* spp.) annually contribute massive amounts of organic material to fresh waters of the North Pacific rim when they spawn and die (Levy 1997). The nutrients and energy provided by spawning salmon appear to increase freshwater and terrestrial ecosystem productivity (Willson et al. 1998; Wipfli et al. 1998; Cederholm et al. 1999), and may subsidize (after Polis et al. 1997) otherwise nutrient-poor Pacific Northwest ecosystems (Cederholm et al. 1999; Gresh et al. 2000). This potentially important linkage from marine to freshwater and terrestrial ecosystems provided by salmon challenges the conventional biogeochemical paradigm that material flows unidirectionally from land to sea (Naiman and Bilby 1998).

Stable isotope analyses have been used to assess trophic structure and diet in marine (e.g., Welch and Parsons 1993; Gould et al. 1997), terrestrial (e.g., Ostrom et al. 1997; Ben-David et al. 1998), and freshwater ecosystems (e.g., Kline et al. 1990; Bilby et al. 1996). Stable isotope values of nitrogen ($\delta^{15}N$) are indicators of an organism's trophic level because the heavier nitrogen isotope accumulates in the consumer with each successive trophic transfer by approximately 3–4‰ (DeNiro and Epstein 1981). Carbon isotope values ($\delta^{13}C$) are indicators of an organism's diet because consumers tend to reflect the carbon isotope values of the food they consume. This means that provided material moves in a predictable way through food chains, stable isotopes can be used to identify the source of nitrogen and carbon for the base of the food web, and can reveal the dominant pathway by which nutrients and energy flow.

Several recent studies have used stable isotopes to quantify the contribution of marine-derived nutrients (MDN) from spawning salmon to freshwater ecosystems (Kline et al. 1990; Bilby et al. 1996; Johnston et al. 1997). The $\delta^{15}N$ and $\delta^{13}C$ values of marine organic material are typically higher than those of terrestrial and freshwater organic material. Adult salmon incorporate marine nutrients during maturation in the ocean and are thereby enriched with the heavier isotopes of nitrogen and carbon. Salmon entering fresh water retain their marine isotopic signature because they do not feed in fresh waters, and therefore remain isotopically distinct from terrestrially derived organic material (Kline et al. 1990). Consequently, stable isotopes can be used to trace MDN through freshwater ecosystems, and in conjunction with mass-balance equations (e.g., Kline et al. 1990), quantify the contribution of marine-derived nitrogen or carbon to freshwater food webs. Using these equations, previous studies have estimated that salmon can contribute 17–30% (Bilby et al. 1996) to >50% (Kline et al. 1990) of the nitrogen, and 23–40% (Bilby et al. 1996) of the carbon present in freshwater organisms.

The objectives of this study were to determine the amount of MDN incorporated into different trophic levels and to delineate pathways of carbon and nitrogen transfer in southeastern Alaska stream food webs. Stable isotope ratios of several different trophic levels were compared between artificial streams to which salmon carcasses were added, and natural streams in which salmon carcasses were either naturally present or were artificially placed. No previous studies of Pacific salmon involving stable isotopes (e.g., Kline et al. 1990; Bilby et al. 1996) have used artificial streams, alone or in combination with natural stream studies. The novel combination of experimental approaches used in our study offered a unique opportunity to investigate MDN incorporation and flow in stream food webs for two reasons. First, artificial and natural streams differ in terms of scale, control, replication, and realism. Second, a stream receiving a single influx of salmon carcasses could exhibit different responses than a stream that has received salmon spawners repeatedly over the years.

Materials and methods

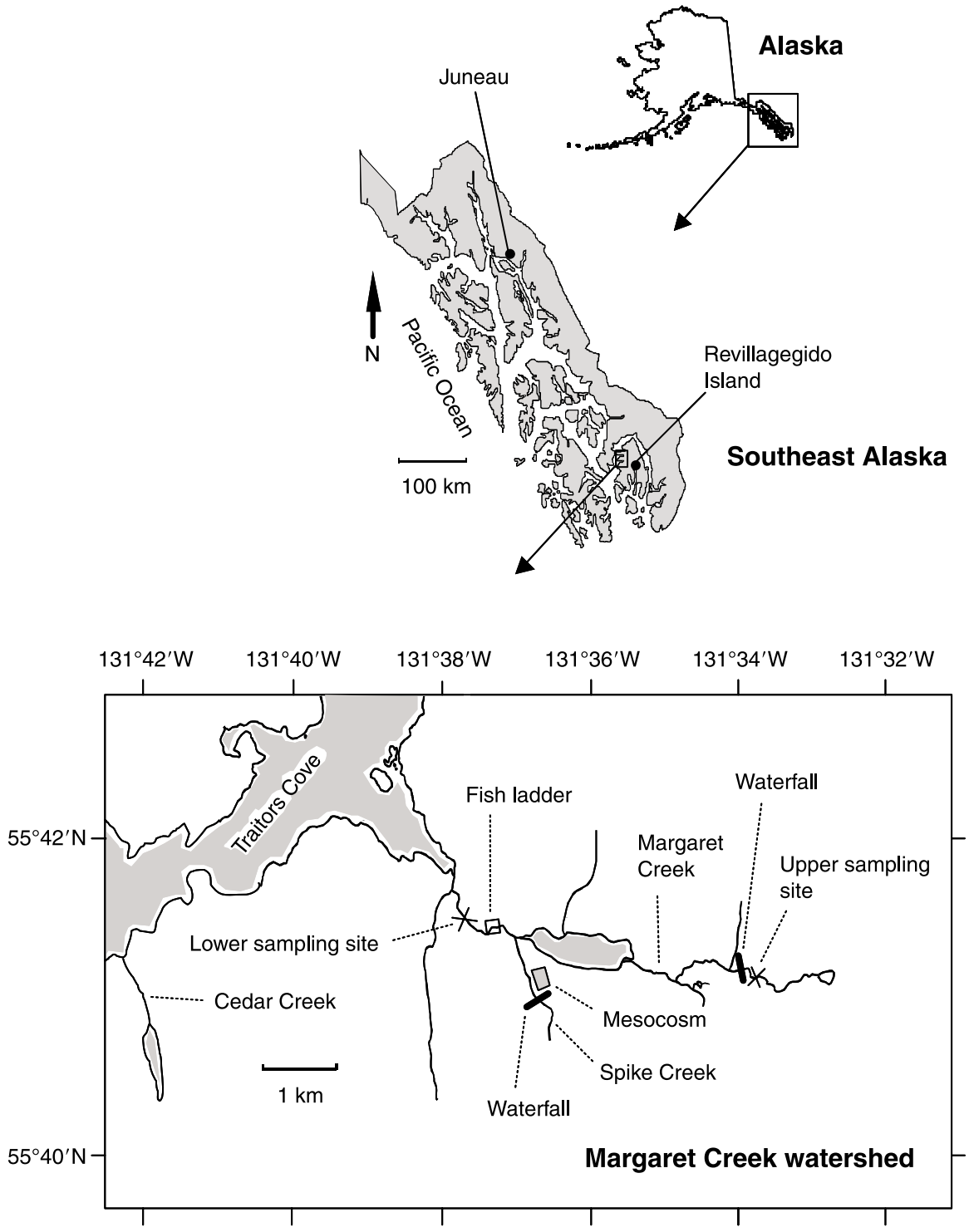
Study sites

The study took place on Revillagigedo Island, southeastern Alaska (Fig. 1). Two study streams (Margaret Creek and Spike Creek) were within the Margaret Creek watershed (55°41′N, 131°36′W), and a third stream (Cedar Creek) was located 4 km along the coast. Third-order Margaret Creek served as our naturally enriched stream. The lower portion of the watershed supports anadromous runs of pink (*Oncorhynchus gorbuscha*), chum (*Oncorhynchus keta*), and coho (*Oncorhynchus kisutch*) salmon, steelhead (*Oncorhynchus mykiss*) and cutthroat (*Oncorhynchus clarki*) trout, and Dolly Varden char (*Salvelinus malma*). Since construction of a fish ladder on lower Margaret Creek in 1990, anadromous fish have had access to the upper watershed (Bryant et al. 1999). However, a waterfall upstream of the fish ladder serves as a natural fish barrier. Thus, a site below the fish ladder on Margaret Creek and a site above the waterfall were chosen to represent salmon-carcass-enriched and control reaches, respectively (Fig. 1). Second-order Cedar Creek contains cutthroat trout, Dolly Varden char, and three-spine stickleback (*Gasterosteus aculeatus*), but no anadromous salmon. Cedar Creek is similar (e.g., in gradient, riparian vegetation, stream geomorphology, and biota) to many tributaries of Margaret Creek, and thus was chosen to artificially enrich with salmon. The artificial stream mesocosm was located adjacent to Spike Creek in the Margaret Creek watershed.

Mesocosm study

The mesocosm consisted of 36 straight, once-through artificial stream channels, each 250 cm long and 18 cm wide (six per table), that were gravity fed with Spike Creek water from above a waterfall that acts as a barrier to fish migration (Wipfli et al. 1998). Discharge for each channel averaged 0.45 ± 0.03 $L \cdot s^{-1}$. Each channel contained a pool that was supplied with natural mineral substrata and wood blocks for fish cover. Water temperature during the experiment was measured at 1-h intervals using a data logger in the single outflow flume for all channels, which averaged 9.1°C (range 5.4–12.7°C) during the experiment. Natural colonization by benthic organisms via drift from Spike Creek was allowed for 26 days before the experiment began. Three young-of-the-year (age 0) juvenile coho salmon (45–68 mm fork length) captured from Spike Creek were placed in each channel on 16 August 1998. Four treatment levels of pink salmon carcass (tissue and eggs) were used in this study: 0, 1, 2, and 4 carcasses$\cdot m^{-2}$ (or 0, 1.86, 3.72, and 7.44 $kg \cdot m^{-2}$). In the other two channels of each table an additional control and 3 carcasses$\cdot m^{-2}$ treatment were applied but were not sampled for stable isotopes. Treatment levels were within

Fig. 1. Location of study sites in southeastern Alaska, U.S.A.



the natural range of spawner densities for Margaret Creek (1–20 carcasses·m^{-2} or ~2–36 kg·m^{-2}, Wipfli et al. 1999). Treatments were randomly assigned to channels in each table using a Latin square design. Tissue chunks were occasionally and partially macerated by hand to simulate the physical processing of carcasses observed in local streams.

Epilithic biofilm, aquatic macroinvertebrates, and juvenile coho salmon were sampled 23–24 October 1998 for stable isotopes. Biofilm and macroinvertebrates were sampled from channels with the highest carcass loading (4 carcasses·m^{-2}) and from control channels (0 carcasses·m^{-2}). Biofilm was scraped with a razor blade from the outflow gutter at the end of each channel, filtered onto pre-ashed glass fiber filters, and dried at 60°C. Macroinvertebrates were collected from each channel by agitating the substrate and collecting drifting material in nets attached to channel outflows. We sampled common macroinvertebrates representing three functional feeding groups: the shredder *Zapada* spp. (Plecoptera:

Nemouridae), the predator *Sweltsa* spp. (Plecoptera: Chloroperlidae), and predominantly detritivorous chironomid midges (Diptera: Chironomidae) (Merritt and Cummins 1996). Samples were sorted live and then dried in glass vials at 60°C. To provide sufficient material for isotopic analysis, homogenate samples of 3–100 individuals of each functional feeding group were used. In some cases, the sample mass from a single channel was too small for isotopic analysis, so samples from multiple channels of a single treatment were pooled. Coho were taken from channels, stored frozen, and then dried at 60°C. A 10% (0.1–0.6 g wet mass) subsample of each fish was used for isotope analysis. Previous analyses indicated that the subsample and the whole fish were isotopically indistinguishable (P.H. Ostrom, unpublished data).

Natural stream studies

Naturally enriched stream

Baseflow discharge for Margaret Creek was 3830 $L \cdot s^{-1}$, mean channel wetted width was 16.4 m, and water temperature averaged 9.2°C (6.2–11.6°C). Samples for stable isotope analysis were collected from Margaret Creek on 31 October 1998, above and below the natural fish barrier, eight weeks after the peak of the pink salmon run. Biofilm was brushed and scraped from five rocks into a single container and processed as described above. Macroinvertebrates were sorted as described above and dried at 60°C in glass vials as a homogenate of several individuals (6–50 individuals depending on size). Dorsal muscle tissue of five pink salmon was collected on 20 September 1998 to provide marine isotope reference values.

Artificially enriched stream

Baseflow discharge for Cedar Creek was 9.3 $L \cdot s^{-1}$, mean channel wetted width was 2.2 m, and water temperature averaged 10.9°C (range 7.2–14.8°C) during the experiment. A 155-m reach of Cedar Creek was enriched with pink salmon carcasses downstream from the 420-m control reach. Salmon carcasses were added to the treatment reach on four separate dates: 4, 11, 22, and 26 September 1998, which resulted in a cumulative carcass-loading rate of 0.5 carcasses$\cdot m^{-2}$ (or 0.71 kg wet mass$\cdot m^{-2}$). Cutthroat trout were captured with minnow traps on 28 and 29 October 1998 from control and enriched reaches and were processed as described above. Macroinvertebrates and biofilm were not sampled because the primary focus of this experiment was fish.

Stable isotope analyses

Stable isotopes were analyzed in the Environmental Geochemistry Laboratory at Michigan State University. Prior to isotope analysis, dried biofilm samples were immersed in 10% HCl to remove inorganic carbon. Dried macroinvertebrate samples were ground to a homogenous fine powder. Fish tissue was lipid-extracted prior to homogenization by Soxhlet extraction for 7 h using an azeotropic mixture of chloroform and methanol (Gould et al. 1997). Lipids are depleted in ^{13}C relative to other tissues and can obscure the dietary signal (DeNiro and Epstein 1977). No significant differences were found between a lipid-extracted and non-extracted aliquot for a subset of macroinvertebrate samples (P.H. Ostrom, unpublished data).

Biofilm samples were prepared for isotope analysis by modified Dumas combustion (Macko et al. 1987), and the resulting gases purified by cryogenic gas separation and subsequent isotope determinations were performed using a PRISM (Micromass, Manchester, U.K.) stable isotope ratio mass spectrometer. Samples of macroinvertebrate and fish tissue were analyzed using a Carlo Erba NA 1500 nitrogen–carbon analyzer interfaced with the PRISM mass spectrometer (Wong et al. 1992). Stable carbon and nitrogen isotope ratios were expressed as

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ and $\delta^{15}N$, respectively. The standards were Vienna Peedee Belemnite (VPDB) for carbon and atmospheric N_2 for nitrogen. The reproducibility of these measurements was ≤0.2‰.

The carbon and nitrogen isotope ratios were used in mass balance equations (cf. Kline et al. 1990; Bilby et al. 1996; Johnston et al. 1997) to provide estimates of the percent MDN assimilated by organisms. We used the equation (after Johnston et al. 1997)

$$\%\text{MDN enrichment} = 100(\delta X_{se} - \delta X_c)/((\delta X_s + (TL^*\delta X_e)) - \delta X_c)$$

where δX_{se} is the isotope ratios of the organism in areas enriched with salmon, δX_c is the isotope ratios of the organism in areas without salmon enrichment (control), TL is the correction factor for trophic level (1 for primary consumers and 2 for secondary consumers), δX_s is the isotope ratio of salmon tissue, and δX_e is the isotopic enrichment factor per trophic level (3‰ for nitrogen and 1‰ for carbon).

Statistical analyses

For biofilm and macroinvertebrates from the mesocosm experiment, only the highest carcass treatment (4 carcasses$\cdot m^{-2}$) and control channels were compared. Data were analyzed using *t* tests, with a Bonferroni correction as appropriate. Response variables throughout this study were $\delta^{15}N$ and $\delta^{13}C$ isotope ratios. For coho, we used a randomized-block, split-plot design, replicating four treatments (0, 1, 2, and 4 carcasses$\cdot m^{-2}$) and three subtreatments (coho size) across six blocks. Mesocosm tables served as blocks. Each channel contained one fish from each of three size classes (small, mean = 50 mm; medium, mean = 55 mm; and large, mean = 60 mm), which were experimental subtreatments. Data were analyzed using a General Linear Model procedure (PROC GLM, SAS Institute Inc. 1989) at $\alpha = 0.05$. The effect of fish size and its interaction with treatments was also tested.

Samples of biofilm and macroinvertebrates from Margaret Creek were pooled prior to homogenization to provide sufficient mass for isotope analysis, thereby precluding statistical analysis. We compared the nitrogen and carbon isotope values of cutthroat trout from control and carcass-enriched reaches of Cedar Creek using *t* tests. Although within-stream comparisons for Cedar Creek constitutes pseudoreplication (Hurlbert 1984) because treatments were not replicated, this approach enabled us to evaluate if patterns in isotopic values found in natural streams corresponded to those in artificial streams.

Results

Mesocosm study

In the presence of salmon carcasses, epilithic biofilm was 1.7‰ more enriched with ^{13}C than biofilm in control channels but no significant difference was found in ^{15}N enrichment (Table 1). In carcass-treated channels, significant isotopic enrichment was found with individual macroinvertebrate taxa, and all macroinvertebrate taxa combined (Table 1). Macroinvertebrates from carcass-treated channels were at least 3.9 and 1.8‰ more enriched with ^{15}N and ^{13}C, respectively, than were macroinvertebrates in control channels. Chironomid midges were the most enriched with ^{15}N, whereas the shredder *Zapada* was the most enriched with ^{13}C. The predator *Sweltsa* was the least enriched with both ^{15}N and ^{13}C. Juvenile coho salmon from all carcass-treated channels were significantly enriched with ^{15}N and ^{13}C compared with fish from control channels (Table 1). Isotopic values of fish in treated channels differed from control fish by an average of 2.3‰ for $\delta^{15}N$ and 2.2‰ for $\delta^{13}C$. The $\delta^{15}N$ and $\delta^{13}C$ values of juvenile coho did not respond significantly to carcass loading levels beyond 1 carcass·m^{-2} (Fig. 2). Results for carbon isotope values showed a significant interaction between carcass loading and coho size class ($p < 0.05$, Fig. 3), suggesting that at lower carcass loading densities, larger juvenile coho became relatively more ^{13}C-enriched with the presence of carcasses than did other size classes.

Natural stream studies

Although not tested statistically, epilithic biofilm from below the barrier in Margaret Creek appeared more isotopically enriched than biofilm above the barrier by 5.4‰ for $\delta^{15}N$ but only 0.9‰ for $\delta^{13}C$ (Table 1). Macroinvertebrate taxa from below the fish pass also appeared to be enriched with ^{15}N and ^{13}C compared with the same taxa from above the fish barrier (Table 1). Isotope values below the barrier were higher by at least 4.2‰ for $\delta^{15}N$ and 0.6‰ for $\delta^{13}C$. Chironomid midges were the most enriched with both ^{15}N and ^{13}C, whereas the shredder *Zapada* was the least isotopically enriched. In Cedar Creek, $\delta^{15}N$ and $\delta^{13}C$ values for cutthroat trout were significantly higher (Table 1) in the carcass-treated reach than in the control reach, with isotope values elevated by 2.1‰ for $\delta^{15}N$ and 2.7‰ for $\delta^{13}C$.

Degree of MDN enrichment

Biofilm in salmon-treated mesocosm channels was not enriched with ^{15}N but $\delta^{13}C$ isotope values indicated that 26% of biofilm carbon came from salmon carcasses (Table 1). In Margaret Creek, however, biofilm nitrogen isotope ratios showed higher enrichment (73%) than did carbon isotope ratios (22%). In the mesocosm, estimates of percent MDN assimilation were similar for carbon and nitrogen for both the predator *Sweltsa* and the shredder *Zapada*, although *Zapada* assimilated more MDN than did *Sweltsa* (Table 1). Assimilation estimates for chironomid midges were higher with marine nitrogen than carbon. In Margaret Creek, estimates of percent MDN assimilation for both *Sweltsa* and *Zapada* were higher with nitrogen than carbon, but overall were higher for *Sweltsa* than *Zapada*. In contrast, assimilation estimates for chironomid midges were similar for nitrogen and carbon. Fish from the mesocosm and Cedar Creek assimilated more marine-derived carbon (34–37%) than marine-derived nitrogen (20–22%).

Discussion

Influence of Pacific salmon on ecosystem structure and function

The massive influx of organic material transported by Pacific salmon into fresh water can profoundly influence the structure and function of recipient freshwater ecosystems (Cederholm et al. 1999). Enrichment of streams by spawning salmon is believed to be part of a positive feedback loop (Wipfli et al. 1998), critical for sustaining freshwater productivity, including salmon populations themselves (Cederholm et al. 1999). The density of carcasses required to maintain freshwater productivity has yet to be established (Wipfli et al. 1999), and there may be limits to the amount of MDN that can be incorporated by stream food webs (Wipfli et al. 1999; Bilby et al. 2001). Stable isotope analyses, by providing estimates of MDN enrichment of various food web components under different salmon spawning densities, should help determine the amount of salmon material needed to sustain stream productivity (Johnston et al. 1997; Bilby et al. 2001).

Flows of marine N and C in freshwater food webs

The ^{15}N enrichment of epilithic biofilm was difficult to interpret, but we present some plausible explanations based on the current isotopic literature. Epilithic biofilm appeared to assimilate a modest amount of marine-derived carbon, probably reflecting the assimilation of dissolved carbon by heterotrophs, trapping of salmon particles within the biofilm matrix, or sorption of MDN onto rock surfaces (Bilby et al. 1996). However, $\delta^{13}C$ values of biofilm could also have been altered by increased algal growth rates stimulated by carcass nutrients, resulting in reduced ^{13}C discrimination during photosynthesis (Laws et al. 1995). Wipfli et al. (1998) documented more biofilm ash-free dry mass (AFDM) and chlorophyll *a* in artificial and natural streams in the presence of salmon carcasses in these same systems, and found that algae were a small component of the biofilm layer (Wipfli et al. 1999).

In the mesocosm, the $\delta^{15}N$ values of epilithic biofilm in carcass-treated channels were lower than those in control channels and could not be used to estimate percent MDN contribution. This could be explained by isotopic discrimination (i.e., fractionation) during nitrogen cycling, which can influence the $\delta^{15}N$ of algae (Fogel and Cifuentes 1993). When nitrogen is not limiting, inorganic nitrogen depleted in ^{15}N is used preferentially (Altabet et al. 1991; Ostrom et al. 1997), a possible scenario in mesocosm channels if salmon carcasses increased dissolved inorganic nitrogen concentrations (see Bilby et al. 1996). Nitrification results in the preferential incorporation of ^{14}N into nitrate (Fogel and Cifuentes 1993) and is an important process in streams (Strauss and Lamberti 2000), including salmon spawning streams (R.T. Edwards, Pacific Northwest Research Station, USDA Forest Service, 2770 Sherwood Lane, Juneau, AK 99801, personal communication). The low $\delta^{15}N$ values for biofilm could therefore reflect utilization of a ^{15}N-depleted nitrate pool, as

Table 1. $\delta^{15}N$ and $\delta^{13}C$ isotope values, results of statistical comparisons (p values), and percent marine-derived nutrient (MDN) enrichment for different trophic levels or taxa in the three study components (mesocosm, Margaret Creek, and Cedar Creek).

	$\delta^{15}N$				$\delta^{13}C$			
Taxa	No MDN present	MDN present	p	% MDN	No MDN present	MDN present	p	% MDN
Mesocosm								
Epilithic biofilm	8.0 (1.1)	6.3 (0.1)	0.210	–[a]	–27.2 (0.1)	–25.5 (0.1)	<0.001	26
Zapada	1.1 (0.2)	6.3 (0.5)	<0.001	36	–28.7 (0.2)	–25.2 (0.4)	0.001	39
Sweltsa	3.9 (0.2)	7.8 (0.5)	0.002	26	–26.3 (0.1)	–24.5 (0.2)	0.002	23
Chironomidae	3.1 (0.5)	11.2 (0.4)	<0.001	64	–25.6 (0.6)	–22.5 (0.3)	0.010	52
Macroinvertebrates (all taxa combined)	2.7 (0.4)	8.4 (0.8)	<0.001	42	–26.9 (0.5)	–24.1 (0.4)	<0.001	38
Coho salmon (age 0)	8.5 (0.3)	10.8 (0.2)	0.001	22	–25.3 (0.6)	–23.1 (0.2)	0.005	34
Margaret Creek								
Epilithic biofilm	5.3	10.7	NT	73	–24.7	–23.8	NT	22
Zapada	0.6	4.8	NT	28	–29.2	–28.6	NT	7
Sweltsa	4.0	11.0	NT	48	–27.4	–25.1	NT	25
Chironomidae	–0.5	6.6	NT	44	–28.6	–25.0	NT	40
Macroinvertebrates (all taxa combined)	1.4	7.5	NT	40	–28.4	–26.2	NT	24
Pink salmon (adult)	—	12.7 (0.4)	—	—	—	–20.6 (0.5)	—	—
Cedar Creek								
Cutthroat trout (age 1+)	8.3 (0.1)	10.4 (0.2)	<0.001	20	–25.9 (0.5)	–23.2 (0.2)	0.002	37

Note: All isotope values are means (1 standard error), which for coho were averaged across all mesocosm carcass treatments. NT, not tested statistically.

[a]Mass balance equations did not provide a solution (i.e., produced a negative value).

Fig. 2. Mean $\delta^{15}N$ (■) and $\delta^{13}C$ (▲) values for juvenile coho salmon (*Oncorhynchus kisutch*) in mesocosm channels with different salmon carcass loadings. Different letters indicate significant differences ($p < 0.01$). Error bars represent 1 standard error ($n = 3$).

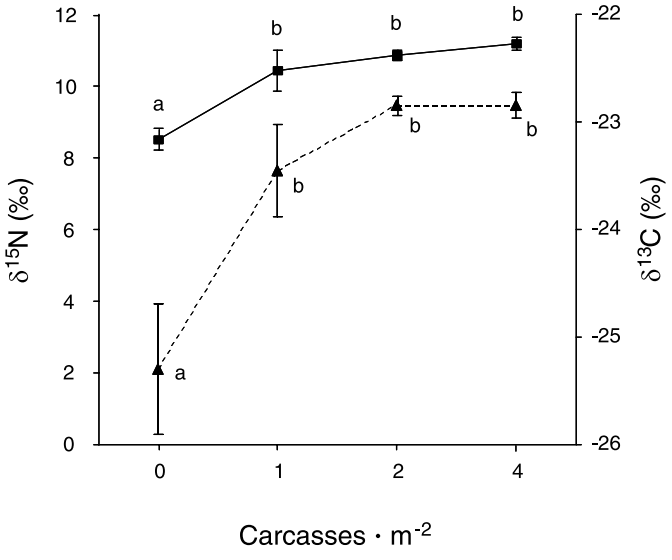
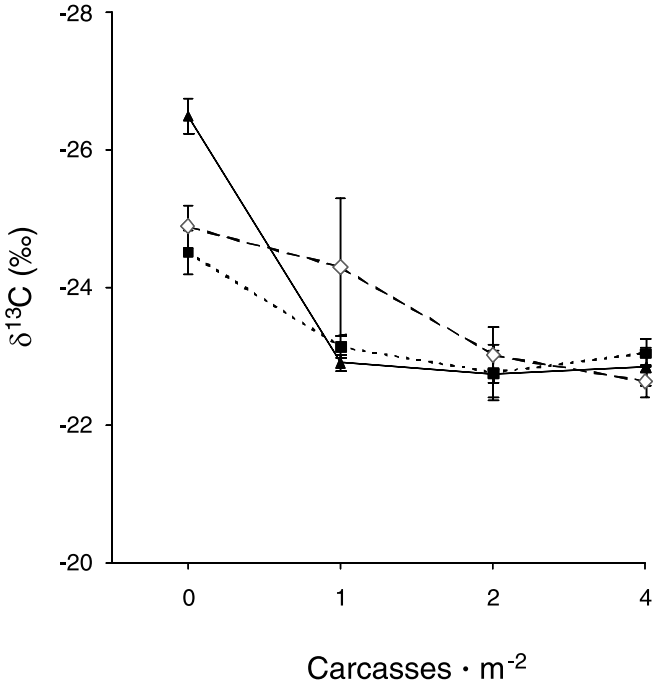


Fig. 3. Mean $\delta^{13}C$ values for three different class sizes (▲, large; ■, medium; ◇, small) of juvenile coho salmon (*Oncorhynchus kisutch*) in mesocosm channels with different salmon carcass loadings. Error bars represent 1 standard error ($n = 3$).



has been observed with algae in other freshwater environments (McCusker et al. 1999).

In contrast to the mesocosm, $\delta^{15}N$ values for epilithic biofilm in Margaret Creek appeared to be higher in the presence of salmon, suggesting assimilation of marine-derived nitrogen. However, estimates of percent MDN assimilation for carbon were lower (22%) than those for nitrogen (73%), suggesting that the biofilm incorporated more marine-derived nitrogen than carbon. This could reflect different pathways of material flow for autotrophs and heterotrophs. Whereas both autotrophs and heterotrophs take up dissolved inorganic or organic nitrogen, only heterotrophs are likely to assimilate nitrogen from marine-derived particulate matter

(Fenchel et al. 1998). Thus, if autotrophs account for a large portion of the epilithic biofilm community, then estimates of MDN incorporation should be lower for carbon than for nitrogen, as we found in Margaret Creek. Overall, our results show that uptake of MDN by biofilm is more complex than previous studies suggest.

Benthic macroinvertebrates exhibited a range of MDN enrichment amongst taxa. Relative to other consumers, isotope values for *Zapada* in the absence of salmon were low in both the mesocosm and Margaret Creek, suggesting similar food sources. *Zapada* nitrogen isotope values likely reflect ingestion of alder (*Alnus* spp.) leaf fragments. Alder are common riparian plants in the Margaret Creek watershed and have $\delta^{15}N$ values near 0‰ because they are nitrogen fixers (Johnston et al. 1997). In mesocosm channels, *Zapada* derived about 40% of their nitrogen and carbon from salmon carcasses, suggesting a more direct pathway of MDN incorporation. In Margaret Creek, *Zapada* assimilated much more marine-derived nitrogen than carbon, suggesting a less direct pathway of MDN incorporation. MDN assimilation by a shredder, like *Zapada*, requires an uptake mechanism operating through leaf biofilm, like biofilm uptake of ammonium and (or) nitrate by autotrophs and heterotrophs. Differences in percent MDN assimilation based on carbon versus nitrogen isotope ratios for consumers may reflect the relative proportion of autotrophs and heterotrophs within the biofilm at the base of the food web.

Of the consumers we considered, the isotope ratios of chironomid midges appeared to be the most isotopically enriched. Chironomids from above the salmon barrier had very low $\delta^{15}N$ values, which like *Zapada* probably reflected the abundant alder in the upper Margaret watershed or influence of ^{15}N-depleted nitrate derived from nitrification within the system. Relative to individuals from the upper Margaret Creek watershed, chironomids in control channels of the mesocosm were enriched with ^{15}N, suggesting that nitrification did not control isotope values at the base of the food web and perhaps were reflecting food sources other than alder in the mesocosm. It is possible that these individuals were either predators or collector–gatherers consuming a detrital food resource unique to the mesocosm. This is consistent with Merritt and Cummins (1996), who suggest that although most chironomids are detritivores, some are herbivores or predators. Chironomids in both carcass-treated channels and lower reaches of Margaret Creek assimilated marine-derived nitrogen and carbon, suggesting that salmon tissue was an important component of the detrital pool and perhaps was directly ingested. Chironomids are likely to be important for the incorporation of MDN into stream food webs, given their strong assimilation of MDN, rapid colonization of carcasses (Chaloner et al. 2002), and importance as prey to salmonids (Wipfli 1997).

Predatory *Sweltsa* stoneflies appeared to have higher isotope ratios than did *Zapada*, especially in Margaret Creek. As with *Zapada*, the isotopic similarity of *Sweltsa* from mesocosm control channels and upper Margaret Creek suggests that food sources were similar. In carcass-treated channels, *Sweltsa* assimilated a similar amount of marine-derived nitrogen and carbon, and MDN were likely incorporated indirectly through prey. Below the salmon barrier of Margaret Creek, the lower assimilation of marine-derived nitrogen by *Sweltsa* compared with *Zapada* and chironomids in the mesocosm was probably related to less efficient energy and nutrient transfer with increasing trophic level. The disparity between nitrogen and carbon isotope enrichment may reflect differences in pathways of material flow at the base of the food web.

Overall, assimilation of MDN by fish was lower but more consistent among taxa than was found with macroinvertebrates. Juvenile coho in control channels were enriched with both ^{15}N and ^{13}C relative to macroinvertebrates, reflecting their primary food source of aquatic and terrestrial invertebrates (Wipfli 1997). Cutthroat trout from Cedar Creek were isotopically similar to juvenile coho in the mesocosm, suggesting a similar diet. Within carcass-enriched streams, assimilation of MDN by coho and cutthroat trout was less than invertebrates (up to 37%), perhaps resulting from trophic transfer inefficiencies. Alternatively, low MDN assimilation may reflect variable levels of MDN assimilation by invertebrates in their diet. Both coho and cutthroat trout appeared to derive less nitrogen than carbon from salmon carcasses, and overall less nitrogen from carcasses than invertebrates. This could reflect differential assimilation or loss of marine-derived carbon and nitrogen during fish metabolism, which is known, for example, to take place during starvation. The largest juvenile coho in the mesocosm showed the most carbon isotope enrichment at the lowest carcass treatment, suggesting that larger fish benefit most from carcass enrichment. This probably reflects the foraging hierarchies that often become established in groups of juvenile coho (Nielsen 1992). Such hierarchies would mean that larger individuals would have access to carcasses, which are likely to be areas of highest invertebrate abundance (Chaloner et al. 2002) and where invertebrates probably incorporate the most MDN.

Comparison among study approaches and with previous studies

Our study was unique in that it combined data from artificial and natural streams to trace MDN incorporation and flow in stream food webs under both controlled and natural conditions. We showed that both producers and consumers can incorporate marine carbon and nitrogen, which is consistent with Kline et al. (1990) and Bilby et al. (1996), but in contrast with Johnston et al. (1997), who found virtually no marine carbon enrichment. We observed that MDN flow and incorporation into food webs differed between natural and artificial streams to some degree. For example, biofilm in the natural stream incorporated marine nitrogen, whereas mesocosm biofilm did not. Significantly, we found no evidence in artificial streams of a relationship between carcass loading levels (i.e., spawner densities) and marine isotope enrichment. Wipfli et al. (1999) did not find a significant response by macroinvertebrate densities or biofilm AFDM to carcass loading but did find a significant response by biofilm chlorophyll *a*. Johnston et al. (1997) and Bilby et al. (2001) both reported a positive relationship between spawner densities and ^{15}N enrichment of invertebrates and fish. However, Johnston et al. (1997) found increased isotopic enrichment up to ~4 kg wet mass·m^{-2}, whereas Bilby et al. (2001) found enrichment only up to 0.10–0.15 kg wet mass·m^{-2}. Such a

relationship could also exist in southeastern Alaska streams, but at carcass loading levels much lower than those used in the mesocosm, and more similar to those given by Bilby et al. (2001). A system could become so "saturated" with MDN that no further isotopic enrichment takes place, which would explain why juvenile coho were only marginally more enriched with MDN than were cutthroat trout despite carcass loadings in the mesocosm that were much higher (~4.3 kg wet mass·m^{-2}) than in Cedar Creek (~0.7 kg wet mass·m^{-2}).

The incorporation and subsequent flow of MDN through stream food webs likely reflects conditions unique to natural and artificial streams, as well as the location of each individual study (i.e., Kline et al. 1990; Bilby et al. 1996). Physically and biologically, natural streams are much more complex than artificial streams, resulting, for example, in more potential pathways for MDN uptake. Such complexity probably also makes comparisons among different studies as well as among different streams difficult. Natural streams likely present a wider range of nutrient and food resources than the simplified environments of artificial streams. For example, leaves were probably less abundant and more fragmented in mesocosm channels than in natural streams. The nature of salmon enrichment probably also differed between the mesocosm and natural streams. In Margaret Creek, for example, salmon were alive, and therefore excreted nutrients as waste products before dying, whereas the mesocosm channels and Cedar Creek received only dead fish. Not only were spawner densities effectively higher in the mesocosm than Cedar Creek, but the mesocosm also did not experience spates that probably flush out much salmon material from natural streams. Given the levels of enrichment of some trophic levels, in ours as well as in other studies (Kline et al. 1990; Bilby et al. 1996), there is clear evidence that a significant amount of MDN is retained within natural streams.

Invertebrate consumers in our study were highly variable in MDN enrichment, in contrast to fish consumers. This is consistent with previous studies (e.g., Kline et al. 1990; Bilby et al. 1996; Johnston et al. 1997) and may reflect an overall consistency in fish diets as compared with those of invertebrates. Diet could determine whether marine nutrients are incorporated through consumption of tissue or eggs, or through less direct pathways, such as consumption of biofilm or invertebrates enriched with MDN. A potentially important aspect of diet is the elemental stoichiometry (e.g., C:N and N:P) of food items, which can influence the isotopic composition of a consumer (Adams and Sterner 2000). Growth has also been shown to have a positive influence on the isotopic composition of fish; fast-growing individuals become more isotopically similar to their food than slow-growing individuals (Hesslein et al. 1993). That there were little differences in isotopic composition above a certain threshold of carcass loading probably reflects upper limits on fish growth.

Mechanisms of isotopic fractionation and movement within food webs will be important topics for future research. For example, isotopic fractionation could have masked the degree of MDN assimilation by mesocosm biofilm. Ultimately, better understanding of such mechanisms will lead to a more refined understanding of the pathways by which marine nutrients and energy become incorporated into stream food webs. Two general pathways of incorporation have been proposed: (*i*) consumption by fish and invertebrates of salmon tissue, and (*ii*) uptake by microbial biofilms of mineralized or inorganic nutrients excreted by fish or leached from carcasses (Kline et al. 1990; Bilby et al. 1996). Our results and those of previous studies (e.g., Kline et al. 1990; Bilby et al. 1996) indicate that both of these pathways are probably involved in the incorporation of MDN into stream food webs. However, the relative importance of individual pathways, operating in different streams and watersheds, remains unclear (Wipfli et al. 1999). Such information is crucial for determining how to maintain or restore both stream productivity and salmon populations (Johnston et al. 1997). For example, stable isotopes could help evaluate the uptake of dissolved inorganic nutrients of terrestrial origin to replace MDN that was once delivered by spawning salmon (Ashley and Slaney 1997). Overall, a better understanding of the role that marine nutrients and energy play in freshwater ecosystems will likely improve management strategies for salmon and the streams in which they spawn.

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