

# Effects of spawning Pacific salmon on the isotopic composition of biota differ among southeast Alaska streams

ALEXANDER J. REISINGER, DOMINIC T. CHALONER, JANINE RÜEGG<sup>1</sup>, SCOTT D. TIEGS<sup>2</sup> AND GARY A. LAMBERTI

Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, U.S.A.

## SUMMARY

1. Adult Pacific salmon (*Oncorhynchus* spp.) transport marine nutrients to fresh waters and disturb sediments during spawning. The relative importance of nutrient fertilisation and benthic disturbance by salmon spawners can be modulated by environmental conditions (e.g. biological, chemical and physical conditions in the catchment, including human land use).

2. To determine the importance of the environmental context in modifying the uptake and incorporation of salmon-derived material into stream biota, we measured the nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotopic composition of benthic algae (i.e. epilithon) and juvenile coho salmon (*Oncorhynchus kisutch*) in seven streams across a timber-harvest gradient (8–69% catchment area harvested), both before and during the salmon run. Conditional bootstrap modelling simulations were used to assess variability in the response of epilithon and juvenile coho salmon to spawning salmon.

3. In response to spawning salmon, epilithon exhibited enrichment in both  $\delta^{15}\text{N}$  (mean: 1.5‰) and  $\delta^{13}\text{C}$  (2.3‰). Juvenile coho were also enriched in both  $\delta^{15}\text{N}$  (0.7‰) and  $\delta^{13}\text{C}$  (1.4‰). Conditional bootstrap models indicate decreased variation in data as spatial replication increases, suggesting that the number of study sites can influence the results of Pacific salmon isotope studies.

4. Epilithon isotopic enrichment was predicted by environmental conditions, with  $\delta^{15}\text{N}$  enrichment predicted by stream temperature and timber harvest ( $R^2 = 0.87$ ) and  $\delta^{13}\text{C}$  enrichment by discharge, sediment size, timber harvest and spawner density ( $R^2 = 0.96$ ). Furthermore, we found evidence for a legacy effect of salmon spawners, with pre-spawner  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of both epilithon and juvenile coho predicted by salmon run size in the previous year.

5. Our results show that the degree of incorporation of salmon-derived nitrogen and carbon differs among streams. Furthermore, the environmental context, including putative legacy effects of spawning salmon, can influence background isotopic concentrations and utilisation of salmon-derived materials in southeast Alaska salmon streams. Future studies should consider the variation in isotopic composition of stream biota when deciding on the number of study sites and samples needed to generate meaningful results.

**Keywords:** *Oncorhynchus*, periphyton, resource subsidy, southeast Alaska, stable isotopes

## Introduction

Pacific salmon (*Oncorhynchus* spp.) can have important ecological effects on stream ecosystems in which they spawn. Salmon spawners deliver an ecosystem resource subsidy (Polis, Power & Huxel, 2004) in the form of

excretory products, gametes and carcasses that can increase primary production (Chaloner *et al.*, 2004, 2007) and growth of stream-resident fish (Bilby *et al.*, 1998; Wipfli *et al.*, 2003). Spawning salmon can also act as ecosystem engineers (Wright & Jones, 2006) by scouring and displacing sediment and benthic organisms during

Correspondence: Alexander J. Reisinger, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, U.S.A.  
E-mail: areisin1@nd.edu

<sup>1</sup>Present address: Janine Rüegg, Division of Biology, Kansas State University, Manhattan, KS 66506, U.S.A.

<sup>2</sup>Present address: Scott D. Tiegs, Department of Biological Sciences, Oakland University, Rochester, MI 48309, U.S.A.

upstream migration and redd construction (Peterson & Foote, 2000; Moore, Schindler & Scheuerell, 2004). The ecological effects of salmon thus represent a balance between fertilisation and disturbance that, in turn, can be modulated by the environmental context (i.e. the ambient chemical, physical and biological conditions; after Janetski *et al.*, 2009) of a given ecosystem.

Stable isotope analyses are a useful tool in ecology (Schindler & Lubetkin, 2004; Fry, 2006), providing important information about ecosystem structure (Vander Zanden, Cabana & Rasmussen, 1997) and function (Peterson *et al.*, 2007). Despite the broad agreement about the processes that contribute to isotopic fractionation (Fry, 2006), uncertainty remains about the importance of specific environmental factors that control isotopic enrichment and fractionation in fresh waters (but see Finlay, Power & Cabana, 1999; Finlay, 2004). In part, this uncertainty reflects the limited number of isotopic studies at the landscape scale (i.e. isoscapes, after Bowen, 2010), which encompass sufficient environmental heterogeneity to draw general conclusions regarding environmental influences on isotopic enrichment and fractionation. Such uncertainties about stable isotopes apply to studies of Pacific salmon.

Adult salmon accumulate most of their biomass in the ocean and the distinct isotopic composition of marine material allows stable isotopes to be used as tracers of the movement of salmon-derived material into and through freshwater and terrestrial food webs (Schindler & Lubetkin, 2004; Fry, 2006). The presence of salmon spawners has been shown to alter the isotopic composition of freshwater biota, including benthic algae (i.e. epilithon) (Kline *et al.*, 1990; Holtgrieve *et al.*, 2010), macroinvertebrates (Chaloner *et al.*, 2002) and stream-resident fish (Bilby, Fransen & Bisson, 1996; Reichert, Greene & Bilby, 2008). Furthermore, the magnitude of the change in isotopic composition (i.e. isotopic enrichment or depletion) has been used to estimate the quantity of salmon-derived nitrogen (N) and carbon (C) assimilated into food webs (Bilby *et al.*, 1996; Chaloner *et al.*, 2002). However, many salmon stable isotope studies have been of either limited spatial extent (1–2 streams, Kline *et al.*, 1990; Bilby *et al.*, 1996; Chaloner *et al.*, 2002), or considered only one trophic level (Reichert *et al.*, 2008; Holtgrieve *et al.*, 2010; Verspoor, Braun & Reynolds, 2010). Background variability in data, especially due to low spatial resolution, can be addressed using conditional bootstrap modelling (Dixon, 2001). Such modelling can increase the confidence of study outcomes while providing insights into study designs (cf. Rügge *et al.*, 2012). Janetski *et al.* (2009) argued that studies undertaken at larger spatial scales are needed to make broader generalisations about the role of salmon-

derived material in freshwater ecosystems. Such studies also need to analyse the variability of predictor and response variables, and consider the consequences for study design, especially given the variety of environmental conditions of native salmon spawning streams.

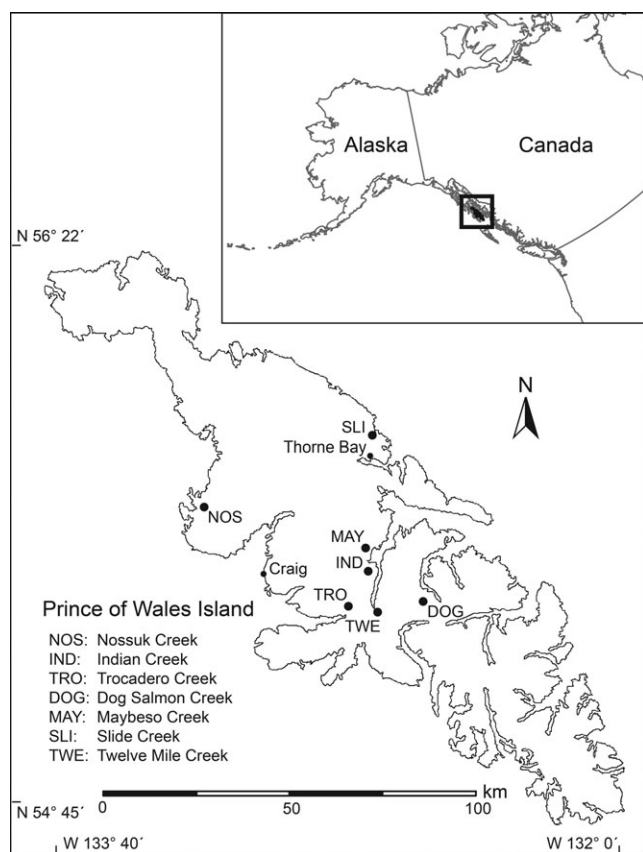
Only recently has there been an explicit attempt to understand the role of the environmental context in determining the ecological effects of spawning salmon, including salmon studies in general (Tiegs *et al.*, 2008; Rügge *et al.*, 2012) as well as those specifically using stable isotopes (Holtgrieve *et al.*, 2010; Verspoor *et al.*, 2010). Variation in the environmental context among streams can be driven by human land-use change. Timber harvest is a prevalent land use in the native range of salmon (including southeast Alaska; Bryant & Everest, 1998) that can alter stream hydrology (Tonina *et al.*, 2008), water chemistry (Gravelle *et al.*, 2009), geomorphology (Jackson *et al.*, 2007) and sediment size (Platts *et al.*, 1989). Such changes in the environmental context could alter the capacity of a stream to retain and incorporate salmon-derived material. For example, reduced sediment size can shift the net ecological influence of salmon from enrichment to disturbance because smaller sediments are more prone to displacement and scour (Tiegs *et al.*, 2008; Holtgrieve *et al.*, 2010). Furthermore, changes in the environmental context could obscure detection of the broader ecological effects of salmon spawners. For example, changes in hydrology can alter the isotopic composition of epilithon, independent of biological mechanisms (Finlay *et al.*, 1999).

The goal of our study was to determine how the environmental context influences the uptake and incorporation of salmon-derived material by stream biota. To do so, we measured the N ( $\delta^{15}\text{N}$ ) and C ( $\delta^{13}\text{C}$ ) stable isotope composition of producer and consumer trophic levels (epilithon and juvenile coho salmon, *Oncorhynchus kisutch*) of seven southeast Alaska streams. We predicted that (i)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of epilithon and juvenile coho salmon would be higher 'during' the salmon run than 'before' the salmon run, and (ii) the extent of isotopic enrichment would be related to specific components of the environmental context, including factors altered by land-use change. We further assessed whether the spatial scale, specifically within-stream sample size and number of streams, would influence the ability of our study to detect isotopic enrichment of stream-resident biota.

## Methods

### Study sites

Our study was conducted in a temperate rainforest ecosystem of southeast Alaska. We studied seven streams



**Fig. 1** Location of study streams on Prince of Wales Island, southeast Alaska, U.S.A.

located on Prince of Wales Island (POW; Fig. 1), located in catchments with 8–69% of the catchment harvested of timber in the past 50 years, thus exhibiting a broad environmental gradient (Table 1, see Tiegs *et al.*, 2008; Rüegg *et al.*, 2012 for more information). Per cent timber harvest in the catchment was determined using ArcGIS<sup>TM</sup> (Esri, Redlands, CA, U.S.A.) and data provided by the USDA Forest Service and the southeast Alaska GIS Library (<http://seakgis.alaska.edu>). We used the area of

timber harvest upstream of our study reach to calculate per cent timber harvest, in contrast to Tiegs *et al.* (2008) and Rüegg *et al.* (2012) who reported total area harvested throughout the entire catchment of the same streams. All study streams receive runs of pink (*Oncorhynchus gorbuscha*), chum (*Oncorhynchus keta*) and coho (*O. kisutch*) salmon. Spawner abundances were estimated by counting salmon in 4-m-wide belt transects that were spaced every 10 m of the 300-m study reach, accounting for approximately 40% of the study reach (see Tiegs *et al.*, 2008 for full description). Spawner abundances were then scaled to the entire stream and converted to density using average stream width. Median sediment size for each stream was determined by surveying five haphazardly selected particles at each 10-m transect along the 300-m study reach using a gravelometer ( $n = 150$  particles per reach).

### Sample collection and processing

Streams were sampled for epilithon and juvenile coho twice in 2007, before the salmon run (June) and during the salmon run (October). The 'during' sampling took place after peak spawner densities were observed, but live fish and carcasses were still present in the channel.

In each stream, epilithon was collected from five haphazardly selected rocks, of typical size for the stream (mean dimensions: 12.5L × 8W × 5H cm), from four riffles before the salmon run ( $n = 20$  per stream) and from three riffles during the salmon run ( $n = 15$  per stream). The entire rock surface was scrubbed using synthetic brushes. Slurries from all rocks in one riffle were pooled, generating four and three pooled replicate samples per stream for before and during sampling periods, respectively. The epilithon slurry was then split, with a known volume filtered (Whatman GF/F<sup>®</sup>; Whatman PLC, Maidstone, Kent, U.K.) and stored frozen for later chlorophyll *a* (chl *a*) analysis. The remaining slurry was centrifuged, the precipitate dried at 60 °C and then stored frozen for

**Table 1** Characteristics of study streams

Site Name (Identifier)	Spawner density (# m <sup>-2</sup> )	Catchment area (km <sup>2</sup> )	Timber harvest (%)	Temperature <sup>*,†</sup> (°C)	Median sediment size (mm)	Baseflow discharge (m <sup>3</sup> s <sup>-1</sup> )
Indian (IND)	0.37	25.9	8.2	9.91 (4.25–13.55)	45	0.27
Trocadero (TRO)	0.11	44.8	8.5	9.63 (4.43–13.25)	64	0.48
Nossuk (NOS)	0.09	19.5	8.8	9.93 (3.65–13.19)	64	0.09
Dog Salmon (DOG)	0.94	37.3	18.1	6.98 (0.54–12.28)	90	0.41
Maybeso (MAY)	0.72	38.7	24.7	9.01 (3.26–12.73)	45	0.61
Slide (SLI)	0.20	26.0	57.8	10.60 (2.74–14.65)	64	0.28
Twelve-Mile (TWE)	0.51	31.1	68.5	9.46 (4.51–12.33)	23	0.52

\*Mean (range).

†Measured continuously using Onset<sup>®</sup> temperature dataloggers from 1 June to 31 October 2007.

isotopic analysis. Rock surface areas were determined by wrapping rocks in aluminium foil and then using a mass-surface area relationship (Steinman, Lamberti & Leavitt, 2006).

Pink salmon represented the major spawning run in POW streams (>90% of total spawners), but coho salmon are the dominant juveniles rearing in the streams; pink and chum salmon emigrate to the ocean shortly after hatching, whereas coho spend up to 2 years in their natal stream. Juvenile coho salmon ( $n = 5$  individuals of similar size; 50–60 mm nose-to-fork length) were collected from each stream both before and during the spawning run using baited minnow traps. Traps were baited with salmon eggs held in perforated whirlpaks® (Nasco, Fort Atkinson, WI, U.S.A.) that attract fish but prevent egg consumption from changing the isotopic composition of trapped fish (cf. Chaloner *et al.*, 2002). Coho were euthanised, weighed for mass, measured for length (nose to fork) and stored frozen until processed in the laboratory. Adult pink salmon were collected from each stream using hook-and-line ( $n = 5$  females per stream) to establish the isotopic endpoint of marine nutrients. Representative tissue cross-sections of pink salmon were taken from the head (snout to gill plate) and body (before dorsal fin to anal opening) and stored frozen for isotopic analysis.

In the laboratory, epilithon filters were submerged in ethanol and analysed fluorometrically for chlorophyll *a* (Steinman *et al.*, 2006), followed by ashing to determine ash-free dry mass (AFDM; Tiegs *et al.*, 2008). Chlorophyll *a* and AFDM were expressed per unit rock area based on sample volume and rock surface area. The remaining epilithon, as well as juvenile coho and adult pink salmon steaks, were thawed in preparation for stable isotope analysis. All samples were rinsed with 1.0 N HCl to remove carbonates associated with stream water in samples (Bosley & Wainright, 1999); juvenile coho were eviscerated prior to acidification. Acidified samples were then dried at 60 °C and homogenised into a fine powder. Juvenile coho and adult spawners were lipid-extracted after homogenisation using a 2 : 1 azeotropic chloroform/methanol solvent (Folch, Lees & Stanley, 1957) and re-dried at 60 °C. Lipids were removed because they can exhibit low  $\delta^{13}\text{C}$  compared to other biochemical components of organisms (DeNiro & Epstein, 1977). All processed samples were stored in pre-ashed scintillation vials at –20 °C prior to isotopic analyses.

Samples were analysed for stable isotopes using a Carlo Erba Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ, U.S.A.) coupled to a Finnigan Delta+ Mass Spectrometer (Thermo Scientific, Waltham, MA, U.S.A.) at the Center for Environmental Science and Technology at the

University of Notre Dame. Data were deemed acceptable if the standard deviation of acetanilide standards during the run was <0.2 per mil (‰). The standard deviations for acetanilide standards were 0.090 and 0.112 ‰ for N and C, respectively. Stable isotope ratios of N ( $\delta^{15}\text{N}$ ) and C ( $\delta^{13}\text{C}$ ) were expressed as

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

where  $R$  is  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$  for  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$ , respectively.

The term 'isotopic enrichment' is used to refer to the difference between before- and during-spawner isotopic composition due to variable background isotopic values among sites:

$$X_{\text{enrich}} = (\delta X_{\text{during}} - \delta X_{\text{before}}) \quad (2)$$

where  $X_{\text{enrich}}$  is the enrichment seen in  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$ ,  $\delta X_{\text{during}}$  is the mean  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  during the salmon run, and  $\delta X_{\text{before}}$  is the mean  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  from a site before the salmon run. Due to  $\delta^{13}\text{C}$  values being inherently negative, eqn 2 results in a negative value when enrichment occurs, and therefore,  $\delta^{13}\text{C}$  enrichment values were subsequently multiplied by –1. This approach differs from previous research (Kline *et al.*, 1990; Chaloner *et al.*, 2002) since those studies were concerned with the contribution of salmon-derived material to stream biota and thus used a mixing model, whereas our intent was to predict the change in isotopic composition due to salmon spawners.

### Statistical analyses

A two-way analysis of variance (ANOVA,  $\alpha = 0.05$ ; Zar, 2009) was used to determine whether the salmon run and study stream influenced the isotopic composition of epilithon and juvenile coho salmon. The two factors were time of sampling relative to the salmon run (i.e. before or during the run) and study stream. Response variables were  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of epilithon or juvenile coho in per mil (‰). As appropriate, Tukey's HSD post hoc test ( $\alpha = 0.05$ ; Zar, 2009) was used to evaluate specific contrasts between pairs of study streams. To confirm that adult spawner isotopic composition did not differ among streams, and thus was not driving differences in isotopic composition, one-way ANOVAs ( $\alpha = 0.05$ ; Zar, 2009) were conducted with  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  of adult pink salmon as the response variables and study stream as the factor. All data were assessed for normality and homogeneity of variance, but no transformations were needed to meet ANOVA assumptions.

The importance of environmental factors for determining the isotopic composition and enrichment of epilithon and juvenile coho was evaluated using multiple linear



regression (MLR) with Akaike Information Criterion for small sample sizes ( $AIC_c$ ; Burnham & Anderson, 2002) used to select the best model. Separate model selections were performed for the four different response variables: epilithon  $\delta^{15}\text{N}$ , epilithon  $\delta^{13}\text{C}$ , juvenile coho  $\delta^{15}\text{N}$  and juvenile coho  $\delta^{13}\text{C}$ . For each response variable, the three models examined were (i) before-salmon isotope composition, (ii) during-salmon isotopic composition and (iii) isotopic enrichment (i.e. the difference in isotopic composition between before salmon and during salmon; see eqn 2). Environmental factors included were water temperature, timber harvest, median sediment size, spawner density and discharge in the full model (Table 1), and then,  $AIC_c$  was used to select the best model. Factors included in the full model were based upon time period analysed in the model. For example, spawner density from the previous year was used as a predictor for the 'before' MLR to test for a potential 'legacy' salmon effect from one year to the next (*sensu* Verspoor *et al.*, 2010). Time-period-specific discharge and temperature were used for the before-salmon and during-salmon MLRs, and the change in discharge and temperature between the two time periods was used in the enrichment MLRs.

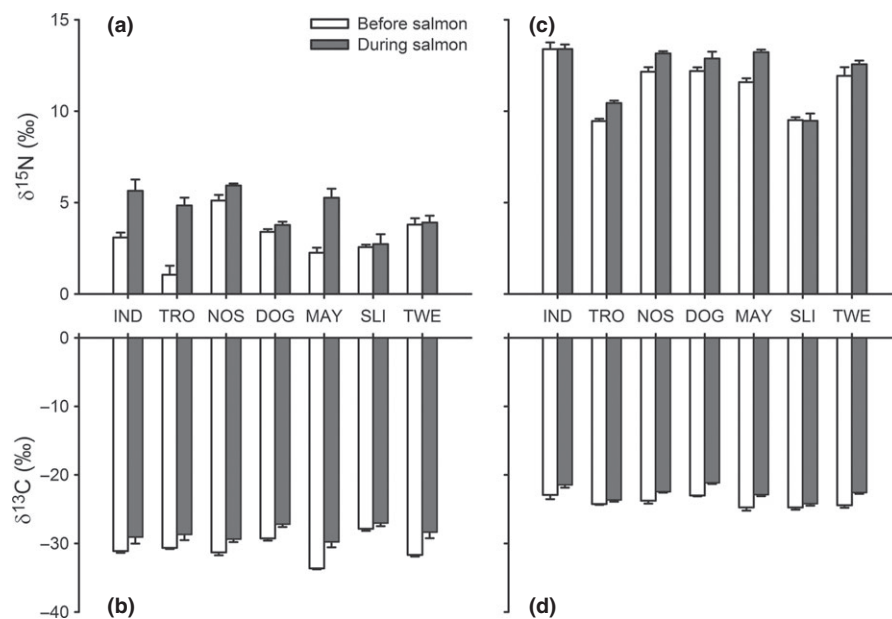
To assess the influence of replication and stream selection on the isotopic composition of epilithon and juvenile coho salmon, conditional bootstrapping models were run with our original data (Dixon, 2001). Iterations were set to randomly select 1–7 streams while allowing for

replacement (i.e. seven different bootstrapping models per time period and trophic level; cf. Rügge *et al.*, 2012). After data selection, the mean  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were calculated separately for before and during salmon, as well as isotopic enrichment for epilithon or juvenile coho (i.e. 42 models). Selection and mean calculation was repeated 1000 times for each model, and the 95% confidence interval (CI) was determined. Throughout the manuscript, data are presented as mean  $\pm$  standard error except for bootstrap model results, which are reported in terms of 95% CIs. All analyses were performed using R 2.8.0 (Crawley, 2007; R Development Core Team, 2010).

## Results

### Isotopic composition and enrichment of stream biota

The isotopic composition of epilithon changed with the arrival of Pacific salmon spawners, and the extent of this change was stream-specific. Epilithon  $\delta^{15}\text{N}$  increased from  $3.04 \pm 0.57\text{‰}$  (mean  $\pm$  SE) before the salmon run to  $4.58 \pm 0.68\text{‰}$  during the run (Fig. 2a), while  $\delta^{13}\text{C}$  increased from  $-30.80 \pm 0.69\text{‰}$  to  $-28.50 \pm 0.40\text{‰}$  (Fig. 2b). Presence of spawning salmon and stream were both significant factors in the  $\delta^{15}\text{N}$  of epilithon (two-way ANOVA:  $F_{1,35} = 64.2$ ,  $P < 0.001$  and  $F_{6,35} = 14.5$ ,  $P < 0.001$ , respectively). A significant interaction (two-way ANOVA:



**Fig. 2** Mean ( $\pm$ standard error)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for epilithon (a, b) and juvenile coho salmon (c, d), before (white) and during (grey) the salmon run for all streams sampled. Streams are ordered left to right, from lowest to highest proportion of catchment removed by timber harvest.

$F_{6,35} = 8.9$ ,  $P < 0.001$ ) suggests that enrichment is stream-specific (Fig. 3a). Presence of spawning salmon and stream were also significant factors in the  $\delta^{13}\text{C}$  of epilithon (two-way ANOVA:  $F_{1,35} = 79.7$ ,  $P < 0.001$  and  $F_{6,56} = 19.2$ ,  $P < 0.001$ , respectively), but no significant interaction was observed between the two main factors (Fig. 3b).

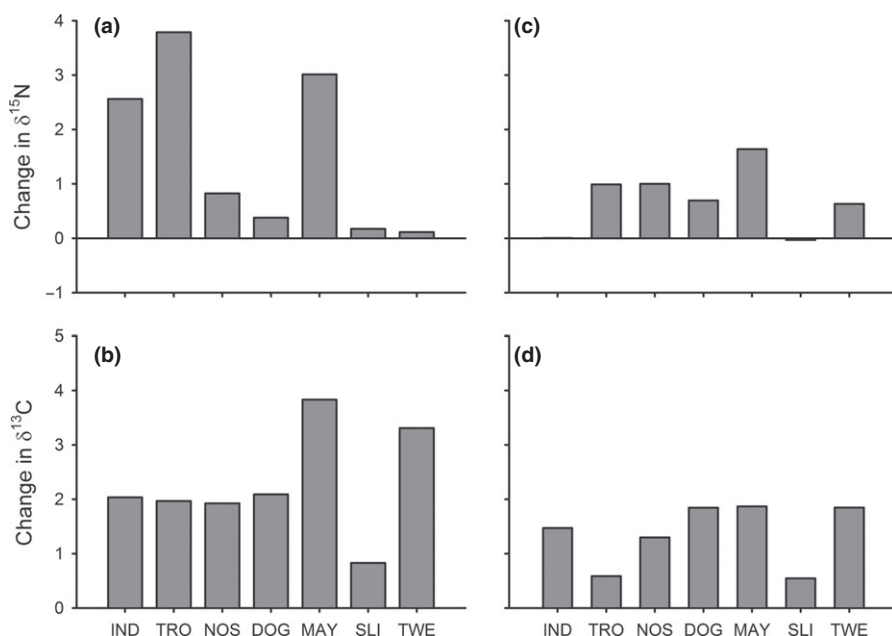
The isotopic composition of juvenile coho salmon also changed with the arrival of spawners, and the extent of this change was again stream-specific. Juvenile coho  $\delta^{15}\text{N}$  increased from  $11.46 \pm 0.55\text{‰}$  (mean  $\pm$  SE) to  $12.16 \pm 0.59\text{‰}$  (Fig. 2c) and  $\delta^{13}\text{C}$  increased from  $-23.98 \pm 0.77\text{‰}$  to  $-22.63 \pm 0.41\text{‰}$  (Fig. 2d). Presence of spawning salmon and stream were both significant factors in the  $\delta^{15}\text{N}$  of juvenile coho (two-way ANOVA:  $F_{1,56} = 24.3$ ,  $P < 0.001$  and  $F_{6,56} = 61.5$ ,  $P < 0.001$ ). A significant interaction (two-way ANOVA:  $F_{6,56} = 2.3$ ,  $P = 0.037$ ) suggests that the extent of isotopic enrichment of juvenile coho depended upon the stream (Fig. 3c). Salmon spawner presence and stream were both significant factors in the  $\delta^{13}\text{C}$  of juvenile coho (two-way ANOVA:  $F_{1,56} = 62.4$ ,  $P < 0.001$  and  $F_{6,56} = 15.9$ ,  $P < 0.001$ ) but, as with epilithon  $\delta^{13}\text{C}$ , no significant interaction existed between the main factors (Fig. 3d).

Changes in isotopic composition of epilithon and juvenile coho in response to the presence of salmon spawners showed similarities and differences. Epilithon  $\delta^{15}\text{N}$  (grand mean of increase:  $1.55 \pm 0.58\text{‰}$ ) (Fig. 3a) increased less than epilithon  $\delta^{13}\text{C}$  ( $2.28 \pm 0.37\text{‰}$ ) (Fig. 3b).

Similarly, juvenile coho  $\delta^{15}\text{N}$  ( $0.70 \pm 0.22\text{‰}$ ) (Fig. 3c) increased less than juvenile coho  $\delta^{13}\text{C}$  ( $1.35 \pm 0.22\text{‰}$ ) (Fig. 3d). Overall, epilithon exhibited more enrichment than juvenile coho.

#### Environmental influences on isotopic enrichment

The isotopic composition of epilithon was predicted by certain environmental factors under specific circumstances. The  $\delta^{15}\text{N}$  of epilithon before salmon spawners was best predicted by temperature and spawner density from the previous year ( $R^2 = 0.56$ ), and  $\delta^{13}\text{C}$  of epilithon before salmon was best predicted by temperature, spawner density from the previous year, per cent timber harvest and median sediment size ( $R^2 = 0.85$ ) (Table 2). During salmon spawning, epilithon  $\delta^{15}\text{N}$  was best predicted by a model ( $R^2 = 0.99$ ) which included per cent timber harvest, sediment size, discharge and the current year's spawner density (Table 2), whereas  $\delta^{13}\text{C}$  was not explained by any environmental factor considered (Table 2). The isotopic enrichment of epilithon  $\delta^{15}\text{N}$  (i.e. difference between before and during spawning) was positively related to water temperature and negatively to per cent timber harvest ( $R^2 = 0.87$ , Table 2). The  $\delta^{13}\text{C}$  enrichment of epilithon was negatively related to discharge, sediment size and per cent timber harvest and positively to spawner run density ( $R^2 = 0.96$ , Table 2).



**Fig. 3** Change in isotopic ratios of epilithon (a, b) and juvenile coho salmon (c, d) between before and during the salmon run for all streams sampled. Streams are ordered left to right, from lowest to highest proportion of catchment removed by timber harvest.

**Table 2** Models selected by AIC<sub>c</sub> to predict isotopic ratios of epilithon and juvenile coho salmon during the various time periods of the study

Response	Time period	Factors in best model	R <sup>2</sup>
Epilithon $\delta^{15}\text{N}$	Before	T (+), 2006 spawners (+)	0.56
	During	Harvest (-), sediment (-), Q (-), 2007 spawners (+)	0.99
	Enrichment	T (+), harvest (-)	0.87
Epilithon $\delta^{13}\text{C}$	Before	T (+), 2006 spawners (-), harvest (+), sediment (+)	0.85
	During	None	NA
	Enrichment	Q (-), 2007 spawners (+), sediment (-), harvest (-)	0.96
Juvenile coho salmon $\delta^{15}\text{N}$	Before	2006 spawners (+), Q (-), harvest (-), sediment (+)	0.98
	During	None	NA
	Enrichment	None	NA
Juvenile coho salmon $\delta^{13}\text{C}$	Before	T (+), harvest (-), 2006 spawners (+)	0.91
	During	Harvest (-), Q (-), T (-), sediment (-)	0.94
	Enrichment	2007 spawners (+), Q (-)	0.76

T, temperature; harvest, timber harvest; 2006 and 2007 spawners, spawner densities; Q, discharge; sediment, sediment size.

The isotopic composition of juvenile coho was also explained by environmental factors but again under specific circumstances. Before the salmon run, juvenile coho  $\delta^{15}\text{N}$  was best predicted by spawner density in the previous year, discharge, sediment size and timber harvest ( $R^2 = 0.98$ , Table 2), while  $\delta^{13}\text{C}$  was best predicted by water temperature, timber harvest and the previous year's spawner density ( $R^2 = 0.91$ , Table 2). During the salmon run,  $\delta^{15}\text{N}$  of juvenile coho was not predicted by environmental factors, whereas the best model for  $\delta^{13}\text{C}$  included timber harvest, discharge, temperature and sediment size ( $R^2 = 0.94$ , Table 2). Isotopic enrichment of juvenile coho  $\delta^{15}\text{N}$  was not predicted by any environmental factor, whereas  $\delta^{13}\text{C}$  was best predicted by the current year's spawner density and discharge ( $R^2 = 0.76$ , Table 2).

#### Variability of isotopic composition

Confidence intervals of epilithon isotopic composition were smaller during the salmon run compared to before salmon for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Not surprisingly, higher replication provided a more accurate representation of the observed mean. The largest reduction in the CI of epilithon isotopic composition occurred when the number of streams sampled increased from one to two, reducing the CI for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  by approximately 30% before salmon and approximately 20% during salmon (Fig. 4a,b). The largest % reduction in the variability of epilithon  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  enrichment also occurred when the number of streams increased from one to two, reducing the CI by approximately 30% (Fig. 5a,b).

The CI for juvenile coho isotopic composition was larger during the salmon run for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (Fig. 4c,d). The greatest CI reduction in the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of juvenile coho before the salmon run occurred when the

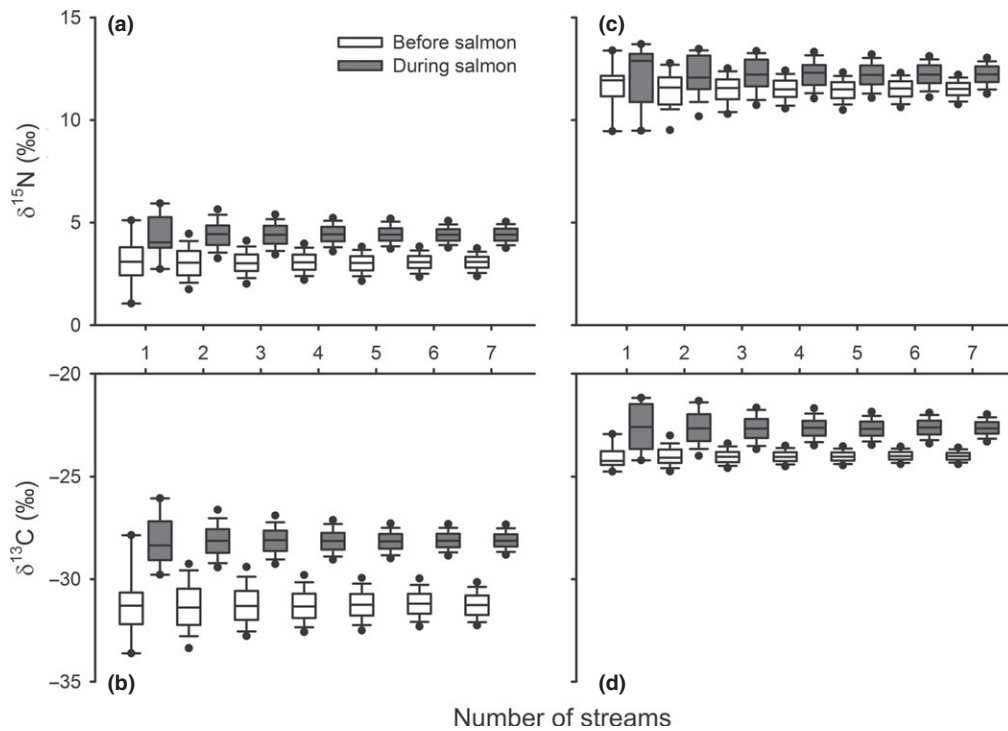
number of streams sampled increased from two to three, resulting in an approximately 20% decrease in the CI. During the salmon run, the largest reduction in CI occurred when replication was increased from one to two streams, reducing the CI by approximately 30% (Fig. 4c,d). The variability of enrichment was reduced most by increasing the number of streams from one to two, which reduced the CI for juvenile coho by approximately 35% (Fig. 5c,d).

## Discussion

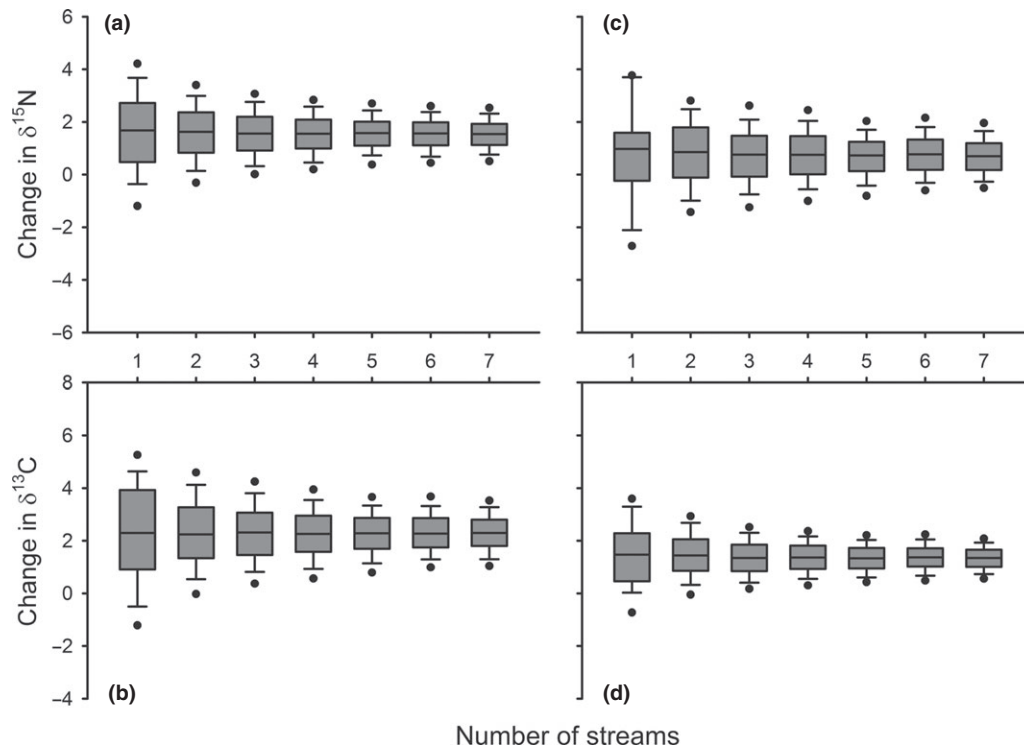
### *Uptake and incorporation of salmon-derived nutrients by stream biota*

Epilithon became isotopically enriched in the presence of Pacific salmon spawners in all of our study streams. Enrichment of epilithon  $\delta^{15}\text{N}$  (1.5‰) was somewhat lower than observed in other studies in southeast Alaska (1.9–4.5‰; Kline *et al.*, 1990; Chaloner *et al.*, 2002) or Washington (1.9–2.2‰; Bilby *et al.*, 1996; Claeson *et al.*, 2006). The enrichment of epilithon  $\delta^{13}\text{C}$  (2.3‰), however, was similar to other studies in southeast Alaska (1.3–6.5‰; Kline *et al.*, 1990; Chaloner *et al.*, 2002) and Washington (0.0–3.4‰; Bilby *et al.*, 1996; Claeson *et al.*, 2006). Previous southeast Alaska studies were limited to one or two sites, and discrepancies in isotopic enrichment found between studies may reflect large regional variation. Such variation was evident in our study and the broader literature (Janetski *et al.*, 2009), but is not captured by the sampling design of most studies. Isotopic variation might be expected for epilithon, given the potential diversity of organisms present in biofilm as compared with higher trophic levels such as fish.

Juvenile coho also became isotopically enriched in the presence of salmon spawners in most of our study



**Fig. 4** The  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for epilithon (a, b) and juvenile coho salmon (c, d), before (white) and during (grey) salmon run, as modelled by bootstrapping with 1000 iterations. Each box represents the median (horizontal line) and the 75% (box), 90% (whisker) and 95% (dots) confidence intervals.



**Fig. 5** The change in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for epilithon (a, b) and juvenile coho salmon (c, d), as modelled by bootstrapping with 1000 iterations. Each box represents the median (horizontal line) and the 75% (box), 90% (whisker) and 95% (dots) confidence intervals.



streams. However, the overall enrichment of juvenile coho  $\delta^{15}\text{N}$  (0.7‰) was lower than in previous studies in southeast Alaska (2.0–2.6‰; Kline *et al.*, 1990; Chaloner *et al.*, 2002; Graham, 2006) or Washington (2.8–3.5‰; Bilby *et al.*, 1996, 1998). Similarly, enrichment of juvenile coho  $\delta^{13}\text{C}$  (1.4‰) was lower than in previous studies (i.e. 2.1–4.5‰ from the same studies as above). Closer inspection of data from previous studies reveals potential for much larger variation (e.g. <1‰ to >5‰ for  $\delta^{15}\text{N}$  in Bilby *et al.*, 2001). Such variation among studies, as well as between trophic levels and chemical elements, could provide insights into the mechanisms underpinning the ecological effects of spawning salmon on stream ecosystems.

Overall, isotopic composition of epilithon and juvenile coho was broadly consistent with previous studies and reflected the putative trophic level [epilithon ( $\delta^{13}\text{C}$  of –30‰ and  $\delta^{15}\text{N}$  of 3‰) and juvenile coho ( $\delta^{13}\text{C}$  of –24‰ and  $\delta^{15}\text{N}$  of 11‰)]. However, epilithon was more enriched than juvenile coho, and C was more enriched than N. Previous studies of two or more trophic levels (Kline *et al.*, 1990; Bilby *et al.*, 1996; Chaloner *et al.*, 2002; Graham, 2006) found that higher trophic levels underwent more consistent enrichment than lower trophic levels. Similarly, in these and other studies (Bilby *et al.*, 1998; Reichert *et al.*, 2008), epilithon showed similar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  enrichment, whereas juvenile coho exhibited greater  $\delta^{15}\text{N}$  enrichment relative to  $\delta^{13}\text{C}$ .

Our results provide insights into the pathways of uptake and incorporation of salmon-derived nutrients in stream ecosystems (Kline *et al.*, 1990; Bilby *et al.*, 1996). In the indirect pathway, salmon-derived nutrients are either mineralised through excretion by live fish, or leached from carcasses and eggs as decomposition products. Inorganic nutrients and dissolved organic material then enter the food web through assimilation by epilithon constituents. In the direct pathway, consumers assimilate nutrients from ingested tissue and eggs. In our study, both epilithon and juvenile coho displayed an increased  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , suggesting the involvement of both pathways (cf. Kline *et al.*, 1990; Bilby *et al.*, 1996). However, making absolute inferences about the relative importance of different pathways is difficult because of the complexities and uncertainties of isotope biogeochemistry (Fry, 2006). For example, physical processes such as water velocity can alter  $\delta^{13}\text{C}$  (Finlay *et al.*, 1999). However, changes in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in stream reaches lacking salmon (Kline *et al.*, 1990; Claeson *et al.*, 2006; Graham, 2006) suggest that non-salmon isotopic fractionation probably explains only a portion of the increase evident in salmon reaches. Furthermore, increased epilithon  $\delta^{13}\text{C}$  is consistent with responses to salmon tissue used in nutrient limitation

experiments, suggesting that heterotrophs are important for the assimilation of salmon nutrients (Rüegg *et al.*, 2011).

#### *Environmental controls on utilisation of salmon-derived material*

We found that several environmental factors influenced the uptake and incorporation of salmon-derived nutrients by epilithon. Salmon-derived N uptake by epilithon increased with temperature and decreased with the proportion of the catchment harvested of timber. Increased water temperature can stimulate epilithon metabolism, which in turn increases N assimilation (Reay *et al.*, 1999) and thus demand for salmon-derived N. Timber harvest could have decreased N enrichment of epilithon through altered stream geomorphology, especially reduced sediment size (Platts *et al.*, 1989), which increases susceptibility to spawner-mediated disturbance (Tiegs *et al.*, 2008). Although sediment size has negatively predicted the absolute value of epilithon  $\delta^{15}\text{N}$  during a salmon run (cf. Holtgrieve *et al.*, 2010), sediment size does not necessarily predict the magnitude of change in response to salmon spawners (cf. Verspoor *et al.*, 2010). Removing timber from catchments has multiple environmental effects, many of which were not explicitly analysed in our study. For example, timber harvest has been shown to increase the likelihood of bed-scouring events (Tonina *et al.*, 2008), which would increase the impact of disturbance by high discharge events on stream epilithon in catchments with more timber harvest. Future mechanistic studies may reveal more direct impacts of timber harvest on the ecological effects of salmon. In our study, change in epilithon  $\delta^{13}\text{C}$  was explained by differences in sediment size, timber harvest, discharge and spawner densities of the current run, suggesting that C enrichment of epilithon is driven by the availability of salmon-derived material.

Several studies have suggested that stream ecosystems can become saturated with salmon-derived material (Bilby *et al.*, 2001; Chaloner *et al.*, 2002). Beyond this 'saturation threshold', ecosystems no longer respond to further increases in spawner densities. We found that spawner density affected epilithon and juvenile coho salmon  $\delta^{13}\text{C}$  enrichment as well as epilithon  $\delta^{15}\text{N}$  during the run. Our study streams can exhibit nutrient limitation and a range of changes in dissolved nutrients in response to salmon spawners (Levi *et al.*, 2011; Rüegg *et al.*, 2011). Nutrient limitation may override other environmental factors, such as light or temperature, which might otherwise control the uptake of salmon-derived N or C. Although most of our study streams may have been below

the nutrient saturation threshold prior to the arrival of salmon, predicting alleviation of nutrient limitation by salmon is challenging (Rüegg *et al.*, 2011), and could vary from year to year as environmental conditions change (cf. Janetski *et al.*, 2009).

We found evidence of a spawner legacy effect for both trophic levels and both isotopes. The spawner run density in the previous year was included in the best model for before-salmon  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for both epilithon and juvenile coho, suggesting that salmon-derived material can be stored in, or returned to, the stream (Fellman *et al.*, 2008; Rex & Petticrew, 2008). Evidence of such a legacy effect has been found before. For example,  $\delta^{15}\text{N}$  of epilithon in multiple sites within a British Columbia catchment was best predicted by cumulative spawner abundance from the previous 4 years (Verspoor *et al.*, 2010). Additionally, macroinvertebrates exhibited higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in a spawning section of a stream relative to an upstream control reach prior to salmon spawners arriving in the stream (Graham, 2006). Interannual environmental variation may alter background  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of biota in ecosystems receiving salmon-derived material (Satterfield & Finney, 2002), but such a consistent legacy effect suggests that spawner-derived material may remain in, or return to, a stream over a longer period than previously suspected (this study; Fellman *et al.*, 2008; Rex & Petticrew, 2008). The mechanisms underlying the importance of legacy effects remain unclear but probably include the retention, immobilisation and storage of salmon-derived material.

Salmon-derived material could be retained or stored within a stream through many different processes. One potential mechanism is flocculation, which has been shown to be an important process for transformation and retention of salmon-derived N and C within streams (Rex & Petticrew, 2008). Such flocculated material could be resuspended during high flow events or disturbance associated with salmon redd construction. Another potential mechanism is deposition of salmon-derived material on to the floodplain, and subsequent re-entry of isotopically enriched N and C into the main channel during high discharge (Fellman *et al.*, 2008). Although such legacy effects have been suggested before (Graham, 2006; Verspoor *et al.*, 2010), further study is needed to determine the extent and mechanisms associated with this process.

#### *Studying the ecological effects of salmon spawners*

Ecosystem variability is an important challenge to understanding the ecological effects of salmon. For example, temperature and discharge, which both influenced isotopic composition, can vary among reaches, streams and ecore-

gions, but also among seasons and years. Indeed, although our seven study streams were separated by <50 km, we found as much variation as more widely dispersed studies (e.g. >200 km in Reichert *et al.*, 2008; >90 km in Verspoor *et al.*, 2010). One explanation for differences among and within studies is variation in the physical, biological and chemical environmental context, especially associated with variable run timing across the native salmon range. For example, in southeast Alaska, salmon runs peak in August and September (Kline *et al.*, 1990; Chaloner *et al.*, 2002), whereas in Washington runs peak in late November (Bilby *et al.*, 1996, 1998). Variability in conditions can influence epilithon responses to salmon spawners (Rüegg *et al.*, 2012) and could make broader generalisations difficult across the native range of salmon. However, such variability could be taken into account through combined temporal and spatial controls (Janetski *et al.*, 2009).

Use of controlled experiments in laboratory or field mesocosms (cf. Chaloner *et al.*, 2002) should be encouraged to provide a mechanistic understanding of salmon effects, reflecting a similar call made for stable isotope laboratory experiments in general (Gannes, O'Brien & Martínez del Río, 1997; Martínez del Río *et al.*, 2009). Such controlled experiments are needed because of the complexities of isotope biogeochemistry and the logistical challenge of finding comparable streams to study. Experiments could establish which pathways of uptake and incorporation have primacy, and whether such primacy shifts with changes in environmental conditions over space and time. For example, seasonally low temperatures could reduce the digestive efficiency of juvenile coho, causing starvation with implications for isotopic composition (Gannes *et al.*, 1997; Martínez del Río *et al.*, 2009).

One further approach for evaluating the use of stable isotopes in ecology, especially the role of variability, is conditional bootstrap modelling (Rüegg *et al.*, 2012). Our use of conditional bootstrap modelling underscores the importance of spatial scale in salmon studies, especially as scale may influence the interpretation of a study. For example, our modelling suggests that if only one stream had been studied, we may not have found evidence for isotopic enrichment (i.e. the 95% CI of isotopic composition before and during salmon overlapped if only one stream was used in the model). Although two to three streams appeared sufficient to capture most of the variation within the region we studied, further research is needed to establish this for both intra- and inter-regional variability. For example, a minimum of seven streams were needed to establish the response of epilithon to spawning salmon over a 1-year study within one region (Rüegg *et al.*, 2012). Conditional bootstrap modelling may

facilitate inter-regional comparisons and allow broader generalisations about the ecological effects of salmon by fully characterising the inherent variability present in salmon studies. A fuller picture of variability is important because, for example, our study was undertaken on an island where the close proximity of study streams probably meant less variability than on the mainland or over the native range of salmon. Our study results should be considered conservative, and generalising beyond our study area should be done with caution. However, our results demonstrate the potential of such modelling for interpreting isotope studies, especially given the complexity of isotope biogeochemistry.

Recent isotopic studies of epilithon and fish have considered only N (Reichert *et al.*, 2008; Holtgrieve *et al.*, 2010). This perhaps reflects the more complicated sample preparation for C (i.e. the need to acidify or lipid-extract samples), or that salmon-mediated changes in dissolved C have rarely been observed (reviewed by Janetski *et al.*, 2009). However,  $\delta^{13}\text{C}$  was informative to our study, providing further evidence for the potential legacy effect observed before the salmon run. Therefore, both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  should be studied in combination to provide a clearer picture of the ecological effects of spawning salmon on stream ecosystems.

In conclusion, we found evidence from N and C stable isotopes that uptake and incorporation of salmon-derived material by stream biota was discernible, but varied among streams and trophic levels. Such variation reflected contrasts in environmental factors that influence epilithon (stream temperature, timber harvest) and juvenile coho salmon (discharge and spawner densities). Enrichment was more pronounced lower in the food web (i.e. epilithon) than higher in the food web (i.e. stream-resident fish). However, no single factor within a year appeared to drive isotopic enrichment, either for a given trophic level or across streams. We found evidence that the size of the salmon run in one year can influence the isotopic signature of stream-resident biota prior to the salmon run in the following year, suggesting a legacy effect that has eluded previous studies. Identifying important temporal and spatial factors in the incorporation of salmon-derived nutrients is critical for understanding the effect that salmon can have on freshwater ecosystems.

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