

## Marine Subsidies in Freshwater: Effects of Salmon Carcasses on Lipid Class and Fatty Acid Composition of Juvenile Coho Salmon

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**Abstract.**—Returning adult salmon represent an important source of energy, nutrients, and biochemicals to their natal streams and may therefore have a quantitative effect on the energy levels of stream-resident salmonids. We tested this hypothesis by constructing simulated streams for coho salmon *Oncorhynchus kisutch* to which we added 0, 1, and 4 carcasses/m<sup>2</sup> (0, 0.71, and 2.85 kg wet mass/m<sup>2</sup>) of pink salmon *O. gorbuscha*. After 60 d we evaluated the lipid class and fatty acid composition of rearing coho salmon from the simulated streams; the lipid content and triacylglycerols of the coho salmon increased with increasing carcass density whereas phospholipids decreased. Increased amounts of triacylglycerols accounted for most of the lipid increase. In addition to increasing in concentration, the fatty acid composition of the triacylglycerols also changed with carcass density. Triacylglycerols of juvenile coho salmon from the control streams had significantly higher omega-3 : omega-6 ratios as a result of fivefold and sixfold increases in the concentrations of eicosapentanoic and docosahexanoic fatty acids, respectively. These data demonstrate an immediate nutritional benefit resulting from the introduction of salmon carcasses in juvenile coho salmon rearing habitat and indicate the utility of fatty acid and lipid class analysis for examining the effects of marine-derived nutrients on juvenile salmonids.

The annual arrival of thousands of tons of adult salmon to streams around the North Pacific represents a value to resident fish that may have been largely overlooked (Levy 1997). Recent effort has focused on examining the fate of marine-derived nitrogen, phosphorus, and carbon (Kline et al. 1990; Wipfli et al. 1998, Wipfli et al. 1999; Bilby et al. 2001) and establishing the role of these elements as fertilizers (Ben-David et al. 1998). However, stream-resident parr of coho salmon *Oncorhynchus kisutch* have been observed consuming eggs and carcass tissue of pink salmon *O. gorbuscha*, suggesting that the marine-derived biomass may also provide a source of nutrition to resident fishes (Koski and Kirchofer 1984; Bilby et al. 1998). Wipfli et al. (2003) reported higher growth rates of juvenile coho salmon that were exposed to salmon carcasses and eggs in Alaska.

Potential nutritional elements provided by re-

turning salmon include lipids, which represent a valuable energy source for juvenile fish. A 1,500-g adult pink salmon delivers approximately 60 g of lipid when it arrives in a small coastal stream (Gende 2002), or at least 57 kcal of energy (Anthony et al. 2000). Therefore, a moderately sized pink salmon escapement of a few thousand individuals can represent a substantial source of energy to resident fish. This marine-derived biomass has been shown to increase the biomass of periphyton and density of stream invertebrates (Wipfli et al. 1998). Thus, the annual return of adult salmon represents a potential boon to resident fish, by providing flesh and eggs for direct consumption (Bilby et al. 1998) or by increasing the availability of rearing coho prey (Wipfli et al. 1999).

Lipids consumed by juvenile coho salmon, regardless of their source, are either used immediately or stored for later use. Surplus lipids are stored primarily as triacylglycerols (TAG) in salmonids (Sheridan et al. 1983). Triacylglycerols are constructed from three fatty acid chains bound to a glycerol backbone. When exogenous supplies of energy fail to meet endogenous demand, a fatty acid is hydrolyzed from the TAG and catabolized for energy. Thus, the amount of lipid devoted to

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TAG indexes the amount of surplus energy available to an individual and thereby provides a measure of its nutritional condition (Gatten et al. 1983; Fraser 1989).

A large number of fatty acids can compose TAG, including those that cannot be synthesized by juvenile coho salmon (Saddler et al. 1966). Fatty acids that cannot be synthesized are generally referred to as the essential fatty acids because they are required to maintain cell integrity and immune function (Sargent et al. 1999). Although these fatty acids have an important value as components of structural lipids, they are also stored without modification in TAG (Olsen et al. 1991). Thus, the fatty acid composition of TAG also reveals the nutritional condition by indicating the amount of essential nutrients held in reserve.

The ultimate sources of essential fatty acids are the plants and bacteria that form the base of the local food web (Olsen 1999). Consequently, examination of the fatty acid composition of storage lipids may provide general information regarding the ultimate source of stored energy. For example, differences in the relative amounts of omega-3 and omega-6 fatty acids in freshwater and marine fish (Henderson and Tocher 1987) are generally believed to relate to differences in the ultimate sources of these essential fatty acids (Napolitano 1999). Values for the ratio of omega-3 to omega-6 fatty acids in freshwater fish are typically less than 4.0, whereas those of saltwater fish normally range between 5.0 and 15.0 (Henderson and Tocher 1987). Increased omega-3 : omega-6 ratios in marine fish can be attributed to increased amounts of docosahexanoic (22:6[n-3])<sup>2</sup> and eicosapentanoic (20:5[n-3]) acids relative to linolenic (18:2[n-6]) and arachidonic (20:4[n-6]) acids. Consequently there are substantial differences in the relative amounts of these fatty acids in juvenile salmonids and returning adults (Sheridan et al. 1985). However, consumption of adult tissues by juveniles should alter the juvenile fatty acid composition from one characteristic of a terrestrial energy source towards one more characteristic of a marine source.

<sup>2</sup> Fatty acids are often categorized by the number and locations of their double bonds. Fatty acids with the first double bond located three bonds from the methyl end of the chain are designated as omega-3 fatty acids; likewise, omega-6 fatty acids have a the first double bond located six bonds from the methyl end. In this report, fatty acids are identified as *xx:a(n-b)*, where *xx* refers to the number of carbons in the chain, *a* to the number of double bonds, and *b* to the location of the first double bond from the methyl end.

In this study we set out to test whether stream-resident fish derive direct nutritional benefits from the presence of salmon carcasses in their rearing habitat. We did this by relating various carcass densities to the lipid content and composition of juvenile coho salmon. In addition, we examined the fatty acid composition of TAG to determine if the presence of a marine energy source could be detected in the tissues of parr exposed to carcasses. The data in this paper are drawn from a much larger study that involved a broader range of carcass loads and recorded a greater number of responses than those reported here. These responses to increased carcass density include invertebrate density, juvenile coho salmon growth (Wipfli et al. 2003), and isotopic enrichment (Chaloner et al. 2002a).

### Methods

This study was part of a larger experiment wherein six carcass treatments were applied, but the cost precluded lipid analysis of all treatments. The carcass loads chosen for lipid analysis represent the extremes of the range of treatments applied.

*Coho salmon rearing conditions.*—To evaluate the effects of carcasses on coho salmon lipid composition, different densities of pink salmon carcasses were placed into artificial streams along with three coho salmon parr (44–67 cm fork length). The artificial streams (channels; 237 cm long × 18 cm wide × 12.5 cm deep) were filled with 16 L of gravel substrate (diameter ranged from 4 cm to 20 cm; see Wipfli et al. 1998) obtained from a nearby streambed the previous year. The gravels were thoroughly rinsed before experiments began. Each channel was supplied with unfiltered water from the nearby stream that had no adult salmon present. Water flowed through each channel for 26 d before the fish and carcasses were introduced to allow for colonization by benthic invertebrates. Flow rates averaged 0.45 L/s and temperature ranged between 5.4°C and 12.7°C.

Carcass treatments nominally represented densities of 0, 1, and 4 female carcasses per square meter. Treatments were established by distributing 150-g chunks of pink salmon carcass and 27-g clusters of eggs throughout each channel. Fifty-three live female pink salmon were collected on August 16 and euthanized; their ovaries were removed and divided into egg clusters. Carcass chunks were also cut from the freshly killed females between the pectoral girdle and anus. Four carcass chunks and egg clusters, representing a

single carcass, were scattered along the length of each channel. There were six replicate channels for each of the treatments. The carcass pieces were macerated by hand on days 24, 38, and 52 of the experiment to simulate the physical fragmentation effects of freshets observed in nearby natural streams. Maceration left tissue fragments scattered on and within the substrate; egg clusters largely remained intact. Some suspended tissue fragments were lost from the channels. Wipfli et al. (2003) provide details on experimental design and sampling procedures.

Wild juvenile coho salmon were collected from a nearby stream, weighed, individually marked, and placed into the channels on August 16, 1998. The fish were not artificially fed during the study but could forage on adult salmon tissue, natural drift, and benthic prey in the channels. After 60 d (i.e., on October 24, 1998), all surviving fish were removed, weighed, and placed in a freezer maintained at  $-20^{\circ}\text{C}$ . Fish were frozen individually, and retained at this temperature for approximately 3 months before lipid extraction and analysis began.

*Lipid extraction from rearing coho salmon.*—Coho salmon lipids were extracted using a modification of Folch's method as outlined by Christie (1982). Whole coho salmon parr were homogenized and extracted in a solution of 33% methanol and 66% chloroform. The extract was vacuum filtered and the solid residue reextracted and filtered. The two filtrates were combined in a separatory funnel and a liquid-liquid extraction was performed using an aqueous solution of 0.88% potassium chloride (88 g/100 mL) at one quarter of the total volume of filtrate. The bottom layer of the resulting biphasic solution was collected and reextracted with a fresh potassium chloride solution. After this second extraction, the purified lipid was collected, and its volume was reduced under a vacuum to 5 mL via a rotary evaporator. A 2-mL aliquot was withdrawn to gravimetrically determine the lipid mass. Measurements of lipid mass are expressed as the percentage of the wet tissue mass composed of lipid. The remaining 3 mL were retained for further analysis.

*Lipid class separations.*—Lipids were separated into lipid classes by high-pressure liquid chromatography and quantified with an evaporative light-scattering detector, and their TAG fractions were retained for further analysis. Separated lipid classes included TAG, sterols, monoacylglycerols, diacylglycerols, free fatty acids and wax/sterol esters. In addition, five phospholipid classes were

resolved: phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin and lyso-phosphatidylcholine. Concentrations of these were summed to find the total phospholipid content. Concentrations of the classes are expressed as percentages of total lipid. Detailed analysis of the lipid class composition provided an opportunity to examine the degree of hydrolyzation that had occurred in the samples after their long storage period. Subsequently, only the TAG fraction was retained for further analysis. No analysis was performed to determine the degree of oxidation.

The high-pressure liquid chromatography method, based on Christie (1985), used a 3- $\mu\text{m}$  silica column and a solvent gradient to resolve the lipid classes. The solvent gradient consisted of hexane:chloroform (1:1) at injection, followed by iso-octane:tetrahydrofuran (99:1), isopropanol:chloroform (4:1), and isopropanol:water (1:1). After separation, TAG was split between the detector and a sample vial (60:40) with a proportioning valve. Toluene and the antioxidant 3,5-di-tert-butyl-4-hydroxytoluene were added to the aliquot retained in the vial. The volume of this sample was reduced to 1 mL by evaporation under nitrogen, and the sample was stored at  $-20^{\circ}\text{C}$  for later transesterification and fatty acid analysis. Lipid class concentrations were determined from a three-point calibration curve using calibration standards normalized to an internal standard (1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N,N-dimethyl). The calibration standards included myristate, cholesterol, triolein, monolein, 21:0, choline, plant phosphatidylinositol, egg phosphatidylethanolamine, and bovine phosphatidylserine.

*Fatty acid composition of coho salmon triacylglycerols.*—Triacylglycerol samples collected from the high-pressure liquid chromatography column were initially transesterified to fatty acid methyl esters following methods described in Christie (1982). The TAG samples, diluted with toluene and antioxidant, were mixed with a 1.5% solution of  $\text{H}_2\text{SO}_4$  in methanol then heated at  $80^{\circ}\text{C}$  for 2 h. A 5% NaCl solution was added during cooling, and the cooled solution was extracted twice with hexane. Each time the extract was washed with 2% potassium carbonate in deionized water and then dried by pouring the contents through a column loaded with  $\text{Na}_2\text{SO}_4$ . The purified fatty acid methyl esters were collected in a clean vial, and the volume adjusted under nitrogen to make 1 mL.

The concentrations of 29 fatty acids were measured via temperature-programmed gas chromatography and mass spectrometry. The fatty acid

TABLE 1.—Average  $\pm$  SE weight (g), lipid content, and lipid class composition of coho salmon after rearing with different densities of pink salmon carcasses. Percent lipid is expressed relative to whole wet mass; lipid classes represent percentages of total lipid, where WE = wax and cholesterol esters, TAG = triacylglycerols, ST = sterols, FFA = free fatty acids, and  $\Sigma$ PL = sum of all phospholipids detected. Lipid classes also examined but not detected include monoacylglycerols, diacylglycerols, phosphatidylinositol, lyso-phosphatidylcholine, and sphingomyelin.

Carcasses/ m <sup>2</sup>	Weight	% Lipid	Lipid class composition (%)				
			WE	TAG	ST	FFA	$\Sigma$ PL
0	1.45 $\pm$ 0.12	1.5 $\pm$ 0.1	3.7 $\pm$ 0.6	5.3 $\pm$ 2.3	15.0 $\pm$ 2.2	35.1 $\pm$ 4.1	40.0 $\pm$ 4.7
1	2.56 $\pm$ 0.28	4.8 $\pm$ 0.4	11.1 $\pm$ 0.6	42.2 $\pm$ 3.4	13.9 $\pm$ 1.2	21.8 $\pm$ 3.2	10.9 $\pm$ 1.1
4	3.21 $\pm$ 0.30	5.7 $\pm$ 0.3	12.2 $\pm$ 0.7	52.9 $\pm$ 3.4	12.3 $\pm$ 1.8	15.7 $\pm$ 2.3	7.0 $\pm$ 1.4

methyl esters were separated on a Hewlett-Packard model 6890 gas chromatograph equipped with a 30-m Omegawax 250 fused silica capillary column. Fatty acid masses were measured with a Hewlett Packard model 5973 mass-selective detector operating in selected ion monitoring mode. Fatty acid concentrations were determined using a five-point calibration curve normalized to deuterated surrogates (19:0 and 23:0). Surrogates were added to samples before transesterification. The calibration curves were developed each day samples were analyzed. Fatty acid concentrations were corrected to reflect the molecular weight differences between the ester and the respective acid. Concentrations were further corrected by subtracting the concentrations for each fatty acid observed in method blanks. Recovery rates of the surrogates were estimated by comparing observed masses to masses of an internal standard (21:0) that was added just before injection. Estimates of analytical error were determined from reference samples processed at the same time as the samples. Fatty acid concentrations are reported as percentage of the total mass of fatty acids observed.

*Experimental design statistical analysis.*—The experiment used a randomized block, split-plot design (Wipfli et al. 2003). Data were analyzed using the general linear model procedure in the Statistical Analysis System (SAS) to test the hypotheses. The three carcass treatments (0, 1, and 4 carcasses/m<sup>2</sup>) were applied to whole channels (plots). Each of these treatments was represented on a given table (block), and a total of six tables were used. Fish size was the subplot factor, and each channel was seeded with three coho salmon: small (1.30  $\pm$  0.04 g), medium (1.86  $\pm$  0.04 g), and large (2.47  $\pm$  0.08 g). Carcass-load effects were examined by comparing the mean response of carcass-treated fish with fish not exposed to carcasses and by contrasting the responses of fish exposed to the two carcass loads.

Responses tested by this design include the pro-

portion of wet weight devoted to lipid and the proportion of lipid devoted to the major storage and structural lipid classes: TAG and the summed phospholipids. We also evaluated the effect of carcass load on the omega-3:omega-6 ratio of the TAG to identify the influence of marine-derived lipid on coho salmon energy reserves. To understand how the ratio was affected, we also examined the percentage of TAG composing 20:5(n-3) and 22:6(n-3) (the two most common omega-3 fatty acids) and 18:2(n-6) (the most common omega-6 fatty acid). All response variables were transformed as necessary to conform to the assumption of homogenous variances and normality.

## Results

### *Lipid Content and Composition*

Juvenile coho salmon in treated channels had significantly more lipid ( $P < 0.001$ ) than fish in the control channels. Fish from the treated channels has similar amounts of lipid, regardless of carcass load ( $P = 0.160$ ). Coho salmon not exposed to carcasses averaged 1.46  $\pm$  0.10% (mean  $\pm$  SE) lipid, compared with 4.75  $\pm$  0.37% for the one-carcass treatment and 5.72  $\pm$  0.34% for the four-carcass treatments. No interaction was detected between fish size and carcass load ( $P = 0.090$ ).

Increases in lipid content among fish exposed to carcasses were accompanied by dramatic differences in the concentrations of storage lipids (Table 1). The percentage of lipid devoted to TAG (the primary lipid storage class) was significantly higher among fish from the treated channels ( $P < 0.001$ ) and increased with carcass density ( $P = 0.007$ ). The percentage of lipid devoted to TAG increased tenfold between fish from the untreated and most heavily loaded channels. No interaction between size and carcass load was detected ( $P = 0.126$ ).

Fish not exposed to carcasses had significantly

TABLE 2.—Fatty acid composition (mean ± SE) of coho salmon triacylglycerols (TAG), expressed as the percent total fatty acids. Fatty acids are designated as *xx:a (n-b)*, where *xx* is the carbon chain length, *a* is the number of double bonds, and *b* is the location of the first double bond from the methyl end. Fatty acids that were not detected (ND) are also listed, as is the total mass of fatty acids recovered.

Fatty acid	Carcasses/m <sup>2</sup>		
	0	1	4
14:0	1.68 ± 0.28	3.25 ± 0.35	2.66 ± 0.18
14:1 (n-5)	0.03 ± 0.03	0.06 ± 0.02	0.07 ± 0.03
15:0	0.38 ± 0.06	0.56 ± 0.04	0.54 ± 0.02
15:1 (n-5)	ND	ND	ND
16:0	13.00 ± 0.33	14.06 ± 0.61	13.54 ± 0.55
16:1 (n-7)	4.48 ± 0.41	6.20 ± 0.42	6.47 ± 0.51
17:0	0.61 ± 0.06	0.53 ± 0.03	0.48 ± 0.02
17:1 (n-7)	0.38 ± 0.06	0.59 ± 0.05	0.72 ± 0.03
18:0	6.50 ± 0.36	4.97 ± 0.19	4.70 ± 0.11
18:1 (n-11)	0.02 ± 0.02	0.14 ± 0.03	0.09 ± 0.02
18:1 (n-9) (cis and trans)	34.41 ± 2.19	23.23 ± 1.22	20.87 ± 0.41
18:1 (n-7)	3.32 ± 0.31	3.68 ± 0.16	3.60 ± 0.18
18:2 (n-6) (cis)	17.23 ± 1.80	7.39 ± 0.70	5.16 ± 0.47
18:2 (n-6) (trans)	ND	ND	ND
18:3 (n-6)	0.67 ± 0.26	0.37 ± 0.06	0.30 ± 0.05
18:3 (n-3)	5.54 ± 1.03	2.74 ± 0.38	1.98 ± 0.20
20:0	0.61 ± 0.06	0.22 ± 0.03	0.14 ± 0.02
20:1 (n-9)	0.79 ± 0.14	2.09 ± 0.29	1.45 ± 0.11
20:2 (n-6)	0.14 ± 0.09	0.34 ± 0.05	0.35 ± 0.04
20:3 (n-6)	0.84 ± 0.18	0.34 ± 0.02	0.29 ± 0.01
20:4 (n-6)	2.32 ± 0.27	1.36 ± 0.06	1.36 ± 0.03
20:3 (n-3)	0.26 ± 0.07	0.33 ± 0.05	0.26 ± 0.02
20:5 (n-3)	1.47 ± 0.25	6.56 ± 0.91	9.11 ± 0.66
22:1 (n-9)	0.19 ± 0.08	0.45 ± 0.08	0.27 ± 0.03
22:2 (n-6)	0.01 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
22:5 (n-3)	1.10 ± 0.45	3.29 ± 0.39	4.30 ± 0.29
22:6 (n-3)	3.31 ± 0.61	16.61 ± 1.94	20.76 ± 1.16
22:0	0.26 ± 0.03	0.09 ± 0.01	0.06 ± 0.01
24:0	0.13 ± 0.03	0.03 ± 0.01	0.03 ± 0.01
24:1 (n-9)	0.32 ± 0.10	0.47 ± 0.06	0.37 ± 0.02
Total saturated fatty acids	23.16 ± 0.74	23.70 ± 1.11	22.15 ± 0.84
Total monosaturated fatty acids	43.95 ± 1.79	36.91 ± 1.43	33.91 ± 0.82
Total omega-3 polyunsaturated fatty acids	11.68 ± 0.96	29.53 ± 3.04	36.42 ± 2.02
Total omega-6 polyunsaturated fatty acids	21.21 ± 1.87	9.86 ± 0.74	7.51 ± 0.50
Omega-3/omega-6	0.61 ± 0.12	3.60 ± 0.60	5.39 ± 0.54
Total mass recovered (ng)	390.91 ± 53.03	500.81 ± 27.19	464.21 ± 35.34

more lipid devoted to structure ( $P < 0.001$ ) than fish exposed to carcasses, as indicated by differences in phospholipid (Table 1). The lipid of coho salmon not exposed to carcasses averaged  $40.0 \pm 4.7\%$  phospholipid, whereas those exposed to the carcasses averaged much less ( $<10.8 \pm 1.4\%$ ) and decreased with carcass density ( $P = 0.012$ ). No interaction between fish size and carcass load was detected ( $P > 0.133$ ).

*Fatty Acid Composition of TAG*

Increased TAG levels among coho salmon exposed to carcasses were accompanied by increased concentrations of omega-3 fatty acids. Coho salmon exposed to carcasses had significantly higher omega-3 : omega-6 ratios ( $P < 0.002$ ) than unexposed fish, and levels increased with carcass den-

sity ( $P = 0.024$ ). No interaction between carcass load and fish size was detected ( $P = 0.913$ ). Ratios increased nearly tenfold between unexposed coho salmon and those exposed to 4 carcasses/m<sup>2</sup> (Table 2).

The large increase the omega-3 : omega-6 ratio resulted from increasing concentrations of 20:5(n-3) and 22:6(n-3) coupled with decreased levels of omega-6 fatty acids. The TAG percentages composing 20:5(n-3) and 22:6(n-3) were five to six times higher in exposed coho salmon than unexposed coho ( $P < 0.001$ ), but did not differ among the exposed groups ( $P > 0.312$ ). In contrast, the TAG percentage composing 18:2(n-6), the most prevalent omega-6 fatty acid, decreased with carcass density ( $P = 0.049$ ). Interactions between fish size and carcass density were not detected in any

of these comparisons ( $P > 0.200$ ). Because of these changes, the summed amounts of omega-6 fatty acids decreased from  $21 \pm 1.9\%$  of TAG in the unexposed to  $7 \pm 0.5\%$  of TAG in the most exposed coho salmon; the percentages of omega-3 fatty acids increased from  $11.7 \pm 1.0\%$  in the unexposed to  $36.4 \pm 2.0\%$  in the most exposed. In addition, the percentage of saturated fatty acids with more than 18 carbons and 18:1(n-9) appeared to be lower among the exposed coho (Table 2) relative to the unexposed coho, but these differences were not tested.

### Discussion

The conditions in the carcass-treated artificial streams led to improved nutritional condition for freshwater-rearing coho salmon, as indicated by increased energy storage and reserves of essential fatty acids. Increased energy reserves are consistent with observations of increased food availability and growth of these same fish (Wipfli et al. 2003). In a previous study, Wipfli et al. (1999) reported the number of chironomids increased linearly with carcass density, and in this study chironomids were the most abundant macroinvertebrate taxon present (Chaloner et al. 2002b). In southeastern Alaska, chironomids can be an important component of coho salmon diets during September (Koski and Kirchofer 1984; Wipfli 1997). Similarly, in Washington Bilby et al. (1998) found increased numbers of macroinvertebrates in streams with carcasses present. In addition to the macroinvertebrates, carcasses and eggs provided an additional source of lipid and protein for coho salmon (Koski and Kirchofer 1984; Bilby et al. 1998).

The increased growth and TAG indicates that the presence of carcasses helps to ameliorate the conflicting demands on energy for growth and storage in juvenile salmonids. To avoid predation, young-of-the-year must use surplus energy to maximize growth and avoid predation (Post and Parkinson 2001). However, that energy allocation must be ultimately be balanced against the need to store energy and forestall starvation during winter. Juvenile coho salmon exposed to carcasses in this study were able to increase their length by at least 14% (Wipfli et al. 2003) while simultaneously increasing their mass-specific energy storage at least 25-fold (i.e., TAG%  $\times$  lipid% from Table 1). This increase would be particularly beneficial to young-of-the-year salmonids overwintering in streams because they can use more than 40% of

their lipid reserves overwinter (Berg and Bremset 1998).

The relatively high levels of free fatty acids observed in these fish indicate that we may have underestimated the TAG and phospholipid levels. Free fatty acids are the products of enzymatic hydrolysis of phospholipid and TAG, and free fatty acids tend to accumulate in frozen samples (Auborg 1999). Increases in free fatty acid levels are usually attributed to the hydrolysis of phospholipid (Bligh and Scott 1966; Braddock and Dugan 1972; Nishimoto et al. 1978; Hardy et al. 1979; Kaneniwa et al. 2000), suggesting our estimates of TAG are less likely to be influenced by hydrolyzation than our phospholipid estimates. In addition, estimates of total lipid are unaffected by hydrolyzation, and variation in lipid content is usually driven by variation in TAG (Sheridan et al. 1983). Therefore, despite uncertainty regarding the true TAG level for each treatment, our conclusion that TAG content increases with carcass load is unaffected.

The fatty acid composition of the TAG demonstrates that coho salmon in the treated channels were consuming material enriched with omega-3 fatty acids, particularly 22:6(n-3). Although the carcass fatty acids were not directly measured, the carcasses were the most likely source of omega-3 fatty acids. This is consistent with results of isotopic analysis revealing enriched levels of  $^{15}\text{N}$  and  $^{13}\text{C}$  in the carcass-exposed fish (Chaloner et al. 2002a). Adult salmon are well-known sources of 22:6(n-3) and 20:5(n-3) (Henderson and Tocher 1987; Ackman 1999). For example, Sasaki et al. (1989) reported 22:6(n-3) and 20:5(n-3) represented more than 10% of the total lipid in fresh adult pink salmon. In Atlantic salmon *Salmo salar* spawning in Norwegian rivers these two fatty acids accounted for nearly 30% of the lipid (Olsen 1999). Insects are another potential lipid source for coho, however aquatic insects typically have very low amounts (<1%) of 22:6(n-3) (Hanson et al. 1985; Bell et al. 1994; Sushchik et al. 2003) indicating they were an unlikely source for 22:6(n-3).

Alternatively, coho salmon could have synthesized 22:6(n-3) and 20:5(n-3) from ingested 18:3(n-3) (Olsen and Ringo 1992). Aquatic insects could be sources of 18:3(n-3) (Hanson et al. 1985; Bell et al. 1994; Sushchik et al. 2003), and increased 22:6(n-3) and 20:5(n-3) might therefore be accounted for by increased prey availability (Wipfli et al. 2003). However, Bell et al. (2001) found that the contribution of synthesized 22:

6(n-3) to the total amount of 22:6(n-3) in Atlantic salmon tissues was about 5% that of dietary contributions. This suggests a negligible effect of insect density on the levels of 22:6(n-3) in the tissues of carcass exposed coho.

The small impact of insects on the levels of omega-3 fatty acids found in the coho salmon is further indicated by comparing the lipid masses represented by insects with those of carcass tissue. In a similar study, chironomid larvae accounted for 97% of the invertebrate biomass in test channels loaded with carcasses and represented an average biomass of 8.6 g in the channels loaded with four carcasses/m<sup>2</sup> (Chaloner et al. 2002b). Chironomids average approximately 15% lipid (Hanson et al. 1985); thus, the lipid available as insects in these channels was approximately 1.3 g. In contrast, these same channels were loaded with approximately 2,400 g of salmon flesh and 432 g of eggs. Senescent female pink salmon average approximately 0.7% lipid and their eggs average 12.5% (Gende 2002), indicating that marine-derived lipids accounted for more than 50 times (70.8 g) the amount represented by insects. This difference is amplified by the fact that marine-derived lipids have proportionately higher levels of omega-3 fatty acids.

Despite the growing body of literature describing the effects of marine-derived nutrients, few studies describe carcass densities under natural circumstances. Estimates derived by dividing the total escapement by the area of the stream obviously inflate carcass density because they fail to account for carcass losses. An alternative estimate can be obtained from Wertheimer et al. (2000), who counted the total number of carcasses deposited in the flood plains of eight pink salmon streams (as part of an effort to estimate total escapement to those southeastern Alaska streams). Their reported ratio of carcass numbers to escapement indicated that between 8% and 40% of the escapement is retained as carcasses in the flood plain. This can be considered an upper limit to the proportion of the run retained in the flood plain because it does not account for number of carcasses lost after they were initially counted. Although area estimates are not available for those streams, these ratios can be applied to an escapement to Sashin Creek, which is within 35 km of the surveyed streams. Sashin Creek has a coho salmon rearing area of approximately 16,557 m<sup>2</sup> (Crone and Bond 1976). It had an escapement of 35,655 pink salmon in the same year as the surveyed streams (Wertheimer et al. 2000). Thus, the upper limit of carcass density in

Sashin Creek for that year probably ranged between 0.17 and 0.86 carcasses/m<sup>2</sup>. Assuming a spawned-out pink salmon carcass weighs approximately 1,200 g, this translates to a range of approximately 200–1,000 g of tissue/m<sup>2</sup>. In contrast, Bilby et al. (1998) reported carcass densities ranging between 3 and 10 g/m<sup>2</sup> for two southwestern Washington streams.

Although carcass densities in wild streams are likely to differ from those applied in this study, it is important to note that the greatest difference in response in this study was obtained between unexposed fish and those exposed to 1 carcass/m<sup>2</sup>. We report a 25-fold increase in the mass specific TAG level for fish from these two treatments, and exposed fish weighed nearly twice that of the unexposed fish (Wipfli et al. 2003). Consequently, lower densities than those described here are likely to elicit a measurable response. This was the case for cutthroat trout *O. clarki* and Dolly Varden *Salvelinus malma* that demonstrated increased growth after exposure to a cumulative density of 0.54 carcasses/m<sup>2</sup> in Cedar Creek, Alaska (Wipfli et al. 2003). Carcass density may be quite high in localized stream sections that collect carcasses, and these areas may prove attractive to rearing salmon. For example, Bilby et al. (1998), after seeding short sections of wild streams with coho salmon carcasses at densities ranging between 560 and 710 g/m<sup>2</sup>, recorded significant increases in densities of rearing coho salmon in those sections. Thus, estimates of carcass density based on total stream area may underrepresent the densities experienced by juvenile fish.

The difficulties associated with estimating the carcass density in natural streams reveal the need for metrics that integrate carcass signal strength and effects in juvenile salmonid tissues. Such a metric would obviate the need to estimate density by providing a value that integrates carcass effects on freshwater-rearing salmonids from all possible sources. Isotopic analysis has provided valuable insight into the trophic pathways for marine-derived carbon and nitrogen and offers the potential as a tool for measuring signal strength (Chaloner et al. 2002a). The data presented here reveal fatty acid and lipid class analysis may be another such a tool. Fatty acid analysis may be used for identifying the presence of marine derived lipids in the tissues of juvenile fishes, and examination of the lipid class composition can measure the benefit in terms of energy allocation. These data complement those provided by isotopic studies on carcass exposed juveniles because lipids are typically

depleted in  $^{13}\text{C}$  and removed from tissues before analysis (Chaloner et al. 2002a). Consequently, observations of marine-derived nitrogen and carbon, combined with observations of increased marine-derived fatty acids, give a picture of the overall strength of the carcass influence, whereas lipid class analysis measures the energetic effect.

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